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Emerging contaminants and nutrients recovery by *Picocystis* sp. under continuous culture in contaminated secondary municipal wastewater effluent

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ABSTRACT

This study assesses the growth and the recovery ability of the Chlorophyta *Picocystis* sp., cultivated in wastewater supplemented by a model mixture of emerging contaminants (EMCs). The mixture of EMCs consisted of three pharmaceuticals, diclofenac (DCF), sulfadiazine (SDA), and oxytetracycline (OTC), and the plastics precursor bisphenol A (BPA). Continuous cultures were carried out for 27 days in a set of four columns of photobioreactors connected in series to investigate the daily EMCs and nutrient recovery. *Picocystis* was able to grow in contaminated wastewater with daily biomass productivity of 0.5 ± 0.05 g L^{-1} d^{-1}. *Picocystis* exhibited high daily EMCs removal efficiencies reaching 100 %, 83–92 %, 93–95 %, and 66–70 % of 1 mg L^{-1} initial concentration of OTC, DCF, BPA, and SDA, respectively. Besides, *Picocystis* showed daily nutrient recovery rates of 40 % for nitrogen and total organic carbon and 27 % for phosphorus.

These results pointed to the promising application of *Picocystis* in a continuous bioremediation system for the removal of emerging contaminants and nutrients from wastewaters.

1. Introduction

The accumulation of Emerging Contaminants (EMCs) in the aquatic environment has become a growing concern due to the development of knowledge relating to their ecotoxicological properties [1,2]. EMCs include a wide variety of chemical compounds, such as pharmaceuticals, personal care products, plasticizers, surfactants, and pesticides [3], used daily in large quantities released in the environment mainly via discharges from conventional wastewater treatment plants [1,4].

Since conventional wastewater treatment plants (WWTPs) are not efficient in removing all these types of chemicals, many of these compounds occur in natural water bodies [5,6,7], where they may exert unintended adverse effects on wildlife even at relatively low concentrations at the μg L^{-1} level [8,9]. In fact, pharmaceuticals residues may induce carcinogenicity, teratogenicity, mutagenicity, endocrine-disrupting effects, and reproductive developmental toxicity [9]. Especially, antibiotics residues alter microbial community composition and activities, and induce the emergence of resistance genes and bacteria, even at environmentally low concentrations [9,10]. The plasticizer bisphenol A (BPA) may interfere with the endocrine system leading to adverse effects on the reproductive, neurological, and immunological systems in both human and animals [8]. Consequently, there is a need to develop alternative treatment processes to remove EMCs from wastewaters.

Physical-chemical technologies such as adsorption on activated carbon and advanced oxidation processes (i.e. ozonation, photooxidation, radiolysis, and electrochemical oxidation) have been demonstrated to be effective in EMCs removal [11,12,13]. However, the toxicity of the resulting transformation products and the high operating costs of these technologies limited their use [4]. Other bioremediation processes of EMCs based on the use of microorganisms such as bacteria were widely investigated due to their eco-compatibility and lower costs [14]. Nevertheless, the application of this process could induce genetic resistance in bacteria [8,14,15].

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Recent works reported that many microalgae species could remove several contaminants [16] besides their ability to grow efficiently on wastewaters as a nutrient media [17]. In addition, the feasibility of cultivating microalgae in wastewaters to remove dissolved organic carbon, phosphorus, and nitrogen in municipal, industrial and artificial wastewater has been, widely, demonstrated [18]. Also, microalgae are known to exhibit antibacterial activity, which can boost wastewater disinfection, and enhance the deactivation of pathogens, due to the high pH and O_2 concentrations mediated by photosynthesis [19]. Therefore, the integration of microalgae culture in WWTPs systems could, on the one hand, reduce the amount of contaminants in wastewater effluents, and on the other hand, promote the production of microalgal biomass, using wastewater as a source of nutrients. Such systems are economic, cost-effective, and eco-friendly for the bioremediation of emerging contaminants in wastewater plants.

In this context, microalgae and especially extremophilic species appear to be suitable candidates for the bioremediation of wastewater containing EMCs. These species are known for their tolerance to harsh environmental conditions and high anthropogenic pollution [20,21]. Previous laboratory cultures, using artificial media, showed high growth performance of the alkaliphilic Chlorophyta *Picocystis* sp. exposed to high concentrations of several contaminants such as cadmium [22], bisphenol A [23,24], and diclofenac [25] coupled to high removal efficiency of these contaminants. However, all these studies were performed on a fully artificial medium, each compound being addressed, separately.

