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► **To cite this version:**

Sabrina Ben Ouada, Rihab Ben Ali, Nicolas Cimetiere, Christophe Leboulanger, Hatem Ben Ouada, et al.. Biodegradation of diclofenac by two green microalgae *Picocystis* sp. and *Graesiella* sp. *Ecotoxicology and Environmental Safety*, 2019, 186, pp.109769. 10.1016/j.ecoenv.2019.109769 . hal-02364873

HAL Id: hal-02364873

<https://univ-rennes.hal.science/hal-02364873v1>

Submitted on 20 Jul 2022

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1 **Biodegradation of Diclofenac by two green microalgae: *Picocystis* sp. and**
2 ***Graesiella* sp.**

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20 **Abstract**

21 The aim of the present study was to provide an integrated view of algal removal of diclofenac
22 (DCF). Two isolated microalgal strains *Picocystis* sp. and *Graesiella* sp. were cultivated under
23 different DCF concentrations and their growth, photosynthetic activity and diclofenac
24 removal efficiency were monitored.

25 Results showed that DCF had slight inhibitory effects on the microalgal growth which did not
26 exceed 21% for *Picocystis* and 36% for *Graesiella* after 5 days. Both species showed different
27 patterns in terms of removal efficiency. In presence of *Picocystis* sp., the amounts of removed
28 DCF were up to 73%, 43% and 25% of 25, 50 and 100 mg L⁻¹ respectively; whereas only
29 52%, 28% and 24% were removed in the presence of *Graesiella* at same DCF tested
30 concentrations. DCF removal was insured mainly by biodegradation. To better reveal the
31 mechanism involved, metabolites analyses were performed. Two DCF
32 biodegradation/biotransformation products were detected in presence of *Picocystis*.

33 This study indicated that *Picocystis* performed a satisfactory growth capacity and DCF
34 removal efficiency and thus could be used for treatment of DCF contaminated aqueous
35 systems.

36 **Keywords**

37 Microalgae; Extremophiles; Diclofenac; Removal; Biodegradation; Biotransformation.

38 **1. Introduction**

39 The presence of emerging contaminants as pharmaceutical and personal care products (PPCPs)
40 in the aquatic environment continues to give rise to concern due to their environmental risk
41 and toxicological properties. Indeed, these chemical compounds can cause multiple changes

42 in the physiological state of organisms, and their occurrence in the environment may affect
43 non-target species (**Valavanidis et al., 2014**).

44 Among the most used PPCPs, Diclofenac (DCF) is a non-steroidal anti-inflammatory drug
45 (NSAID) widely prescribed as an antipyretic analgesic. It is often found as a persistent toxic
46 waste and one of the most widely available drugs in the world. Recent studies based on the
47 Intercontinental Marketing Services (IMS) health data (which serves 82% of the global
48 population) from 86 countries estimated that about 1443 ± 58 tons of DCF are consumed
49 globally on an annual basis (**Acuña et al., 2015**). However, this is only an indication of the
50 DCF consumption for human health-related applications and does not include DCF's
51 veterinary uses.

52 A large part of the consumed DCF is excreted in urine and feces in original form so entering
53 municipal wastewater. DCF is ineffectively removed by conventional wastewater treatment
54 plants (WWTPs) (**Langenhoff et al., 2013; Sophia and Lima, 2018**). Thus, it can be
55 discharged into the environment with treated wastewater effluent, recycled water, and
56 wastewater plant sludge. Actually, DCF is detected in several aquatic environments all over
57 the world at concentration ranges from few hundreds to thousands of ng/L (**Lonappan et al.,**
58 **2016**). It may be transported through food chains (**Cuellar-Bermudez et al., 2017**) causing
59 toxic adverse effects on many aquatic organisms, even at environmentally low
60 concentrations (**Xu et al., 2019**).

61 Therefore, it is mandatory to investigate alternative treatments for DCF removal from
62 wastewaters. Among them, those based on the use of microalgae are emerging as a
63 sustainable and economical solution (**Escapa et al., 2017**). Recent studies reported that many
64 microalgae species could remove several pharmaceutical contaminants, including DCF
65 (**Xiong et al., 2018**). Therefore, using microalgae to remove DCF from wastewater could be
66 prospective.

67 To date, only very few studies have been carried out on the assessment of DCF removal using
68 microalgae (**Escapa et al., 2017; Villar-Navarro et al., 2019**). Furthermore, the ability of
69 microalgae to accumulate DCF as well as its biodegradation and/or biotransformation
70 products were not yet investigated. Thus, information regarding DCF effects, removal and
71 biotransformation products in the presence of microalgae are required.

72 Among microalgae, a particular interest is given to the use of extremophilic species in
73 bioremediation systems (**Varshney et al., 2014**). Such organisms are assumed to have
74 specific qualities allowing them to tolerate high concentration of several pollutants and further
75 could be more efficient in the fast contaminant removal from wastewaters (**Peeples, 2014**).

76 The aim of the present work is to evaluate and compare, under laboratory culture conditions,
77 the effects and the removal efficiency of DCF by two extremophilic microalgae strains
78 *Picocystis* sp. and *Graesiella* sp. isolated from two polluted ponds in Tunisia for the purpose
79 of selecting resistant species for their bioremediation use. To fulfill those purposes, the effect
80 of DCF on growth and photosynthesis of both species was assessed together with their
81 abilities to remove and accumulate DCF. Then, the resulting biotransformation and
82 biodegradation products were addressed.

83 **2. Materials and methods**

84 **2.1 Chemicals and reagents**

85 Diclofenac sodium salt ($\geq 98\%$) was purchased from Sigma Aldrich. Ultra-pure water (UPW)
86 was delivered by Elga Pure Lab System (resistivity 18.2 M Ω .cm, COT <50 μ g C/L).
87 Acetonitrile (ACN) with 0.1% formic acid (FA) was purchased from JT Baker (LC-MS grade)
88 and used in association with UPW with 0.1% formic acid.

