



Research Paper

Evaluating membrane bioreactor treatment for the elimination of emerging contaminants using different analytical methods

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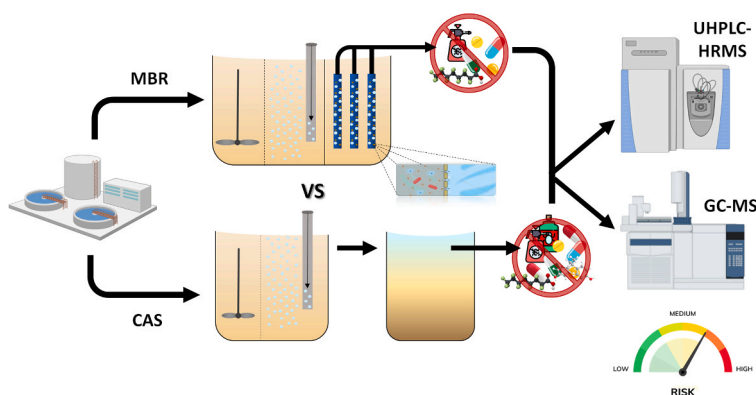
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HIGHLIGHTS

- Suspect screening was useful to identify > 180 CECs in both analysed effluents.
- GC-MS target methodology was suitable to extend the analytical coverage to SVOCs.
- MBR-based treatment showed slightly enhanced removal efficiencies than CAS.
- Both treatments showed similar environmental risk of the non-eliminated compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Since wastewater treatment plants (WWTPs) were not originally designed to eliminate contaminants of emerging concern (CECs), alternative strategies like membrane bioreactor (MBR) technology are gaining importance in achieving effective CEC removal and minimising their environmental impact. In this study, composite wastewater samples were collected from the biggest WWTP in the Basque Country (Galindo, Biscay) and the performance of two secondary treatments (i.e. conventional activated sludge treatment, CAS, and MBR) was assessed. The combination of a suspect screening approach using liquid chromatography tandem high-resolution mass spectrometry (LC-HRMS) and multitarget analysis by gas chromatography-mass spectrometry (GC-MS) allowed the detection of approximately 200 compounds in the WWTP effluents. The estimated removal efficiencies (REs) revealed that only 16 micropollutants exhibited enhanced removal by MBR treatment (RE > 70% or 40 – 60%). The environmental risk posed by the non-eliminated compounds after both treatments remained similar, being anthracene, clarithromycin, bis(2-ethylhexyl) phthalate (DEHP) and dilantin the most concerning pollutants (RQ > 1). The Microtox® bioassay confirmed the MBR's efficiency in removing baseline toxicity, while suggesting a

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similar performance of CAS treatment. These minimal differences between treatments call into question the worthiness of MBR treatment and emphasise the need to seek more efficient alternative treatment methods.

1. Introduction

Population growth has triggered a chain reaction that has increased the production and use of chemicals that, in most cases, end up in the environment. Among those chemicals, the group of so-called contaminants of emerging concern (CECs) deserves special attention in many research studies as the risks CECs could pose in the environment are still unknown [25,37,43]. Pharmaceuticals and personal care products (PPCPs), pesticides, plasticisers and industrial chemicals are, to name a few, some of the chemicals considered as CECs [39]. All those compounds are commonly used in anthropogenic activities and end up in wastewaters [25,35,37,38].

Many efforts are made in wastewater treatment plants (WWTPs) in order to eliminate all CECs and, thereby, minimise their impact on the environment. However, conventional treatments (e.g. conventional activated sludge systems, CAS) implemented in WWTPs were not initially designed to eliminate those compounds and thus, myriads of compounds are continuously released into the environment reaching aquatic ecosystems [25,35,38,42]. To mitigate or minimise the discharge of CECs, various cutting-edge technologies with proven elimination efficiency, such as chlorination, ozonation or UV radiation processes, have recently emerged [4,48]. However, all those mentioned treatments may give rise to the formation of transformation products (TPs) that can exhibit equivalent or even higher levels of toxicity compared to the original compounds [25]. As an alternative, several hybrid elimination techniques combining biological and physicochemical methods have come to the forefront (i.e., constructed wetlands, membrane bioreactors or hybrid reactor systems) [29]. Among them, membrane bioreactor (MBR) technology is gaining popularity, especially in urban wastewater treatment. The appeal of biological membranes, often referred to as “green membranes”, stems from their more eco-friendly nature. Membrane-based technologies entail a reduced environmental footprint, primarily as they are typically derived from renewable materials [45]. However, it is worth noting that this technology was not initially designed for CEC removal. This technology, used as a secondary treatment of wastewater, is a biological process using activated sludge, based on the use of ultrafiltration membranes to separate the active sludge from the residual water [46,47,6]. The sieving effect of the membrane results in the retention of pollutants with a molecular weight exceeding the membranes’ molecular weight cut-off, which improves the interaction efficiency between chemicals and microorganisms [29]. The MBR system offers some benefits over CAS such as a higher solid retention time (SRT), a reduced hydraulic retention time (HRT), minimal space requirements, and efficient sludge removal from the wastewater [47]. Consequently, treated effluents exhibit enhanced quality in terms of several physicochemical parameters (e.g. biochemical oxygen demand (BOD), total suspended solids (TSS) content or turbidity) [46,47,7] and bacteriological contamination (E-coli) [6].

The characteristics of wastewater treated using MBR technology make it feasible to directly use such effluents in some applications without any further treatment. This stands in contrast to effluents treated with conventional biological treatments, which require further treatments for disinfection and TSS content reduction [6]. As a limitation, MBR treatment involves a higher energy demand, higher cleaning and membrane replacement costs and susceptibility to long-term performance issues arising from biofouling. In order to overcome those problems and improve the removal efficiency, several integrated MBR systems and hybrid processes have been combined. These novel technologies encompass advanced oxidation process (AOP), granular MBRs [29], reverse osmosis [22], forward osmosis [11] and electro-osmosis

membrane systems [33], or MBR/Photocatalyst couplings [16,44], among others.

