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Removal processes and estrogenic activity of bisphenol—A and triclosan using microalgae

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ABSTRACT

This study aimed to evaluate the effect of microalgal photoautotrophic treatment on estrogenic activity (EA) and removal process of two emerging contaminants (ECs), bisphenol-A (BPA) and triclosan (TCS), in synthetic wastewater (SWW). The concentration used for BPA (17 mg/L) and TCS (325 μg/L) is the median effective concentration (EC_{50}). Two conditions were evaluated, using a microalgae inoculum of ≈300 and ≈500 mg TSS/L (Total Suspended Solids per liter). For BPA, biodegradation was found to be the removal process contributing to the highest percentage removal, reaching >40 % for both initial microalgae inoculum (≈300 and ≈500 mg TSS/L). For TCS, the highest removal process was photodegradation, with >28 % (sum of direct and indirect removal). However, for TCS it was observed that for TSS ≈ 500 mg/L TSS, sorption (adsorption and absorption) increased by ≈17 % with respect to that determined for TSS ≈ 300 mg/L. Microalgae photoautotrophic treatment, using ≈500 mg TSS/L, resulted in a reduction of EA for TCS (by 33 %); but a 1.13-fold increase of EA for BPA. No EA effect of BPA and TCS was observed at ≈300 mg TSS/L. Both treatments resulted in a removal of >95 % of BPA and ≈86 % of TCS. For direct photodegradation, removals of both BPA and TCS were quantified as 3.8 % and 14.4 %, respectively. However, an increase in EA was observed for both ECs (1.79-fold for BPA and 1.23-fold for TCS). Indirect photodegradation resulted in removals of 26.2 % and 14.1 %, respectively. Additionally, EA showed a 2.4-fold increase for BPA, whilst a 17.99 % decrease was observed for TCS. In conclusion, no linear correlation was observed between EA and EC removals. Microalgae photoautotrophic treatment resulted in high removal efficiencies of TCS and BPA, as well as a decreased EA of TCS.

1. Introduction

The presence of emerging contaminants (ECs) in secondary and tertiary treatment effluents is a water reuse barrier as ECs are not completely removed in treatment plants. [1,2]. Conventional wastewater treatments are not designed to treat organic contaminants. Effluents leaving the plants are considered to be a source of endocrine disruptor chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs) [3].

PPCPs and EDCs induce effects by mimicking or antagonizing the in vivo and in vitro effects of natural estrogens such as 17β-estradiol, which are defined as substances with estrogenic activity (EA). These xenobiotic compounds with EA interact with estrogen receptors (ERs) and cause adverse health effects [6]. The response of EA does not follow a linear pattern [7]. Reports have found that there is no relationship between the intensity of the EA response and the concentration of ECs [7]. Estrogenic effects associated with BPA include testicular and hematopoietic malignancies, susceptibility to mammary and prostate neoplastic lesions, increased incidence of breast cancer, decreased sperm quality and infertility, polycystic ovary syndrome, altered natural development, obesity, cardiovascular disease, and type 2 diabetes [8,9]. Estrogenic effects of TCS exposure are various, including: an increase in uterine weight in mammals [10], adverse effects on cardiovascular systems, spontaneous abortions, fetal malformations, liver stress leading to severe hepatocellular changes [11], hormonal changes, induction of antibiotic tolerance, allergic reactions, neurotoxicity, or suppression of the immune system [12]. In vitro assays using recombinant yeasts are used to determine EA. These yeasts can identify compounds with EA, the most commonly used yeast being genetically modified *Saccharomyces cerevisiae* [13–15]. The yeast assay is called "Yeast Estrogen Screen"

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(YES) and has the disadvantage that it takes three days to obtain results. Modifications of the YES technique have been made to reduce the analysis time to 24 h [14,16] and 12 h [17]. In the modification by Sanseverino et al. [17], the yeast responds to estrogenic compounds that, upon crossing the cell wall and binding to human estrogen receptors, activate the transcription of the LuxA and LuxB genes, which produce the enzyme luciferase that generates light emission. Because of the light emission, the modification of this technique is the Bio-Luminiscent Yeast Estrogen Screen (BLYES) [17]. BLYES have been successfully used worldwide for chemical screening and evaluation, and ECs monitoring in surface water, drinking water and wastewater, providing rapid (typically within hours), cost-effective, high-throughput detection of ECs [18].

Several green microalgae species have been employed in the study of removal of various ECs. They are favored due to their high removal efficiency and recovery as fertilizer, biofuel, and production of high-quality water effluent [19,20]. The removal of BPA by the action of microalgae has been reported, reaching removal percentages of 90 %. Some of the species used are *Chlorella fusca* [21], *Monoraphidium braunii* [22], *Chlamydomonas reinhardtii, Scenedesmus obliquus, Chlorella pyrenoidosa,* and *Chlorella vulgaris* [20]. For TCS removal, the reported percentage removal was 69.3 - ≈ 100 % using *C. pyrenoidosa, Desmodesmus* sp., *S. obliquus* [23]. 69 % removal of tetracycline was achieved using *C. vulgaris* [24], 58.8 % removal of naproxen using *Scenedesmus quadricauda* [25]. A maximum of 13 % removal of ciprofloxacin was achieved using *Chlamydomonas mexicana, Chlamydomonas pitschmanni, C. vulgaris*, and *Ourococus multisporus* [26], with 31–62 % removal of sulfamethazine and 28–47 % for sulfamethoxazole using *S. obliquus* [27].

The main processes that occur in reactors using microalgae for wastewater treatment to remove emerging contaminants are biodegradation, photodegradation, volatilization, bioaccumulation and sorption [4,28,29]. ECs biodegradation is an alternative method for their removal. Here, ECs are broken into smaller molecules that are less toxic or less harmful than the original compound, in a process catalysed by enzymes [30,31]. Sorption of ECs is defined as the removal of substances from the aqueous phase to a biological material. It involves physico-chemical mechanisms such as sorption (the contaminant enters the microalgal cell, only possible in living biomass) and adsorption (the contaminant remains on the cell surface). Biosorption is influenced by contaminant properties such as hydrophobicity, functional groups, pH, temperature, and contact time [30,32–34]. Photodegradation is a process that can be used for the removal of ECs. Direct photodegradation occurs when photons of light are absorbed by an EC, breaking bonds [35,36]. Indirect photodegradation occurs with the generation of free radicals produced during irradiation with sunlight; these free radicals are formed in the presence of dissolved organic matter such as humic and fulvic acids, nitrates and some metal ions [35,36]. Nitrate and nitrite influence radical transformation and ECs degradation due to photolysis of nitrate/nitrite. HO• and reactive nitrogen species can be generated in photolysis of nitrate/nitrite [37].

Furthermore, microalgae can remove environmental estrogens from aquatic environments through processes such as biosorption, bioaccumulation and biodegradation [4,5]. EA removal has been reported for process such as photodegradation and in experiments with microalgae, where the removal process involved in EA reduction are not specified. Photodegradation experiments not involving any other type of treatment include achieved a 95 % removal of EA associated with BPA [38]. Many photodegradation experiments were assisted with chemical oxidation. ECs such as BPA [39–44], estradiol (E2) [41–43,45], 17α-ethynylestradiol (EE2) [41,42,45], 4-NP and 4-octylphenol [42], estrone and estriol [42,45] were removed. The most reported chemical reagents were H_2O_2 [36,37], Fe^{2+}+H_2O_2 [42], TiO_2 + irradiation with ions added as SO_4^{2-}, Cl^- NH_4^+, HPO_4^{2-}, HCO_3^- and NO_3^- [44], chlorination [43]. The EA assay used by the by the aforementioned authors were YES [39–43,46–48], Y2H (Yeast two-hybrid) [38,44,49–51], MCF-7 cells [45], and BLYES [7]. The use of microalgae for the removal of EA has also been studied, but there are few reports of this application. Microalgae species employed were *C. fusca* [21], *Scenedesmus dimorphus* [52], *C. reinhardtii, S. obliquus, C. pyrenoidosa* [20], *C. vulgaris* [1,20], *S. capricornutum, S. quadricauda* [1]. Reported ECs removed by microalgae were BPA [21], E2, EE2 [1,20], estrone, estriol [20]. Among 50 ECs present in wastewater, BPA and TCS were detected [20]. The EA assays used were YES [1,20,21] and *E*-screen [52]. Other treatments to remove EA associated with ECs, including coagulation, chlorination, bromination, oxidation by permanganate and carbon nanotubes have been proposed [46,53–55]. Chlorination has been used to remove the EA associated with bisphenol-A (BPA), triclosan (TCS) and 4-nonylphenol (4-NP) [7,49,50]. In addition to chlorination, bromination has been used to remove EE2 [46]. Oxidation with ozone in the gaseous phase was used to remove BPA, TCS, 4-NP, and EE2, and achieved >77 % of EA reduction [7,47,51]. For other wastewater treatments, BLYES was used to perform a comprehensive study of estrogenic activity in wastewater with the presence of 30 representative ECs (including BPA) in 12 municipal wastewater treatment plants using different treatment processes. High correlation coefficients ($p < 0.001$) were found between BLYES and a chemical analysis including ECs concentration, EC_{50} for 17β-E2 and EC_{50} for each ECs [56].

While there is evidence of adverse effects of ECs on aquatic organisms and human health, little information is available on removal of EA in microalgal wastewater systems, and the contribution of the metabolic process to the EA response of ECs. This study was designed to evaluate the removal process involved on biological treatment using microalgae on EA and on removing TCS and BPA. For the treatment, a microalgal mixed culture containing *S. obliquus* and *Desmodemus* sp. was inoculated into synthetic wastewater (SWW). EA was measured using the yeast-based in vitro BLYES assay and compound removal was determined by Gas Chromatography/Mass Spectrophotometry (GC/MS). For each process involved, concentration of ECs and measurement of EA were performed.

2. Materials and methods

2.1. Reagents

The reagents used for the assays were BPA ≥99 % - Sigma Aldrich 239,658-50G, TCS analytical grade Sigma Aldrich PHR-1338-1G and methanol HPLC grade ≥ 99.9 % Sigma Aldrich 34,860-1 L, 17β-estradiol ≥98 % Sigma Aldrich E8875-250MG. Reagents for minimal medium without leucine and modified uracil (YMM leu- ura-) for yeast growth were purchased from Sigma Aldrich. Additionally, reagents used for the preparation of synthetic wastewater (SWW) and sampling for analysis were analytical grade.

2.2. Microalgae mixed culture and culture conditions

Experiments were carried out at laboratory scale, and microalgae were acclimatized to SWW. The composition of SWW was based on a modified BG-11 medium recipe and the microalgae mixed culture was the same as used in a previous study [57].