This work aimed to assess the ability of *Picocystis* sp. (CINS 23) to grow in domestic wastewater supplemented by a model EMCs mixture consisting of three pharmaceuticals, diclofenac (DCF), sulfadiazine (SDA), and oxytetracycline (OTC), and an industrial chemical, a precursor to important plastics and resins, bisphenol A (BPA). These contaminants are widely used and commonly detected, in water plants, at concentrations ranging from a few hundred to thousands of ng/L [26,27]. First, *Picocystis* growth kinetics were assessed in batch and continuous culture mode. Then, the daily removal efficiency of the studied EMCs (DCF, SDA, OTC, and BPA) as well as the recovery ability of nutrients (nitrogen, phosphorus, and total organic carbon), are evaluated during 27 days' of *Picocystis* continuous culture in wastewater supplemented by EMCs.

2. Materials and methods

2.1. Chemicals and reagents

Analytical grade chemicals were purchased from Sigma Aldrich: diclofenac (>98.0 % purity) oxytetracycline (>95 % purity), sulfadiazine (>98.0 % purity), and bisphenol A (97 %% purity).

The stock solutions of DCF, SDA, OTC, and BPA, with the concentration of 1 g L^{-1}, were prepared, separately, by dissolving an appropriate amount of the compound in ethanol (0.05 %), then, mixed, and stored below the temperature of 5 °C.

2.2. Wastewater effluent collection and media preparation

The wastewater effluent (WW) used in this study was obtained from the effluent of the municipal wastewater treatment plant in Sfax, Tunisia. It consisted of secondary treated wastewater, being subjected to sedimentation followed by aeration and decantation, before its discharge into the sea. For experiments, WW samples were collected in a clean plastic container and settled for one hour to remove excess of the suspended solids materials. Thereafter, WW was immediately frozen at −20 °C until use without further processing. Dissolved nutrient content was analyzed before the experiment (Table 1).

The contaminated media (CWW) was obtained by adding an aliquot of EMCs mixed solution to the WW. The EMCs mixed solution, containing DCF, SDA, OCT, and BPA, was prepared in ethanol (0.05 %) and added to the unsterilized WW immediately before the experiment, to achieve a final concentration of 1 mg L^{-1} of each contaminant. WW without EMCs was set as control.

Table 1
Wastewater characteristics.

Total organic carbon (TOC)	186 ± 25 mg L^{-1}
Total phosphorus (TP)	19 ± 5 mg L^{-1}
Total nitrogen (TN)	71 ± 11 mg L^{-1}
Chemical oxygen demand (COD)	329.6 ± 53.5 mg L^{-1}
Biochemical oxygen demand (BOD)	80 ± 00 mg L^{-1}

2.3. Microalgae strain

The microalgae strain *Picocystis* sp. CINS 23 used was isolated from a household sewage "Essed valley" located in Center East of Tunisia (35°59′23″N, 10°30′10″E) at water pH 11 and maintained in the Tunisian National Institute of Marine Science and Technology (INSTM) microalgae collection. The strain, preliminarily, was cultivated under sterile conditions in Zarrouk medium [28]. *Picocystis* was gradually preacclimated to WW by successive subcultures before the experiment. Precultures, established in 2 L Erlenmeyer flasks, were then used to inoculate each set of 4 columns of photobioreactors connected in series.

2.4. Experimental set-up

Experimental cultures were conducted in a 20 L photobioreactor (PBR) composed of a set of 4 Plexiglas columns connected in series (Fig. 1). Each column has a diameter of 13 cm, a height of 50 cm, and a working volume of 5 L. The 4 columns of the PBR are arranged at different levels and are interconnected from bottom to top by polystyrene tubes (12 mm in diameter) ensuring the gravity circulation of the culture between the columns. A circulation pump (1.3 L min^{-1}) submerged in the last column served the first one, allowing the culture to circulate throughout the system. Culture temperature was maintained at 30 ± 1 °C by incubation of the equipment within a thermostatic chamber. Illumination was provided by six fluorescent tubes delivering light intensity of 75 μmol photons m^{-2} s^{-1} with a 12/12 light/dark cycle. The pH was monitored and maintained near 8.6 using Eutech Instruments pH/ORP electrodes equipped with solenoid valves that were programmed to bubble sterilized CO_2 into individual bioreactors if the pH increased above 8.6.

Cultures in CWW were operated first in batch mode until the end of the exponential growth phase (7 days). Each experimental culture was initially inoculated by approximately 0.8 g L^{-1} dry weight (dw) of *Picocystis* biomass density. *Picocystis* culture in WW without ECs, conducted under the same conditions, was set as control.

