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Environmental persistence, detection, and mitigation of endocrine disrupting contaminants in wastewater treatment plants – a review with a focus on tertiary treatment technologies

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Endocrine disrupting chemicals are a group of contaminants that have severe effects on humans and animals when exposed, like cancer and alterations to the nervous and reproductive systems. The increasing concentrations of several endocrine disrupting chemicals in the environment are strongly related to anthropogenic activities, and as the population grows this problem becomes more relevant. Thus, wastewater is one of the main sources of endocrine disrupting chemicals, and the technologies employed during primary and secondary treatment in wastewater treatment plants cannot remove these contaminants. Due to this, researchers have tried to develop more efficient technologies for tertiary treatment of wastewater and reduce the concentration of endocrine disrupting chemicals discharged into the environment. Some of the most promising technologies include adsorption, ultrafiltration, advanced oxidation processes and biodegradation. The use of nanomaterials as adsorbents, catalysts, membranes and supports has played a key role in enhancing the efficiency of these technologies. The results showed that these technologies have great potential on the lab-scale, and even some of them have already been employed at some wastewater treatment plants. However, there are still some challenges to achieving a global implementation of these technologies, related to reducing the costs of materials and enhancing their current performance. The use of biomass/waste derived carbon materials and implementing hybrid technologies are accessible approaches for their implementation in tertiary treatment.

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Environmental significance

Environmental pollution is a critical issue that requires proper measures for a sustainable environmental health. The current literature heavily reports on the environmental persistence, detection, and mitigation of endocrine disrupting contaminants in wastewater treatment plants. However, there is no detailed review available on tertiary treatment technologies that support the sustainable mitigation of emerging pollutants from water matrices. Herein, an effort has been made to cover this notable literature gap by stressing the efficient abatement of hazardous substances from environmental matrices.

1. Introduction

The accentuated population growth and the consequent industrial development to comply with human needs have led to serious contamination of water.¹ Currently, about 1.2 billion people consume contaminated water.² According to the United Nations in 2025, 2.7 billion people will not have access to potable water.³ Water pollution is an issue of global importance due to the increasing awareness of innumerable pollutants, such as organic and inorganic compounds, which reduce the amount of drinking water as the demand rises.⁴ The discharge

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of millions of liters of water derived from basic human activities (flushing toilets, washing clothes and dishes, and bathing), as well as industrial and hospital activities, into sewer systems generates a very complex matrix that may include diverse emerging contaminants. Most wastewater treatment plants (WWTPs) are not designed to treat contaminants like endocrine disrupting chemicals (EDCs). Due to this, WWTP effluents that are discharged into the environment could have potentially hazardous concentrations of EDCs.^{5,6} EDCs are natural or artificial chemical compounds that affect the proper functioning of the endocrine system in humans as well as animals. They can include hormones (industrial chemicals, veterinary drugs, pesticides, and food additives) as well as compounds that block, interfere, or mimic endogenous hormones, such as certain pharmaceuticals. EDCs alter important physiological activities such as reproduction, growth, and development.7-9

Some pharmaceuticals, like sulfamethoxazole (SMX) and carbamazepine, among others, are reported to be EDCs and can enter water systems after excretion by humans and animals. This generates large-scale contamination and consequently enters the food chain through agricultural crops later accumulating in adipose tissues of animals and humans, generating a public health problem of high concern. Foods of animal origin (meat, milk, eggs, etc.) have a greater risk of containing EDCs as they can be derived from the consumption of contaminated food and water, and farm animals sometimes are fed with illegal levels of hormones to increase their growth.8 The monitoring of EDCs is highly important as they are bioaccumulative compounds and the body's elimination of these compounds is very slow, which leads to their continuous bioaccumulation resulting in many alterations in metabolism, with the negative effects sometimes being difficult to determine, but some of them include a higher risk of cancer, alterations to the reproductive system, and interference in the immune and nervous system function.10-12

Nonylphenols, bisphenol A (BPA), as well as the degradation products of alkylphenol ethoxylate, and steroidal estrogens are

some of the main EDCs that have been detected in wastewater all over the world. Effective treatments that can reduce or eliminate EDCs are important to reduce the biological risks to human health as well as the threat posed to different ecosystems.13 Different technologies for the efficient elimination of EDCs have been studied extensively (tertiary treatment). However, in most WWTPs secondary treatment, normally consisting of trickling filter treatment and activated sludge, is the last step, which is insufficient and fails at eliminating EDCs. This can cause further complications; for example EDCs derived from incomplete degradation can increase estrogenic activity and produce derivative compounds such as octylphenol and nonylphenol.13

This review focuses on the occurrence and transport of EDCs in water and consequently the long-term problems in society and the environment, focusing on the latest detection and treatment developments, conducting an extensive revision of bio-based technologies for tertiary treatment in WWTPs for the removal, degradation and inactivation of EDCs and ensuring the safe reuse of treated wastewater.

EDC sources, occurrence and transport in water, soils, and foods

Although the transportation and accumulation routes in the environment of EDCs are complex, studies have shown that most of them are closely related to human activities. Industrial and WWTP effluents and intensively cultivated agricultural soils are the main sources of contamination with EDCs of water systems.14,15 The cycle of contamination begins when they enter water bodies (surface and underground) and soil (Fig. 1).16

For example, natural and synthetic hormones that are not metabolized are excreted through urine or feces, and thus, sewage transports contaminated wastewater. Other anthropogenic sources are found in consumer products such as bottles and containers of plastic as well as metal, personal care

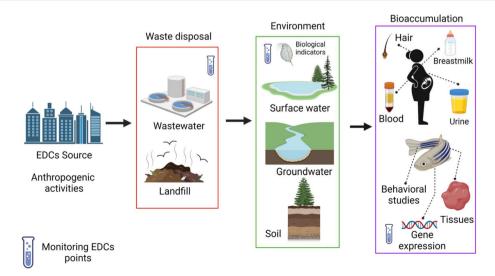


Fig. 1 EDC migration pathway of contamination and proposed sample points for EDC monitoring. Created with https://BioRender.com.

Table 1 Values of IC_{50} and the lowest observed effect concentration (LOEC) of EDCs from different sources or uses

EDC	Source/use	IC_{50} (μ M)	LOEC ($\mu g L^{-1}$)	Ref.
Triclosan	Personal care products	90^a	0.66	147-149
Triclocarban	Personal care products	16	0.28	147, 148 and 150
BPA	Plastics	5	500	151-153
BPB	Plastics	0.93	0.1^b	152, 154 and 155
BPC	Plastics	46^a	15 ^c	152, 156 and 157
Diclofenac	Pharmaceutical	30.7^{a}	1	158-160
Diethyl phthalate	Plastics	7.9	_	161 and 162
Caffeine	Pharmaceutical/food	500	1	158, 163 and 164
	industry			
Chloroacetonitrile	Disinfection products	_	1.60^{b}	165 and 166
Tetrabromobisphenol A	Flame retardant	0.7	0.3 ^c	167-169
ТВР	Flame retardant	4	_	170-172
Glyphosate	Pesticide	30^b	1.7 ^b	173-175
Fipronil	Insecticide	1.6	0.32	176-178
Fenoxycarb	Insecticide	3	27.7	179-181

products (PCPs), detergents, agricultural products (pesticides), food production and other industrial activities (Table 1).¹⁷ Humans can be exposed directly to EDCs by using containers and/or PCPs that contain these chemicals. Also, an indirect path of exposure is followed when these products end their life cycle

and are discarded into landfills. Weathering causes the contained EDCs to be released into the environment and could infiltrate soil and groundwater. When animals or plants come in contact with contaminated soils or water, the bioaccumulation of EDCs may occur, and this creates another

Table 2 EDC occurrence in different water samples (ng L⁻¹)

Sample	EDC	Concentration (ng L^{-1})	Country	Ref
Sea water	BPS	11.3	Romania	182
	BPF	19.7		
	BPA	416		
Estuarine water	Methylparaben	47	China	183
	Triclocarban	117		
	Triclosan	122		
	<i>Ortho</i> -phenylphenol	141		
Surface water	4-Tert-octylphenol	1000	China	184
	Atrazine	8.22	Italy	185
	Nonylphenol	5545	China	184
	Nonylphenol	36.6	Serbia	186
	BPA	233	China	184
	BPA	2180	China	187
	BPA	800	Czech Republic	188
	BPA	105.7	Serbia	186
	BPAF	205	Czech Republic	188
	BPAF	2.58	China	187
	BPS	133		
	Caffeine	3500		189
	DEHP	1094		184
	Triclosan	105		190
WWTP effluent	BPA	3	Mexico	191
	4-Nonylphenol	2.3		
	4-Tert-octylphenol	5		
	17β-estradiol	9.5		
	17α-Ethinyl estradiol	26.8		
Swimming pool	BPA	23.2	China	192
	EE2	78.8		
	4-Tert-octylphenol	2.8		
	Caffeine	39		
	Tributyl phosphate	27	Australia	193

pathway of exposure through the ingestion of contaminated food.