2.3. Experimental design

2.3.1. Removal of ECs by the effect of microalgae

The EC_{50} concentrations determined in a previous study [52] were used to assess the removal of the EA of BPA and TCS after treatment with microalgae. For the microalgae mixed culture formed by *S. obliquus* and *Desmodesmus* sp., BPA = 17 mg/L and TCS ≈ 325 μg/L were used. Experiments were carried out with two different initial inoculum concentrations, ≈300 or ≈500 mg TSS/L. TSS was determined using APHA standard method 2540 D [58]. The culture conditions were 600 mL initial volume, 12/12 h light/dark cycles with a light intensity of 100

μmol/m^2s (cold white light), constant agitation of 150 rpm , room temperature and a time of culture of 15 days in batch experiments. Each experiment was performed in triplicate.

2.3.2. Photodegradation for removal of ECs

To determine the effect of light on the degradation of ECs, controls were performed in the absence of microalgae. In these experiments, the reduction of EA and the concentration of BPA and TCS were monitored. Direct photodegradation, in which light was directly incident on the bonds of the molecules, was evaluated. Indirect photodegradation, in which free radicals are formed that break the bonds of the molecules was also studied [35,36]. For photodegradation, the same EC_{50} concentrations were used for BPA and TCS as for microalgae treatments. For direct photodegradation, ultrapure water type I (electrical resistivity = 18.2 MΩ/cm) was used as the medium. For indirect photodegradation, SWW was used to induce free radical formation. The experimental conditions of volume, agitation, light intensity, temperature, treatment time were the same as those used in the experiments with microalgae. A control was performed with the same characteristics as the direct photodegradation but protected from light. Each experiment was performed in triplicate.

2.4. Sampling for analysis

Samples of 20 mL were taken at the beginning and end (zero and 15 days) of microalgae and photodegradation experiments. Samples were stored at 4 °C, protected from light. To determine the EA and removal of BPA and TCS in the aqueous phase, a sample from microalgae experiments was centrifuged (Hermle Labortechnick GmbH, model Z513K) at 11055 xg (8000 rpm) for 25 min. The supernatant was filtered through a filter with pore size of 0.22 μm (Millex Filter Unit, Ref.: SLGV033NB). After filtration, the analytes were extracted by solid phase extraction (SPE) (SampliQ C18 ODS, 500 mg, Agilent) to elute the compounds, methanol (HPLC grade) was passed through the cartridges. Later, the sample was concentrated with nitrogen gas to a final volume of 300 μL (SPE extract).

To determine the concentration of ECs sorbed by the microalgae cells, the pellet obtained after centrifugation was treated according to the methodology of He et al. [59], the aim is to desorb the sorbed ECs. Only day zero (absence of ECs on microalgae cells) and day 15 (absence of ECs) sorption samples were evaluated. Recovery of the ECs retained in the microalgae cell wall is the first phase of desorption. The pellet was washed twice, for each wash, 6 mL of methanol was added and shaken for 60 s, followed by centrifugation at 11055 xg for 25 min. The supernatant was subjected to filtration (0.22 μm pore size), SPE and concentration under nitrogen (SPE-adsorbed extract). Cell lysis was the second phase of the desorption of the ECs that had crossed the cell wall. To the resulting pellet, 3 mL of a dichloromethane/methanol mixture (1:2 *v*/v) was added and sonicated (Branson 2510 DTH Ultrasonic) for 20 min. The sample was subsequently centrifuged at 11055 xg for 25 min. Washes with the dichloromethane/methanol mixture were performed three times to ensure EC extraction. The supernatant was filtered, passed through SPE and concentrated under nitrogen (SPE-absorption extract).

Samples from photodegradation experiments were filtered through a 0.22 μm pore size filter, passed through SPE, and concentrated as describe previously (photodegradation SPE extract). Photodegradation samples were evaluated on days zero and 15. The aim of this treatment was to make the SPE extract available for both analyses: GC/MS and BLYES determinations.

2.5. Chemical analysis

EA analysis and quantification of BPA and TCS were performed using the SPE extract obtained from samples. The removal of EA and the concentration of TCS and BPA were assessed using an integrated BLYES-GC/MS method [7,17].

2.5.1. GC/MS analysis

Quantification of BPA and TCS was performed by GC/MS using an Agilent 7890 A GC coupled to an Agilent 5975C triple Axis MS detector. The DB5MS column (30 m × 0.25 mm i.d, 0.25 μm film) was used for the analysis and helium as carrier gas. The injection temperature was 250 °C and an injection volume of 1 μL of SPE extract was used. The oven programming was according to that reported by Ma et al. [60]. The analysis was carried out in SIM mode. The characteristic ions were BPA: 65, 91, 119, 213 and 228 *m/z*, while for TCS: 51, 63, 79, 114, 146, 218 and 290 *m/z*. Samples from each removal process were injected in triplicate ($n = 9$).

2.5.2. Estrogenic activity (EA) analysis

EA analyses were carried out according to the methodology used by Orta et al. [7]. *S. cerevisiae* yeast was used [17] which is able to create a bioluminescent response in the presence of estrogenic compounds. Yeast culture was performed overnight in YMM leu- ura- medium at 30 °C and 120 rpm for 12 h until it reached an OD_{600} of approximately 1.0. Appropriate dilutions of the SPE extracts were performed and included a positive control with 17β-estradiol and negative controls with HPLC methanol and ultrapure water type I. Samples were placed in a 96 well assay plate with lid (Costar, Ref. 3917, Lot. 11,522,039). 200 μL of *S. cerevisiae* culture were added to each well of the plate. Bioluminescence was measured using a microplate reader (Biotek FLx800). Luminescence recording was performed with a Gen5™ Microplate Reader and Imager software version 3.02 (BioTek Instruments, Inc.). The calculation of the toxic equivalency of BPA and TCS was performed by dividing the EC_{50} of 17β-estradiol by the EC_{50} of the sample, according to equation no. 1 [7].

$$EEQ = \frac{EC_{50}\ (E2)}{EC_{50}\ (sample) \times CF_{SPE}} \quad (1)$$

where:

- EEQ: estrogen equivalents measured as the relative potency against 17β-estradiol, ng/L.
- EC_{50} (E2): 17β-estradiol EC_{50}, result provided by the luminometer software, in mol/L (M).
- EC_{50} (sample): Sample EC_{50}, result provided by the luminometer software, in mol/L (M).
- CF_{SPE}: Concentration factor from SPE treatment, in this case 66.666 (initial 20 mL of sample concentrated to a final volume of 300 μL). Samples from each removal process were analyzed by triplicate ($n = 9$).

2.6. Mass balance for removal process

From the determination of the concentration of ECs in the medium (residual concentration), absorption, adsorption, and by the action of photodegradation, the mass balance is represented by Eq. (2):

$$X_b = X_i - X_r - X_e - X_a - X_{dp} - X_{ip} - X_{of} \quad (2)$$

where, X_b: biodegraded concentration; X_i: initial concentration; X_r: residual concentration in the aqueous phase; X_e: adsorbed concentration; X_a: absorbed concentration; X_{dp}: concentration degraded by direct photodegradation; X_{ip}: concentration degraded by indirect photodegradation; X_{of}: concentration degraded by other factors (sample from light-protected photodegradation experiment).

The biodegradation concentration (X_b) refers to the difference in the amount of ECs due to the action of the microalgae. The water used, SWW, does not contain any bacteria present in ordinary wastewater; although the experiment was not carried out under sterile conditions, the presence of microorganisms is minimal with respect to the action of

the microalgae. Other factors can include: incrustation of emerging contaminants on the flask walls, slight photodegradation, presence of microorganisms other than those reported (this study was not conducted under sterile conditions) [61]. Sample handling, retention in the SPE cartridge and slight volatilization may also affect the results. These factors have not been considered individually in the removal of ECs.

2.7. Statistical analysis

Graphs were produced using Microsoft Excel Professional Plus 2016, and analysis of variance (ANOVA) was carried out using R Studio 4.1.2 software.

The amount of biomass with two concentrations (TSS ≈ 300 and ≈500 mg/L) and the exposure time with two conditions ($t = 0$ days, 15 days) were considered as factors in the ANOVA test for the experiments with the presence of microalgae. In the case of the photodegradation experiments, the factors to be considered were the exposure time with two conditions (t = 0 days, 15 days) and the type of photodegradation with three factors: direct (medium: Milli-Q water), indirect (medium: SWW) and control (Milli-Q water, protected from light).

3. Results and discussion

BPA and TCS are ECs with estrogenic activity and cause oxidative stress [6,8–12,62]. Atengueño-Reyes et al., [57], reported that the presence of BPA and TCS did not show negative effects on the growth of microalgae (TSS), on the production of biomolecules such as carbohydrates, lipids and proteins, as well as on the amount of chlorophyll "a". In this study, it was also observed that the capacity of microalgae to digest wastewater was not reduced, and in some parameters (nitrates, ammoniacal nitrogen, orthophosphates, total alkalinity) it was observed that the presence of ECs increased the nutrient uptake.

3.1. Total removal percentage of BPA and TCS

The total removal of BPA was 95.1 % ($P < 0.05$), equivalent to 16.172 ± 0.828 mg/L for the initial inoculum of ≈300 mg/L. For the initial inoculum of ≈500 mg/L, the total removal was 95.68 % ($P < 0.05$), corresponding to 16.271 ± 0.703 mg/L. For TCS, the total removal was 61.1 % ($P < 0.05$), equivalent to 192.383 ± 23.031 μg/L for the initial inoculum of ≈300 mg/L; while for the initial inoculum of ≈500 mg/L, the total removal was 86.25 % ($P < 0.05$), equivalent to 271.584 ± 6.362 μg/L.

The removal of emerging contaminants by microalgal action has been reported for microalgae consortia. A consortium consisting of *Stigeoclonium* sp. diatoms, *Chlorella* sp. and *Monoraphidium* sp. was used for the removal of 27 ECs found in urban wastewater. The removal was carried out at pilot scale, achieving a removal of >90 % [19]. Another example of using a microalgae consortium to remove ECs was the use of *Chlorella* sp., *Scenedesmus* sp., *Vorticellides* sp. and *Uronema minutum*. The removal of 2 mg/L of 17β-estradiol in wastewater reached a percentage of 55–100 % at pilot scale and at laboratory scale a removal percentage of 71–100 % was achieved [63]. BPA removal reached a percentage of 20, 46.4, 42.9, and 43 % from initial BPA concentrations of 2, 4, 6, and 8 mg/L, respectively. This was achieved with a consortium of *C. pyrenoidosa, Acinetobacter* sp., *Serratia marcencens*, *Pseudomonas* sp. and bacteria [64].