Secondly, continuous cultures were performed for 27 days. A volume of culture equivalent to the dilution rate (determined in Section 3.1 in the results section) was, daily, collected, using a metering pump, and replaced by an equal volume of WW contaminated by EMCs to maintain the biomass productivity at its maximum level. Furthermore, a non-inoculated CWW was maintained to evaluate the removal without microalgae cells.

2.5. Productivity and dilution rate determination

The biomass concentration density (g L^{-1}) was determined gravimetrically by daily measurements of dry weight. Triplicate samples (10 mL) were filtered over prewashed 0,7 μm glass microfiber filters (GF/F filter, Whatman Plc., UK) and dried overnight at 80 °C. The filters were cooled to room temperature in a desiccator before the weighing.

The daily biomass productivity (g $L^{-1}d^{-1}$) was calculated, during the exponential growth stage, based on the initial (B0, g L^{-1}, on Day 0) and final biomass densities (Bt, g L^{-1}, on Day t) according to the following

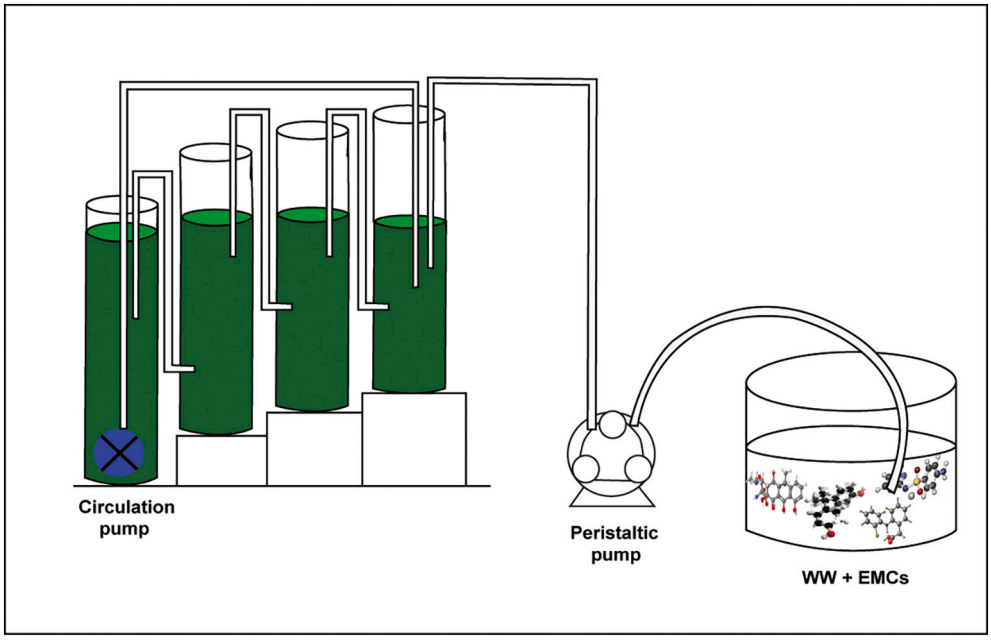

Fig. 1. Schematic of the culture system composed of a set of 4 columns PBRs connected in series.

equation [29]:

$$Biomass\ productivity\ \left(g\ L^{-1}d^{-1}\right) = \frac{(Bt - B0)}{(Tt - T0)}$$

The daily dilution rate was determined at the end of the exponential growth phase using the following equation [30]: $D\ (day^{-1}) = BP_{te}/\ B_{te}$.

Where BP_{te} is the biomass productivity at the end of the exponential growth phase (t_e), B_{te} is the biomass density at t_e.

2.6. Nutrient recovery and EMCs removal evaluation

The daily rate of nutrient recovery and EMCs removal were assessed during the continuous culture (27 days). Each tested component was evaluated separately by determining its concentration in the filtrate according to the following equation [25,31]:

$$removal\,(\%) = \frac{(initial\,concentration\,(day_t) - final\,concentration\,(day_{t+1}))}{initial\,concentration\,(day_t)} \times 100$$

Samples of cultures (300 mL) were collected daily and centrifuged at 10.000 ×*g* for 10 min. The supernatant was filtered through a syringe filter (0.45 μm, Whatman) and stored at 4 °C in the dark before analysis. Aliquots of supernatant were re-filtered through pre-LC-MS 0.2 μm PTFE filters (Whatman, Puradisc) and used for the determination of the residual EMCs in the medium. The same extraction procedure was applied for CWW experiments without microalgae cells, conducted in the same culture conditions, to determine the EMCs removal without *Picocystis*.