Despite the superiority of the MBR technology in improving the physicochemical parameters of wastewater, there is not much information in the literature regarding the efficiency of MBR technology for CECs removal. The observations found in some research works point out the suitability of MBR technology for the elimination of certain pharmaceuticals (e.g. acetaminophen, bezafibrate, enalapril, gemfibrozil, ketoprofen, naproxen, sulfamethoxazole) [30,46,52,8], natural hormones (e.g. estriol, progesterone, testosterone) [30,8], plasticisers (e.g. bisphenol A, nonylphenol) [52] or musk fragrances (e.g. galaxolide, tonalide) [8]. The efficiency of MBR technology was also proven in some effect-based studies [12,7]. However, the evaluation of the efficiency of wastewater treatments is typically limited to the targeted monitoring of pre-selected compounds and thus, unknown compounds that may occur in wastewater remain overlooked. In order to address this gap, suspect and/or non-targeted analysis using high-resolution mass spectrometry enables the determination of a larger amount of compounds present in wastewater samples providing a closer view of the risk for the environment.

Within this context, the objective of this study was to assess the efficiency of the alternative secondary wastewater treatment based on MBR membranes for the elimination of CECs and to compare it with the efficiency rendered by a CAS system. Specifically, the study involved the analysis of CECs within effluent samples of a WWTP located in the Basque Country (Galindo, Biscay). The identification of polar micropollutants was accomplished through liquid chromatography tandem high-resolution mass spectrometry (LC-HRMS), using both suspect screening and subsequent confirmation through a validated target analysis. Multi-targeted analysis was also performed using gas chromatography-mass spectrometry (GC-MS) in order to determine semi-volatile organic compounds (SVOCs). The efficiency of both secondary treatments was evaluated in terms of the removal of micropollutants and the potential toxicity associated with the compounds determined in both effluents. Moreover, an experimental assessment of the baseline toxicity of the compounds present in MBR effluent was conducted using the Microtox® toxicity bioassay.

2. Experimental section

2.1. Reagents and materials

All chemicals and laboratory materials used in this work are compiled in section S1 and Tables S1 and S2 of the Supporting Information (SI).

2.2. Sampling and effluents characterisation

The biggest and the most important WWTP in the Basque Country, Spain, with a classical CAS treatment process and a pilot MBR treatment was selected as a representative WWTP facility in the present study. Composite effluent samples (24 h) were collected during 9 weeks (February-May 2021) in the Galindo WWTP located in Sestao, Biscay, Spain, for the analysis of polar contaminants. In parallel, 21 samples were collected between March 4th and April 29th (n = 7 per treatment) for the analysis of non-polar SVOCs. Three different effluents were sampled during the whole sampling period: (i) effluents corresponding to a primary treatment of wastewater (Prim); (ii) effluents corresponding to a secondary treatment based on a CAS treatment, and; (iii) effluents corresponding to a secondary treatment based on MBR (see detailed information in Section S2 in SI). All samples were stored and

frozen at $-20\text{ }^{\circ}\text{C}$ until their analysis.

Physicochemical parameters data of the effluents was provided by the WWTP laboratory (see detailed information in Section S3 in SI).

2.3. Sample treatment

Water samples were thawed and, once at room temperature, were filtered through cellulose filters ($0.7\text{ }\mu\text{m}$, 90 mm, Whatman; Maidstone, UK). Representative aliquots of 250 mL of the three different effluents were treated in triplicate according to a previously validated method in our research group with slight modifications [28,41] (see details in Section S2 in SI).

2.4. Analysis of compounds by UHPLC-q-Orbitrap

2.4.1. Data acquisition

The analysis of polar compounds was carried out on a Thermo Scientific Dionex Ulti-Mate 3000 UHPLC coupled to a Thermo Scientific Q Exactive Focus quadrupole-Orbitrap mass spectrometer (UHPLC-q-Orbitrap) equipped with a heated electrospray ionisation source (HESI, Thermo-Fisher Scientific, CA, USA). The chromatographic separation and acquisition methods were based on previously developed methods [28,37] detailed in Sections S4–1 in SI.

2.4.2. Data treatment: Suspect analysis

The workflow applied using the Compound Discoverer 3.2 (Thermo-Fisher Scientific) for suspect analysis data treatment was previously reported by González-Gaya et al. [28] (see detailed information in SI). Only Lorentzian peaks were considered. The SusDat NORMAN database (40059 compounds, www.norman-network.net) was used as a suspect list with a fixed error lower than ± 5 ppm in the exact mass. The molecular formulas suggested by the software were only accounted if MS1 was satisfactorily matched (SFit > 30% and isotopic profile > 70%). Minimum peak areas considered were set at 10^6 units for both negative and positive ionisation modes. Additionally, only peaks with sample-to-blank area ratio higher than 10 and chromatographic peak areas with a relative standard deviation (RSD %) lower than 30% within injection replicates ($n = 3$) were further studied. MS2 spectra were compared with mzCloud database (<https://www.mzcloud.org/>), and a match of over 70% was set for the positive identification of the feature. When the standards of the candidates were available, experimental retention time was confirmed with an allowed error of ± 0.1 min. If standards were not available in the laboratory, retention times were estimated from the Retention Time Index (RTI) platform (<http://rti.chem.uoa.gr/>) and candidates were rejected or accepted depending on the statistical difference with the estimated value taking into account the uncertainty of the model built. Finally, features were annotated according to Schymanski and coworkers [51]. Although this scale is numbered from one to five, in this work we annotated compounds up to level 3. Level 1 of identification indicates the highest confidence level, involving features confirmed with standards, whereas level 3 represents the lowest confidence level, encompassing features identified as potential candidates with a known structure, but belonging to a group of isomers.

2.4.3. Data treatment: Multitarget analysis

Quantitative data analysis of the suspects annotated as level 1 was performed using the Tracefinder 4.2 software (Thermo-Fisher Scientific). Target compounds and their instrumental characteristics including molecular formula, ionization mode, retention time (tR) and experimental MS/MS fragments were added to the software library according to studies previously performed by the research group [37]. To avoid false positives, the experimental retention time window was limited to 60 s around the retention time of the pure standard, a mass error equal to or less than 5 ppm, isotopic profile matching at more than 70% and mass accuracy for fragments equal to or less than 5 ppm were considered.

2.5. Analysis of compounds by GC-MS

2.5.1. Data acquisition

The analysis of non-polar SVOCs was performed by means of GC-MS using an Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The chromatographic separation and acquisition parameters were established based on a previously developed method [41,55] (see SI, Sections S4–2).

2.5.2. Data treatment: Multitarget analysis

The MassHunter Qualitative software (v10.0) and MassHunter Quantitative software (v.10.0) (Agilent Technologies) were used for the automatic integration and quantification of the chromatographic peaks.

2.6. Assessment of secondary treatments' efficiency: CAS vs MBR

The efficiency of both secondary treatments was determined in terms of: (i) removal efficiency of detected compounds (RE, %) and, (ii) potential toxicity of the compounds still present in the effluent samples gathered after the assessed treatments.