The use of microalgae monocultures has also been reported for the removal of ECs. In the case of BPA, the use of *C. fusca* resulted in removal percentages >95 % of a BPA concentration range of 10 to 80 μM and 70 % at 160 mM [21]. Zhou et al. [20] removed 50 ECs in municipal wastewater, of which approximately 32 had a removal >50 %. Among these ECs, BPA and TCS were found to be removed by *C. reinhardtii* (14.25 % BPA; 41.72 % TCS), *S. obliquus* (11.2 % BPA; 68.58 % TCS), *C. pyrenoidosa* (0.8555 % BPA; 57.55 % TCS), and *C. vulgaris* (0.953 % BPA; 47 % TCS). Triclosan removal (400 μg/L) has been reported for *C. pyrenoidosa, Desmodesmus* sp. and *S. obliquus* with removal percentages of 69.3, ≈100, and 99.7 %, respectively [23].

Removal of ECs other than BPA and TCS has been reported for species similar to those used in this study. Ding et al. [25] removed naproxen with *S. quadricauda* with removal of 58.8, 72.6, and 1.7 % at initial concentrations of 1, 10, and 100 mg/L, respectively. Xiong et al. [27] removed sulfamethazine and sulfamethoxazole with *S. obliquus*, with removals of 31 to 62 % for sulfamethazine and 28 to 47 % for sulfamethoxazole.

It was observed that the results obtained in this study are similar to the reported capacity of microalgae to remove ECs, although the initial concentration of ECs used in this study exceeds those reported in the cited studies. Therefore, it was concluded that the mixed culture composed of *S. obliquus* and *Desmodesmus* sp. has a BPA removal capacity of >95 % and for TCS the capacity exceeds 60 %.

3.2. Removal process

Bioadsorption, bioaccumulation, biodegradation and photodegradation are the removal processes of ECs. However, it must be considered that the optimal removal processes of ECs by photosynthetic microorganisms are strongly influenced by the characteristics of the ECs and the microalgae specie [29]. The contribution to the removal of BPA and TCS was calculated according to Eq. 2 from the GC/MS quantification of the extracts obtained from the SPE. It was observed that BPA is an EC with a higher percentage of biodegradation (>40 % at both initial TSS) compared to TCS (≈12–20 %). While the main route contributing to the removal of TCS is photodegradation >28 % (sum of direct and indirect) (Table 1).

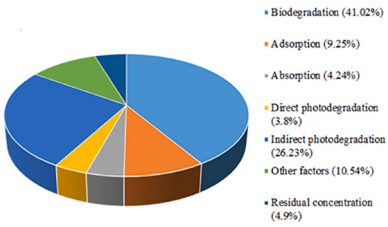
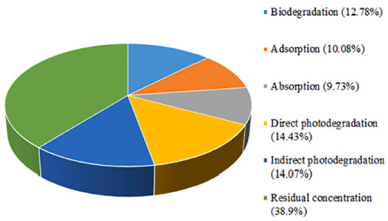
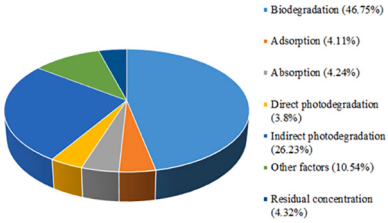
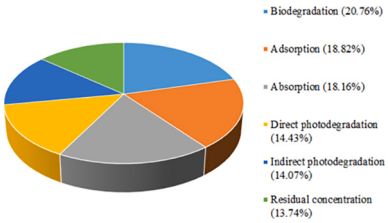
3.2.1. Biodegradation

Studies conducted by other authors show that the BPA removal process for other microalgal species are predominantly via biodegradation [23,36,65,66]. Using *Chlorella sorokiniana*, removal percentages of 38.5, 30.7, and 20.7 were obtained for BPA concentrations of 10, 20, and 50 mg/L, respectively [65]. Wang et al. [36] reported removal percentages of 57, 25, 18 and 26 for BPA concentrations of 1.3, 5.5 and 13.5 mg/L, respectively, which they attributed to the bioactivity of *Desmodesmus* sp. WR1. Ji et al. [66], reported that when BPA was removed by *C. mexicana* and *C. vulgaris* individually, the joint removal process were bioaccumulation and biodegradation. The percentage of BPA removed was 39 and 28 % for *C. mexicana* and *C. vulgaris*, respectively. The biodegradation values reported by other authors differ slightly from those obtained in this study (>40 %), which is consistent with what has been reported for BPA and its high tendency to biodegrade.

Few studies have been conducted on the removal process for triclosan, for example, Wang et al. [23] found that the amount of TCS (400 μg/L) taken up by microalgae was 59.2 % on the first day, while on the seventh day 55 % was quantified for *C. pyrenoidosa* (10^7 cells/mL). For *Desmodesmus* sp. TCS uptake was 39.9 % on the first day, decreased to 14.5 % on the fourth day, and was 2.8 % on the seventh day. *S. obliquus* had an intake of 2.1 % on the first day of exposure and 1 % on the seventh day. The biodegraded amount of TCS studied was quantified in the range of 12–20 % at TSS ≈ 300 mg/L (3.33×10^6 cells/mL) and TSS ≈ 500 mg/L (6.21×10^6 cells/mL). It was observed that the removal percentage reported by Wang et al. [23] was higher than that obtained in the present study. An explanation for this could be due to the initial number of microalgae used, which is 3-fold and 1.61-fold higher compared to TSS ≈ 300 mg/L and TSS ≈ 500 mg/L, respectively.

Other ECs for which removal processes were evaluated are those reported by Matamoros et al. [67]. Caffeine, ibuprofen, galaxolide, tributyl phosphate, 4-octylphenol and tris(2-chloroethyl) were removed by microalgal consortium, mainly *Chlorella* sp. and *Scenedesmus* sp. Matamoros et al. [67] determined the concentration of biodegraded ECs based on the difference between degraded fractions. It was found that

Table 1
Contribution to the removal of BPA and TCS due processes removal.

		Bisphenol-A		Triclosan	
		Initial concentration (X_i) = 17.01 ± 0.48 mg/L		Initial concentration (X_i) = 314.86 ± 36.25 μg/L	
		TSS ≈ 300 mg/L			
Removal process	Eq. 2 term	Concentration (mg/L)	% Removal	Concentration (μg/L)	% Removal
Biodegradation	X_b	6.98 ± 0.20	41.02	40.23 ± 22.86	12.78
Adsorption	X_e	1.57 ± 0.04	9.25	31.75 ± 0.07	10.08
Absorption	X_a	0.72 ± 0	4.24	30.65 ± 6.11	9.73
Direct photodegradation	X_{dp}	0.65 ± 0.08	3.8	45.45 ± 1.90	14.43
Indirect photodegradation	X_{ip}	4.46 ± 0.26	26.23	44.31 ± 17.15	14.07
Other factors	X_{of}	1.79 ± 0.49	10.54	Not detected	Not detected
Residual concentration	X_r	0.83 ± 0.04	4.9	122.48 ± 23.03	38.9
Graphic					
		TSS ≈ 500 mg/L			
Biodegradation	X_e	7.95 ± 0.15	46.75	65.39 ± 22.86	20.76
Adsorption	X_a	0.70 ± 0	4.11	59.26 ± 15.35	18.82
Absorption	X_{dp}	0.72 ± 0.01	4.24	57.18 ± 14.61	18.16
Direct photodegradation	X_{ip}	0.65 ± 0.08	3.8	45.45 ± 1.9	14.43
Indirect photodegradation	X_{of}	4.46 ± 0.26	26.23	44.31 ± 17.15	14.07
Other factors	X_r	1.79 ± 0.49	10.54	Not detected	Not detected
Residual concentration	X_r	0.74 ± 0.01	4.32	43.28 ± 6.36	13.74
Graphic					

Standard deviation is equivalent to 9 measurements (n = 9).

volatilization contributed to 99 % removal for the ECs galaxolide, tributyl phosphate, 4-octylphenol, and tris(2-chloroethyl). While biodegradation was effective for caffeine (59 %) and ibuprofen (95 %).

3.2.2. Photodegradation

Photolysis of BPA (5 mg/L) in the presence of UV-A and UV-C in distilled water degrades 70 % of BPA [42]. Removal of BPA (10, 20, and 50 mg/L) by abiotic factors (microbial action was negligible) was 11.3, 13.2, and 18.8 %, respectively [65]. Koumaki et al. [68], for a BPA concentration of 2 μg/L, obtained a removal <60 % after 110 h of exposure (photodegradation) and 30 % after 163 h of exposure (photolysis), but this removal increased to 90 % in the presence of NO_3^- (10 mg/L). In the same study, the photodegradation of TCS was observed, which presented the highest photodegradation without the presence of nitrate ion (93.55 %), the addition of the ion slightly increased the removal to 98.06 and 96.77 % at nitrate ion concentrations of 1 and 10 mg/L, respectively. Martínez-Zapata et al. [69], found 72 % removal of TCS (2 mg/L) after 19 h of exposure. Wang et al. [36], investigated direct (Milli Q water) and indirect (wastewater) photodegradation for TCS at 150 μg/L; there was a removal of 97 % (direct photodegradation) and 62 % (direct photodegradation) at 4 h of exposure. The author argues that the primary process of TCS photodegradation is direct, i.e. the action of light to break the bonds of the molecule. Wang et al. [36] found that TCS at 400 μg/L had ≈3.28 % removal by photodegradation.

In the present study, it was observed that the greatest removal due to the effect of photodegradation of BPA was indirect, where the chemical species present in the SSW enhanced the percentage removal. A removal rate of 3.8 and 26.23 % was obtained for direct and indirect photodegradation, respectively. As reported by Koumaki et al. [68], the presence of nitrate enhanced the degradation of BPA, where the average initial amount of nitrate was 5.1 mg/L. The interaction of light and nitrate ion forms nitrite radical and $^{\cdot}O^-$ radical; the latter reacts with water to form hydroxyl ion and hydroxyl radical [68]. This explains the higher degradation efficiency in synthetic wastewater. The photodegradation of TCS was 14.43 and 14.07 % for direct and indirect photodegradation, respectively. The difference between the two processes of removal that can be attributed to photodegradation is minimal.