For nutrient analysis: Total nitrogen (TN) was determined according to the Kjeldahl method [32] by mineralization of organic matter, followed by distillation and titration. Total phosphorus (TP) concentration was determined, spectrophotometrically, using the phospho-vanado-molybdate complex method. Total organic carbon (TOC) concentration was analyzed by a total organic carbon analyzer (Shimadzu COT-VCPH/CPN, Kyoto, Japan).

EMCs (DCF, SDA, OTC, and BPA) analysis were performed by UPLC-MS/MS consisting of a Waters (Aquity UPLC) liquid chromatographic system coupled to a mass spectrometer detector (Quattro Premier; Micromass) equipped with an electrospray ionization source and operated with Masslynx V4.1 software. A BEH-C18 chromatographic column (100 mm × 2.1 mm ID; 1.7 μm) was used for EMCs separation. The composition of elution solvents and mobile phase flows are presented in Table 2. The injection volume was 5 μL and the flow rate of injection was 0.4 mL min^{-1}. The MS/MS was working in multiple reaction monitoring (MRM) mode for the detection and quantification of contaminants. The analysis was performed using ESI negative mode for BPA and positive mode for PPCPs. Nitrogen was used as the collision and nebulizing gas. The retention times, collision energy, capillary voltage, monitored ions, and MS parameters of the studied compounds are presented in Table 2. Complete calibration curves were performed at the beginning and the end of each sample batch analysis, and the mean slope value of these curves were used for contaminant quantification. The limit of detection (LOD) and the limit of quantification (LOQ) were 0.180 and 0.547 mg L^{-1}, respectively. For each tested contaminant, a sample blank, a laboratory fortified blank, a duplicate, and positive control were conducted to ensure the accuracy of the sampling, extraction, and analytical procedures. The sample blanks were treated exactly like a sample including exposure to all glassware, equipment, solvents, and reagents to check possible sources of contamination. The concentrations of the tested contaminant in the blank samples were below the LOD. The laboratory fortified blank is a sample blank spiked with known contaminant concentration and extracted following the same procedures as detailed above. The EMCs recoveries were between 89 and 113 %, indicating the applicability of the current extraction method. All reported data were not corrected for recoveries. Further, a calibration verification standard was performed at the beginning of each analytical run and after each group of 10 samples to ensure the stability of the analytical method.

Table 2
MRM parameters for EMCs analyses.

	Retention time (min)	Precursor ion (m z^{-1})	Collision energy (eV)	Capillary voltage (V)
DCF	4.68	296.35 > 214.21	29	23
SDA	1.71	251.36 > 155.93	15	29
OTC	2.73	461.53 > 426.33	19	39
BPA	1.36	227 > 212	20	25

2.7. Statistical analysis

The experiments were carried out using independent duplicate samples with three replicates measurements. The results were presented as mean values ± standard deviation ($n = 6$). The differences in the biomass yield and biomass productivity between experiments were analyzed for significance using one-way ANOVA at a significant level of 0.05.

3. Results

3.1. Picocystis culture in the batch mode

The growth performance of *Picocystis* sp. in terms of biomass concentration (Fig. 2a) and biomass productivity (Fig. 2b) were monitored during 7 days of batch cultures in CWW and WW media. Results confirm that *Picocystis* sp. CINS23 can grow in WW even in the presence of a mixture of the studied EMCs. The addition of the mixture of EMCs did not inhibit the growth of *Picocystis* in comparison with control cultures (WW). Both batch cultures (with and without EMCs) have a similar logistic evolution.