RE (%) of individual micropollutants was estimated considering the concentrations of compounds in the primary and secondary effluents [26,34,38] (see Eq. 1).

$$RE_i \text{ (\%)} = \left(\frac{[i]_{Prim} - [i]_{effluent}}{[i]_{Prim}} \right) \times 100 \quad (1)$$

In the case of the compounds tentatively identified as level 2a [51], a pseudo-quantitative calculation of RE was done using chromatographic peak area instead of concentration [18] (see Eq. 2).

$$RE_i \text{ (\%)} = \frac{Peak \text{ Area, } i_{Prim} - Peak \text{ Area, } i_{Effluent}}{Peak \text{ Area, } i_{Prim}} \times 100 \quad (2)$$

The comparison of concentrations or peak areas of compounds in treated and non-treated samples was done using an independent two sample t-test. The RE % values were only determined for compounds with concentration/areas statistically different at a 95% confidence level (i.e., p-value < 0.05). Additionally, compounds that were not detected above the LOQs were neither considered for efficiency comparison between both technologies to avoid overestimation of risks. Similarly, compounds present at levels < LOQs in the non-treated samples were also excluded to avoid biased calculated values.

For those contaminants with RE > 90% (RSD values $\leq 10\%$) potential transformation products (TPs) were addressed with *in-silico* predictions based on BioTransformer 3.0. Phase I, environmental microbial and abiotic transformation pathways [13] were considered to build the suspect list as described by Alvarez-Mora et al. [3].

Risk quotients (RQs) approach was followed to determine the toxicity potential of the CECs present in both secondary effluents. RQs for each compound were calculated according to the European Union technical Guidance Document as the ratio of the measured environmental concentration (MEC) in samples and their respective predicted no-effect concentration (PNEC) [21]. 95th percentiles of the measured concentrations among different days for each compound were used as MEC values to avoid risk overestimation. The PNEC values were estimated as described by Lopez-Herguedas et al. [37,38] (see details in section S7 in SI).

Toxicity potential of the compounds with a confidence level of 2a was evaluated by calculating their respective ToxPi score based on Feng and collaborators with slight modifications [23] (see Eq. 3).

$$ToxPi \text{ score}_i = \frac{R_i - R_{min}}{R_{max} - R_{min}} + \frac{T_i - T_{min}}{T_{max} - T_{min}} \quad (3)$$

Where, R and T represent logarithmically transformed values for peak areas and reciprocal lowest PNEC ($\log(1/\text{lowest PNEC})$), respectively.

In this case, the 95th percentiles of the detected peak areas among the different days were considered. Endogenous compounds were excluded for the estimation of toxicity potential. Moreover, only tentative candidates detected at a detection frequency larger than 75% in the studied effluent samples were considered for environmental risk assessment.

2.6.1. Microtox® toxicity bioassay

The Microtox assay is based on the measurement of the bioluminescence inhibition of the marine bacteria *Vibrio fischeri* which could be caused by different toxicants [31]. In this case, the toxicity bioassay was performed to experimentally assess the efficiency of the MBR technology for toxicity removal. Acute toxicity of compounds annotated as level 1 and found in MBR effluent samples was determined considering the 95th percentiles of the quantified compounds. Acute toxicity was determined for compounds with RQ values exceeding 0.1, or for compounds with RQ values below 0.1 when calculated with predicted ecotoxicological data. Environmentally non-relevant compounds (RQ < 0.1) not found in any secondary effluents (i.e. CAS and MBR) or detected at low concentrations (< 10 ng/L), were excluded from the analysis. Toxicity assessments of individual compounds were performed using a Microtox® M500 Analyzer (Microbics Corporation, USA) as described by Rodríguez-Rodríguez et al. [49] (see details in Sections S6–1 in SI). Briefly, after adjusting osmolality of each solution or sample to 2% of salinity, the percentage of bioluminescence inhibition was determined by the comparison between a saline control and the diluted sample after an exposure time of 15 min. Saline diluents (NaCl, 2%) and zinc sulphate (0 – 25 mg/L, EC₅₀ = 3 – 5 mg/L) were used as negative and positive controls, respectively. Experiments were performed in triplicate and samples with a mean inhibition value not exceeding 20% were considered non-toxic [14,5].

3. Results and discussion

3.1. Suspect screening by means of UHPLC-q-Orbitrap

The workflow applied for the suspect analysis (described in Section 2.4.2) enabled the identification of 184 compounds annotated at confidence levels 1–3. Detailed information on those compounds, their occurrence (expressed as detection frequency, %) and the minimum, maximum and mean areas in the different analysed treatments (i.e. primary, MBR and CAS) is given in Table S3. Based on the predefined identification criteria, overall, upon confirmation with reference standards, 82 compounds (level 1) were quantified in total. Additionally, 92 candidates were tentatively identified as probable structures (level 2a), and 10 compounds were designated as tentative candidates (level 3). The majority of compounds, comprising over 90% of the total, were consistently detected in effluents from primary and both secondary treatments, exhibiting a similar detection frequency (> 90%). However, concretely, 17 compounds were no longer found after MBR and CAS treatments, as indicated by their occurrence falling below 30% in secondary effluents. Particularly noteworthy among these compounds were the pharmaceuticals acetaminophen and gabapentin (level 1), rosuvastatin and varenicline (level 2a), the pesticide spiroxamine (level 1), the plasticisers bisphenol A and bisphenol S (level 1), and the illicit drug cocaine (level 2a).

3.1.1. Quantification of the suspects annotated as level 1 by UHPLC-q-Orbitrap

A total of 96 compounds including PPCPs, pesticides, industrial agents, phthalates and hormones were quantified in all the analysed effluents: 91 in Prim, 88 in CAS and 81 in MBR (see detailed compound list and concentration values expressed in ng/L in Table S4 in SI). Additionally, fourteen compounds that were not initially detected using suspect analysis workflow were quantified using target analysis in wastewater effluents (see Table S4 in SI).

Several compounds found in the wastewater effluents are included in

the current Watch List proposed by WFD for water quality monitoring [19,27]. Among those compounds, pharmaceuticals like metformin, diclofenac or fluconazole stand out due to their high concentration levels and detection frequencies, underscoring their widespread use in everyday life and thus, constituting a continuous environmental threat.