89 Salts and reagents for medium preparation suppliers are specified in Supplementary material 1.

90 All chemicals used were of analytical grade.

91 2.2 Algal strains and culture conditions

92 Two extremophilic chlorophyta species were investigated in this study: an alkaliphilic
93 *Picocystis* sp. CINS 23 and a thermophilic *Graesiella* sp. CINS 60. The strains have been
94 deposited in the National Institute of Marine Sciences and Technologies Collection.
95 *Picocystis* sp. was isolated from a household sewage “Essed valley” located in Center East
96 of Tunisia (35° 59' 23" N, 10° 30' 10" E) and *Graesiella* sp. from a hot water catchment
97 basin emerging from “Ain Echfa,” a hot spring located in northern Tunisia (36°49' N, 10°34'
98 E). Preliminary laboratory experiments were conducted to define the optimal autotrophic
99 growth conditions: culture media, temperature and light intensity (**Ben Ali et al., 2017; Ben**
100 **Ouada et al., 2018; Mezhoud et al., 2014**). The strains were cultivated separately in batch
101 culture under sterile conditions: *Graesiella* in Bold’s Basal Medium (BBM) (**Bischoff and**
102 **Bold, 1963**) modified according to the Elser concept for freshwater microalgae with C:N:P
103 ratio equal to 166:20:1 (**Elser et al., 2000**), and *Picocystis* in Zarrouk medium (**Zarrouk,**
104 **1966**) (Supplementary material 1). The initial pH was adjusted to 6.8 and 8.4 for BBM and
105 Zarrouk media, respectively. Cultures were maintained in optimal growth conditions at
106 temperature of 30°C and light intensity of 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ under 8/16-h illumination cycle as
107 established by preliminary laboratory experiments. Experimental cultures were conducted at
108 these optimal growth conditions for all the designed experiences.

109 Exponentially growing cultures from *Picocystis* and *Graesiella* were separately inoculated in
110 500 mL Erlenmeyer flasks containing respectively 300 mL of Zarrouk and BBM media, with
111 an initial cell density of 10^6 cells mL^{-1} . Cultures were exposed separately for 5 days to DCF at
112 0 (control), 25, 50, 100 and 200 mg L^{-1} initial concentrations. For each experimental DCF
113 concentration and separately for each tested strain, a series of six Erlenmeyer flask cultures
114 were used in triplicate. Daily, 3 ml of each culture served for the determination of the optical
115 density and the photosynthetic activity. The determination of the residual, adsorbed,

116 accumulated, and biodegraded DCF amounts of 25, 50 and 100 mg L⁻¹ initial tested
117 concentrations, was carried out at the end of the experiment (day 5). Blank controls (without
118 algae cells) served also for the determination of the DCF abiotic removal (Supplementary
119 material 2).

120 The stock solution of diclofenac with the concentration of 1 g L⁻¹ was prepared by dissolving
121 an appropriate amount of the compound in distilled water and it was stored below the
122 temperature of 5 °C. The testing solutions were diluted to the desired concentrations during
123 the experiments.

124 **2.2 Growth inhibition test**

125 Growth was followed spectrophotometrically in each culture condition by measuring the daily
126 changes in OD_{680 nm} during five days of DCF exposure.

127 Growth curves of microalgae population were obtained by plotting OD_{680 nm} values and
128 incubation time. The areas under each growth curve were evaluated using Origin 8.5 software
129 (OriginLab, Northampton, USA). Inhibition of algal activity was expressed as the percentage
130 inhibition (PI%) defined according to **De Orte et al. (2013)** by the following equation:

$$131 \quad \text{PI\%} = \left[\frac{\text{Control area} - \text{Treated area}}{\text{Control area}} \right] \times 100 \quad (\text{Eq. A1})$$

132 Where, Control area and Treated area are the integrated areas under the growth curve of
133 control and treated culture, respectively.

134 **2.3 Photosynthetic parameters**

135 Chlorophyll fluorescence for each culture condition was measured every day using the
136 Aquapen-CAP-C 100 fluorometer (Photon Systems Instruments, Brno, Czech Republic). A 3
137 mL sample was collected daily from each experimental culture and diluted with fresh medium

138 to a final optical density at 680 nm of 0.3. Before the measurement, each micro-algal sample
139 was dark-adapted for 15 min at room temperature to allow the complete re-oxidation of PSII
140 reaction centers. The chlorophyll fluorescence transients (OJIP) were induced by light pulses
141 at a fixed excitation wavelength of 650 nm and intensity of 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and
142 recorded for up to 1 s on a logarithmic time scale. The OJIP-test parameters were determined
143 from transient analysis according to **Strasser et al. (2000)** and the maximal PSII
144 photochemical efficiency (F_v/F_m) was derived, wherein $F_v = F_m - F_o$ is the variable
145 fluorescence, and F_o and F_m are minimal and maximal fluorescence yields in dark-adapted
146 state, respectively.

147 The non-photochemical quenching (NPQ) was measured according to the manufacturer's
148 instructions (protocol NPQ1, Photon Systems Instrument). The intensities of actinic light (450
149 nm) and pulse-saturating light were 300 and 3000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively. The
150 NPQ was calculated as $\left(\left(F_m - F'_m\right)/F'_m\right)$ (**Campbell et al., 1998**), wherein F'_m is the
151 maximal fluorescence in a light-adapted state.

152 **2.4 Determination of DCF removal and degradation intermediates**

153 After 5 days of DCF exposure, 150 mL of each algae culture were centrifuged at 10,000 \times g for
154 10 minutes. The supernatant was used for the determination of the residual DCF in the
155 medium. The cell pellets were washed three times with de-ionized water and then centrifuged.
156 The supernatant was used for the determination of DCF adsorbed on the microalgae surface.
157 The cell pellets were mixed with 2 mL of dichloromethane-methanol (1:2 v/v) and digested
158 by sonication for 45 min (**Ji et al., 2014**). After centrifugation for 10 min at 4500 \times g, the
159 resulting supernatant was conserved to determine the accumulated DCF within the microalgae
160 cells.