3.2.3. Sorption

There are few studies reporting percentage removal by sorption on live (or biologically active) microalgae. Eio et al. [65], using *C. sorokiniana*, reported a percentage removal of 0.05 and 0.11 % for microalgae concentrations of 10^6 and 10^7 cel/mL, respectively, while no BPA was detected at concentrations of 10^4, 10^5 cel/mL. Wang et al. [70] removed TCS (800 ng/L) with *C. pyrenoidosa* (3×10^7 cells/mL) with a removal of 72.3 % after 6 h of exposure. The authors reported that the removal process involved were adsorption and absorption. The major differences between the results of the present study and those reported are the initial concentration of microalgae and the initial concentration of ECs. When comparing the sorption in this study for BPA and TCS, large differences are observed. For BPA, the sorption percentages were 9.25 % for adsorption and 4.24 % for absorption at TSS ≈ 300 mg/L, respectively. For TSS ≈ 500 mg/L, adsorption and absorption

contributed to BPA removal of 4.11 % and 4.24 %, respectively. It was observed that by increasing the initial concentration of microalgae, adsorption decreased by ≈5.14 %, but this percentage is reflected in the increase of biodegradation. This trend indicates the tendency of BPA to biodegrade. In the case of TCS, sorption at TSS ≈ 300 mg/L was quantified at 10.08 % and 9.73 % for adsorption and absorption, respectively; while at TSS ≈ 500 mg/L the adsorption and absorption increased to 18.82 % and 18.16 %, respectively. It was observed that by increasing the initial concentration of microalgae, the sorption, in general, increased by 17.17 %. This increase in the contribution of sorption as a removal process is reflected in the decrease of the residual TCS concentration. If the amount of TCS sorbed is compared against the amount biodegraded and photodegraded, a greater contribution is observed, this is because TCS is a weak acid, lipophilic and non-volatile, it is moderately soluble in water, it is photodegradable, and, being lipophilic, it tends to bioaccumulate in adipose tissue [71–74]. TCS could bioaccumulate in lipids, due to the high polyunsaturated fatty acid content of microalgae [75]. There is an accumulation at the cytoplasmic level, TCS causes oxidative stress that results in lipid peroxidation. This increases membrane permeability, resulting in TCS diffusion [76].

3.3. Estrogenic activity

Results for removal and EA of BPA and TCS in microalgae experiments are shown in Table 2. "Response" in this table refers to the tendency of estrogenic activity to change at different concentrations of ECs. In this regard, Myers and Hessler [77] suggest that ECs do not exhibit a linear pattern in their dose-response relationship. This is because many EDCs with estrogenic activity do not follow this pattern, but instead exhibit non-linear, U-shaped or inverted U-shaped dose-response curves. This pattern means that they can cause toxic effects at high doses, no effect at intermediate doses and adverse effects at low doses or vice versa. Bergamasco et al. [78] report similar patterns for surface water where they analyse the response of estrogenic activity with EC concentrations, using high performance liquid chromatography (HPLC). These authors indicated that BLYES showed higher sensitivity than HPLC, and that for BPA concentrations of 8.1 or 47 ng/L they showed the same response for BLYES: 0.2 ng/L EEQ of 17β-estradiol.

The final BPA percentage removal reached 95.1 % and 95.68 % ($P < 0.05$) at initial concentrations of ≈300 and ≈500 mg TSS/L, respectively. The EA of BPA at day 15 of exposure increased 7-fold and 1.13-fold ($P < 0.05$) at initial levels ≈300 and ≈500 mg TSS/L, respectively. The TCS at day 15 of exposure was quantified as 61.10 % and 86.25 % ($P > 0.05$) of removal at ≈300 and ≈500 mg TSS/L, correspondingly. The EA increased 4.37-fold and decreased 0.33-fold ($P < 0.05$) at ≈300 and ≈500 mg TSS/L, respectively. The removal rates achieved in this study for BPA and TCS are consistent with a recent review published by Sun et al. [5] which indicated that in laboratory studies, the removal efficiency of environmental estrogens by microalgae was in most cases >80 %.

With respect to the concentration of ECs sorbed by adsorption and absorption, the estrogenic activity showed an increase for both ECs with respect to that determined on day zero. BPA was the EC that showed the highest increase in EEQ 17β-estradiol (ng/L). While TCS had the lowest increase in EEQ 17β-estradiol (ng/L) with respect to day zero.

There are few studies in which EA is determined by removal of ECs. There are 4 studies that use microalgae, but do not attribute EA response to a specific removal process. As for photodegradation, few studies have determined EA; most studies focus on chemically assisted photodegradation. This study determined the response of the EA for each removal process, thus providing an idea of how each removal process modifies the variation in the EA.

3.3.1. Removal of estrogenic activity by of microalgae treatment

The data obtained in this study indicate that the percentage of removal of emerging contaminants was about 95 % for BPA and slightly >86 % for TCS when photoautotrophic treatment with microalgae (≈500 mg TSS/L) was applied. Experiments reported using microalgae to reduce EA are limited. Table 3 shows reported studies where EA is removed by different treatments such as microalgae, oxidation and photodegradation. In the reviewed reports (Table 3), *C. fusca* completely removed the EA of BPA [18] and the removal of BPA concentration ranging from 70 – >95 %. The removal of EA for 50 ECs, including BPA and TCS, was performed individually for *C. reinhardtii, S. obliquus, C. pyrenoidosa* and *C. vulgaris*. BPA removal was >98 % and TCS removal ranged from 31.41 to 58.27 % for these four microalgae species [20]. Similar results to those reported in the literature were observed in the present study, as BPA removal was >95 % by treatment with a microalgae mixed culture containing *S. obliquus* and *Desmodemus* sp. It is possible that the high BPA removal is due to the fact that BPA is less toxic

Table 2
Removal of BPA and TCS. Response of estrogenic activity for each removal process identified.

Removal process	TSS (mg/L)	Bisphenol-A				Triclosan			
		Removal of BPA		Estrogenic activity		Removal of TCS		Estrogenic activity	
		mg/L	%	EEQ 17β-estradiol (ng/L)	Response	μg/L	%	EEQ 17β-estradiol (ng/L)	Response
Total	≈300	16.17 ± 0.04	95.1	0.157 ± 0	7-fold increase	192.39 ± 23.03	61.1	0.546 ± 0	4.37-fold increase
	≈500	16.27 ± 0.01	95.68	0.025 ± 0.01	1.13-fold increase	271.59 ± 6.36	86.25	0.090 ± 0.02	0.333-fold decrease
Adsorption	≈300	1.57 ± 0.04	9.25	0.191 ± 0.07	8.51-fold increase	31.75 ± 0.07	10.08	0.714 ± 0.07	5.89-fold increase
	≈500	0.70 ± 0	4.11	0.387 ± 0.08	15.25-fold increase	59.26 ± 15.35	18.82	0.208 ± 0.04	1.71-fold increase
Absorption	≈300	0.72 ± 0	4.42	0.098 ± 0.02	4.36-fold increase	30.65 ± 6.11	9.73	0.183 ± 0.02	1.51-fold increase
	≈500	0.72 ± 0.01	4.24	0.127 ± 0.02	4.99-fold increase	57.18 ± 14.07	18.16	0.247 ± 0.05	2.03-fold increase
DP	———	0.65 ± 0.08	3.8	0.049 ± 0	1.79-fold increase	45.45 ± 1.90	14.43	0.185 ± 0.01	1.23-fold increase
IP	———	4.46 ± 0.26	26.23	0.04 ± 0.01	2.4-fold increase	44.31 ± 17.15	14.07	0.140 ± 0.03	0.17-fold decrease
Other factors	———	1.79 ± 0.5	10.54	0.026 ± 0	1.08-fold increase	ND	ND	ND	ND

The estrogenic response was calculated according to what was quantified on day zero of the experiment.
DP: direct photodegradation; IP: indirect photodegradation; ND: not detected.

Table 3
Microalgae treatment, photodegradation and oxidation to remove emerging contaminants (ECs) and estrogenic activity (EA).

Treatment	EC	Concentration EC	Medium	Conditions	EA Assay	Removal EC (%)	Removal EA (%)	Ref.
Microalgae culture								
S. dimorphus	17α-E2 E1 E2 E3	5 μg/L	Municipal WW	MC: 40 mg/L HRT: 8 days 12:12 h light/dark LI: 100 $\mu E/m^2$ s	*E*-screen	10–20	30 % @ 24 h	[52]
S. capricornutum *S. quadricauda* *C. vulgaris*	EE2 E2	3 mg/L	BG-11	MC: 3×10^7 cel/mL 12:12 h light/dark LI: 201.5 $\mu mol/m^2$ s	YES	E2 (91), EE2 (83) E2 (73), EE2 (57) E2 (92), EE2 (55)	80 64 68	[1]
C. fusca	BPA	10–160 μM	Bristol	LI: 0–36 W/m^2 8:16 h light/dark HRT: 168 h	YES	70 % @ 160 >95 % @ 10–80	≈100	[21]
C. reinhardtii *S. obliquus* *C. pyrenoidosa* *C. vulgaris*	BPA TCS	BPA: 20145.6 ng/L TCS: 41.7 ng/L	Raw WW	MC: 0.05 mg/L Chl-a 12:12 h light/dark LI: 60 $\mu mol/m^2$ s HRT: 7 days	YES	BPA (98.57) TCS (58.27) BPA (98.88) TCS (31.41) BPA (99.14) TCS (42.44) BPA (99.04) TCS (52.99)	46.15 81.19 64.1 56.41	[20]
Mixed culture *S. obliquus* and *Desmodesmus* sp.	BPA TCS	BPA 17 mg/L TCS 325 μg/L	SWW	12:12 h light/dark LI ≈ 100 $\mu mol/m^2$ s CT: 15 days MC ≈ 300 mgTSS/L MC ≈ 500 mgTSS/L	BLYES	BPA: 95.1 TCS: 61.1 BPA: 95.68 TCS: 86.25	BPA: 7.02-fold increase TCS: 4.37-fold increase BPA: 1.13-fold increase TCS: ≈33.35 %	This study
Photodegradation + oxidation	4-OP, 4-NP, BPA, estrone, E2, EE2, E3	100 μg/L	Secondary effluent WW	12 W/m^2 Fe^{2+} = 20, 40, 60 mg/L H_2O_2 = 100, 200, 300 mg/L	YES	80 @ 20 mg/L Fe^{2+} and 100 mg/L H_2O_2	0–62 @ UV + Fe^{2+} + H_2O_2	[42]
	E2 EE2	3 μg/L	DW, type I and II	UV 0–12,000 mJ/cm^2 500 μg/L H_2O_2	YES	EE2: 95 % @ 5000 mJ/cm^2 E2: 99 % @ 4000 mJ/cm^2	95 %	[41]
	E2	500 μg/L	DW Secondary effluent WW	Irradiance: 2.1 mW/cm^2 Chlorination 10 mg/L	YES	NR	DW 97.2 @ 5 min + UV + Cl 96.2 @ 5 min + Cl WW 78.3 @ 5 min + UV + Cl 49.1 @ 5 min + Cl	[48]
	E1, E2, EE2, E3	250 μg/L	Tap water	UV + TiO_2 HRT: 4 h	MCF-7 cells	90 % @ 3 h	≈85 @ 3 h	[45]
	BPA	520 μmol/L	UPW Effluent WW	Irradiation 4.25 × 10^{-6} E^{-1} 750 mL total volume 500 μM H_2O_2	YES	Without H_2O_2: UPW: 7.3 WW: 8.8 With H_2O_2: UPW: 35 WW: 28	50 % @ 120 min	[40]
	BPA	100 μM	MQW	Radiation UVB 0–100 J/cm^2	Y2H	NR	ND	[38]
	BPA	100 ng/L	MQW Secondary effluent WW	Irradiance: 0.7 W TiO_2 0.8 g/L	Y2H	NR	≈100 % @ TiO_2 + 50 min + 0.2 mM (SO_4^{2-}, Cl^-, NO_3^-) ≈48.2 % @ TiO_2 + 50 min+ NH_4^+ ≈39.5 % @ TiO_2 + 50 min+ HPO_4^{2-} ≈78.3 % @ TiO_2 + 50 min+ HCO_3^-	[44]
	BPA	100 μg/L	MQW Secondary effluent WW	Chlorination 0.2–2 mg/L Irradiance 6 W	YES	MQW: 18 and 70 for UVA and UVC respectively, @ 120 min WW: 66 @ 0.2 mg/L Cl, 3 min 99 @ 2 mg/L, 3 min	<QL (26 μg/L)	[43]
	BPA	100 μg/L	UPW	Irradiance 6 W 3–30 mg/L H_2O_2 HRT: 5 min	YES	70 @ UVA 18 @ UVC	93.35 @ pH = 5 99.19 @ pH = 7 <DL @ pH = 9	[39]
	BPA TCS	BPA: 17 mg/L TCS = 325 μg/L	MQW	12:12 h light/dark LI: ≈100 $\mu mol/m^2$ s HRT: 15 days	BLYES	BPA: 3.8 TCS: 14.43	BPA: 1.79-fold increase TCS: 1.23-fold increase	This study