There are regulations for these pollutants in most countries; however, it has been determined that limits typically exceed recommended concentrations. 18 Some examples of EDCs found in water samples are expressed in Table 2. Even though several EDCs, their effects and promising degradation alternatives have been studied, changes in our consumption patterns, the development of new products and technology patterns could lead to unknown EDCs being discharged into the environment.

Environmental and health effects of EDCs

The presence of EDCs in the environment is a global concern because of the effects on wildlife as well as on animals for human consumption. Some EDCs are not only bioaccumulated but also biotransformed; for example, tetrabromobisphenol A exposure promoted gametogenesis and altered sex hormone levels in mussels, altering their life cycle and population dispersal.19 Also, EDC exposure alters neurodevelopment and behavior in living organisms, reducing growth in juvenile organisms, and disrupting some metabolic functions of organs like the liver (disrupts the transcript abundance of liver receptors and leptin) as in the case of Zebrafish exposed to venlafaxine.20 Also, EDCs can affect the offspring of population exposed to these pollutants, where the offspring of zebrafish exposed to flutolanil is affected in its development (gonad endocrine disruption, decreased reproduction and short body length) and its mortality rate is increased.21 Some EDCs pose a higher risk to some species over others; for example, estradiol, methyl triclosan and triclosan (TCS) showed an elevated risk for surface water aquatic species.22 Therefore, it is necessary to conduct punctual and specific EDC monitoring, to act in a timely manner and understand the consequences of their contamination in the environment.

Some metals such as aluminum and manganese can act like EDCs, and the accumulation of these metals reduces relative fecundity in Astyanax altiparanae, and this accumulation in some cases is accelerated due to the availability of metals which causes the acidification of aquatic environments, making these metals more available for organisms than in alkaline environments.23 Therefore, the monitoring and removal of some metals must be performed not only at WWTPs but also in environments with high levels of these metals. Another exposure pathway to EDCs is through PCPs, as they are disposed through wastewater, sanitary landfills and other disposal ways that do not have enough treatment processes for their effective removal, leading to the discharge of EDCs in water and soil.

Furthermore, human exposure to EDCs is due to not only contact with contaminated environments but also their consumption of food and drinking water (Table 5). Fish consumption is a route of EDC exposure, di (2-ethylhexyl) phthalate bioaccumulated in Epinephelus coioides and Platycephalus indicus (widely consumed fish species from Persian Gulf), especially in liver tissue. Because of this, consuming at least two fish-based meals per week may result in a moderate health risks.24 The EDC bioaccumulation by fish occurs not only due to their presence in biological tissue but also due to the ingestion of plastics and microplastics that also act as vectors of EDCs in aquatic ecosystems mainly by sorption processes.^{25,26}

Therefore, EDCs are consumed by humans causing alterations in the endocrine system that can affect the life quality of populations. For example, prenatal exposure to some EDCs (organochlorines and glycol ethers), directly or indirectly, may increase the risk of hypospadias. Also, prenatal exposure to phenols is associated with an increased risk of cryptorchidism.27 A continuum mapping methodology was employed to determine the concentration of EDCs in maternal blood samples. It was found that higher levels of PBDEs in maternal blood were associated with a lower birth weight and higher levels of PCBs, and PFAS were associated with increased birth weight, demonstrating that exposure to these pollutants already has a negative effect on the health of the population.28 It is important to know that postnatal children's EDC exposure can cause neurodevelopment effects, and these effects are related to the duration of exposure as well as the type of EDC.²⁹ With respect to the importance of the stage of development where the exposure exists, the effects of EDC exposure during puberty are increases in anxious behavior and a reduction in social interaction.30 Additionally, phthalate metabolites were found in the adult population. They include bisphenols and pesticides in urine and hair samples, demonstrating that there is

Table 3 Recent studies on the degradation of endocrine disruptors using different nanomaterials in photocatalysis and enzyme-mimicking strategies

Nanomaterial	EDC	Degradation strategy	Degradation efficiency (%)	Time of reaction (min)	Ref.
CQDs/BiOBr nanosheets	BPA	Photocatalysis	\sim 55	180	194
CQDs/BiOBr	BPA	Photocatalysis	73	150	195
CQDs/polymer carbon nitride	BPA	Photocatalysis-PMS activation	95	30	105
$N-CQD/SiO_2$	BPF	Photocatalysis-PDS activation	14.3	120	196
$N-CQD/CeZrO_2$	BPF	Photocatalysis-PDS activation	34.9	120	196
$N-CQD/Al_2O_3$	BPF	Photocatalysis-PDS activation	81.5	120	196
CQDs/Bi ₂ WO ₆	BPA	Photocatalysis	\sim 55	120	197
CQDs/Bi ₂ MoO ₆	BPA	Photocatalysis	54	120	198
CNQDs	BPA	Photocatalysis	\sim 100	80	199

Table 4 Biodegradation of different EDCs

		Efficiency					
EDCs	Organism	(%)	Time	Concentration	Ref.		
BPA	Pleurotus ostreatus and Pleurotus pulmonarius	100	1 h	$100~{ m mg~L}^{-1}$	200		
BPA	Pleurotus ostreatus and Pleurotus pulmonarius	85	1 h	200 mg L^{-1}	201		
Nonylphenol	Ganoderma lucidum	80	50 min	5 ppm	202		
Triclosan	Ganoderma lucidum	45	50 min	5 ppm			
BPA	Pycnoporus sanguineus (CS43) and	100	<24 h	$20~{ m mg~L^{-1}}$	137		
	Trametes versicolor (commercial laccase)						
Carbaryl	Pseudomonas putida KT2441	100	<30 h	50 mg L^{-1}	203		
Carbofuran	Pseudomonas putida KT2440	100	<30 h	50 mg L^{-1}	204		
Chloropyrifos	Pseudomonas putida KT2444	100	<30 h	$50~\mathrm{mg~L^{-1}}$	205		
Di (2-ethyl hexyl) phthalate	Pleurotus ostreatus	100	504 h		206		
Fanpropathrin	Pseudomonas putida KT2443	100	<30 h	$50~{ m mg~L^{-1}}$	207		
Parathion	Pseudomonas putida KT2442	100	<30 h	$50~{ m mg~L^{-1}}$	208		
Carbofuran	Cupriavidus sp. ISTL7	98	96 h	400 ppm	209		
Congo red	Oudemansiella canarii	80	<24 h	50 mg L^{-1}	210		
17α-Ethynylestradiol	Water-sediment microcosms (bacteria from	44-99	60 days	$2 \mu g g^{-1}$	211		
	Proteobacteria, Actinobacteri, Acidobacteria,						
	Chloroflexi, Nitrospirae, and Bacteroidetes phyla)						
Estrone	Haematococcus pluvialis	97	40 days	5 mg L^{-1}	212		
Estrone	Selenastrum capricornutum	80	40 days	$5~{ m mg~L^{-1}}$			
Estrone	Scenedesmus quadricauda	97	40 days	5 mg L^{-1}			
17β-Estradiol	Haematococcus pluvialis	100	40 days	5 mg L^{-1}			
17β-Estradiol	Selenastrum capricornutum	100	40 days	5 mg L^{-1}			
17β-Estradiol	Scenedesmus quadricauda	100	40 days	5 mg L^{-1}			
17β-Estradiol	Chlorella vulgaris	100	40 days	5 mg L^{-1}			
17α-Ethynylestradiol	Haematococcus pluvialis	>85	40 days	5 mg L^{-1}			
17α-Ethynylestradiol	Scenedesmus quadricauda	>85	40 days	$5~{ m mg~L^{-1}}$			
17α-Ethynylestradiol	Microbial community (Proteobacteria,	15-90	70 days	$50 \ \mu { m g \ L^{-1}}$	213		
	Firmicutes, and Nitrospirae)		•	. 0			
17α-Ethynylestradiol	Lentinula edodes	100	21 days	200 μg	214		
Testosterone	Lentinula edodes	100	21 days	50 μg			

Table 5 EDC occurrence in different drinking water (ng L^{-1}) and food (ng L^{-1}) samples