161 The same extraction procedure was applied for media without algae cells at all tested DCF
162 concentrations exposed to the same culture conditions during five days to determine the
163 abiotic removal of DCF.

164 Total DCF removal percentage was calculated according to the following equation:

$$165 \quad \text{DCF removal (\%)} = \frac{(\text{initial DCF concentration} - \text{final DCF concentration})}{\text{initial DCF concentration}} \times 100 \quad (\text{Eq. A2})$$

166 Biotransformation/biodegradation percentage (Bp) of DCF by microalgae was calculated
167 according to the following equation (Li et al., 2009):

$$168 \quad \text{Bp(\%)} = (CI - Ar - Aa - Ad - Ac) \times 100 / CI \quad (\text{Eq. A3})$$

169 Where CI is the initial amount of DCF, Ar is the residual amount in the medium, Aa is the
170 amount of abiotic removal, Ad is the amount adsorbed to the algal cells and Ac is the amount
171 accumulated on algal cells. Specific removal of DCF (RS) was calculated according to the
172 following equation:

$$173 \quad \text{RS (mg g}^{-1}\text{)} = \frac{(Ad + Ac + Bp)}{dw} \quad (\text{Eq. A4})$$

174 Where Ad is the DCF amount adsorbed to the algal cells, Ac is the amount accumulated on
175 algal cells, Bp is the biodegraded amount and dw the dry weight of microalgae cells (g L⁻¹).

176 **DCF Analysis**

177 DCF analysis was carried out using a Waters (Aquity UPLC) liquid chromatographic system
178 coupled to a mass spectrometer detector (Quattro Premier; Micromass) equipped with an
179 electrospray ionization source. Chromatographic separation was performed on a BEH-C18
180 chromatographic column (100 mm × 2.1 mm ID; 1.7 μm). LC elution was performed with
181 100% acetonitrile as mobile phase A and an ultrapure water 9:1 acetonitrile (v/v) as mobile

182 phase B. The pH of the eluent was adjusted to 2 with 0.1 % formic acid. Gradient elution at a
183 flow rate of 0.4 mL min⁻¹ consisted in 0% A (100% B) from 0 to 1 min, followed by a linear
184 increase to 90% A (10% B) in 4 min and held for one minute; finally, from 6 to 7 min, mobile
185 phase A decreased back to 0%. Nitrogen was used as the collision and nebulizing gas. The
186 injection volume was 5 µL. The analysis was performed using ESI positive mode under the
187 following conditions: capillary, 23 V; collision, 29 eV; column temperature, 45°C; sample
188 temperature 5°C. The multiple-reaction monitoring (MRM) mode was applied for the DCF
189 detection and quantification. The retention time is 4.47 min and MRM parameters: parent and
190 daughter ions are 296.35 and 214.21 Da, respectively.

191 Complete calibration curves were performed at the beginning and at the end of the sample set,
192 the mean slope value of these curves was used for DCF quantification. The limit of detection
193 (LOD) and the limit of quantification (LOQ) were 0.180 and 0.547 mg L⁻¹, respectively.

194 For the detection of DCF degradation intermediates, the full scan (FS) mode was performed,
195 in a scan range of 60 to 500 m/z. Spectra were recorded with an average of 20-25 scans under
196 identical experimental conditions. The reference full scan spectrum is presented in Fig. 1. The
197 structural identity of each biodegradation product was performed with the LC-MS/MS
198 fragmentation analysis. The analytical device was controlled by Micromass MassLynx 4.1
199 software.

200 **2.5 Statistical analysis**

201 Mean and standard deviation values of the three biological replicates were calculated for each
202 treatment and for the control. In all cases, data are given as mean value with standard
203 deviation. Two-way ANOVA was used to analyze the effect of DCF tested concentrations and
204 culture duration on growth and photosynthetic activities and one-way ANOVA was
205 performed to determine the significant differences between removal efficiencies of studied

206 species among DCF tested concentrations, using IBM SPSS Statistic 21.0.0 software. A P-
207 value < 0.05 was considered statistically significant. When significant differences were
208 observed, means were compared using the Post Hoc test for Two-way ANOVA analysis at a
209 level of significance of 0.05 (P<0.05). For the DCF removal efficiencies, the significance of
210 differences between the control and treated samples was analyzed by the one-way ANOVA
211 analysis using the multiple-range Duncan test at the same level of significance (P<0.05).

212 **3. Results**

213 **3.1 Effect of DCF on microalgae growth**

214 The effect of DCF concentrations on *Picocystis* sp. and *Graesiella* sp. growth during 5 days of
215 exposure was evaluated by the growth inhibition percentage (PI%) (Table 1). Results showed
216 that the DFC has slight effect on both species. The PI of *Picocystis* sp. and *Graesiella* sp. did
217 not reach 40 % for both species during the whole period of exposure and even at the highest
218 DCF tested concentration (200 mg L⁻¹). The maximum PI recorded was 21% for *Picocystis*
219 and 36% for *Graesiella* implying that *Picocystis* was relatively more tolerant to DCF.
220 Furthermore, a negative PI was observed when *Picocystis* was exposed to 25 and 50 mg/L
221 DCF, which reflects a stimulation of the microalgae growth by 4 and 21 %, respectively.