(*continued on next page*)

Table 3 (*continued*)

Treatment	EC	Concentration EC	Medium	Conditions	EA Assay	Removal EC (%)	Removal EA (%)	Ref.
			SWW			BPA: 26.23 TCS: 14.07	BPA: 2.41-fold increase TCS: 17.99 %	
Oxidation	EE2	10 μM	MQW	Ozone dose 5–24 μM 2 min	YES	99.72	98.5 @ 19 μM O_3	[47]
	EE2	10 μM	Buffer fosfates	0–28 μM Cl^- and Br^-	YES	NR	> 87	[46]
	4-NP	500 μg/L	SRW	Chlorination 1.3 mg/L	Y2H	84 @ 10 min	30 @ 10 min At 60 and 120 min no AE	[50]
	4-NP	4 mg/L	MQW	Ozone dose 1.5 mg/L	YES	NR	≈100 @ 10 min	[51]
	BPA 4-NP TCS	1000 ng/L	Well water	Ozone dose gas phase 1, 2 and 3 mg/L 1, 5, 10 min Chlorination 0.2, 1 and 1.5 mg/L 10 min	BLYES	BPA: 98.7 4-NP: 79.3 TCS: 97 BPA: 86.2 4-NP: 94.3 TCS: 97.8	BPA:77–96 4-NP: 93–99 TCS: 94–96 BPA: 81–94 4-NP: 95–97 TCS: 92–99	[7]
	BPA	500 μg/L	SRW	Chlorination 1.46 mg/L	Y2H	80 @ 10 min	25.77, 9.42 and 4.08 at 10, 30 and 60 min, respectively	[49]

BPA: Bisphenol-A; TCS: Triclosan; MC: microalgae concentration; LI: light intensity; DW: deionized water; WW: wastewater; SWW: synthetic wastewater; SRW: synthetic raw water; UPW: ultrapure water; MQW: Milli Q water; Y2H: Yeast two-hybrid; QL: quantification limit; DL: detection limit; NR: no reported; ND: no detected; E1: estrone; E2: 17β-estradiol; EE2: 17α-ethynylestradiol; E3: estriol; 17α-E2: 17α-estradiol; 4-OP: 4-octylphenol; 4-NP: 4-nonylphenol, HRT: hydraulic retention time; Chl-a: chlorophyll "a"; UVA: ultaviolet light A; UVB: ultaviolet light B, UVC: ultaviolet light C; CT: culture time.

than TCS, according to the EC_{50} median lethal dose previously found by Atengueño-Reyes et al. [57] for BPA (17 mg/L) and TCS (325 μg/L), which were also used in this study. Total EA removal (associated with all 50 ECs found in wastewater) was 46.15 %, 81.19 %, 64.1 %, and 56.41 % for *C. reinhardtii, S. obliquus, C. pyrenoidosa,* and *C. vulgaris*, respectively [20]. Wu et al. [1] obtained reductions of 68 %, 64 %, and 80 % for *C. vulgaris, S. quadricauda*, and *S. capricornutum* to remove EE2 and E2, with an initial inoculum of 3×10^7 cells/mL. In contrast, the removal rates of the ECs ranged from 55 % to 92 %. Zhou et al. [20] reported a removal of ECs and EA of 10–20 % and 30 % respectively. The authors used the microalgae *S. dimorphus* to remove estrogens. Compared to the current study, it was found that at initial ≈300 mg TSS/L, the EA was multiplied with respect to day zero; however, at initial ≈500 mg TSS/L, the EA was only reduced by 33.35 % for TCS. This suggests that the higher the initial microalgae concentration, the lower the EA. This is consistent with results reported by Wu et al. [1] and what was observed in this study: increased cell density of the microalgal culture achieves a higher removal of EA. Biodegradation has the property of breaking down molecules into others that have no toxicity or less toxicity than the original molecule [31]. However, experiments in the presence of BPA at ≈500 mg TSS/L showed a lower EA than that recorded at ≈300 mg TSS/L, confirming the trend that the higher the cell density of microalgae, the lower the EA. In this situation, the important role of microalgal species in the removal of environmental estrogens has also been investigated, as some ECs can be toxic to some microalgal species, limiting their removal [5,29]. Therefore, for the application of microalgae in wastewater treatment plants, the species used must have a high level of antioxidant enzymes in order to resist the toxic effects of high concentrations of environmental estrogens. Although effective results have been obtained at the laboratory level, the application of microalgae in wastewater treatment plants involves the interaction and thus the co-metabolism of other microorganisms, such as bacteria [4,29]. It is therefore recommended to consider the effect of bacteria in the removal process, as in the presence of environmental estrogens, the intermediates produced by bacteria to degrade environmental estrogens may be toxic to microalgae [5]. Other factors that affect the removal of estrogenic activity by microalgae in wastewater treatment plants and have a strong influence on their efficiency include temperature, pH and chemical oxygen demand, retention time, and sufficient light to support the growth of photosynthetic microorganisms [29].

3.3.2. Removal of estrogenic activity by photodegradation

BPA removal in the photodegradation experiments was observed to be 3.8 % ($P > 0.05$), 26.23 % ($P < 0.05$) and 10.54 % due to direct, indirect and control (protected from light) photodegradation. Factors not evaluated during the experiment may explain the decrease in BPA in the control photodegradation. EA was shown to increase 2.4-fold in the indirect photodegradation experiments, while direct photodegradation showed a 1.79-fold increase and the control showed a 6.19 % increase. The removal of TCS was 14.434 % ($P < 0.05$) and 14.074 % ($P > 0.05$) for direct and indirect photodegradation, respectively. In the case of TCS, no decrease in the initial amount of TCS was observed in the photodegradation control. However, EA showed a decrease of 17.99 % ($P > 0.05$) for indirect photodegradation and an increase of 23.33 % ($P > 0.05$) for direct photodegradation. A possible explanation for increase in EA for BPA in direct photodegradation could be the formation of by-products that may be more toxic than the initial contaminant by-products that may be favored in indirect photodegradation [79], while TCS shows a greater decrease in EA because it is extremely susceptible to degradation by the action of light [74]. Table 3 compares the efficiency of EA removal by microalgae and photodegradation. There are no studies in the literature that evaluate the contribution of all removal processes or that do not specify each removal process. In this table, only the effect of microalgae is compared, and in independent experiments, photodegradation is compared.

From Table 3, the only study focusing on photodegradation, Mutou et al. [38], tested the degradation of BPA in milli-Q water, in which no EA was detected at the end of the experiment. The studies summarized in Table 3 tested the combination of photodegradation with TiO_2, H_2O_2, chlorination and iron. In these studies, the removal of emerging contaminants ranged from 7.3 to 99 % and the EA was quantified after treatments from 0 to ≈100 % removal, while the initial concentrations of emerging contaminants ranged from 100 ng/L to 500 μg/L. Other studies using oxidation with chemical agents also used in disinfection, such as chlorine and ozone, reported removals of emerging contaminants from 79.3 % to 99.72 %, which are higher than those reported in

other studies. Quantification of EA in chlorination and ozonation treatments is reported from >4 % to ≈100 %. Concentrations of the emerging contaminants tested ranged from 1000 ng/L to 4 mg/L [7,46,47,49–51].

According to the European Community document COM (2011)876, the threshold concentration of 17β-estradiol at which no effects are observed in aquatic organisms is 0.4 ng/L [2,80]. In the case of BPA, the 17β-estradiol equivalents on day 15 of treatment were 0.1572 ng/L and 0.0254 ng/L for ≈300 and ≈500 mg TSS/L, respectively. During the photodegradation experiments, the 17β-estradiol equivalents were 0.0489, 0.04 and 0.0257 ng/L for indirect, direct and control photodegradation, respectively. For TCS EA, after 15 days of treatment, 17β-estradiol equivalents of 0.53 and 0.0895 ng/L were observed for microalgae experiments at ≈300 and ≈500 mg TSS/L, respectively. After photodegradation, the 17β-estradiol equivalents were 0.1404 and 0.1846 ng/L for indirect and direct photodegradation, respectively. Of the results obtained, only the EA recorded for TCS at ≈300 mg TSS/L was higher than what is considered safe by the European Community. Studies have reported predicted no-effect concentrations (PNECs) of estrogen equivalents of 0.1–0.4 ng/L for prolonged exposures and 0.5–2 ng/L for short exposures [81]. Zhou et al. [82], reported a PNEC of 20 ng/L for TCS. Adeel et al. [83], indicated that adverse effects in humans occur at lower concentrations than reported previously, and therefore a daily intake of 0–50 ng/kg body weight is established. The concentration at which there is no observable effect level (NOEL) is 0.3 mg/day/kg body weight [83].