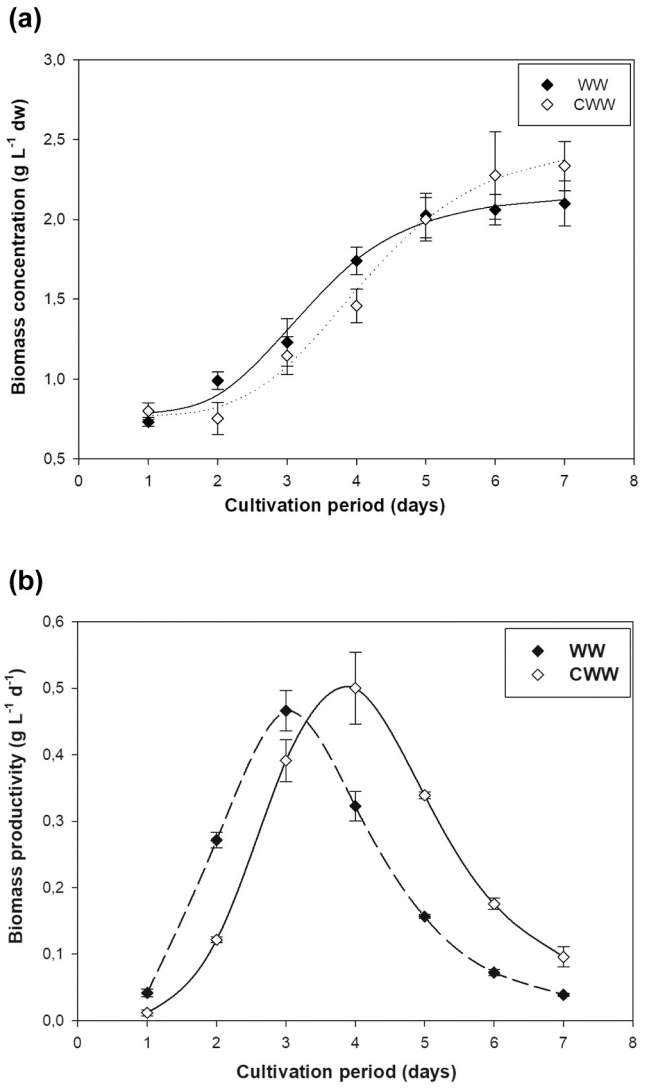

Fig. 2. Variation of *Picocystis* (a) biomass concentrations ($g\,L^{-1}$ dw) and (b) biomass productivity ($g\,L^{-1}\,d^{-1}$) under batch culture in ⋄ CWW and ◆ WW. Dots correspond to experimental data and lines (continuous and dashed lines) correspond to fittings by the logistic kinetic model during batch culture. Error bars represent standard deviation (n = 6).

Picocystis, under culture in CWW, showed a maximum biomass concentration of $2.33 \pm 0.15\,g\,L^{-1}$ at the end of the culture period, non-significantly different from that of culture in WW without contaminants ($2.10 \pm 0.14\,g\,L^{-1}$). The maximum daily biomass productivity of about $0.50 \pm 0.05\,g\,L^{-1}\,d^{-1}$ was recorded after 3–4 days with a non-significant difference for both cultures in CWW and WW media. Consequently, the maximal relative biomass productivity derived in culture in CWW was evaluated at about 34 % d^{-1}.

3.2. Picocystis continuous culture in CWW

3.2.1. Picocystis growth

Picocystis continuous culture in CWW was carried out for 27 days, adopting a daily dilution ratio (34 %) equivalent to the maximum relative biomass productivity obtained in the batch CWW culture. The biomass density was stabilized ($1.42\,g\,L^{-1}$) during the whole experimental period ensuring constant daily biomass productivity of $0.44\,g\,L^{-1}\,d^{-1}$ (Fig. 3).

Once the *Picocystis* growth rate in CWW was stabilized under the continuous culture, the daily uptake of nutrients and EMCs could be assessed.

3.2.2. Nutrient recovery

During the 27 days of the continuous culture in the CWW medium, the average daily amount of total phosphorus (TP) was close to 27 % of the initial TP amount ($19\,mg\,L^{-1}$). It achieved a maximum of 42 % on the 15th day and then stabilized to ~24 % for the rest of the experimental period (Fig. 4a). The average daily amount of total nitrogen (TN) recovery was near 40 % of the initial TN amount ($71\,mg\,L^{-1}$) during the first 5 days and then stabilized to ~23 % for the rest of the experimental period (Fig. 4b). The average daily amount of total organic carbon (TOC) recovery was near 40 % of initial TOC ($186\,mg\,L^{-1}$) during the 27 days of the continuous experimental culture (Fig. 4c).

3.2.3. EMCs removal

Removal percentages of EMCs from CWW in continuous cultures with and without microalgal inoculum are summarized in Table 3. Results show high EMCs removal amounts from CWW by *Picocystis*. In presence of *Picocystis*, the daily amounts of removed EMCs reached up to 100 % for OTC, 83–92 % for DCF, 66–70 % for SDA, and 93–95 % for BPA.