222 **3.2 Effect of DCF on microalgae photosynthetic activity**

223 The effect of DCF on the photochemical efficiency of photosystem II (Fv/Fm) and the non-
224 photochemical quenching (NPQ), an indicator of the excess-radiant energy dissipation to heat
225 in PSII antenna complexes during light-adapted state, of *Picocystis* and *Graesiella* were
226 determined during 5 days of DCF exposure at 25, 50, 100 and 200 mg L⁻¹ initial DCF
227 concentrations. Compared to the controls, the Fv/Fm ratios of *Picocystis* treated cultures were
228 not inhibited during the first 3 days of DCF exposure (Fig. 2). A slight decrease was recorded

229 only at the end of the exposure duration (4th and 5th day) in *Picocystis* cultures exposed to
230 high DCF concentrations (50 - 200 mg L⁻¹). For *Graesiella*, no inhibitory effect on Fv/Fm
231 ratios was recorded during the whole experiment period regardless the DCF tested
232 concentrations. Contrariwise, an increase in the NPQ values of both species was observed
233 when exposed to high DCF tested concentrations (50 - 200 mg L⁻¹) as compared to the
234 controls especially since the third day of DCF exposure (Fig. 3). After 5 days' exposure to
235 200 mg L⁻¹ DCF, the NPQ values of *Picocystis* and *Graesiella* treated cells were about two
236 folds of the control groups.

237 **3.3 DCF removal from the culture medium**

238 The DCF removal by *Picocystis* and *Graesiella* was investigated at 25, 50 and 100 mg L⁻¹
239 DCF after 5 days of treatment (Table 2). Blank controls (without algae cells) were also
240 investigated. Both species showed different patterns in terms of removal efficiency. In
241 presence of *Picocystis* sp., the amounts of removed DCF were up to 73%, 42% and 25% of 25,
242 50 and 100 mg L⁻¹, respectively; whereas only 52%, 28% and 24% were removed in the
243 presence of *Graesiella* at the same DCF concentrations.

244 DCF removal in blank controls did not exceed 8% regardless of initial concentrations
245 indicating that abiotic losses were negligible, and the observed DCF decrease was mainly due
246 to the removal by microalgae.

247 The quantity of DCF adsorbed and accumulated within the microalgae cells did not exceed
248 also 0.5% in all tested concentrations for both studied species (Table 2) that implies that the
249 DCF removal by microalgae was insured mainly by biotransformation/biodegradation.

250 As illustrated in table 2, the DCF Biotransformation/biodegradation percentage (Bp) in the
251 presence of *Picocystis* were about 69%, 36% and 21% of 25, 50 and 100 mg L⁻¹ DCF,

252 respectively. For *Graesiella*, the Bp were 44%, 21% and 18% of DCF at the same tested
253 concentrations.

254 The specific removal (RS) of DCF by *Picocystis* was between 12.39 and 18.96 mg g⁻¹ for 25-
255 100 mg L⁻¹ initial DCF concentrations. In *Graesiella*, the maximal RS was 12.23 mg g⁻¹
256 observed for 100 mg L⁻¹ initial DCF concentration.

257 **3.4 Characterization of biodegradation products**

258 DCF degradation products were separated by HPLC and characterized by on-line electrospray
259 ionization–ion trap mass spectrometry (ESI–MS). In the presence of *Picocystis*, the
260 comparison of chromatograms obtained (c) with and (a) without microalgae (Fig. 4) showed
261 the appearance of two additional peaks at retention times of 1.21 and 3.82 min, respectively.
262 The first one, labeled DP1 in Fig. 5a, yielded a molecular ion at m/z 312 Da and two
263 additional ion fragments at m/z 266 and 230. The MS2 fragmentation spectrum of m/z 312
264 revealed a fragment ion at m/z 266 corresponding to the loss of one CO₂ molecule, and the
265 MS3 fragmentation spectrum of m/z 266 showed a fragment ion at m/z 230 corresponding to
266 the DCF dechlorination. The difference in mass between the molecular ion of the product DP1
267 (312 Da) and DCF (296 Da) was 16 Da, which might be related to the hydroxylation of the
268 compound. The addition of an hydroxyl to the molecule supported the lower retention time
269 observed for the compound by increasing the molecule’s polarity (**Rigobello et al., 2013**).
270 Although information provided by LC-MS instruments is not enough to establish the precise
271 position of the OH groups in the molecule, knowledge of the reactivity of the different species
272 and the cited literature allowed us to propose probable structure as seen in Fig. 5a.
273 The second peak, labeled DP2 in Fig. 5b, showed a molecular ion at m/z 152 Da and two
274 additional ion fragments at m/z 134 and m/z 106. The MS2 fragmentation of m/z 152 revealed
275 a fragment ion at m/z 134 corresponding to the dehydration by an intramolecular cyclization,

276 and the MS3 fragmentation spectrum of m/z 134 showed a fragment ion at m/z 106
277 corresponding to the loss of one CO molecule. The difference of 144 Da between diclofenac
278 and DP2 is consistent with a loss of the dechlorinated benzene ring as proposed in Fig. 5b.
279 In the presence of *Graesiella*, no difference was recorded between the EIS-MS
280 chromatograms obtained with and without microalgae.

281 4. Discussion

282 Microbial removal of pollutants relies on a trade-off between tolerance of the organisms to the
283 toxicity of the targeted compounds, and their efficiency to effectively sequester or degrade
284 them. To date only few studies on DCF toxicity to microalgae were reported, mostly on
285 freshwater cyanobacteria and chlorophyta species (Supplementary material 3). The measured
286 sensitivities to diclofenac spread from 84.5% growth inhibition in the freshwater
287 cyanobacterium *Cylindrospermopsis raciborskii* exposed to 0.1 mg L⁻¹ (Bácsi et al., 2016) to
288 50% growth inhibition (EC50) in the marine chlorophyceae *Dunaliella tertiolecta*
289 (DeLorenzo and Fleming, 2008) exposed to 185.7 mg L⁻¹ DCF. Comparatively, *Picocystis*
290 and *Graesiella* were characterized in the present study by a high tolerance to DCF, since an
291 exposure up to 200 mg L⁻¹ did not reach 40% inhibition for both strains. This behavior could
292 at least partly attributed to the origin of the model organisms, considering that extremophile
293 algae have already reported to be tolerant to chemical contamination (Ben Ali et al., 2017;
294 Ben Ouada et al., 2018; Rehman and Shakoori, 2004). Furthermore, growth stimulation up
295 to 21% was observed in *Picocystis* exposed to 50 mg L⁻¹ DCF, as reported for other
296 chlorophyta strains exposed to 25 mg L⁻¹ DCF (Escapa et al., 2017). This behavior is
297 consistent with potential use of DCF as organic carbon source, since chlorophyta species were
298 shown able to photo-heterotrophic metabolism when culture medium was complemented by
299 organic substrates (Ratha et al., 2013; Zili et al., 2015).