Studies have shown that cell proliferation, and hence biomass productivity, is limited by several environmental parameters, including DO, Dissolved Oxygen; COD, Chemical Oxygen Demand; pH, Hydrogen Potential; HRT, Hydraulic Retention Time; NH_4^+, Ammonia; NO_3^-, nitrogen nitrates; PO_4^{3-}, orthophosphates; T (°C), Temperature (degrees Celsius); solar irradiance is Watts per square metre W/m^2 [84]. In these outdoor systems, the lower biomass productivity is directly related to the removal rate of ECs, which is also affected by operational parameters such as light penetration and mixing, dissolved oxygen (DO), pH, temperature and time. Therefore, further outdoor studies are essential to overcome these limiting challenges for efficient and sustainable removal of ECs. Removing ECs from wastewater using microalgae could be an environmentally friendly water treatment process that could simultaneously treat other contaminants from the wastewater or be integrated into existing Wastewater Treatment Plants. In addition, the microalgal biomass produced could be valorised to produce various value-added products, making this approach more affordable and sustainable. However, several challenges need to be overcome to commercially explore this approach and integrate the circular economy aspect while developing microalgal-based ECs removal [4].

4. Conclusions

The highest percentage of BPA removal was due to biodegradation (>40 %). It was observed that by increasing the initial concentration of microalgae (from TSS ≈ 300 mg/L to ≈500 mg/L, BPA =17 mg/L, lab-scale) there was an ≈6 % increase in biodegradation. Indirect photodegradation (presence of ions in the SWW) was the second removal pathway contributing to the decrease in BPA, light intensity condition was ≈100 μmol/m^2 s. In the case of TCS, both direct and indirect photodegradation contributed >28 % to the removal with same light intensity condition aforementioned. As with BPA, as the initial number of microalgae increased (from TSS ≈ 300 mg/L to ≈500 mg/L, TCS ≈ 325 μg/L, lab-scale), biodegradation increased by ≈8 %. Another effect that the increase in microalgae had on the removal of TCS was that the amount removed by sorption increased by 17.17 %.

EA removal was no >33.35 % for TCS at ≈500 mg TSS/L microalgae. Removal percentages for both types of photodegradation were very similar (just over 14 %) for TCS. However, the highest EA removal reduction was for TCS under indirect photodegradation conditions. In the case of BPA, an increase in EA was observed even though BPA removal was >95 %. The removal of BPA in photodegradation experiments was higher for BPA with 26.23 % removal in indirect photodegradation. One reason for the increase in EA is that the degradation of the molecules, leads to the formation of other molecules which have greater toxicity than the original molecule. Photoautotrophic treatment with the mixed culture of the microalgae *S. obliquus* and *Desmodemus* sp. resulted in a higher removal efficiency of TCS and BPA from wastewater compared to photodegradation. Although there is no linear relationship between TCS and BPA concentration and EA behavior, a decrease for TCS was observed when treated with microalgae. Similarly, compared to photodegradation, microalgae treatment showed a lower increase in EA for BPA.

The use of microalgae to reduce estrogenic activity is an area of research that has yet to be fully explored. This study provides a prospecting of how the removal processes (biodegradation, biosorption, and photodegradation) are involved in the removal of ECs and in modifying the trend of estrogenic activity. The concentrations used for BPA and TCS in this study were the EC_{50} reported for the mixed culture used. The reported EC_{50} are much higher in magnitude than those reported in the literature for wastewater. Another area of opportunity would be to scale up this type of treatment to a pilot scale where the scale-up behavior could be observed in modifying the trend of estrogenic activity.

Ethical approval

This is an observational study. The Ethics Committee of the Environmental Engineering Laboratory, Institute of Engineering, National Autonomous University of Mexico, has confirmed that ethical approval is not required because no pathogenic organisms were used in the experiments.

Consent to participate or publish

Not aplicable.

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CRediT authorship contribution statement

Karina Atengueño-Reyes: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Sharon B. Velásquez-Orta:** Writing – review & editing, Supervision. **Isaura Yáñez-Noguez:** Writing – review & editing, Supervision, Resources. **Ignacio Monje-Ramírez:** Writing – review & editing, Supervision. **María Teresa Orta-Ledesma:** Writing – review & editing, Validation, Supervision, Funding acquisition, Formal analysis, Conceptualization.

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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References

[1] P.H. Wu, H.Y. Yeh, P.H. Chou, W.W. Hsiao, C.P. Yu, Algal extracellular organic matter mediated photocatalytic degradation of estrogens, Ecotox. Environ. Safe. 209 (2021) 111818, https://doi.org/10.1016/j.ecoenv.2020.111818.

[2] J. Zdarta, L.N. Nguyen, K. Jankowska, T. Jesionowski, L.D. Nghiem, A contemporary review of enzymatic applications in the remediation of emerging estrogenic compounds, Crit. Rev. Environ. Sci. Techno. 52 (2021) 1–30, https://doi.org/10.1080/10643389.2021.1889283.

[3] J.L. Chen, S. Ravindran, S. Swift, N. Singhal, Changes in estrogenicity and micropollutant concentrations across unit processes in a biological wastewater treatment system, Water Sci. Technol. 77 (5–6) (2018) 1673–1682, https://doi.org/10.2166/wst.2018.047.

[4] S. Mehariya, P. Das, M.I. Thaher, M. Abdul Quadir, S. Khan, S. Sayadi, A. H. Hawari, P. Verma, S.K. Bhatia, O.P. Karthikeyan, A. Zuorro, H. Al-Jabri, Microalgae: a potential bioagent for treatment of emerging contaminants from domestic wastewater, Chemosphere 351 (2024) 141245, https://doi.org/10.1016/j.chemosphere.2024.141245.

[5] X.L. Sun, Y. Wang, H.Q. Xiong, S.T. Wang, Y.C. Fang, H. Xiang, Removal of environmental estrogens from wastewater by microalgae under the influence of bacteria, Jour. Clean. Prod. 414 (2023) 137635, https://doi.org/10.1016/j.jclepro.2023.137635.

[6] G. Bittner, M. Denison, C. Yang, M. Stoner, G. He, Chemicals having estrogenic activity can be released from some bisphenol A-free, hard and clear, thermoplastic resins, Environ. Health 13 (2014) 103, https://doi.org/10.1186/1476-069X-13-103.

[7] M.T. Orta-Ledesma de Velásquez, J. Aguirre-Londoño, I. Yáñez-Noguez, Assessing the estrogenic activity of EDCs and human risks of groundwater after ozonation and chlorination, Ozone Sci. Eng. 42 (3) (2019) 244–254, https://doi.org/10.1080/01919512.2019.1653753.

[8] D.D. Seachrist, K.W. Bonk, S.M. Ho, G.S. Prins, A.M. Soto, R.A. Keri, A review of the carcinogenic potential of bisphenol A, Reprod Toxicol (Elmsford, N.Y.) 59 (2016) 167–182, https://doi.org/10.1016/j.reprotox.2015.09.006.

[9] J. Moreman, O. Lee, M. Trznadel, A. David, T. Kudoh, C.R. Tyler, Acute toxicity, teratogenic, and estrogenic effects of bisphenol a and its alternative replacements bisphenol S, bisphenol F, and bisphenol AF in zebrafish embryo-larvae, Environ. Sci. Technol. 51 (21) (2017) 12796–12805, https://doi.org/10.1021/acs.est.7b03283.

[10] C.F. Wang, Y. Tian, Reproductive endocrine-disrupting effects of triclosan: Population exposure, present evidence and potential mechanisms, Environ. Pollut. 206 (2015) 195–201, https://doi.org/10.1016/j.envpol.2015.07.001 (Barking, Essex: 1987).

[11] A.C. Marques, M. Mariana, E. Cairrao, Triclosan and its consequences on the reproductive, cardiovascular and thyroid levels, Int. J. Mol. Sci. 23 (19) (2022) 11427, https://doi.org/10.3390/ijms231911427.

[12] K.S. Yoon, S.J. Kwack, In vitro and in vivo estrogenic activity of triclosan, J. Toxicol. Environ. Health Sci. Part A. 84 (19) (2021) 800–809, https://doi.org/10.1080/15287394.2021.1944940.

[13] E.J. Routledge, J.P. Sumpter, Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen, Environ. Toxicol. Chem. 15 (1996) 241–248, https://doi.org/10.1002/etc.5620150303.

[14] P. de Boever, W. Demare, E. Vanderperren, K. Cooreman, P. Bossier, W. Verstraete, Optimization of a yeast estrogen screen and its applicability to study the release of estrogenic isoflavones from a soygerm powder, Environ. Health Perspect. 109 (2001) 691–697, https://doi.org/10.1289/ehp.01109691.

[15] R. Céspedes, M. Petrovic, D. Raldúa, U. Saura, B. Piña, S. Lacorte, P. Viana, D. Barceló, Integrated procedure for determination of endocrine-disrupting activity in surface waters and sediments by use of the biological technique recombinant yeast assay and chemical analysis by LC–ESI-MS, Anal. Bioanal. Chem. 378 (3) (2004) 697–708, https://doi.org/10.1007/s00216-003-2303-5.

[16] M. Wagner, J. Oehlmann, Endocrine disruptors in bottled mineral water: total estrogenic burden and migration from plastic bottles, Environ. Sci. Pollut. Res. Int. 16 (2009) 278–286, https://doi.org/10.1007/s11356-009-0107-7.

[17] J. Sanseverino, R.K. Gupta, A.C. Layton, S.S. Patterson, S.A. Ripp, L. Saidak, M. L. Simpson, T.W. Schultz, G.S. Sayler, Use of Saccharomyces cerevisiae BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds, Appl. Environ. Microbiol. 71 (8) (2005) 4455–4460, https://doi.org/10.1128/AEM.71.8.4455-4460.2005.

[18] T. Xu, A. Young, J. Narula, G. Sayler, S. Ripp, High-throughput analysis of endocrine-disrupting compounds using BLYES and BLYAS bioluminescent yeast bioassays, in: S. Ripp (Ed.), Bioluminescent Imaging. Methods in Molecular Biology vol. 2081, 2020, https://doi.org/10.1007/978-1-4939-9940-8_3. Humana, New York, NY.

[19] V. Matamoros, R. Gutierrez, I. Ferrer, J. Garcia, J.M. Bayona, Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: a pilotscale study, J. Hazard. Mater. 288 (2015) 34–42, https://doi.org/10.1016/j.jhazmat.2015.02.002.