The daily removal in the absence of *Picocystis* of all tested EMCs

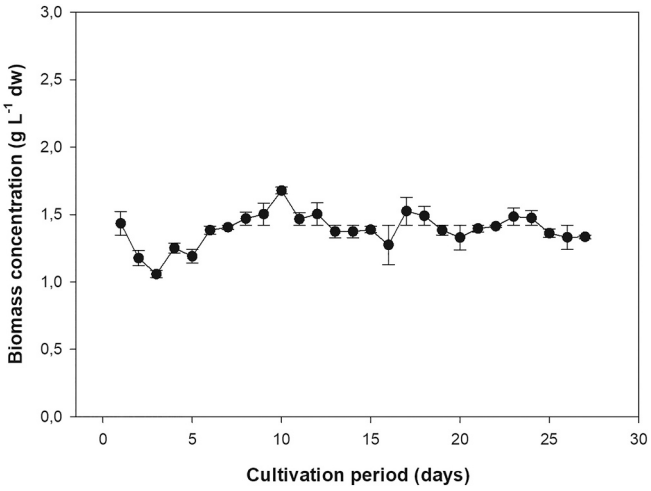

Fig. 3. *Picocystis* biomass evolution under continuous fed-batch culture in CWW at the initial contaminant concentrations ($1\,mg\,L^{-1}$). Error bars represent standard deviation (n = 3).

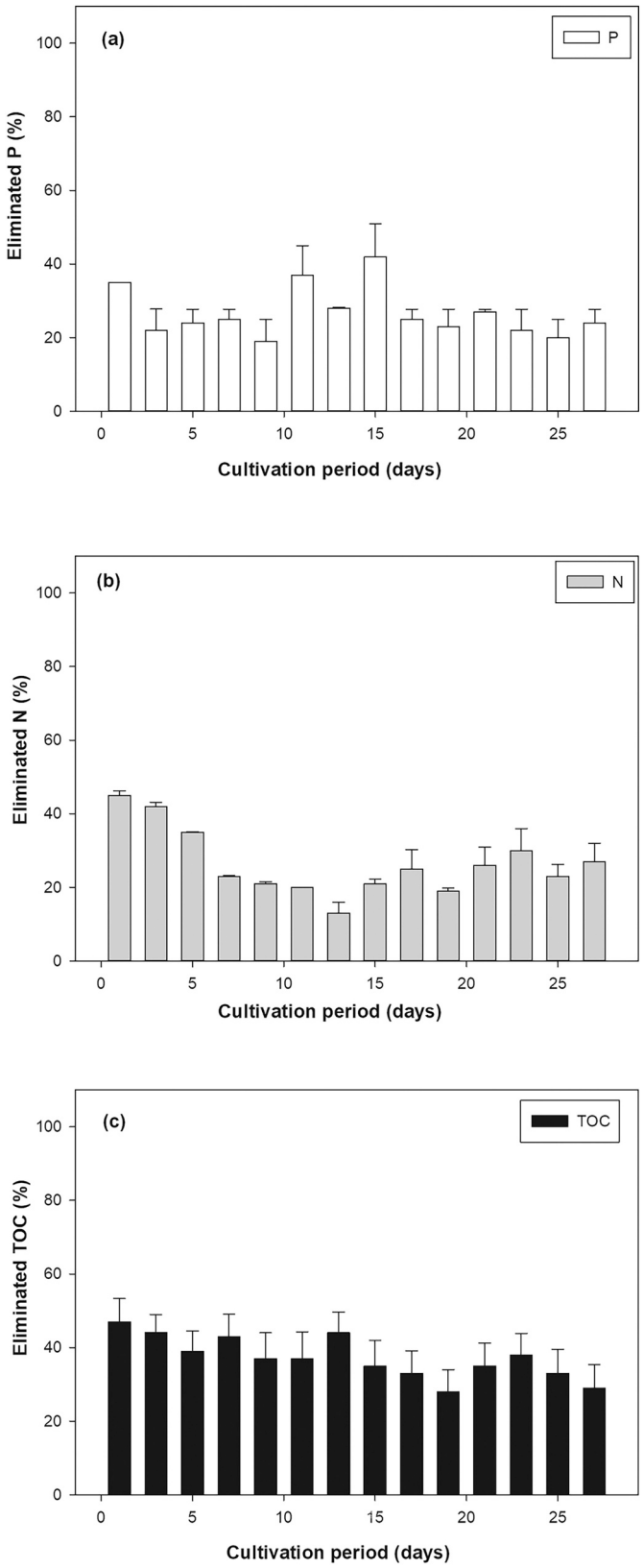

Fig. 4. Daily nutrient removal by *Picocystis* during continuous fed-batch culture in CWW: (a) total phosphorus (P); (b) total nitrogen (N) and (c) total organic carbon (TOC). Error bars represent standard deviation (n = 3).