300 Toxic chemicals often target the photosynthetic apparatus in microalgae (**Choi et al., 2012**),
301 but raw *Picocystis* and *Graesiella* PSII potentials (Fv/Fm) were not altered by DCF exposure
302 in the present study. To date, no toxicity of DCF to PSII integrity was reported for
303 microalgae, whereas higher plants were found sensitive (**Kummerová et al., 2016**).
304 Comparatively, the non-photochemical quenching NPQ, an indicator of the excess-radiant
305 energy dissipation during light-adapted state, increased for both species after DCF exposure.
306 The same trend was reported in river biofilms and in plant species (**Kummerová et al., 2016**),
307 and suggests an adaptive mechanism in *Picocystis* and *Graesiella* to protect PSII antenna and
308 dissipate the excess of absorbed energy (**Müller et al., 2001**).

309 The high tolerance of *Picocystis* and *Graesiella* to DCF could be related to their high DCF
310 removal abilities. Among many studies about chemical remediation by microalgae, little
311 attention was paid to NSAIDs including DCF. **Matamoros et al. (2015a; 2015b)**, **Xiong et al.**
312 **(2018)** and **Zhou et al. (2014)** demonstrated that microalgal inoculation in wastewaters
313 enhanced pharmaceutical removal efficiencies of treatment systems up to 80%. In laboratory
314 monocultures, the amount of DCF removed by green microalgae were about 20 to 80 %, at
315 DCF initial concentration $\leq 25 \text{ mg L}^{-1}$ (**Escapa et al., 2017**). These values are comparable to
316 DCF removal efficiencies of both *Graesiella* (52%) and *Picocystis* (73%) at 25 mg L^{-1} initial
317 DCF concentration. Even at higher initial concentrations, reaching 100 mg L^{-1} , *Picocystis* and
318 *Graesiella* were able to remove about 25-42% and 24-28%, respectively; after 5days of
319 exposure, which demonstrated their high DCF removal abilities; even when compared to other
320 biological treatments (**Langenhoff et al., 2013**).

321 In the present work, a decrease of DCF initial concentrations was observed in the controls
322 without algae suggesting abiotic degradation, most likely by photooxidation (**Michael et al.,**
323 **2014**). However, abiotic loss of DCF did not exceed 8% at all tested concentrations, and

324 biotic removal is the most probable process in accordance with literature data (**Escapa et al.,**
325 **2017**).

326 Biotic removal can be passive, by hydrophobic binding on microalgal cell walls or to the
327 extracellular polysaccharides (**Xiong et al., 2018**), and active when accumulation and
328 biotransformation/biodegradation occur within cells. In the present results, less than 1% of
329 DCF was removed by adsorption and in equal amount by intracellular accumulation. Since
330 EPS can be released into the medium (**Mezhoud et al., 2014**), it is possible to hypothesize
331 that free EPS contributed significantly to DCF removal. Taking into account the method used
332 in this study, the proportion of adsorbed DCF could be higher than that estimated.
333 Nevertheless, **Bácsi et al. (2016)** demonstrated in several microalgae that DCF was not
334 permanently bound to the membranes, and that no intracellular accumulation occurred, in
335 accordance to the data presented here. As reduction of DCF concentration in the cultures
336 cannot be balanced by adsorbed and/or accumulated DCF in the cells, these results suggest
337 that biodegradation processes occurred in both *Picocystis* and *Graesiella* cultures. So far, little
338 is known about microalgae that degrade diclofenac and the involved biodegradation pathways.
339 In this study, two degradation products of DCF were detected in *Picocystis* cultures, a
340 hydroxylated compound and a mono-aromatic product. Hydroxy diclofenac has already been
341 reported as a major product of DCF metabolism in bacteria, fungi and microalgae, with a
342 toxicity reduction compare to the parent product (**Domaradzka et al., 2015; Escapa et al.,**
343 **2018**). The mono-aromatic derivative results from the loss of the benzene dechlorinated ring,
344 as detected by **Cooper and Song (2012)** in advanced oxidation processes of DCF.

345 However, no DCF metabolites were detected in *Graesiella* cultures despite the high decrease
346 in initial DCF concentrations observed. This can be either due to analytical issues
347 (metabolites below detection limits, procedures not suitable for unforeseen compounds, ...) or
348 kinetic factors, as the hypothesis of total mineralization cannot be excluded.

349 There is no evidence that the biodegradation is intracellular and known biodegradation
350 processes involve the production of reactive oxygen species (ROS) in the medium as
351 consequence of photosynthesis, and resulting photo-oxidation or hydroxylation of
352 contaminants **(Oral and Kantar, 2019)**. Extracellular enzymes are also identified in
353 extracellular matrix of microalgae involved in chemicals hydrolysis **(Otto et al., 2015)**.
354 Indeed, EPS can form a hydrated biofilm matrix acting as an external digestive system where
355 extracellular enzymes close to cells, allowing them to metabolize organic compounds
356 **(Flemming and Wingender, 2010; Xiong et al., 2018)**. In this context, DCF byproducts
357 formed could be physically trapped or included in EPS matrix and escape analysis, which
358 need further investigation.