[20] G. Zhou, G. Ying, S. Liu, L. Zhou, Z. Chen, F. Peng, Simultaneous removal of inorganic and organic compounds in wastewater by freshwater green microalgae, Environ Sci Process Impacts 16 (8) (2014) 2018–2027, https://doi.org/10.1039/C4EM00094C.

[21] T. Hirooka, H. Nagase, K. Uchida, Y. Hiroshige, Y. Ehara, J. Nishikawa, T. Nishihara, K. Miyamoto, Z. Hirata, Biodegradation of bisphenol a and disappearance of its estrogenic activity by the green alga Chlorella fusca var. vacuolate, Environ. Toxicol. Chem. 24 (8) (2005) 1896–1901, https://doi.org/10.1897/04-259r.1.

[22] C.E. Gattullo, H. Bährs, C.E.W. Steinberg, E. Loffredo, Removal of bisphenol a by the freshwater green alga Monoraphidium braunii and the role of natural organic matter, Sci. Total Environ. 416 (2012) 501–506, https://doi.org/10.1016/j.scitotenv.2011.11.033.

[23] S. Wang, K. Poon, Z. Cai, Removal and metabolism of triclosan by three different microalgal species in aquatic environment, J. Hazard. Mater. 342 (15) (2018) 643–650, https://doi.org/10.1016/j.jhazmat.2017.09.004.

[24] I. de Godos, R. Muñoz, B. Guieysse, Tetracycline removal during wastewater treatment in high-rate algal ponds, J. Harzad. Mater. 229 (2012) 446–449, https://doi.org/10.1016/j.jhazmat.2012.05.106.

[25] T. Ding, K. Lin, M. Yang, L. Bao, J. Li, B. Yang, J. Gan, Biodegradation of triclosan in diatom Navicula sp.: kinetics, transformation products, toxicity evaluation and the effects of pH and potassium permanganate, J. Hazard. Mater. 344 (2018) 200–209, https://doi.org/10.1016/j.jhazmat.2017.09.033.

[26] J.Q. Xiong, M.B. Kurade, J.R. Kim, H.S. Roh, B.H. Jeon, Ciprofloxacin toxicity and its co-metabolic removal by a freshwatermicroalga Chlamydomonas mexicana, J. Hazard. Mater. 323 (2017) 212–219, https://doi.org/10.1016/j.jhazmat.2016.04.073.

[27] J.Q. Xiong, S. Govindwar, M.B. Kurade, K.J. Paeng, H.S. Roh, M.A. Khan, B. H. Jeon, Toxicity of sulfamethazine and sulfamethoxazole and their removal by a green microalga, Scenedesmus obliquus, Chemosphere 218 (2019) 551–558, https://doi.org/10.1016/j.chemosphere.2018.11.146.

[28] S.N. Tolboom, D. Carrillo-Nieves, M.J. Rostro-Alanis, R. de la Cruz Quiroz, D. Barceló, H.M.N. Iqbal, R. Parra-Saldivar, Algal-based removal strategies for hazardous contaminants from the environment - a review, Sci. Total Environ. 665 (2019) 358–366, https://doi.org/10.1016/j.scitotenv.2019.02.129.

[29] B. Narindri Rara Winayu, H. F. Cheng, H. T. Hsueh, H. Chu, Removal of endocrine disruptor compounds, CO_2 fixation, and macromolecules accumulation in Thermosynechococcus sp. CL-1 cultivation, J. Biotech., 373, (2024) 1–11. https://doi.org/10.1016/j.jbiotec.2023.06.004.

[30] R. Gondi, S. Kavitha, R. Yukesh Kannah, O. Parthiba Karthikeyan, G. Kumar, V. Kumar Tyagi, J. Rajesh Banu, Algal-based system for removal of emerging pollutants from wastewater: a review, Bioresour. Technol. 344 (Pt B) (2022) 126245, https://doi.org/10.1016/j.biortech.2021.126245.

[31] J.L. Zhou, L. Yang, K.X. Huang, D.Z. Chen, F. Gao, Mechanisms and application of microalgae on removing emerging contaminants from wastewater: a review, Bioresour. Technol. 364 (2022) 128049, https://doi.org/10.1016/j.biortech.2022.128049.

[32] G.M. Gadd, Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment, J. Chem. Technol. Biotechnol. 84 (1) (2009) 13–28, https://doi.org/10.1002/jctb.1999.

[33] J.Q. Xiong, M.B. Kurade, B.H. Jeon, Can microalgae remove pharmaceutical contaminants from water? Trends in biotechnol. 36 (1) (2018) 30–44, https://doi.org/10.1016/j.tibtech.2017.09.003.

[34] M. Bilal, T. Rasheed, J.E. Sosa-Hernandez, A. Raza, F. Nabeel, H.M. Iqbal, Biosorption: an interplay between marine algae and potentially toxic elements - a review, Mar. Drugs 16 (2) (2018) 65, https://doi.org/10.3390/md16020065.

[35] Z.N. Norvill, A. Shilton, B. Guieysse, Emerging contaminant degradation and removal in algal wastewater treatment ponds: identifying the research gaps, J. Hazard. Mater. 313 (2016) 291–309, https://doi.org/10.1016/j.jhazmat.2016.03.085.

[36] Y. Wang, F.A. Roddick, L. Fan, Direct and indirect photolysis of seven micropollutants in secondary effluent from a wastewater lagoon, Chemosphere 185 (2017) 297–308, https://doi.org/10.1016/j.chemosphere.2017.06.122.

[37] Y. Wu, L. Bu, X. Duan, S. Zhu, M. Kong, N. Zhu, S. Zhou, Mini review on the roles of nitrate/nitrite in advanced oxidation processes: radicals transformation and products formation, J. Clean. Prod. 273 (2020) 123065, https://doi.org/10.1016/j.jclepro.2020.123065.

[38] Y. Mutou, Y. Ibuki, Y. Terao, S. Kojima, R. Goto, Change of estrogenic activity and release of chloride ion in chlorinated bisphenol a after exposure to ultraviolet B, Biol. Pharm. Bull. 29 (10) (2006) 2116–2119, https://doi.org/10.1248/bpb.29.2116.

[39] F.P. Chaves, G. Gomes, A. Della-Flora, A. Dallegrave, C. Sirtori, E.M. Saggioro, D. M. Bila, Comparative endocrine disrupting compound removal from real wastewater by UV/cl and UV/H_2O_2: effect of pH, estrogenic activity, transformation products and toxicity, Sci. Total Environ. 746 (2020) 141041, https://doi.org/10.1016/j.scitotenv.2020.141041.

[40] M. Neamțu, F.H. Frimmel, Degradation of endocrine disrupting bisphenol a by 254 nm irradiation in different water matrices and effect on yeast cells, Water Res. 40 (20) (2006) 3745–3750, https://doi.org/10.1016/j.watres.2006.08.019.