Overall, the concentration of the compounds was notably higher in the primary effluent samples compared to those found in secondary effluent samples (i.e. CAS and MBR). Concretely, the widely used over-the-counter drugs acetaminophen or naproxen, commonly used to treat headaches or relieve fever, were found at 89,504 and 17,312 ng/L (mean concentration values), respectively. In line with other studies, the stimulant caffeine was found at concentrations as high as 76,087 ng/L, with nearly 100% detection frequency in the primary effluent [10,17,38]. Although it was not detected in any of the secondary effluents, the low fluctuation in the concentrations found in the primary effluent point to daily consumption patterns of the population of caffeine-containing products such as coffee or soft drinks. In addition, high concentrations of plasticisers that are ubiquitously found in commonly used products or materials such as BPA (9979 ng/L) or caprolactam (29,248 ng/L) were also detected.

3.2. Multitarget analysis by GC-MS

Multitargeted GC-MS analysis revealed the presence of 15 compounds in the analysed samples (see Table S5 in SI). These chemicals encompass PAHs including acenaphthylene and phenanthrene, musk fragrances such as cashmeran and traseolide, pesticides like lindane, the phthalate bis 2-ethylhexyl phthalate (DEHP), which is already included in WFD priority list, and the plastic additive bis(2-ethylhexyl)adipate (DEHA). Several compounds detected in this study have already been regulated, particularly those categorised as carcinogenic compounds (category 1B), including benz[*a*]anthracene, benzo[*a*]pyrene or naphthalene [21]. Furthermore, some others have been included in priority lists for hazardous substances like anthracene, DEHP or hexachlorocyclohexane [20].

Prominently, compounds with the highest concentration levels and frequency of detection in the primary effluent, observed in 100% of the samples, were the pesticide lindane and its analogue beta-hexachlorocyclohexane (β -HCH) (mean concentrations of 1906 and 18,097 ng/L, respectively), the plasticizer DEHP (mean concentration of 1679 ng/L), and the PAH phenanthrene (mean concentration of 1612 ng/L). It should be noted that, although a decrease in the concentration of contaminants can be observed, the presence of these aforementioned compounds remained ubiquitous in all the analysed samples (i.e. frequency of detection of 100% in both CAS and MBR effluents).

The Monitoring Report of the Hydrological Plan of the Eastern Cantabrian Hydrological District showed annual exceedances of quality standards for DEHP, lindane and naphthalene (detected in this study) from 2014 to 2017 [1]. In most cases, the detection of such compounds in water was identified as an isolated phenomenon. However, the 2020 Basque Water Agency (URA) report indicated chronic lindane presence in the Ibaizabal estuary (where Galindo WWTP is located), potentially linked to historical waste disposal by local companies [2]. Moreover, the minimum levels for lindane and its main by-product β -HCH detected in this study (0.622 and 4.22 μ g/L in the CAS effluent, respectively and 0.23 and 4.2 μ g/L in the MBR effluent, respectively) exceeded the defined environmental quality standards for inland surface waters (maximum admissible concentration and annual average of 0.02 and 0.04 μ g/L, respectively) [9]. Although lindane levels seem to be reduced through the MBR system (mean concentration of 0.5 μ g/L) compared with CAS (mean concentration of 3 μ g/L), future sampling campaigns are needed to ensure whether the potential risks posed by lindane are gradually diminishing.

3.3. Secondary treatments comparison

3.3.1. Removal efficiency

According to the criteria specified in Section 2.6, the RE was determined for approximately 160 compounds analysed by UHPLC-q-Orbitrap and GC-MS (see Table S6). Based on the calculations, the majority of the detected compounds, approximately 70% of the total, exhibited poor REs (i.e. RE < 40%). These results are in line with other worldwide studies [18,26,32,38]. Furthermore, the poor removal of some CECs (e.g. carbendazim, clarithromycin, DEP, terbutryn or triphenylphosphate) in CAS effluents samples was also observed in a previous study of the research group [38]. All these results reinforce the need of reaching a contamination reduction at source, which may involve a rational drug usage or the ban of certain non-biodegradable products, as a first option before the application of effluent treatments.

In the present work, the efficiency of the alternative MBR-based secondary treatment and the comparison with the conventional one implemented (i.e. CAS) was also assessed. In this sense, 29 of the 160 total compounds detected in primary effluent were largely removed (RE > 70%) by both CAS and MBR treatments, while 6 other compounds were partially removed (REs between 40% and 70%). Fig. 1 shows the cumulative concentrations of the compounds detected in the analysed effluents classified by families. A clear trend of elimination of the compounds is observed after both secondary treatments, with analgesics, steroid hormones, preservatives, photosensitisers and antineoplastics standing out as the most concentrated ones and the most prone to be eliminated.

On the other hand, the MBR treatment achieved enhanced removal of 16 additional micropollutants, such as the pharmaceuticals atenolol and bezafibrate or the pesticides propamocarb and lindane. For 12 of those compounds, the elimination rates increased by nearly 100% (RE > 70%), while the REs for the remaining 4 compounds were between 40% and 60% (see Table S5 in SI). As can be seen in Fig. 1, there is a slight improvement in the elimination of certain families including stimulants, plasticisers, non-steroidal anti-inflammatories (NSAIDs), insecticides and steroid hormones, facilitated by the MBR system.

As reported in other studies, the removal of CECs is predominantly driven by hydrophobic interactions in both secondary treatments [29,30,52]. Neutral pH conditions enabled the elimination of compounds such as acetaminophen, caffeine, genistin or methylparaben, which exhibited removal rates exceeding 95%. This could be attributed to their hydrophobicity at pH 7.2 (see Fig. S1), resulting in heightened adsorption onto the sludge, and thus improved their RE. However, ionised compounds displayed varying REs. Regarding the positively charged compounds, CAS treatment failed to achieve effective removal, whereas MBR treatment unexpectedly favoured the elimination of certain compounds. Conversely, in the case of negatively charged compounds, CAS treatment seemed to be superior (see Fig. S1 and Table S6). Ionised compounds were expected to show low removal efficiency with MBR treatment due to the molecular charge leading to an enhanced repulsion between the microbial sludge and the membrane, hampering the adsorption process; however, highly varied RE values were observed, as reported in other studies [30].

On the other hand, the elevated biomass concentration may have facilitated the degradation rate of some micropollutants [29,30,47], including some pharmaceuticals [8], plasticisers [52] and hormones [30]. Moreover, the higher SRT and lower HRT associated with MBR systems may have further increased the degradation capacity of compounds [30,47].

Based on the RE results, MBR technology could be effective for the elimination of some compounds. Nevertheless, according to the literature, the repeatability of RE of the MBR technology is not assured [8]. This phenomenon could be attributed to variations in the presence of readily biodegradable organic matter in wastewater, that may influence the microbial degradation of certain compounds [15]. Additionally, possible reversible reactions of certain compounds and/or their metabolites that can occur during the treatment may lead to an increase in environmental levels [24]. Furthermore, enzymatic cleavage of glucuronide conjugates, as observed with compounds like carbamazepine, could also be the reason for the release of parent compounds into the environment [53].