359 **5. Conclusion**

360 Based on IC50 values, *Picocystis* and *Graesiella* were, relatively, tolerant to DCF compared
361 to other chlorophyta species as reported in the literature. The growth of both species was
362 inhibited by less than 40% even at 200 mg L⁻¹ DCF. Interestingly, *Picocystis* and *Graesiella*
363 exhibited high DCF removal efficiencies reaching 73% and 52% of 25 mg L⁻¹ as initial
364 concentration, respectively.

365 The main proportion of DCF removal (69% and 44% of 25 mg L⁻¹ initial DCF concentration)
366 was insured by biodegradation processes. Two biodegradation products of DCF were
367 identified in the presence of *Picocystis*: hydroxy-diclofenac and a mono-aromatic derivative
368 of DCF. In *Graesiella* culture, no metabolites were detected despite the high DCF
369 biodegradation percentage, this may be associated either to low metabolites concentrations
370 below the detection limit of the analytical method used or to the total mineralization of
371 diclofenac. Moreover, the role of EPS in removal processes may be investigated. Further
372 research on the biodegradation pathway and the toxicity assessment of the bioproducts still

373 required. The obtained results open up promising prospects for the application of the
374 microalgae here considered, mainly *Picocystis*, in DCF bioremediation systems. The high
375 tolerance of studied species allows their use in continuous flow culture systems particularly in
376 the case of bioremediation of wastewaters, characterized by lower DCF concentrations.
377 Therefore, we can concentrate or degrade the pollutant faster and with low culture volume.
378 Although, confirmation of these results in pilot-scale at environmental conditions still needed.

379 **Acknowledgements,**

380 Authors are thankful to the French Research Institute for Development (IRD) for financing
381 the Ph.D stipend of Sabrine Ben Ouada under the Laboratory LMI Cosys-med project.

382 The support of this work under “Contrat Programme of Laboratory of Environmental
383 Bioprocesses” by The Tunisian Ministry of Higher Education and Scientific Research is also
384 gratefully acknowledged.

385 **References**

- 386 **Acuña, V., Ginebreda, A., Mor, J. R., Petrovic, M., Sabater, S., Sumpter, J., Barceló, D.,**
387 **2015.** Balancing the health benefits and environmental risks of pharmaceuticals:
388 Diclofenac as an example. *Environ. Int.* 85, 327–333. doi:10.1016/j.envint.2015.09.023
- 389 **Bácsi, I., B-Béres, V., Kókai, Z., Gonda, S., Novák, Z., Nagy, S.A., Vasas, G., 2016.**
390 Effects of non-steroidal anti-inflammatory drugs on cyanobacteria and algae in
391 laboratory strains and in natural algal assemblages. *Environ. Pollut.* 212, 508–518.
392 <https://doi.org/10.1016/j.envpol.2016.02.031>
- 393 **Ben Ali, R., Ben Ouada, S., Chouchene, L., Messaoudi, I., Ben Ouada, H., Othmane, A.,**
394 **2017.** Cadmium effect on physiological responses of the tolerant Chlorophyta specie
395 *Picocystis* sp. isolated from Tunisian wastewaters. *Environ. Sci. Pollut. Res.* 24, 1803–
396 1810. <https://doi.org/10.1007/s11356-016-7950-0>
- 397 **Ben Ouada, S., Ben Ali, R., Leboulanger, C., Zaghden, H., Choura, S., Ben Ouada, H.,**
398 **Sayadi, S., 2018.** Effect of Bisphenol A on the extremophilic microalgal strain *Picocystis*
399 sp. (Chlorophyta) and its high BPA removal ability. *Ecotoxicol. Environ. Saf.*, 158, 1-8.
400 <https://doi.org/10.1016/j.ecoenv.2018.04.008>
- 401 **Bischoff, H.W., Bold, H.C., 1963.** Some soil algae from Enchanted Rock and related algal
402 species, in: Austin, T. (Ed.), *Phycological Studies IV*. University of Texas Publication
403 6318, pp. 1–95.
- 404 **Campbell, D., Hurry, V., Clarke, K., Gustafsson, P., Oquist, G., 1998.** Chlorophyll
405 fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol.*
406 *Biol. Rev.* 62, 667–83.
- 407 **Choi, C.J., Berges, J.A., Young, E.B., 2012.** Rapid effects of diverse toxic water pollutants
408 on chlorophyll a fluorescence: variable responses among freshwater microalgae. *Water*
409 *Res.* 46 (8), 2615–2626. <http://dx.doi.org/10.1016/j.watres.2012.02.027>

410 **Cooper, W.J., Song, W., 2012.** Advanced oxidation degradation of diclofenac. IAEA Rep.
411 46, 168–179.

412 **Cuellar-Bermudez, S.P., Aleman-Nava, G.S., Chandra, R., Garcia-Perez, J.S.,**
413 **Contreras-Angulo, J.R., Markou, G., Muylaert, K., Rittmann, B.E., Parra-Saldivar,**
414 **R., 2017.** Nutrients utilization and contaminants removal. A review of two approaches of
415 algae and cyanobacteria in wastewater. *Algal Res.* 24, 438–449.
416 <https://doi.org/10.1016/j.algal.2016.08.018>

417 **de Orte, M.R., Carballeira, C., Viana, I.G., Carballeira, A., 2013.** Assessing the toxicity
418 of chemical compounds associated with marine land-based fish farms: The use of mini-
419 scale microalgal toxicity tests. *Chem. Ecol.* 29, 554–563.
420 <https://doi.org/10.1080/02757540.2013.790381>

421 **DeLorenzo, M.E., Fleming, J., 2008.** Individual and mixture effects of selected
422 pharmaceuticals and personal care products on the marine phytoplankton species
423 *Dunaliella tertiolecta*. *Arch. Environ. Contam. Toxicol.* 54, 203–210.
424 <https://doi.org/10.1007/s00244-007-9032-2>