[41] E.J. Rosenfeld, P.J. Chen, S. Kullman, K.G. Linden, Destruction of estrogenic activity in water using UV advanced oxidation, Sci. Total Environ. 377 (1) (2007) 105–113, https://doi.org/10.1016/j.scitotenv.2007.01.096.
[42] L.G.R. da Silva, E.P. Costa, M.C.V. M., T. Starling, M.C.V.M. dos Santos Azevedo, S. E.C. Bottrel, R.O. Pereira, A.L. Sanson, R.J.C.F. Afonso, C.C. Amorim, LED irradiated photo-Fenton for the removal of estrogenic activity and endocrine disruptors from wastewater treatment plant effluent, Environ. Sci. Pollut. Res. 28 (2021) 24067–24078, https://doi.org/10.1007/s11356-021-12359-1.
[43] E.M. Saggioro, F.P. Chaves, L.C. Felix, G. Gomes, D.M. Bila, Endocrine disruptor degradation by UV/chlorine and the impact of their removal on estrogenic activity and toxicity, Int. J. Photoenergy. 2019 (2019) 1–9, https://doi.org/10.1155/2019/7408763.
[44] W. Zhang, Y. Li, Y. Su, K. Mao, Q. Wang, Effect of water composition on TiO_2 photocatalytic removal of endocrine disrupting compounds (EDCs) and estrogenic activity from secondary effluent, Int. J. Photoenergy. 215-216 (2012) 252–258, https://doi.org/10.1016/j.jhazmat.2012.02.060.
[45] R.N. Padovan, C. Xavier, E.B. Azevedo, L.S. Carvalho, A.J. de Santos Neto, F. M. Lanças, E.B. Azevedo, Degradation of hormones in tap water by heterogeneous solar TiO_2-photocatalysis: optimization, degradation products identification, and estrogenic activity removal, J. Environ. Chem. Eng. 9 (6) (2021) 106442, https://doi.org/10.1016/j.jece.2021.106442.
[46] Y. Lee, B.I. Escher, U. von Gunten, Efficient removal of estrogenic activity during oxidative treatment of waters containing steroid estrogens, Environ. Sci. Technol. 42 (17) (2008) 6333–6339, https://doi.org/10.1021/es7023302.
[47] M.M. Huber, T.A. Ternes, U. von Gunten, Removal of estrogenic activity and formation of oxidation products during ozonation of 17alpha-ethinylestradiol, Environ. Sci. Technol. 38 (19) (2004) 5177–5186, https://doi.org/10.1021/es035205x.
[48] M. Li, B. Xu, Z. Liungai, H.Y. Hu, C. Chen, J. Qiao, Y. Lu, The removal of estrogenic activity with UV/chlorine technology and identification of novel estrogenic disinfection by-products, J. Hazard. Mater. 307 (2016) 119–126, https://doi.org/10.1016/j.jhazmat.2016.01.003.
[49] J.Y. Hu, T. Aizawa, S. Ookubo, Products of aqueous chlorination of bisphenol a and their estrogenic activity, Environ. Sci. Technol. 36 (9) (2002) 1980–1987, https://doi.org/10.1021/es011177b.
[50] J.Y. Hu, G.H. Xie, T. Aizawa, Products of aqueous chlorination of 4-nonylphenol and their estrogenic activity, Environ. Toxicol. Chem. 21 (10) (2002) 2034–2039, https://doi.org/10.1002/etc.5620211005.
[51] Q. Sun, S. Deng, J. Huang, G. Yu, Relationship between oxidation products and estrogenic activity during ozonation of 4-nonylphenol, Ozone Sci. Eng. 30 (2) (2008) 120–126, https://doi.org/10.1080/01919510701861276.
[52] Y. Zhang, M.Y. Habteselassie, E.P. Resurreccion, V. Mantripragada, S. Peng, S. Bauer, L.M. Colosi, Evaluating removal of steroid estrogens by a model alga as a possible sustainability benefit of hypothetical integrated algae cultivation and wastewater treatment systems, ACS Sustain. Chem. Eng. 2 (11) (2014) 2544–2553, https://doi.org/10.1021/sc5004538.
[53] K. Schenck, L. Rosenblum, T.E. Wiese, L. Wymer, N. Dugan, D. Williams, H. Mash, B. Merriman, T. Speth, Removal of estrogens and estrogenicity through drinking water treatment, J. Water Health 10 (1) (2012) 43–55, https://doi.org/10.2166/wh.2011.135.
[54] P.H.G.D. Cunha, B.M. Souza-Chaves, D.M. Bila, J.P. Bassin, C.D. Vecitis, M. Dezotti, Insights into estrogenic activity removal using carbon nanotube electrochemical filter, Sci. Total Environ. 678 (2019) 448–456, https://doi.org/10.1016/j.scitotenv.2019.04.342.
[55] J. Yang, G. Wen, J. Zhao, X. Shao, J. Ma, Oxidation of bisphenol A by permanganate: reaction kinetics and removal of estrogenic activity, Water Sci. Technol 67 (8) (2013) 1867–1872, https://doi.org/10.2166/wst.2013.068.
[56] J. Zhou, X. He, Z. Zhang, G. Wu, P. Liu, D. Wang, P. Shi, X.X. Zhang, Chemical-toxicological insights and process comparison for estrogenic activity mitigation in municipal wastewater treatment plants, Water Res. 253 (2024) 121304, https://doi.org/10.1016/j.watres.2024.121304.
[57] K. Atengueño-Reyes, S.B. Velásquez-Orta, I. Yáñez-Noguez, I. Monje-Ramírez, P. Mijaylova-Nacheva, A. Chávez-Mejía, M.T. Orta-Ledesma, Microalgal consortium tolerance to bisphenol A and triclosan in wastewater and their effects on growth, biomolecule content and nutrient removal, Ecotox. Environ. Safe. 262 (2023) 115117, https://doi.org/10.1016/j.ecoenv.2023.115117.
[58] American Public Health Association, American Water Works Association, Water Environment Federation, (2023). Standard methods for examination of water and wastewater. https://www.standardmethods.org/.
[59] N. He, X. Sun, Y. Zhong, K. Sun, W. Liu, S. Duan, Removal and biodegradation of nonylphenol by four freshwater microalgae, Int. J. Environ. Res. and Public health. 13 (12) (2016) 1239, https://doi.org/10.3390/ijerph13121239.
[60] J.Z. Ma, C.Z. Zhu, J. Lu, Y. Lei, J.Z. Wang, T.H. Chen, Photochemical reaction between triclosan and nitrous acid in the atmospheric aqueous environment, Atmos. Environ. 157 (2017) (2017) 38–48, https://doi.org/10.1016/j.atmosenv.2017.03.011.
[61] K.Y. Lam, Z.H. Yu, R. Flick, A.J. Noble, E. Passeport, Triclosan uptake and transformation by the green algae Euglena gracilis strain Z, Sci. Total Environ. 833 (2022) 155232, https://doi.org/10.1016/j.scitotenv.2022.155232.
[62] A. Azizullah, S. Khan, G. Gao, K. Gao, The interplay between bisphenol A and algae–a review, J. King Saud. Univ. Sci. (2022), https://doi.org/10.1016/j.jksus.2022.102050, 102050.
[63] E. Palardé, A. Hom-Díaz, P. Blánquez, M. Martínez-Alonso, T. Vicent, N. Gaju, Effect of cultivation conditions on b-estradiol removal in laboratory and pilot-plant photobioreactors by an algal-bacterial mixed culture treating urban wastewater, Water Res. 137 (2018) 86–96, https://doi.org/10.1016/j.watres.2018.02.060.
[64] W. Fu, X. Li, Y. Yang, D. Song, Enhanced degradation of bisphenol A: influence of optimization of removal, kinetic model studies, application of machine learning and microalgae-bacteria consortia, Sci. Total Environ. 858 (Pt 2) (2023) 159876, https://doi.org/10.1016/j.scitotenv.2022.159876.
[65] E.J. Eio, M. Kawai, C. Niwa, M. Ito, S. Yamamoto, T. Toda, Biodegradation of bisphenol a by an algal-bacterial system, Environ. Sci. Pollut. Res. 22 (2015) 15145–15153, https://doi.org/10.1007/s11356-015-4693-2.
[66] M.K. Ji, A.N. Kabra, J. Choi, J.H. Hwang, J.R. Kim, R.A.I. Abou-Shanab, Y.K. Oh, B. H. Jeon, Biodegradation of bisphenol a by the freshwater microalgae Chlamydomonas mexicana and Chlorella vulgaris, Ecol. Eng. 73 (2014) 260–269, https://doi.org/10.1016/j.ecoleng.2014.09.070.
[67] V. Matamoros, E. Uggetti, J. García, J.M. Bayona, Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study, J. Hazard. Mater. 301 (2016) 197–205, https://doi.org/10.1016/j.jhazmat.2015.08.050.
[68] E. Koumaki, D. Mamais, C. Noutsopoulos, M.C. Nika, A.A. Betsou, N.S. Thomaidis, A. Eftaxias, G. Stratogianni, Degradation of emergeing contaminants from water under natural sunlight: the effect of season, pH, humics acids and nitrate and identification of photodegradation by-products, Chemosphere 138 (2015) 675–681, https://doi.org/10.1016/j.chemosphere.2015.07.033.
[69] M. Martínez-Zapata, C. Aristizabal, G. Penuela, Photodegradation of the endocrine-disrupting chemicals 4n-nonylphenol and triclosan by simulated solar UV irradiation in aqueous solutions with Fe (III) and in the absence/presence of humic acids, J. Photochem. Photobiol. A Chem. 251 (2013) 41–49, https://doi.org/10.1016/j.jphotochem.2012.10.009.
[70] S. Wang, X. Wang, K. Poon, Y. Wang, S. Li, H. Liu, S. Lin, Z. Cai, Removal and reductive dechlorination of triclosan by Chlorella pyrenoidosa, Chemosphere 92 (11) (2013) 1498–1505, https://doi.org/10.1016/j.chemosphere.2013.03.067.
[71] M.W. Pannu, G.S. Toor, G.A. O'Connor, P.C. Wilson, Toxicity and bioaccumulationof biosolids-borne triclosan in food crops, Environ. Toxicol. Chem. 31 (2012) 2130–2137, https://doi.org/10.1002/etc.1930.
[72] S. Mathews, S. Henderson, D. Reinhold, Uptake and accumulation of antimicrobials, triclocarban and triclosan, by food crops in a hydroponic system, Environ. Sci. Pollut. Res. Int. 21 (9) (2014) 6025–6033, https://doi.org/10.1007/s11356-013-2474-3.
[73] G.S. Dhillon, S. Kaur, R. Pulicharla, S.K. Brar, M. Cledón, M. Verma, R. Y. Surampalli, Triclosan: current status, occurrence, environmental risks and bioaccumulation potential, Int. J. Environ. Res. Pub. He. 12 (2015) 5657–5684, 10.3390%2Fijerph120505657.
[74] L.W. Olaniyan, N. Mkwetshana, A.I. Okoh, Triclosan in water, implications for human and environmental health, SpringerPlus 5 (1) (2016) 1639, https://doi.org/10.1186/s40064-016-3287-x.
[75] B.R. Kumar, G. Deviram, T. Mathimani, P.A. Duc, A. Pugazhendhi, Microalgae as rich source of polyunsaturated fatty acids, Biocatal. Agric. Biotechnol. 17 (2019) 583–588, https://doi.org/10.1016/j.bcab.2019.01.017.
[76] C.C. Tang, T.Y. Wang, R. Wang, C. Varrone, Z. Gan, Z.W. He, Z.H. Li, X.C. Wang, Insights into roles of triclosan in microalgal-bacterial symbiosis system treating wastewater, Bioresour. Technol. 385 (2023) 129331, https://doi.org/10.1016/j.biortech.2023.129331.
[77] P. Myers, W. Hessler, Does the dose make the poison? Extensive results challenge a core assumption in toxicology, in: Environmental Health News, 2007. Accessed, https://endocrinedisruption.org/assets/media/documents/2007-04-30_does_the_dose_make_the_poison.pdf.
[78] A.M. Bergamasco, M. Eldridge, J. Sanseverino, F.F. Sodré, C.C. Montagner, I. C. Pescara, W.F. Jardim, G.A. de Umbuzeiro, Bioluminescent yeast estrogen assay (BLYES) as a sensitive tool to monitor surface and drinking water for estrogenicity, JEM 13 (11) (2011) 3288–3293, https://doi.org/10.1039/c1em10464k.
[79] H.T. Nguyen, Y. Yoon, H.H. Ngo, A. Jang, The application of microalgae in removing organic micropollutants in wastewater, Crit. Rev. Environ. Sci. Technol. 53 (6) (2020) 723–732, https://doi.org/10.1080/10643389.2020.1753633.
[80] A.C. Johnson, E. Dumont, R.J. Williams, R. Oldenkamp, I. Cisowska, J.P. Sumpter, Do concentrations of ethinylestradiol, estradiol, and diclofenac in European rivers exceed proposed EU environmental quality standards? Environ. Sci. Technol. 47 (21) (2013) 12297–12304, https://doi.org/10.1021/es4030035.
[81] B. Jarošová, L. Bláha, J.P. Giesy, K. Hilscherová, What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? Environ. Int. 64 (2014) 98–109, https://doi.org/10.1016/j.envint.2013.12.009.
[82] S. Zhou, C. di Paolo, X. Wu, Y. Shao, T.M. Seiler, H. Hollert, Optimization of screening-level risk assessment and priority selection of emerging pollutants - the case of pharmaceuticals in European surface waters, Environ. Int. 128 (2019) 1–10, https://doi.org/10.1016/j.envint.2019.04.034.
[83] M. Adeel, X. Song, D. Francis, Y. Yang, Environmental impact of estrogens on human, animal and plant life: a critical review, Environ. Int. 99 (2017) 107–119. https://doi.org/https://doi.org/10.1016/j.envint.2016.12.010.
[84] S. B. Velásquez-Orta, I. Yáñez-Noguez, I. M. Ramírez, M. T. O. Ledesma, Pilot-scale microalgae cultivation and wastewater treatment using high-rate ponds: a meta-analysis. Environ Sci Pollut Res Int. Advance online publication. https://doi.org/10.1007/s11356-024-34000-7.