Sample	EDC	Concentration (ng g ⁻¹)	Country	Ref.
Drinking water	DDT	0.275^{a}	India	215
Drinking water	ү-НСН	55.7 ^a	India	215
Drinking water	Nonylphenol	7.9^{a}	Serbia	186
Drinking water	BPA	35.6^{a}	Serbia	186
Drinking water	BPA	57 ^a	Spain	216
Drinking water	Methylparaben	119^a	Spain	216
Drinking water	Caffeine	5.2^{a}	Italy	217
Drinking water	Naproxen	278 ^a	Spain	216
Apple	DDE	0.023	India	215
Apple	ү-НСН	0.44	India	215
Tomato	DDT	2.34	India	215
Tomato	ү-НСН	0.77	India	215
Wheat	DDT	23.2	India	215
Wheat	ү-НСН	3.16	India	215
Rice	DDE	1.46	India	215
Rice	ү-НСН	1.59	India	215
Canned tuna	BPA	69.1	European Union	218
Plaice small	Triclosan	142.4	European Union	218
Mussels	BPA	54.7	European Union	218
Mussels	Triclosan	42.26	European Union	218
Mussels	BDE100	0.22	European Union	219
Mussels	Methylparaben	0.080	European Union	219
Mullet	Carbamazepine	1.1	Brazil	220

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a bioaccumulation of EDCs in humans. EDCs are especially found in hair samples, illustrating chronic exposure.31

EDCs from pharmaceutical sources and their metabolites affect biota as well as humans. Such compounds including levonorgestrel (contraceptive) were found in WWTP effluents and in aquatic environments. It was found that low concentrations of levonorgestrel and its fractions caused a reduction in pregnancy maintenance in some aquatic species and humans.³² Also, it was found that alkylphenol (nonylphenol and oxylphenol) and perfluoroalkyl substance exposure in women is associated with endometrial (the most common gynecological cancer in the United States) and breast cancer development. 33,34 Exposure can also aggravate some diseases such as COVID-19, where some biological pathways may be dysregulated by EDCs and function as contributors to COVID-19 severity, additionally increasing the side effects of the infection.35 To take care of population health and the environment, protocols for the removal and treatment of EDCs currently found in the world should be implemented as well as promoting new methods of waste treatment, to prevent the cycle of this contamination from continuing to occur.

Endocrine disruptors: methods of analysis and detection by biosensors

EDC concentrations in wastewater are low, on the order of ng L^{-1} or $\mu g L^{-1}$ and the most used techniques for their detection and quantification EDCs are the traditional analysis techniques such as high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS), ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) and gas chromatography time-of-flight mass spectrometry (GC-TOF-MS).36,37 These techniques have a high sensitivity of detection and reproducibility, but require many steps of sample preparation, large sample volumes, long times of analysis, expensive consumables, and equipment. Electrochemical sensors have been reported as a new and rapid application procedure for the detection and screening of EDCs and other toxic compounds in wastewater, with very low detection limits, high sensitivity and low cost, Fig. 2.37,38

Biosensors have been developed using cells, specific molecules, aptamers, antibodies, DNA, and model organisms,

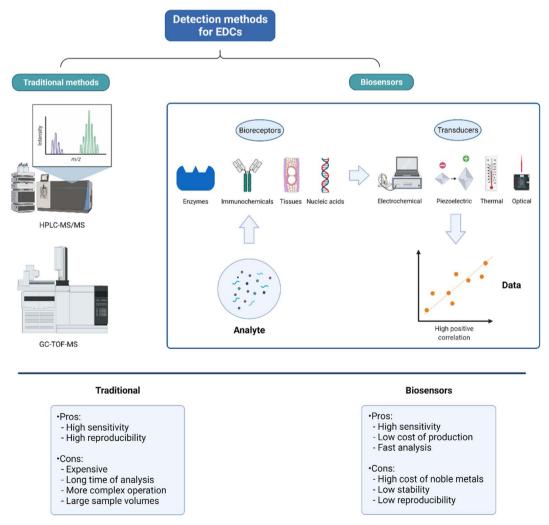


Fig. 2 Comparative between EDC detection methods, and a diagram of how biosensors operate. Created with https://BioRender.com.

among others, and these sensors have been applied for the detection of different EDCs. Transducer sensors have been developed based on carbon nanomaterials such as graphene oxide (GO) and carbon nanotubes, metal oxide nanomaterials, noble metal nanomaterials, polymers and other materials. 8,39,40 In a study, the authors developed a method for the electrochemical detection, quantification, and removal of BPA through low-cost carbon felt with a detection limit of $4.78 \times 10^{-4} \, \mu M$. The detection of BPA in real water samples demonstrated a sensitivity between 98.4 and 101%, and the sensor was reusable. An electrochemical sensor developed with covalentorganic frameworks (COFs), named CTpPa-2, using galvanostatic charge and discharge, differential, and cyclic pulse voltammetry, allowed the detection of BPA and bisphenol S (BPS) with detection limits of 0.02 and 0.09 μM, respectively. 42 Most of the studies on the development of biosensors for the detection of EDCs focus on the detection of BPA and analogs, and some estrogens such as 17β-estradiol (E2).8,43

5. Fate and removal of endocrine disruptors in conventional WWTPs

The problem posed by EDCs in wastewater is the lack of specific degradation methods with high efficiencies. In developing countries most of the WWTPs are only equipped with basic treatment stages that cannot remove a wide range of contaminants such as EDCs. 44,45 WWTPs typically consist of two stages: primary treatment and secondary treatment. 46,47 However, some contaminants are not removed during those stages and tertiary treatment is required (Fig. 3).

During the first stage of treatment, physical processes are employed for the separation of solids from water. In traditional WWTPs, the primary treatment is composed of a screening process, grit removal and sedimentation. During the screening, solid waste is separated by using a device with uniform-sized openings that retain coarse materials. Grit chambers are used for the settling of sand and gravel to avoid further accumulation or damage to the mechanical instruments. Finally, during the sedimentation process, sedimentation tanks or primary settlers are employed to enable the organic suspended solids to settle as sludge.^{46,47}

After primary treatment most pathogens and contaminants are still in the stream. Secondary treatment focuses on the degradation of the biological content, mainly by using aerobic and anaerobic treatment systems. 47,48 The most common aerobic systems are trickling filters and activated sludge. In the trickling filters there is a fixed medium where microbes grow, forming biofilms that can consume the organic matter, 49,50 while in the activated sludge process a high concentration of microorganisms clumped as small particles in suspension degrade the organic material. 51-53 On the other hand, the anaerobic systems employed during the secondary treatment are based on anaerobic digestion. In this process, the organic matter is transformed into a mixture of biogas and sludge. The most common anaerobic treatments are an up-flow anaerobic sludge bed (UASB), expanded granular sludge bed (EGSB), and anaerobic fixed film reactor (AFFR).47

The concentration of organic pollutants in the effluents after these stages varies depending on several factors, such as the initial concentration and the susceptibility of each pollutant to be degraded by the employed techniques. However, in most cases the concentration of pollutants in WWTP effluents is within the range of ng L^{-1} to $\mu g L^{-1}$. Nevertheless, for contaminants like EDCs even low concentrations might represent an environmental risk as most of them have potential for bioaccumulation. The tertiary treatment has been implemented for enhanced removal of the remaining contaminants in wastewater.

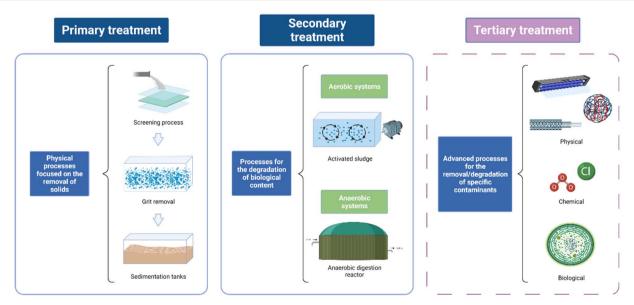


Fig. 3 Schematic of WWTP stages and their role in water decontamination. Created with https://BioRender.com.