Moreover, potential transformation/degradation products (see

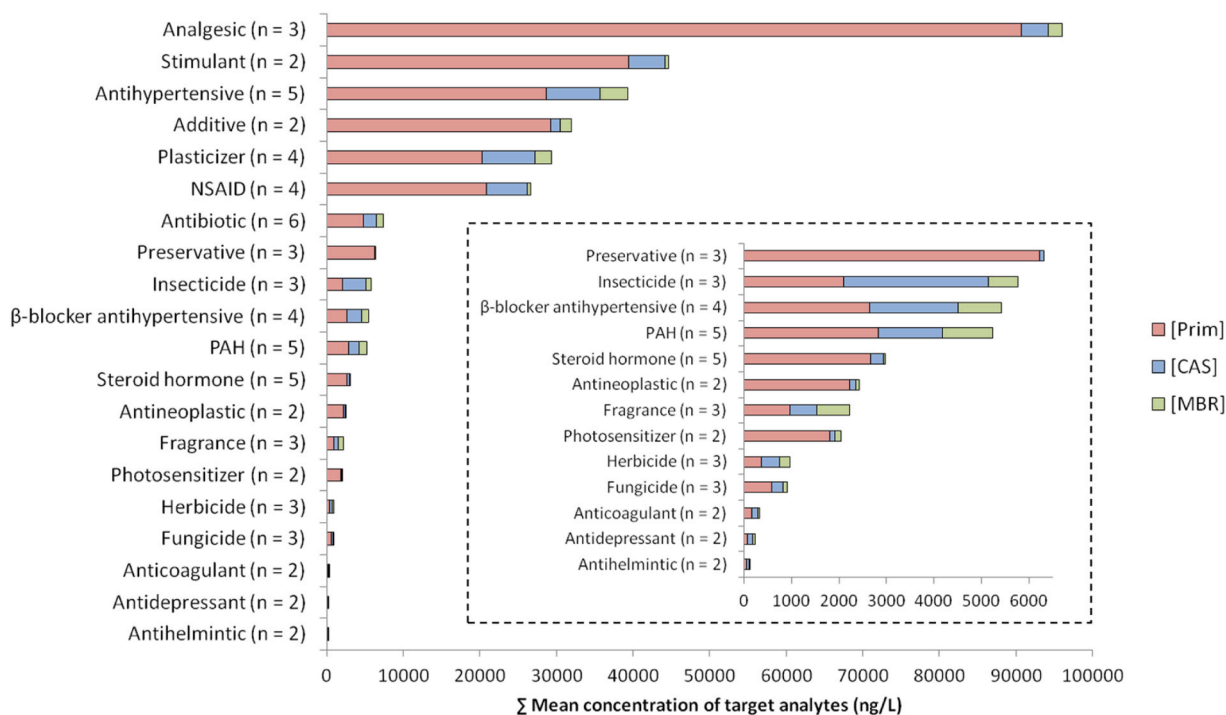


Fig. 1. Sum of mean concentrations (ng/L) of the compounds considered for the calculation of RE (%) classified by their family. Graph delimited by dotted lines includes sum concentrations of families in a zoomed scale. n represents the number of compounds included in each family.

Section 2.6) of the highly removed compounds by both treatments were analysed to ensure the achievement of their complete removal (see Table S6). Notably, none of the TPs predicted for those compounds (see Section 2.6) were detected through suspect analysis, suggesting the potential high efficiency of the evaluated secondary treatments in removing certain compounds.

Based on all the previous results and observations it is clear that the search for new solutions that enhance the removal of chemicals with different physicochemical properties is still required. To face this problem, several studies pointed out the benefits of coupling MBR and photocatalytic-based methods in order to improve the quality of treated wastewater [44,50] and to remove CECs like pesticides [36] or pharmaceuticals [40]. As an alternative, the use of nanomaterials as catalysts (i.e., TiO₂ nanotubes) in elimination processes is widely recommended [40]. Recent studies are moving towards the development of innovative photocatalysis technologies including the combination of TiO₂ with carbon quantum dots (CQDs) [54] or the substitution with WO₃ [50] with the main aim of improving REs of a wide variety of chemicals.

3.3.2. Toxicity potential

The potential toxicity of the most frequently detected compounds (n = 148, detection frequency > 75%) present in both secondary effluents was estimated to assess the potential environmental risk associated with these compounds that will eventually reach the environment (see Table S7). Concentrations of the compounds quantified by the targeted analysis were considered for the RQ calculation, whereas for tentatively identified compounds (level 2a) peak areas were used for the ToxPi calculation (Fig. 2). Based on the estimated individual RQs, overall, the levels of detected compounds, comprising over 80% of the total, in both effluents posed a negligible environmental risk (RQ < 0.1) (Fig. 2A, Table S8).

Individual RQs estimated for each treatment reflect that although in general, higher RQ values were determined for CAS effluents, both treatments share the list of compounds exceeding the threshold of 1. Notably, this list includes pharmaceuticals like clarithromycin and dilantin, the PAH anthracene and the plasticiser DEHP as the compounds of the highest concern (Fig. 3, Table S8). These results suggest the lack of efficiency of both treatments in reducing the environmental levels of specific toxicity drivers to a level that would not impact on the good ecological status. However, it should be noted that MBR treatment improved the removal of some compounds in particular. For instance, pharmaceuticals such as azithromycin, furosemide and telmisartan, as well as the pesticide lindane, exhibited a decreased potential toxicity

from high (RQ > 1) to moderate (0.1 < RQ < 1) levels through MBR treatment (see more details in Fig. S2 and Table S9 in the SI).

Additionally, when it comes to the calculated ToxPi scores, all the detected compounds presented lower values in the MBR effluent in comparison to those obtained in the CAS effluents, which suggests that MBR may be more suitable for mitigating the environmental risk of the anthropogenic-origin contaminants (Fig. 2B, Table S8). It is important to note, however, that the calculation of ToxPi relies on chromatographic peak areas, which may result in an over-/underestimation of the calculated risks. Therefore, the use of chemical standards would be necessary for the accurate quantification of the concentrations and thus make an appropriate comparison between the metrics RQ and ToxPi.