Table 3
Daily removal percentages of OTC, SDA, BPA and DCF by *Picocystis* sp. recorded at 7th, 15th and 27th day of continuous culture in CWW at the initial concentrations of 1 mg L^{-1} of each contaminant. Error bars represent standard deviation ($n = 3$).

	Total removal (%)			Removal without *Picocystis* (%)
	Day 7	Day 15	Day 27	
1 mg L^{-1} OTC	100 ± 0.00	100 ± 0.00	100 ± 0.00	4.9 ± 1.32
1 mg L^{-1} SDA	68.61 ± 1.7	70.22 ± 2.1	66.54 ± 3.5	3.3 ± 1.05
1 mg L^{-1} BPA	95.20 ± 4.5	93.68 ± 7.5	95.40 ± 5.7	4 ± 1.53
1 mg L^{-1} DCF	92.00 ± 2.8	83.96 ± 4.9	90.52 ± 3.8	2.6 ± 1.08

under the same experimental conditions did not exceed 5 % (Table 3).

4. Discussion

Several studies have suggested that sterilization of wastewater, an energy-consuming and expensive process, is necessary to eliminate the bacteria and the viruses that may inhibit microalgae growth [33]. Bohutskyi et al. [34] found that most of the tested seaweed species were unable to develop, effectively, in unsterilized secondary effluents. Similarly, Yu et al. [35] showed that the growth of *Chlorella ellipsoidea* was inhibited in unsterilized secondary household effluents.

In this study, the unsterilized secondary wastewater used supported, perfectly, the *Picocystis* growth requirements, with a maximum growth rate of 0.5 ± 0.05 g L^{-1} day^{-1} comparable to and even greater than that commonly found in cultures of other Chlorophyta species, using artificial media [6,36]. These results confirm once more the exceptional tolerance of *Picocystis* to extreme conditions [22,23,24,25].

The wastewater used in this work is highly concentrated in nutrients. Nitrogen and phosphorus levels (62.7–80.3 and 6.5–21.9 mg L^{-1}, respectively) are higher in comparison to those of wastewater used in previous studies [37,38]. Such high levels pose an environmental problem, in particular when the secondary treated wastewater is discharged into the sea, but constitutes a source of nutrients for the production of microalgal biomass, as shown by the high *Picocystis* productivity using this water as the sole nutrient source. Furthermore, the daily recovery rates of total phosphorus (TP) and nitrogen (TN) by *Picocystis* were approximately 24 to 40 % of the initial amounts in CWW (TN = 186 ± 25 mg L^{-1} and TP = 19 ± 5 mg L^{-1}). Escapa et al. [6] and Aslan and Kapdan [39] reported that *Chlorella* species could remove up to 100 % of nitrogen and phosphorus from synthetic media. But such removal efficiencies were obtained at initial N and P amounts three times lower than those of the wastewater used in this study and they accounted for a total of 9–10 days of batch culture.

According to Nurdogan and Oswald [40], Cho et al. [41], and Ji et al. [42], a nutrient elimination by phosphorus precipitation and nitrogen evaporation could occur at high pH values (9–11). Since in our work, the pH was set at 8.6 we could assume that the nutrient precipitation and/or evaporation was limited and thus microalgal assimilation into biomass was the major mechanism behind nutrient recovery. The daily TN recovery amount recorded in the present work was approximately 57 mg TN L^{-1} day^{-1}, which was equivalent to 13 % of the daily biomass produced. This confirms that most of the removed nitrogen was converted into biomass and that the abiotic loss was negligible in our experimental conditions. On the other hand, the reduction in TOC (≈40 %) suggests that *Picocystis* can metabolize organic carbon in addition to photosynthesis, as it is well known in many Chlorophyta species [6].

The ability of *Picocystis* to grow and survive in unsterilized wastewater contaminated with the mixture of EMCs could be related to its high EMCs removal capacity. Several studies reported the ability of several microalgae species to uptake and metabolize complex molecules

such as pharmaceuticals and endocrine-disrupting chemicals [43,44]. The possible mechanisms involved include bioadsorption, bioaccumulation, and intracellular and extracellular biodegradation or biotransformation [1]. Passive adsorption may occur through the hydrophobic binding effect of proteins on the microalgae cell wall or the physical trapping of contaminants within or throughout the extracellular polymeric substances (EPS) [16,45]. Complete or partial biodegradation of pollutants may occur via different enzymatic reactions such as hydroxylation, carboxylation, oxidation, hydrogenation, glycosylation, demethylation, ring cleavage, decarboxylation, dehydroxylation, and bromination [16,43,45].