Table 6 Lab-scale treatments for EDC removal

Treatment method	Pollutant	Initial concentration	Removal efficiency (%)	Ref.
Adsorption with powdered activated carbon and coagulation	Bisphenol F	1.5 ng L ⁻¹	95.8	78
	BPA	1.74 ng L^{-1}	99.2	
	2,4	<10 ng L ⁻¹	>95.5	
	dihydroxybenzophenon	e		
	Estrone	$< 2.75 \text{ ng L}^{-1}$	96.4 ^j	
	Triclosan	<50 ng L ⁻¹	63.7 ^m	
	Ibuprofen	$< 0.984 \text{ ng L}^{-1}$	99.9	
	Carbamazepine	$< 0.0308 \text{ ng L}^{-1}$	99.9	
Adsorption with powdered activated carbon and ultrafiltration	Carbamazepine	169 ng L^{-1}	60.2	
	Oxybenzone	$< 2.8 \text{ ng L}^{-1}$	80.5^{g}	
	Caffeine	$< 0.666 \text{ ng L}^{-1}$	99.9 ^e	
Powdered activated carbon in a membrane bioreactor	Caffeine	500 ng L ⁻¹	>98	221
	Azithromycin	500 ng L^{-1}	>99	
	Ofloxacin	500 ng L^{-1}	86	
	Naproxen	500 ng L^{-1}	>99	
	Theophylline	500 ng L^{-1}	99.9	
UV-solar/H ₂ O ₂	BPA	$25~\mathrm{mg~L^{-1}}$	77.40	222
Tube-in-tube membrane microreactor for photochemical ${\rm UVC/H_2O_2}$ oxidation systems	Oxytetracycline	2 mg L^{-1}	36	108
Tube-in-tube membrane microreactor with a UVC/H ₂ O ₂ /TiO ₂ system	17β-Estradiol	$100~\mu g~L^{-1}$	48	109
	17α-Ethinylestradiol	$101~\mu { m g}~{ m L}^{-1}$	30	
Fluidized bed reactor with stabilized laccase in porous silica	BPA	$25~\mathrm{mg~L^{-1}}$	90	134
Microalgae-bacteria consortium in photobioreactors lit with a low intensity light emission diode	Sulfamethoxazole	50 μg L ⁻¹	54.34	223

5.1 Tertiary treatment

WWTPs that include tertiary treatment are usually found in more developed countries and their aim is to ensure that wastewater has enough quality to be reused or to reduce the negative effects when it is discharged into the environment. 56,57 This stage is focused on the removal of specific contaminants with advanced techniques, which are classified as chemical, physical, and biological processes.⁵⁸ Ozonation, UV radiation, chlorination and reverse osmosis are among the most employed techniques in WWTPs.58 The selection of the treatment should be based on the characteristics of the target contaminant or contaminants. Although there are several technologies proven with high effectiveness on the lab scale, most of them are not yet employed in WWTPs, in most cases due to a significant reduction of their effectiveness when escalated or a high cost of operation. However, there are studies where different technologies for EDC removal were evaluated, and the results have shown that some of them could be implemented soon in the tertiary treatment for the removal of EDCs at WWTPs (Table 6).

The selection of the technologies for EDC removal/ degradation described henceforward was done based on the number of studies, where they were employed on the lab scale or pilot plants and their actual utilization in WWTPs. The studies described include evaluations in synthetic solutions, real wastewater samples, and spiked water and wastewater samples.

5.1.1 Adsorption. Adsorption is one of the most employed and studied technologies for the removal of contaminants from water. This technique is based on the adhesion of a contaminant (adsorbate) to the surface of a solid material (adsorbent) due to diverse physical or chemical interactions. 59 One advantage of this technology is its versatility, as it can remove a wide range of contaminants, mainly due to the variety of adsorbents that can be employed. Most common adsorbents include carbon materials, COFs, metal-organic frameworks (MOFs), nanoparticles, ceramics, natural adsorbents, and composites.60,61 The removal efficiency of the adsorbents varies depending on the target contaminant, the physicochemical characteristics of the adsorbent, matrix, dose of adsorbent and concentration of the contaminant.

MOFs are usually reported as highly efficient adsorbents for a wide range of inorganic and organic contaminants, including diverse EDCs.⁶² For example, a study evaluated MIL-53 (Crbased active cite MOF) for BPA removal from synthetic solutions. The authors reported a maximum adsorption capacity of 421 mg g^{-1} when the initial concentration of the prepared solutions was within a range of 30-80 ppm.63

MOFs based on aluminum are among the most employed for the adsorption of EDCs. For example, MIL-53 with an Al-based active site and a mesostructured MIL-53(Al) were also employed for the removal of BPA from synthetic solutions. 64 The initial concentrations were between 25 and 250 $\mathrm{mg}~\mathrm{L}^{-1}$ and the maximum adsorption capacity was 325 mg g⁻¹ for MIL-53(Al) and 465 mg $\rm g^{-1}$ for the mesostructured MIL-53(Al). Another Al-based MOF was employed for the adsorption of BPA, 17αethynylestradiol (EE2) and perfluorooctanoic acid (PFOA) from synthetic solutions.⁶⁵ Furthermore, a competitive adsorption analysis was carried out to determine the selectivity of the adsorbent. According to the results, the maximum adsorption capacities at equilibrium were 138.4 mg g $^{-1}$ for BPA, 200.4 mg g $^{-1}$ for EE2, and 169.2 mg g $^{-1}$ for PFOA. The adsorption mechanism was probably driven by electrostatic and hydrophobic interactions. It is demonstrated that Al-based MOFs can remove EDCs efficiently, with maximum adsorption capacities around 100 mg g $^{-1}$, depending on the target contaminant.

MOFs with active sites based on other elements have also been reported. For example, a study evaluated the adsorption of BPA from synthetic solutions using two different MOFs, one Febased (MIL-53) and the other Ni-based (Ni-MOF). The authors evaluated the effect of the physicochemical characteristics of the material, pH, adsorbent dose, contaminant concentration, and contact time. Even though the specific surface area of Ni-MOF (21.71 m² g $^{-1}$) was higher than that of MIL-53 (11.39 m² g $^{-1}$), the former one had a better adsorption performance. The adsorbent dose was the parameter with a higher effect on the removal of BPA, followed by pH, contaminant concentration, and time. The experiments showed that the adsorption mechanism adjusted to a monolayer formation system, and the maximum adsorption capacity of MIL-53 was 88.6 mg g $^{-1}$.

MOFs have also been employed for the adsorption of EDCs like TCS,⁶⁷ ciprofloxacin,⁶⁸ carbamazepine,⁶⁹ or dimethyl phthalate,⁷⁰ with maximum adsorption capacities above 100 mg g⁻¹ in most cases. The reported results demonstrate that MOFs are promising materials with high potential to be implemented in the tertiary treatment of wastewater for the removal of EDCs. However, the main challenge that hinders their application in WWTPs is the scalation of the MOFs' synthesis, as their efficacy is strongly dependent on their structure; therefore cheap synthesis methods may lead to a structure with more defects. Also, the cost is a major drawback as the price per kilogram of the most efficient MOFs could be in the range of hundreds or even thousands of dollars.⁷¹

Recently, magnetic COFs have been evaluated as adsorbents for the removal of diverse EDCs. The results have shown that these materials have great potential as adsorbents of EDCs due to their high adsorption capacity. In a study by ref. 72, a magnetic COF composite was employed for the adsorption from synthetic solutions of atrazine and chlorpyrifos, pesticides classified as EDCs. The authors reported that the COF had an adsorption capacity of 54 mg g^{-1} for atrazine and 270 mg g^{-1} for chlorpyrifos, and both results were superior to those reported in the literature for other nanomaterials such as MOFs or activated carbon. Additionally, desorption studies were performed showing a recovery of >90% of the pesticides, with no major effects on the adsorption capacity of the composite up to the fifth cycle. In another study, the removal of sodium diclofenac (DCF) from water was evaluated by using two magnetic COF composites, where the effect of the material's functionalization with hydroxyl groups on its adsorption capacity was assessed by density functional theory (DFT).73 The authors evaluated the performance of a hydroxyl group-modified COF (OH-COF), and the results showed an adsorption capacity of 203.4 mg g⁻¹ calculated by using the Langmuir model. Reusability analysis demonstrated that this material maintained its original adsorption capacity after 5 cycles.

The removal of BPA by using COFs has also been evaluated, as a study used a magnetic modified COF for BPA adsorption from synthetic solutions and DFT and molecular dynamics (MD) were employed to elucidate the followed adsorption mechanism. The authors reported that the COF had a BPA adsorption capacity of 114.97 mg $\rm L^{-1}$ at pH 6 and 298 K. The results of the MD and DFT showed that the aldehyde groups in the COF and the BPA were interacting by hydrogen bindings.

COFs are promising materials with desirable characteristics for an adsorbent, like high surface area, well defined porous structure, and numerous active sites. However, correspondingly to MOFs, the major challenge to full-scale application is their cost. Also, in contrast to MOFs and other adsorbents, some key aspects of COFs related to their interaction mechanisms, stability and synthesis routes are not well documented. These drawbacks have hindered their application in WWTPs in the actuality; however they remain as a promising alternative that needs to be comprehensively studied.