Concretely, ToxPi scores ranged from 1.06 (observed for the pharmaceutical abacavir, used in combination with other HIV medicines) to 2.04 (observed for the chelating agent edetol) in the CAS effluent samples. In contrast, values calculated for the MBR effluent samples did not exceed 1.63 (specifically, for the muscle relaxant methocarbamol). Among the compounds present in the CAS effluent, edetol and 4-formylaminoantipyrine (a TP of pyrazolones), and to a lesser extent pharmaceuticals like tapentadol (an opioid agonist with analgesic effect), cyproheptadine (antihistaminic), methocarbamol and venlafaxine (antidepressant), and the industrial chemical centralite, stood out as the most highly ranked compounds. On the other hand, although in MBR effluent methocarbamol remained one of the most concerning compounds, the ToxPi score was slightly lower.

Nevertheless, the lack of experimental toxicity data available leaves no other choice than using predicted values based on *in-silico* tools, which may lead to an over-/underestimation of the determined environmental risk.

3.3.3. Toxicity assessment

The Microtox® toxicity bioassay was performed to assess the baseline toxicity of various compounds detected in the MBR effluent samples, thereby ensuring the toxicity removal efficiency of the MBR treatment according to the criteria specified in Section 2.6.1. (see Table 1).

As can be observed in Fig. 4, all individual compounds tested yielded non-toxic measurements, with bioluminescence inhibition remaining below 20% (see Section 2.6.1). These observations indicate the absence of adverse effects under the experimental conditions. Among the tested compounds, the pharmaceutical diclofenac, the PAH anthracene and the plasticiser DEHP stood out as the ones inducing the highest bioluminescence inhibition.

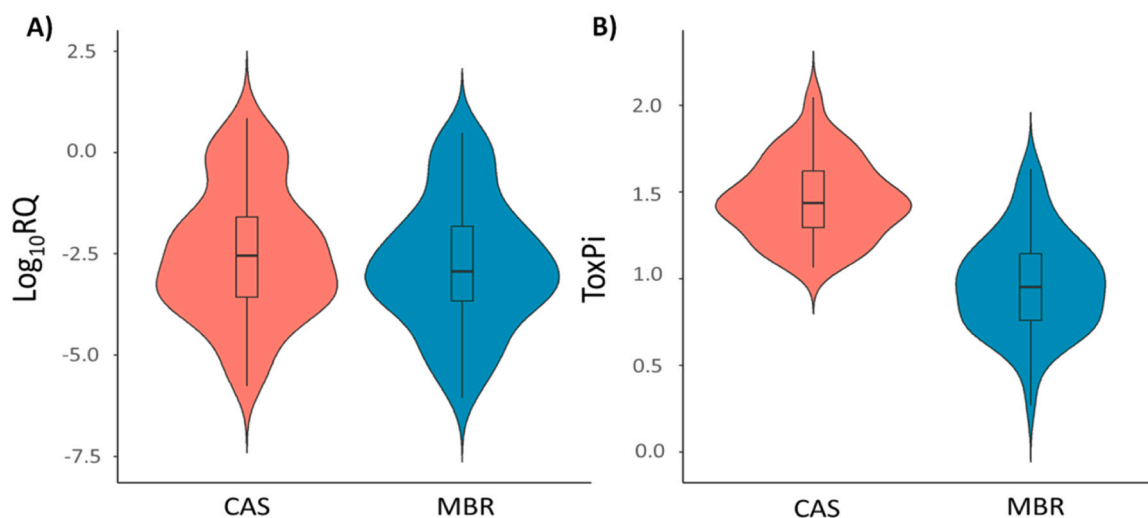


Fig. 2. Violin plots of the most frequently detected compounds representing their potential toxicity: A) RQ values calculated for confirmed compounds (level 1) in logarithmic scale; B) ToxPi values calculated for tentatively identified compounds (level 2a).

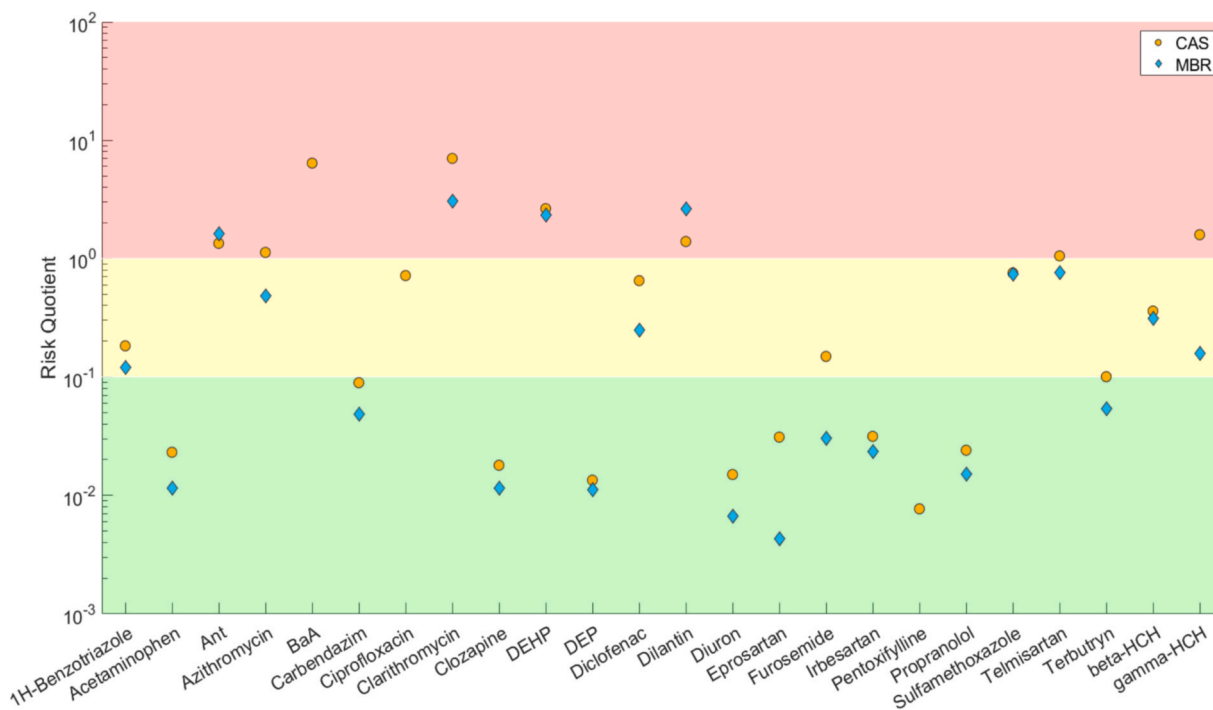


Fig. 3. Calculated RQs for detected compounds in both secondary effluents (i.e. CAS and MBR) by target analysis considering the 95th percentile of the detected concentration (only some compounds are shown as an example).