425 **Domaradzka, D., Guzik, U., Wojcieszynska, D., 2015.** Biodegradation and
426 biotransformation of polycyclic non-steroidal anti-inflammatory drugs. *Rev. Environ. Sci.*
427 *Biotechnol.* 14, 229–239. <https://doi.org/10.1007/s11157-015-9364-8>

428 **Elser, J.J., Fagan, W.F., Denno, R.F., Dobberfuhl, D.R., Folarin, A., Huberty, A.,**
429 **Interlandi, S., Kilham, S.S., McCauley, E., Schulz, K.L., Siemann, E.H., Sterner,**
430 **R.W., 2000.** Nutritional constraints in terrestrial and freshwater food webs. *Nature* 408,
431 578–580. <https://doi.org/10.1038/35046058>

432 **Escapa, C., Coimbra, R.N., Paniagua, S., García, A.I., Otero, M., 2017.** Comparative
433 assessment of pharmaceutical removal from wastewater by the microalgae *Chlorella*
434 *sorokiniana*, *Chlorella vulgaris* and *Scenedesmus obliquus*. In: *Biological Wastewater*

435 treatment and resource recovery, Robina Farooq and Zaki Ahmad (Eds.), Intech, pp. 99-
436 117 (DOI: 10.5772/66772).

437 **Escapa, C., Torres, T., Neuparth, T., Coimbra, R. N., García, A. I., Santos, M. M., Otero,**
438 **M., 2018.** Zebrafish embryo bioassays for a comprehensive evaluation of microalgae
439 efficiency in the removal of diclofenac from water. *Sci. Total Environ.* 640-641, 1024–
440 1033. doi:10.1016/j.scitotenv.2018.05.353

441 **Flemming, H.-C., Wingender, J., 2010.** The biofilm matrix. *Nat. Rev. Microbiol.* 8(9), 623-
442 633.

443 **Ji, M.-K., Kabra, A.N., Choi, J., Hwang, J.-H., Kim, J.R., Abou-Shanab, R. a. I., Oh, Y.-**
444 **K., Jeon, B.-H., 2014.** Biodegradation of bisphenol A by the freshwater microalgae
445 *Chlamydomonas mexicana* and *Chlorella vulgaris*. *Ecol. Eng.* 73, 260–269.
446 <https://doi.org/10.1016/j.ecoleng.2014.09.070>

447 **Kummerová, M., Zezulka, S., Babula, P., Tríska, J., 2016.** Possible ecological risk of two
448 pharmaceuticals diclofenac and paracetamol demonstrated on a model plant *Lemna*
449 *minor*. *J. Hazard. Mater.* 302, 351–361. <https://doi.org/10.1016/j.jhazmat.2015.09.057>

450 **Langenhoff, A., Inderfurth, N., Veuskens, T., Schraa, G., Blokland, M., Kujawa-**
451 **Roeleveld, K., Rijnaarts, H., 2013.** Microbial removal of the pharmaceutical
452 compounds ibuprofen and diclofenac from wastewater. *Biomed Res. Int.* 2013, 1–9.
453 <https://doi.org/10.1155/2013/325806>

454 **Li, R., Chen, G.Z., Tam, N.F.Y., Luan, T.G., Shin, P.K.S., Cheung, S.G., Liu, Y., 2009.**
455 Toxicity of bisphenol a and its bioaccumulation and removal by a marine microalga
456 *Stephanodiscus hantzschii*. *Ecotoxicol. Environ. Safe.* 72, 321–328.

457 **Lonappan, L., Brar, S.K., Das, R.K., Verma, M., Surampalli, R.Y., 2016.** Diclofenac and
458 its transformation products: Environmental occurrence and toxicity - A review. *Environ.*
459 *Int.* 96, 127–138. <https://doi.org/10.1016/j.envint.2016.09.014>

460 **Matamoros, V., Gutiérrez, R., Ferrer, I., García, J., Bayona, J.M., 2015a.** Capability of
461 microalgae-based wastewater treatment systems to remove emerging organic
462 contaminants: A pilot-scale study. *J. Hazard. Mater.* 288, 34–42.
463 <https://doi.org/10.1016/j.jhazmat.2015.02.002>

464 **Matamoros, V., Uggetti, E., García, J., Bayona, J.M., 2015b.** Assessment of the
465 mechanisms involved in the removal of emerging contaminants by microalgae from
466 wastewater: A laboratory scale study. *J. Hazard. Mater.* 301, 197–205.
467 <https://doi.org/10.1016/j.jhazmat.2015.08.050>

468 **Mezhoud, N., Zili, F., Bouzidi, N., Helaoui, F., Ammar, J., Ben Ouada, H., 2014.** The
469 effects of temperature and light intensity on growth , reproduction and EPS synthesis of a
470 thermophilic strain related to the genus *Graesiella*. *Bioprocess. Biosyst. Eng.*
471 37(11),2271-80. doi: 10.1007/s00449-014-1204-7.

472 **Michael, I., Achilleos, A., Lambropoulou, D., Torrens, V.O., Pérez, S., Petrović, M.,**
473 **Barceló, D., Fatta-Kassinos, D., 2014.** Proposed transformation pathway and evolution
474 profile of diclofenac and ibuprofen transformation products during (sono)photocatalysis.
475 *Appl. Catal. B Environ.* 147, 1015–1027. <https://doi.org/10.1016/j.apcatb.2013.10.035>

476 **Müller, P., Li, X.-P., Niyogi, K.K., 2001.** Non-photochemical quenching a response to
477 excess light energy. *Plant Physiol.* 125, 1558–1566.
478 <https://doi.org/10.1104/pp.125.4.1558>

479 **Oral, O., Kantar, C., 2019.** Diclofenac removal by pyrite-Fenton process: Performance in
480 batch and fixed-bed continuous flow systems. *Sci. Total Environ.* 664, 817-823.
481 <https://doi.org/10.1016/j.scitotenv.2019.02.084>.