Another group of adsorbents are carbon-based nanomaterials, which are among the most used for adsorption. This is mainly due to their characteristics, such as high specific surface area, stability, and highly porous structure. Graphenebased materials such as GO, reduced graphene oxide (rGO), single-walled carbon nanotubes (SWCNTs), and multi-walled carbon nanotubes (MWCNTs) are a very popular group of carbon materials with diverse applications. For example, some reports have shown promising results using GO as an absorbent. A study evaluated the removal of EE2 from synthetic solutions using a rGO magnetic composite (rGO/Fe₃O₄).⁷⁵ The authors found out that the EE2 adsorption kinetics from water by the magnetic composite fitted well with a pseudo-secondorder model. The adsorption capacity was 37.33 mg g⁻¹ at a pH of 6, and after five regeneration cycles only 20% of the initial capacity was lost. Magnetic composites, composed of carbon polyhedrons with Co crystals and rGO nanosheets as the support, were evaluated as adsorbents for the removal of BPA from synthetic solutions.76 The results showed that the Co-C/ rGO composites had desirable characteristics for adsorbents, such as high surface area (284.4 m² g⁻¹) and highly porous structure. The adsorption capacity for BPA was 175.1 mg g^{-1} , and the authors reported that the adsorption mechanism was probably due to π - π interactions. Regeneration analyses showed that the composite could retain 83.7% of its capacity after four cycles.

SWCNTs have also been reported as adsorbents for EDC removal from water. A study evaluated the adsorption of BPA and EE2 on SWCNTs from synthetic seawater and brackish water. The authors reported that SWCNTs had higher adsorption capacity for EE2 (34.6–35.71 mg g $^{-1}$) than for BPA (13.39–16.05 mg g $^{-1}$), probably due to hydrophobic interactions as the main adsorption mechanism. The effect of pH was also studied, and the results showed that an increase in the pH from 3.5 to 11 reduced the adsorption of BPA, but it had no effect on EE2. Furthermore, as the concentration of dissolved organic carbon (DOC) increased, the adsorption of both BPA and EE2

decreased linearly. Despite the high performance and adsorption capacity of graphene-based materials like rGO and SWCNT composites for the adsorption of diverse EDCs, the cost of industrial scale synthesis hinders their application as adsorbents in WWTPs. Nevertheless, these materials have been reported as electrocatalysts and photocatalysts when supported with different types of nanoparticles or doped with heteroatoms. The application of graphene-based materials as adsorbents might not be the best option for WWTPs, but they have great potential in advanced oxidation processes (AOPs) and as electrodes for electrochemical sensing.

Other carbon materials like activated carbon, biochar, and even raw biomass have been extensively evaluated as adsorbents, mainly due to their variety, great performance and relatively low cost compared to other nanostructured carbon materials. In a study, the authors investigated treatment technologies for removing a few new micropollutants found in real wastewater.78 Powdered activated carbon (PAC) adsorption as a regularly used consolidated water treatment technology for the removal of organic micropollutants was the target of this study. Hybrid methods that combined PAC with a coagulant (PAC/L) or ultrafiltration (PAC/U) were developed. Also, a traditional Fe(III) based coagulant (FeCl3) as well as a novel natural coagulant derived from bean seeds, Phaseolus vulgaris, were used to demonstrate PAC/L. The results showed that using PAC (20 mg L⁻¹) and natural coagulant (37.5 L L⁻¹) together yielded the highest removal efficiency for bisphenol F, BPA, 2,4-dihydroxybenzophenone, estrone (E1), TCS, ibuprofen, and carbamazepine, with values ranging from 60 to 95.5%. Combined PAC/UF, on the other hand, achieved removal efficiencies ranging from 60 to 99.9%. Adsorption on activated carbon has the advantage of not producing new compounds and having high removal efficiency. When used in conjunction with membranes, they also possess a disinfecting function. The authors discovered, however, that its adsorption capability varies from batch to batch and that the cost of technology implementation, such as carbon synthesis and waste management, is considerable.

The use of non-pyrolyzed biomass has also been assessed in some studies as a low-cost and simple adsorbent. The adsorption of tetracycline (TC), SMX and from synthetic solutions using sulfonated saw dust was evaluated in a study.79 The results showed that the activated biomass had higher adsorption capacity than commercial adsorbents for SMX and BPA, 295.06 and 263.75 mg g^{-1} , respectively. Meanwhile for TC the adsorption capacity of 270.53 mg g^{-1} was close to that of other available adsorbents.

Some WWTPs have already employed activated carbon in tertiary treatment; however there are still challenges to overcome regarding their selectivity in real wastewater samples. Supporting different types of nanoparticles on their surface is a promising approach to enhance the adsorption performance compared to regular carbon materials. Another promising alternative for this group of materials is the generation of lowcost hybrid materials, like nanobiocatalysts by the immobilization of bacteria or enzymes. This approach could exploit the

adsorption capabilities of these low-cost adsorbents, while helping to overcome some drawbacks of using free biocatalysts.

5.1.2 Flocculation/precipitation. Flocculation is a process where a chemical agent is added to a solution, which promotes the bonding between particles, thus generating larger aggregates that can be removed easily.80,81 This method needs to be complemented with another separation technology, such as filtration or decantation. A study was carried out to evaluate the removal of 4-nonylphenol (4NP) from water with the coexistence of natural organic matter (NOM) and suspended inorganic particles using a polymeric cellulose-based flocculant (CMCND).82 The results showed that the removal was up to 79% at a pH of 4, 35 °C and flocculant dosage of 40 mg L^{-1} . The coexistence of NOMs and kaolin had a synergistic effect on 4NP removal. Flocculation, ozonation, adsorption and chlorination were compared to evaluate their efficiencies in the removal of diverse EDCs (Choi et al., 2006). The flocculation method had the highest removal efficiency for di(2-ethylhexyl)phthalate (DEHP, 53%), di-n-butyl phthalate (DBP, 49%), and 4-heptylphenol (DEP, 46%), while for the rest of the EDCs the efficiency varied from 0 to 7%.

Flocculation is a widely employed technology for the removal of contaminants from water. However, most of the studies are focused on the removal of ionic contaminants, and their application in the removal of EDCs is not extensively documented. Most of the reported studies for EDC removal have shown minor efficiencies compared to other technologies like adsorption, thus making flocculation not suitable for a main removal technology. However, further research could be done focusing on coupling flocculation with other removal technologies to increase its efficiency. This could be particularly helpful for existing WWTPs with flocculation technology.

5.1.3 Advanced filtration. The definition of filtration is a physical process that is employed for the separation of one or more substances from another, like solids, liquids, and/or gases, using a separating medium or filter.83 In a study, the authors evaluated the removal of EDCs by ultrafiltration (UF) and ozonation in a synthetic secondary effluent and the effects of effluent organic matter (EfOM).84 The removal percentage of EDCs by UF without EfOM was within the range of 7–27%, while in the presence of EfOM the removal percentage increased in the range of 3-42%. Another study evaluated the addition of SWCNTs in the UF structure for the removal of steroid hormone micropollutants from synthetic solutions.85 With a dose of SWCNT of 2 g m⁻² the removal was in the range of 50-75% at neutral pH and short residence times (0.08-7.1 s). Another study was carried out where the retention of diverse EDCs from synthetic solutions by using nanofiltration (NF)/reverse osmosis (RO) membranes was evaluated, considering factors like the membrane type, solute size, and solute-membrane interaction.86 The high desalting membranes retained >90% of semivolatile organic compounds (SVOCs) but not volatile organic compounds (VOCs) using 0.3 MPa. Moreover, the low desalting membrane could reject up to 80% of VOCs.

Membrane technologies have been successfully implemented in water treatment, especially RO for water purification. However, its application in WWTPs has been hindered by the costs of the membranes and the very low pressures required for their operation. The membrane technology could be evaluated for assisting other technologies where higher water quality standards are required, or even as a quaternary treatment stage.

5.1.4 Chlorination. This method is usually employed in WWTPs for the disinfection of wastewater by adding Cl₂ or Ca(OCl)₂/NaOCl to previously treated wastewater.⁸⁷ This treatment is an oxidative process that is not commonly used in the removal of organic or inorganic contaminants, mainly due to the formation of toxic byproducts. 87,88 However, there are a few studies where the chlorination method was evaluated for the removal of EDCs. For example, a study assessed the effectiveness of chlorination for removing BPA, TCS, and 4NP and its ethoxylates named NP1EO and NP2EO.89 The results showed that the removal of BPA, 4NP, and TCS was in the range of 60-80%. However, the removal efficiency was 37% and 52% for NP1EO and NP2EO, respectively. Nevertheless, toxicity experiments showed that the chlorination produced byproducts, 2,4,6-trichlorophenol, trichloro-BPA, tetrachloro-BPA, and chloroform, were 50-100 times more toxic than the original contaminants.