Table 1

RQ values in each of the studied effluents and the concentration levels tested (95th percentiles of the detected concentrations) of the compounds evaluated in the Microtox® toxicity bioassay. Values in green, yellow and red indicate negligible, moderate and high environmental risks, respectively.

Compounds	Abbreviation	Solvent	RQ		Conc. Tested (ng/L)
			CAS	MBR	
1H-Benzotriazole		MeOH	0.182805	0.119555	203
2-Hydroxybenzothiazole	OBT	MeOH	0.001020	0.000277	4
Azithromycin		AcN	1.126985	0.482776	9
Caprolactam		MeOH	0.000299	0.000232	59
Clarithromycin		DMSO	7.022555	3.053805	6
Diclofenac		MeOH	0.650502	0.249888	11
Dilantin		DMSO	1.392956	2.646919	14
Efavirenz		MeOH	0.000823	0.000216	3
Eprosartan		MeOH	0.031049	0.004323	5
Gabapentin		MeOH	0.000002	0.000001	22
Hydrochlorothiazide		MeOH	0.000009	0.000007	12
Irbesartan		MeOH	0.031490	0.023379	42
Lorazepam		MeOH	0.000213	0.000183	12
Losartan		MeOH	0.006450	0.001153	3
Primidone		MeOH	0.000221	0.000155	14
Sulfamethoxazole		AcN	0.754807	0.743348	20
Telmisartan		MeOH	1.052779	0.758243	61
Terbutryn		MeOH	0.100388	0.053721	0.9
Tramadol		EtOH	0.004620	0.003379	46
Valsartan		MeOH	0.000487	0.000235	40
Anthracene	Ant	DCM-Benzene (1:1)	1.343569	1.620567	6
Bis 2-ethylhexyl phthalate	DEHP	MeOH	2.642275	2.342361	45
Cashmeran	DPMI	IPA	0.000344	0.000743	16
beta-hexachlorocyclohexane	β-HCH	IPA	0.359730	0.311566	221
Lindane	γ-HCH	ACE	1.590095	0.158009	24

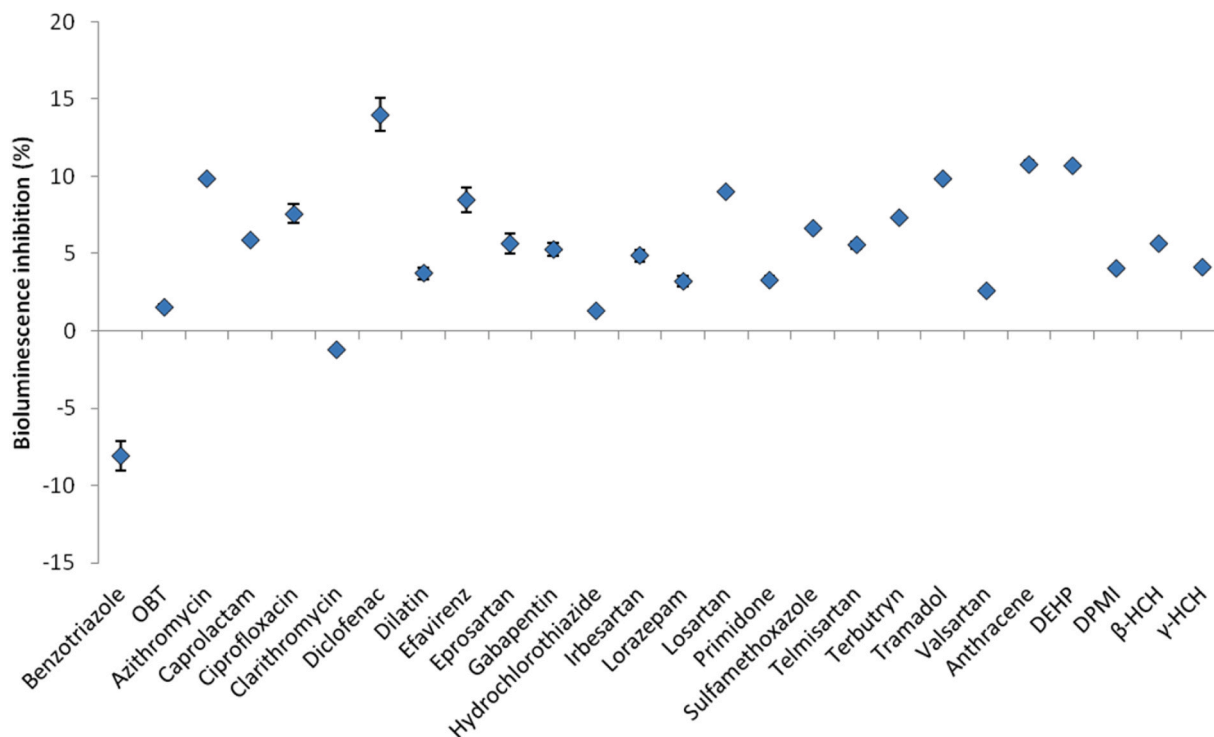


Fig. 4. Bacterial luminescence inhibition of the individually tested compounds in the Microtox® toxicity bioassay. Error bars correspond to the standard deviation (SD) of the three replicates.

Given that interactions between chemicals could lead to an increase in toxicity, mixture effects were also evaluated. For that purpose, those compounds detected at level 1 were mixed in the ratios of concentration found in the wastewater samples. Moreover, considering the non-specificity of the bioassay, potential effects caused by the sample matrix were also assessed spiking an aged WWTP secondary effluent with the mixture of the detected compounds.

As individually tested compounds, the evaluation of mixture toxicity revealed negligible effects in bacteria luminescence inhibition (see Fig. 5). Bioluminescence inhibition remained below the defined safety threshold (< 20%) for both artificial mixtures (i.e. prepared in water and

aged effluent). Interestingly, in the case of the mixture prepared in the aged effluent, there was a noticeable enhancement in luminescence. Thus, apparently, these last conditions were favourable for the bacteria’s survival, possibly attributed to an increase in nutrient availability within the aged secondary effluent.