Among many studies about wastewater remediation by microalgae, little attention was paid to EMCs removal. To date, the main reported studies targeting the EMCS remediation by microalgae consider one contaminant at a time and use artificial media [6,23,24,25,46,47]. Published results on the EMCs removal by microalgae in synthetic wastewater have revealed different efficiencies depending on the contaminant and the microalgae species. For example, de Wilt et al. [48] reported that *Chlorella sorokiniana* grown in synthetic domestic wastewater could recover up to 60–100 % of diclofenac, ibuprofen, paracetamol, and metoprolol. Recently, López-Serna et al. [4] studied the removal of five pharmaceuticals and personal care products from synthetic wastewater under two novel algal-bacterial photobioreactor settings. These authors determined maximum removal above 94 % for ibuprofen, 52 % for naproxen, 98 % for salicylic acid, and 100 % for triclosan and propylparaben.

The EMCs removal in real WW was rarely investigated. Hom-Diaz et al. [47] showed that 60 to 80 % of hormones were removed from anaerobic digestate centrate by the microalgae species *Selenastrum capricornutum* and *Chlamydomonas reinhardtii*.

In our case, *Picocystis* under continuous culture in CWW showed an ability to remove daily up to 70 % of SDA and achieved complete removal of OTC. Antibiotics removal efficiencies varying from 24 to 99 % were recorded after 40 days' culture of *Haematococcus*, *Scenedesmus*, *Chlorella,* and *Chlamydomonas* species in pre-sterilized wastewaters, as reviewed by Xiong et al. [49].

DCF was included in the EU Water Framework Directive watch list alongside the priority substances [50]. High variation in DCF removal rates has been noted in municipal WWTPs as reviewed by Vieno and Sillanpää [51]. Our results showed that <3 % of DCF was degraded or transformed in absence of *Picocystis* while the daily DCF removal from continuous *Picocystis* culture in CWW was about 84 to 92 % of an initial concentration of 1 mg L^{-1}. These findings are consistent with previous studies [25,52], using synthetic media.

Additionally, *Picocystis* was able to remove up to 95 % of bisphenol A, daily, from CWW at an initial concentration of 1 mg L^{-1}. Hom-Diaz et al. [47] reported complete removal of BPA by *Selenastrum capricornutum* and *Chlamydomonas reinhardtii* from wastewater digestate. BPA removal efficiencies (40–88 %) were found for microalgae cultivated in synthetic media [23,24,53,54] but after 5 and 16 days of exposure to BPA concentrations (0.01–75 mg L^{-1}).

In this study, *Picocystis* under continuous culture in CWW showed the ability to remove daily up to 70 % of SDA, up to 90 % of DCF and BPA, and removed completely OTCat 1 mg of initial concentration of each contaminant. As the concentration of these EMCs in natural water bodies is generally of the order of ng/L to μg L^{-1} [8,9], the obtained results point to the promising application of *Picocystis* in a continuous bioremediation system for the total removal of emerging contaminants from wastewaters.

5. Conclusion

Our results show that *Picocystis* was able to grow in wastewater as the sole source of nutrients and in presence of a mixture of EMCs. High biomass productivity was obtained by the recovery of nearly 27–40 % of N, P, and TOC, dissolved in the wastewater. This proportion could probably be increased either by recycling the effluents recovered after filtration of the biomass or by extending the residence time in the reactor to two or three days.

Picocystis exhibited high EMCs removal efficiencies reaching >70 %. Total elimination of experimented EMCs could also be ensured given that the concentration of tested contaminants in wastewater is typically below 1 mg L^{-1}. However, the confirmation of these results at the pilot scale under natural conditions is still necessary. In this case, the use of the reactor composed of columns connected in series is advantageous because it allows the extension of the volume of culture by the connection of other additional units and/or by the increase in the dimensions of each unit while using a single recycling pump.

CRediT authorship contribution statement

R.B.A, S.B.A, H.B.A and S·S conceived and designed the study. S.B.A carried out the experiment and R.B.A analyzed the data. A.J. contributed to analysis. R.B.A, S.B.A and H.B.A contributed to the interpretation of the results. R.B.A. wrote the original draft and revised manuscript with the support of H.B.A. H.B.A, S·S, and C.L performed project supervision. All authors read and commented on the draft manuscript. All authors agreed to the final version.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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