Another study evaluated the effect of chlorination on the estrogenic/antiestrogenic activities of biologically treated water. The authors found out that the chlorination processes increased the antiestrogenic activity and decreased the estrogenic activity of wastewater. This is possibly related to the formation of harmful disinfection byproducts (DBPs), which are considered as EDCs.

5.1.5 Advanced oxidation process. AOPs are chemical treatments where the oxidation of contaminants, especially organic compounds, is carried out by adding powerful oxidizing agents. The aim of these processes is to degrade the contaminants into products that are easier to handle. 91,92 A study carried out by ref. 93 evaluated five different structural phases of MnO₂ for the oxidation of different EDCs (E1, estriol (E3), E2, EE2, and BPA) in the presence of methanol in synthetic solutions. The δ -MnO₂ phase had the highest reactivity towards the oxidation of E1, and after 120 min at pH 3 it was completely removed. For the rest of the EDCs, the overall performance of all structural phases of MnO₂ was very poor (>45%). A hybrid nanofiltration/ catalytic oxidation system was evaluated for the removal of BPA using synthetic solutions (Kim et al., 2008). For the oxidation, the authors employed iron(III) tetrasulfopthalocyanine (Fe(III)-TsPc) in the presence of H_2O_2 . At pH 4.5, > 90% of BPA was oxidized after 3 min. The hybrid system had an overall efficiency of 95% compared to the 72% of the NF-only system. Another study investigated a Fenton-like oxidation of DBP from a spiked landfill leachate using as catalyst natural sediments and sediments loaded with Fe, Cu and Mn.94 The impregnated sediments with 5% of Fe, Cu and Mn had the highest catalytic activity. The degradation of DBP from leachate 1 was 95% at pH 3.0, a concentration of DBP of 5 mg L⁻¹, H₂O₂ dose of 250 mM L^{-1} , sediment dose of 5 g L^{-1} and contact time of 240 min.

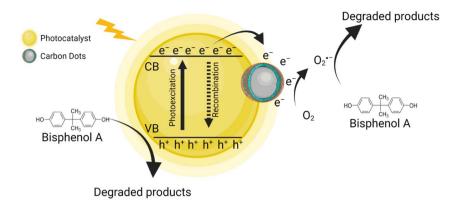
Ozonation is an advanced oxidation technology where ozone (O_3) is added to water, and due to its powerful oxidation capacity, the ozone produces reactive oxygen species that can degrade a wide range of compounds and microorganisms.⁹⁵⁻⁹⁷

This technology has also been employed in several WWTPs with promising results for the degradation of diverse contaminants, including some EDCs.

A study where the ozonation conditions were optimized for the removal of E1 and E2 from synthetic solutions was carried out by ref. 98. The experiments showed that the optimal conditions were an ozone concentration of 4 mg $\rm L^{-1}$, residence time of 5 min, and pH 6. Under these conditions, about 90% of E1 and 95% of E2 were removed. Ozone and ozone/hydrogen peroxide treatments were evaluated to remove gemfibrozil and ibuprofen from spiked treated sewage effluent by ref. 99. A complete removal of gemfibrozil and 80% removal of ibuprofen was achieved by both ozonation and AOP using $\rm O_3/H_2O_2$. Ozonation is one of the most promising approaches for degrading EDCs in WWTPs, as some plants already have the infrastructure for this technology. It is required to optimize the operation parameters to ensure higher degradation efficiency on real wastewater samples.

5.1.5.1 Photodegradation. This includes a group of processes where molecules are transformed or degraded by the absorption of photons from the UV-visible spectrum.100 Typically, three photodegradation types are distinguished: photolysis, photooxidation and photocatalysis. Heterogeneous photocatalysis is an AOP and considered a very effective strategy for wastewater treatment (Fig. 4).101 The traditional AOPs are based on the generation of hydroxyl radicals ('OH) as the main oxidant, which can react with a wide range of pollutants at high reaction rate constants. 101,102 In contrast, persulfate-based AOPs generate sulfate radicals (SO₄ -), which have a higher redox potential and a longer half-life in comparison to 'OH, enhancing the degradation of pollutants. 103,104 Ref. 105 activated peroxymonosulfate (PMS) - a commonly used persulfate - through photocatalysis of a carbon dot (CD) functionalized carbon nitride composite for the degradation of BPA from synthetic solutions under visible light. They evaluated the photocatalytic performance of the photocatalyst without CDs (termed "PCN") and the photocatalyst with CDs (termed "PCNC-2"); the removal efficiency was 10% and 65% under visible light for 30 minutes, respectively. The authors suggest that the notably enhanced performance can be attributed to improved light absorption, inhibition of electron/hole recombination, and an enlarged surface area by the CD introduction. In the presence of PMS, the removal efficiency significantly increased to 24% and 95% with PCN and PCN-2, respectively. Similarly, other authors have reported enhanced photocatalytic systems using nanomaterials.

A nanostructured poly(dimethylsiloxane) (PDMS) modified iron/Nafion/silica composite was studied as a catalyst in the catalytic UV oxidation of E1, E2, E3 and EE2 from synthetic solutions. The degradation of E1 achieved was greater than 90% after 60 min using 8.5 mg L⁻¹ H₂O₂. The addition of the catalyst showed better conversion of E1 compared to UV or H₂O₂. Another study evaluated the degradation of 4NP from synthetic solutions by using a MIL-100(Fe)/ZnFe₂O₄/PCN (porous carbon nitride) composite as a catalyst under visible light irradiation. The authors prepared different composites varying the mass ratio of ZnFe₂O₄/PCN (ZC) to evaluate their catalytic activity. The most active ZC composite was used to



Graphical explanation of how CD assisted photocatalysis works for BPA degradation. Created with https://BioRender.com.

produce MIL/ZC composites by adding MIL-100(Fe) in different ratios. The most active photocatalyst was 30MIL/ZC2, as it achieved 99.84% of 4NP degradation after 120 min and a dose of 30 mg of catalyst. Table 3 shows representative studies for the degradation of endocrine disruptors using different nanomaterials.

Other photooxidative methods have been advanced and improved with the coupling of membrane reactors. This is the case proposed by the study carried out by ref. 108, where a tubein-tube membrane microreactor for photochemical UVC/H2O2 oxidation systems was developed. The main innovation of this system is the radial injection of H₂O₂ into the annular reaction zone through a porous membrane, resulting in a more uniform distribution of the injected chemical across the whole reactor length. The annulus of the proposed new reactor is made up of a ceramic UF membrane inner tubing and a concentric quartz outer tubing. The UF membrane is used to deliver small volumes of H₂O₂ into the reactor's annulus as a dosing device. UVC light is given in the annulus, where the polluted solution to be removed flows, using four mercury lamps mounted externally to the outer tube. The polluted solution's helical motion around the membrane shell side improves H2O2 radial mixing. For a synthetic solution and urban wastewater supplemented with oxytetracycline, the photochemical UVC/H2O2 process had an efficiency of 36% and 7%, respectively. The H₂O₂ dosage in this system was 15.8 mg L⁻¹. Ref. 109 also designed a UVC/ H₂O₂/TiO₂ system-based tube-in-tube membrane reactor for degrading hormones from synthetic solutions. In this investigation, 48 and 30% of E2 and 17α-ethinylestradiol were found, respectively.

Ref. 110 compared direct photolysis, AOP, and photooxidation processes for the degradation of *n*-butylparaben (BP), 4-toctylphenol (OP), trenbolone (TB), and boldenone (BD) from synthetic solutions. The photolysis results showed that BD had the fastest rate of decomposition as the concentration was halved after 2 s. For BP and TB, the degradation was less efficient as it took 7.5 and 26 min, respectively, to achieve 50%. Another study assessed the photolysis of BPS from synthetic solutions considering factors such as pH, source light, and the presence of other substances.111 The authors found that the process was UV-driven. With a 40 W UV-lamp, 50% of degradation was achieved after 43.1 min. The ions in water had different effects in the process, as chloride and ferric enhanced the photodegradation, while nitrate and phosphate decreased the rate of degradation. Ref. 112 studied an AOP using a solardriven system using H2O2, for the degradation and mineralization of BPA from synthetic solutions. The optimal conditions (pH = 3, BPA concentration = 25 mg L^{-1} , and H_2O_2 concentration = 350 mg L^{-1}) allowed a BPA degradation of 77.4% and mineralization of 38.2%. Another study assessed the degradation of BPA from synthetic solutions by a photo-Fenton degradation process using persulfate (PS) and Fe²⁺. 113 The authors found that using PS and Fe2+ in a solar-driven process was the most efficient approach compared to others. A complete degradation was achieved after 5 min using a 30 W lamp and PS/ BPA ratio of 20. Ref. 114 conducted a study where a comparison between ozonation, UV-assisted ozonation (O₃/UV), and photocatalytic ozonation for the degradation of parabens (methyl-, ethyl-, propyl-, butyl-, and benzyl-) from synthetic solutions was conducted. The authors evaluated the effect of parameters like pH and time. For O₃/UV under optimal conditions (time: 45 min and pH 9), the removal efficiency was 65% for methylparaben, 62.5% for ethylparaben, 60% for propylparaben, 58% for butylparaben, and 53% for benzylparaben.