The experimental observations confirm the low baseline toxicity of the compounds released into the aquatic environment after the MBR treatment. Likewise, interactions between not completely eliminated compounds seemed to pose negligible toxic effects. Similarly, considering the minimal differences in concentrations found in both treatments (most of them falling within the same order of magnitude), a

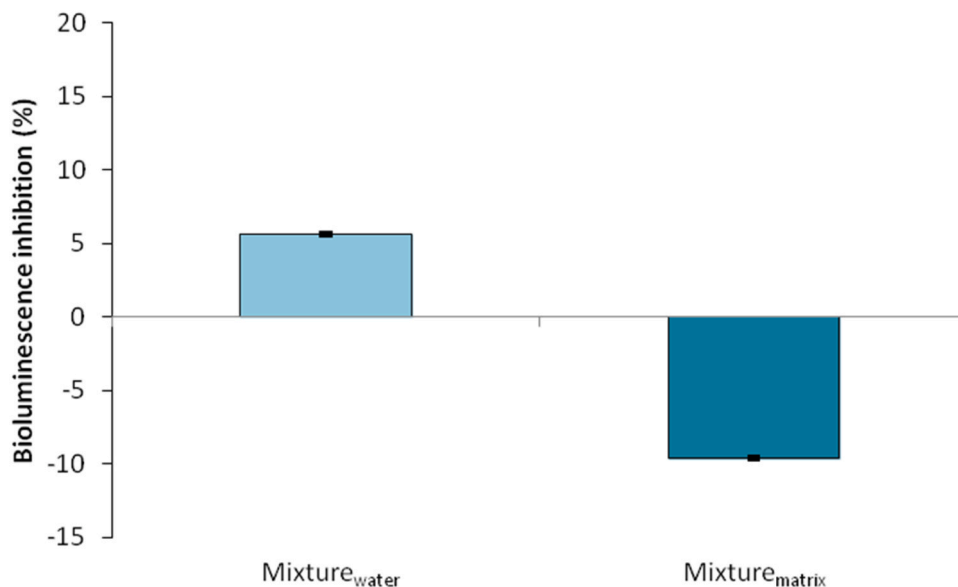


Fig. 5. Bacterial luminescence inhibition of the artificial mixtures tested in the Microtox® toxicity bioassay. Error bars correspond to the standard deviation (SD) of the three replicates.

comparable performance of the CAS treatment in removing baseline toxicity could be suggested.

Differences between calculated (i.e. RQ values) and experimentally tested (i.e. Microtox bioassay) toxicity potential of the compounds point out the need to employ a battery of bioassays covering different endpoints. This comprehensive approach is essential to avoid limiting the assessment of risk associated with detected compounds. Although the RQ metric is a widely used prioritisation strategy for the identification of the most concerning compounds, it fails to mimic the real complexity of natural ecosystems, potentially overlooking unanticipated interactions. Therefore, it should be complemented with experimental toxicity tests to gain a deeper understanding of the toxicity mechanisms of CECs and thus, obtain a more comprehensive characterisation of chemical contamination in the environment. In this regard, Bertanza and collaborators evaluated the toxicity removal efficiency of MBR treatment relative to CAS by performing eleven ecotoxicological bioassays (i.e. baseline toxicity, estrogenic activity, genotoxicity and carcinogenicity), where they pointed out the efficiency of MBR for estrogenicity removal [7]. Likewise, Choi et al. reinforced the superiority of the MBR treatment in reducing estrogenic activity [12].

On the other hand, differences between both approaches may also be attributed to the non-detected compounds, but equally could contribute to toxicity. In this sense, alternative approaches for CECs prioritisation should be followed to enable the identification of the most environmentally relevant compounds in complex matrices. These approaches may include Effect-Directed Analysis (EDA) or Toxicity Identification Evaluation (TIE) strategies.

4. Conclusions

This study compared the efficiency of MBR technology, a greener and innovative approach, and the widely-used CAS treatment for removing CECs. Overall, the present work provides empirical evidence of MBR technology's similar and limited performance for CEC removal compared to CAS treatment with a comprehensive target and non-target analysis, and thus, raises doubts about the worthiness of its implementation due to its high costs and energy demand.

The mass balance assessment revealed that only 18% and 27% of the compounds initially detected in the primary effluent were efficiently removed (i.e., RE > 70%) using CAS and MBR treatments, respectively, with the latter exhibiting slightly improved elimination rates, likely due to enhanced microbial activity and/or higher SRT and lower HRT. However, most of the CECs persisted at elevated concentrations in MBR-treated effluent, posing a potential continuous environmental risk.

The potential risks of the non-eliminated compounds by either treatment were statistically equivalent. While most of the compounds were detected at a safe concentration for the aquatic ecosystem (RQ < 0.1), highly hazardous ones (RQ > 1) were generally unaffected by MBR treatment. Notably, the potential toxicity of these non-eliminated compounds, determined via Microtox® toxicity bioassay, indicated no short-term toxicity to bacteria from individual compounds or mixtures.

This study supports the need to develop other treatments to enhance the removal of CECs, with MBR/photocatalytic-based methods emerging as a potential solution, as well as to implement effect-based methods covering a wider range of endpoints. Moreover, it underscores the need for adopting new prioritisation strategies, such as Effect-Directed Analysis (EDA), which would allow us to identify toxicologically relevant compounds.

CRedit authorship contribution statement

Narao Lopez-Herguedas: Investigation, Formal analysis, Writing – original draft, Visualization, Writing – review & editing. **Mireia Irazola:** Investigation, Formal analysis, Supervision, Writing – review & editing. **Iker Alvarez-Mora:** Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **Leire Mijangos:** Investigation, Formal

analysis, Writing – review & editing. **Dennis Bilbao:** Investigation, Formal analysis. **Nestor Etxebarria:** Supervision, Resources, Funding acquisition. **Olatz Zuloaga:** Supervision, Methodology, Funding acquisition, Data curation. **Maitane Olivares:** Supervision, Methodology, Conceptualization, Formal analysis, Data curation, Resources, Writing – review & editing. **Ailette Prieto:** Supervision, Methodology, Conceptualization, Formal analysis, Data curation, Resources, Writing – review & editing.

Environmental Implication

Contaminants of emerging concern (CECs) primarily enter the environment via wastewater treatment plants, since conventional treatments struggle to remove them completely. Thereby, the development of more effective methods is essential. Among the different emerging treatments, membrane bioreactors (MBR) have gathered special attention as promising alternatives for mitigating the environmental impact of CECs. However, this multidisciplinary approach revealed that MBR performs similarly to conventional treatments. Considering the substantial economic and energy expenses of MBR technology, it may not represent an efficient enough solution for widespread implementation and further research is required to find more effective alternatives.

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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2023.132833](https://doi.org/10.1016/j.jhazmat.2023.132833).

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