In general, AOPs are one of the most developed technologies for the implementation of WWTPs. Studies have shown high degradation efficiencies in most cases, and because of the variety of existing AOPs, these technologies can degrade most of the reported EDCs. Some AOPs based on photodegradation have already been successfully implemented in WWTPs and showed promising degradation efficiencies of EDCs. Yet, it is required to develop low-cost and highly efficient photocatalysts to spread their implementation in less developed countries.

5.1.6 Biological - biosorption and biodegradation. Biosorption consists in the passive intake of contaminants by biomass, inactivated or dead, through physicochemical processes. 115,116 This method has been widely employed in the removal of heavy metals and other inorganic contaminants;117,118 however there are several studies that have shown promising results for the removal of EDCs. The triphenyltin (TPT) mechanisms of biosorption and biodegradation by Stenotrophomonas maltophilia were evaluated in a study. 119 The

authors found that the first step involved the adsorption on the cell surface. At 0.5 mg $\rm L^{-1}$ concentration of TPT and 0.3 g $\rm L^{-1}$ of viable cells, the biosorption efficiency was 3.8% after 10 days. After biosorption, the TPT was degraded intracellularly with an efficiency of 77.8% and an overall removal efficiency of 86.2%.

Another study evaluated the role of extracellular polymeric substances (EPSs) produced by cyanobacteria *Microcystis aeru-ginosa* in the biosorption of phenanthrene (PHE). According to the results, EPS had a positive effect on the bioadsorption by *M. aeruginosa*, and with higher temperatures more PHE binding with EPS was observed. The kinetics of adsorption showed that cyanobacteria alone reached equilibrium after 24 h, while loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) reached equilibrium in less than 2 h. The adsorption capacity at equilibrium for *M. aeruginosa* was 6.78 µg mg⁻¹, while for TB-EPS and LB-TPS it was 9.47 and 12.31 µg mg⁻¹, respectively. The adsorption mechanism was mainly attributed to electrostatic and hydrophobic interactions.

The dried biomass of three kinds of microalgae, red, brown, and green, was employed as a biosorbent for the removal of benzene and toluene, evaluating the effects of ionic strength and temperature. 121 The ionic strength had a neglectable effect on the surface charge of the biomass, and the increase in temperature enhanced the adsorption and desorption processes. Brown microalgae registered the highest adsorption capacity for both benzene and toluene with 112 and 28 mg g^{-1} , respectively. London forces and hydrophobic interactions are likely to guide the adsorption mechanism. Another study evaluated phycoremediation of water using different species of intertidal macroalgae for the removal of BPA from coastal waters.122 The authors used natural seawater and fresh Ulva pertusa, Ulva prolifera, Sargassum horneri, Gymnogongrus flabelliformis, Gracilaria lemaneiformis, and Codium fragile for the biosorption experiments. The results showed that all the studied algal species obtained above 80% of removal after 8 h, while for BPA only *U. pertusa* achieved approximately 100% after 36 h. A pilot-scale experiment was carried out using *U. pertusa*, as it was the algae with the best removal efficiency, and wastewater from a mariculture system. According to these results, BPA and nonylphenol could be efficiently removed after three cycles with an initial concentration of 100 µg L-1 for both contaminants. Furthermore, a field investigation found that in the intertidal zones with a higher presence of *U. pertusa* the EDC concentration was much lower. The removal mechanism was mainly driven by biosorption.

Agro-industrial wastes are another promising alternative, as there are studies that have shown their potential to be used as bio-sorbents for the removal of several pollutants, including EDCs. This alternative for the revalorization of wastes has been extensively studied in recent years due to the growing interest in the application of circular economy principles.

A corncob agro-industrial residue was employed for the biosorption of BPA from water, evaluating the effect of BPA concentration and varying pH. 123 The maximum adsorption capacity of corncob ground fibers was 51.25 mg g $^{-1}$ after 20 min, and the results were fitted to the monolayer adsorption model of Langmuir. The overall adsorption efficiency of the

biosorbent was approximately 90%. The authors found that adsorption efficiency was higher at lower pH; however, there was no significant difference between the adsorption at pH 3, 5 and 7. At pH 9 there was significantly lower efficiency. For the BPA concentration, increasing the initial concentration resulted in a lower removal efficiency.

Another agro-industrial byproduct with potential as a biosorbent is rice husk (RH). A study evaluated the adsorption capacity of RH for removing E1, EE2 and E3 from water, evaluating the effect of pH, temperature, and competitive adsorption.124 The optimal pH and temperature conditions were neutral pH and 298 K. The equilibrium was reached after 60 min for E1 and 120 min for EE2 and E3. Kinetics experiments showed that the E1, E3, and multiple-contaminant systems fitted the pseudo-first-order model, while EE2 fitted well with both pseudo-second order and pseudo-first order models. The authors attributed this to the active sites limiting the process. Single-contaminant system adsorption data were fitted to the Langmuir model, with adsorption capacities of 2.698 mg g^{-1} for E1, 1.649 mg g^{-1} for EE2, and 0.979 mg g^{-1} for E3. Nevertheless, multi-contaminant system adsorption data did not fit into any isotherm model, probably due to the lack of competition between the hormones.

Biodegradation can be defined as the process where biological systems convert a substance into a simpler molecule by a simple or a sequence of biochemical reactions. There are plenty of studies where biodegradation of different pollutants from water is assessed, as shown in Table 4. Another study evaluated the biodegradation of nonylphenol, 4-tert-octylphenol (4tOP), and 2,4-dichlorophenol (2,4-DCP) by *Thielavia* sp. HJ22. The kinetics of removal showed that after 8 h 80%, 95% and 100% of degradation were achieved for 2,4-DCP, nonylphenol and 4tOP, respectively. The authors evaluated the degradation mechanisms and concluded that the laccase production increased the degradation rate.

The biosorption and biodegradation of TPT affected its coexisting ions. This mechanism was further accelerated by biodegradation using Brevibacillus.129 The pH had significant effects on the biosorption of TPT, where higher removal efficiencies were obtained in a pH range of 6.0-9.0. The removal of TPT at an initial concentration of 0.5 mg L^{-1} and a biomass dose of 0.3 g L^{-1} was 60% after 5 days. The effect of adding H₂O₂, glucose and rhamnolipid was evaluated. Increasing glucose concentration between 0 and 0.5 mg L^{-1} also increased the efficiency; however at higher concentrations the opposite effect was observed. Adding H₂O₂ at 6 mmol L⁻¹ increased the TPT degradation to a maximum of 86.8%; nevertheless with higher concentrations the efficiency started to decrease. A study evaluated the degradation of DBP by Rhodovulum sp. DBP07 and the effect of biostimulation, modifying nutrient sources, pH, and temperature, and studying their effect on the degradation efficiency. 130 The authors reported that a maximum degradation of 70.2% was achieved with an initial DBP concentration of 600 mg L⁻¹. Glucose was the nutrient with the most favorable effect on the cell growth and degradation rate, removing 79.8% of a 5 g L⁻¹ concentration, and 77.4% of 2 g L⁻¹. Another study employed three different marine algae (Cylindrotheca

Closterium, Dunaliella salina and Chaetoceros muelleri) for the biodegradation of diethyl phthalate (DEP) and DBP and to evaluate how a coexisting system would affect the degradation rates.131 The authors reported that in single contaminant systems, DBP degradation was slower than DEP degradation, while in coexisting systems the DBP degradation was faster. This was associated with the inhibition of DEP degradation by DBP. In the coexisting system, the DBP degradation efficiencies for algae were 40% for D. salina, 47.1% for C. muelleri, and 93.1% for C. Closterium.

A study evaluated the degradation of natural estrogens E1, E2, and E3, and synthetic estrogen EE2 with an aerobic reactor enriched with a bacterial community from activated sludge. 132 In the system, the hormones were used as the carbon sources for the bacteria, and later sequencing analysis was performed to identify the bacteria in the system. The results showed a degradation efficiency above 98% for the natural hormones after 24 h, while EE2 degradation reached 84.5% after 34 days. The authors reported Emticicia, Nubsella, and Sphingobacterium as the bacteria responsible for the degradation of hormones. Another study assessed an enhancement of microbial degradation of E2 hormone by Shewanella oneidensis by adding biochar from reed straw as a possible promoter of the metabolization of the contaminant. 133 The authors evaluated the effect of the temperature of pyrolysis of biochar on the removal efficiency, and the effect of using aerobic or anaerobic degradation. According to the results, the highest removal efficiency of E2 was 95% by anaerobic degradation using S. oneidensis with 500 °C biochar after 120 h.

Biosorption and biodegradation have shown great potential for the removal of EDCs, due to the wide variety of sorbents that can be employed, their versatility and high performance. The major challenges are related to the use of microorganisms, as it requires time-consuming processes and their production, storage and handling are far more complex than technologies where microorganisms are not required. Thus, the use of agroindustrial waste-based sorbents and immobilized instead of free microorganisms is a very promising research avenue, especially for in situ water remediation.

5.1.7 Enzymatic transformation. The use of enzymes and their biocatalytic activity have been a strategy for removing pollutants in wastewater. However, these biocatalytic processes are usually limited by the lack of stability of the enzyme, the half-life time, and the feasibility of commercial application viability. Therefore, immobilization techniques have been implemented to improve its catalytic characteristics and reuse. Ref. 134 used stabilized laccase in mesoporous silica to build a high-efficiency BPA treatment system in a fluidized bed reactor. Laccase is one of the most robust biocatalysts, with a wide range of uses in environmental processes such detecting and treating chemical contaminants and colors, as well as pharmaceutical elimination.¹³⁵ Silica particles are very attractive, as an immobilization matrix, because they have a wellordered porous structure, high surface area, thick walls, and good hydrothermal resistance. The biotransformation of BPA, in the circulating fluidized bed reactor with immobilized laccases in silica, was very fast. 90% of BPA was transformed in 8

h.134 Another study reported the degradation of BPA with free and immobilized laccases, 136 obtaining around 56 and 89% degradation in the presence of the mediator acetosyringone in 120 min at 25 °C, respectively. Ref. 137 developed a novel biocatalytic system that reached 100% degradation of BPA in 24 h, with immobilized laccase in a multi-channel ceramic membrane bioreactor. A study used magnetic copper nanoflowers as supports for the immobilization of laccase to evaluate the degradation of BPA and to improve the reutilization and separation.138 The laccase-loaded magnetic nanoflower (MNF) characterization showed that the enzymes were attached to magnetic nanoparticles through electrostatic forces. The removal efficiency reached 100% degradation of BPA in less than 5 min. Cyclability experiments showed that the MNF efficiency after 5 cycles was 95%, demonstrating high performance and cyclability for BPA degradation.

The removal of EE2 was assessed using Phanerochaete chrysosporium and Lentinula edodes, and the role of laccases in the transformation of the contaminant.139 The efficiency of degradation by P. chrysosporium was lower than that of L. edodes, with 43% and 50%, respectively. Also, it was observed that there was no enzymatic activity of laccase in the case of P. chrysosporium, and thus it was assumed that L. edodes had higher degradation efficiency due to the laccase activity. According to this, the authors modified the culture conditions to enhance the laccase production by L. edodes, resulting in an EE2 removal efficiency of 80%. The metabolites produced by the EE2 oxidation with purified laccase and those produced by laccase production-enhanced L. edodes cultures were the same, confirming that the EE2 degradation was carried out by laccase. The enzyme-mimicking strategy to degrade endocrine disruptors emerged as a necessity to overcome the drawbacks presented on natural enzymes (Fig. 5). Natural enzymes have been applied as an effective strategy to treat endocrine disruptors.140 For example, oxidases can catalyze estrogens to produce phenoxyl radical intermediates leading to the subsequent formation of homo-oligomers with a lower biotoxicity and bioavailability. 141,142 However, their application is limited by the low stability and high-cost manufacture and storage of natural enzymes. 143 Consequently, artificial nanozymes with simultaneous enzymatic and nanomaterial properties have been developed, being useful for environmental remediation purposes.141,144

As representative work,145 prepared a nanozyme termed "CH-Cu" through a hydrothermal synthesis method, using Cys-His dipeptide and CuCl2 as precursors. The catalytic performance on the degradation of chlorophenol and bisphenol pollutants was tested on the CH-Cu nanozyme and compared to that of natural laccases. The authors concluded that the CH-Cu nanozyme exhibited a higher capability to degrade those compounds, since CH-Cu nanozyme degraded approximately 90% of the representative compounds of chlorophenols and bisphenols, while the degradation efficiency of laccase was around 70% for the same pollutants under the same conditions. Similarly,146 evaluated six common nanozymes for the degradation of estrogen compounds, demonstrating that the dehydrogenase-like activity of gold nanoparticles (AuNPs) was

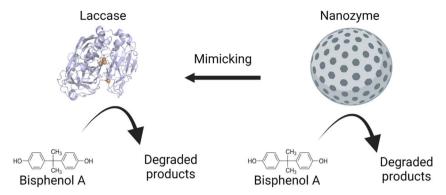


Fig. 5 Representation of laccase-mimicking process for the degradation of BPA by nanozymes. Created with https://BioRender.com.

useful to degrade E2. Moreover, they demonstrated the size effect on the catalytic activity evaluating the degradation performance for E2 of AuNP nanozymes of 13 nm and 5 nm. Smaller nanoparticles presented a higher catalytic performance for E2 degradation caused by a higher catalytically active site density.

Future prospects and challenges

The widespread use of previously discussed technologies in WWTPs or their escalation from the lab-scale to real WWTPs is strongly related to the implementation of new nanomaterials. Either as adsorbents, catalysts, membranes or supports for immobilization, there is a need to develop and implement low-cost and efficient nanomaterials that enhance the degradation/removal of EDCs reported by current technologies. Although there are novel and highly efficient nanomaterials and technologies that could significantly reduce the discharge of EDCs into the environment, in most cases the cost and complex synthesis methods hinder their implementation at WWTPs.

Nevertheless, there are promising alternatives that could serve as a transition point between current methodologies and groundbreaking technologies. For example, carbon materials derived from biomass or waste are low-cost and efficient materials that can be used in several physical, chemical or biological treatment technologies. Also, there are diverse studies focused on modifications that could enhance their performance according to their application. However, an important challenge to overcome could be the reduction of the heterogeneity of these materials and providing standardized escalation routes to synthesize these materials.

In addition, an integrated approach using hybrid treatment technologies and real time monitoring of EDCs should be prioritized. As it has been proven, in most cases the conjunction of different technologies significantly enhances the degradation of EDCs. Furthermore, real time analysis of concentrations will allow us to adjust to the optimal parameters of hybrid systems.

7. Conclusions

Wastewater and industrial effluents are some of the main sources for EDC contamination. As currently most of the WWTPs are not prepared to remove EDCs from wastewater, it is required to implement more strict regulations on the monitoring, use and removal of EDCs.

Due to the complex transportation pathways of EDCs, it is required to develop accessible alternatives for portable detection devices and real time monitoring of the concentration of EDCs, which will allow a faster and easier identification of the sources of contamination and determine the best strategies for the mitigation of environmental impact.

Adsorption, AOPs and enzymatic transformation are some of the technologies that are closer to being widely implemented at WWTPs for efficient EDC removal. Due to their properties, carbon-based nanomaterials have a main role in enhancing the performance of these technologies. Additionally, the use of waste or biomass derived materials could help to reduce the gap and make advanced wastewater treatment technologies more accessible all the world.

Author contributions

Jesús Alfredo Rodríguez-Hernández: writing – original draft preparation, visualization, and conceptualization. Rafael G. Araújo: writing – original draft preparation, visualization, supervision, and conceptualization. Itzel Y. López-Pacheco: writing – original draft preparation. Laura Isabel Rodas-Zuluaga: writing – original draft preparation. Reyna Berenice González-González: writing – original draft preparation and visualization. Lizeth Parra-Arroyo: writing – original draft preparation. Juan Eduardo Sosa-Hernández: writing – review & editing. Elda M. Melchor-Martínez: writing – review & editing. Damià Barceló: writing – review & editing. Lorenzo M. Pastrana: writing – review & editing. Hafiz M. N. Iqbal: writing – review & editing. Roberto Parra-Saldívar: writing – review & editing and funding acquisition.

Conflicts of 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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