482 **Otto, B., Beuchel, C., Liers, C., Reisser, W., Harms, H., Schlosser, D., 2015.** Laccase-like
483 enzyme activities from chlorophycean green algae with potential for bioconversion of
484 phenolic pollutants. *FEMS Microbiol. Lett.* 362 (11), fnv072.

485 <http://dx.doi.org/10.1093/femsle/fnv072>.

486 **Peeples, T.L., 2014.** Bioremediation using extremophiles, in: Microbial biodegradation and
487 bioremediation. Elsevier, pp. 251–268. [https://doi.org/10.1016/B978-0-12-800021-](https://doi.org/10.1016/B978-0-12-800021-2.00010-8)
488 [2.00010-8](https://doi.org/10.1016/B978-0-12-800021-2.00010-8)

489 **Ratha, S.K., Babu, S., Renuka, N., Prasanna, R., Prasad, R.B.N., Saxena, A.K., 2013.**
490 Exploring nutritional modes of cultivation for enhancing lipid accumulation in
491 microalgae. J. Basic Microbiol. 53, 440–450. <http://dx.doi.org/10.1002/jobm.201200001>.

492 **Rehman, A., Shakoori, A.R., 2004.** Tolerance and uptake of cadmium and nickle by
493 *Chlorella* sp ., isolated from tannery effluents. Pakistan J. Zool. 36, 327–331.

494 **Rigobello, E.S., Dantas, A.D.B., Di Bernardo, L., Vieira, E.M., 2013.** Removal of
495 diclofenac by conventional drinking water treatment processes and granular activated
496 carbon filtration. Chemosphere 92, 184–191.
497 <https://doi.org/10.1016/j.chemosphere.2013.03.010>

498 **Sophia A., C., Lima, E. C., 2018.** Removal of emerging contaminants from the environment
499 by adsorption. Ecotoxicol. Environ. Safe. 150: 1–17. doi:10.1016/j.ecoenv.2017.12.026

500 **Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000.** The fluorescence transient as a
501 tool to characterize and screen photosynthetic samples, in: Yunus, M., Pathre, U.,
502 Mohanty, P. (Eds.), Probing photosynthesis: Mechanism, regulation and adaption.
503 London, pp. 445–483.

504 **Valavanidis, A., Vlachogianni, T., Loridas, S., Fiotakis, C., 2014.** An emerging
505 environmental problem disposed medicinal active products pharmaceuticals, antibiotics,
506 and disinfectants in the aquatic environment and toxicological considerations.
507 Pharmakeftiki 26(III), 78-98.

508 **Varshney, P., Mikulic, P., Vonshak, A., Beardall, J., Wangikar, P.P., 2014.** Extremophilic
509 micro-algae and their potential contribution in biotechnology. Bioresour. Technol.

510 <https://doi.org/10.1016/j.biortech.2014.11.040>

511 **Villar-Navarro, E., Baena-Nogueras, R. M., Paniw, M., Perales, J. A., Lara-Martín, P.**
512 **A., 2018.** Removal of pharmaceuticals in urban wastewater: High rate algae pond
513 (HRAP) based technologies as an alternative to activated sludge based processes. *Water*
514 *Res.* 139: 19-29.doi:10.1016/j.watres.2018.03.072

515 **Xiong, J.-Q., Kurade, M. B., Jeon, B.-H., 2018.** Can microalgae remove pharmaceutical
516 contaminants from water? *Trends Biotechnol.* 36(1): 30–44.
517 doi:10.1016/j.tibtech.2017.09.003

518 **Xu, M., Huang, H., Li, N., Li, F., Wang, D., Luo, Q., 2019.** Occurrence and ecological risk
519 of pharmaceuticals and personal care products (PPCPs) and pesticides in typical surface
520 watersheds, China. *Ecotoxicol. Environ. Safe.* doi:10.1016/j.ecoenv.2019.01.131

521 **Zarrouk, C., 1966.** Contribution à l'étude d'une Cyanophycée, influence de divers facteurs
522 physiques et chimiques sur la croissance et la photosynthèse de "*Spirulina maxima*"
523 (Setch et Gardner) Geitler. PhD thesis, University of Paris, France.

524 **Zhou, G.-J., Ying, G.-G., Liu, S., Zhou, L.-J., Chen, Z.-F., Peng, F.-Q., 2014.**
525 Simultaneous removal of inorganic and organic compounds in wastewater by freshwater
526 green microalgae. *Environ. Sci. Process. Impacts* 16, 2018.
527 <https://doi.org/10.1039/C4EM00094C>

528 **Zili, F., Mezhoud, N., Trabelsi, L., Chreif, I., Ben Ouada, H., 2015.** Fatty acid composition
529 of the thermophilic *Gloeocapsa gelatinosa* under different combinations of temperature,
530 light intensity, and NaNO₃ concentration. *J. Appl. Phycol.* 27, 97.
531 <https://doi.org/10.1007/s10811-014-0296-4>

532

533 **Figures caption**

534 **Fig. 1.** Full scan spectrum of the DCF reference solution (DCF in ultra-pure water) in a scan
535 range of 60 to 500 m/z

536 **Fig. 2.** Variation in the PSII photochemical efficiency (F_v/F_m) of (a) *Picocystis* sp. and (b)
537 *Graesiella* sp. during 5 days of DCF exposure. Data are represented as mean \pm SD (n=3).
538 Same letters indicate no significant difference according to Post Hoc test ($P \geq 0.05$) (uppercase
539 letters for culture duration and lowercase letters for tested concentrations).

540 **Fig. 3.** Variation in the non-photochemical quenching (NPQ) of (a) *Picocystis* sp. and (b)
541 *Graesiella* sp. under exposure to 0, 25, 50, 100 and 200 mg L⁻¹ DCF for 5 days. Data are
542 represented as mean \pm SD (n=3). Same letters indicate no significant difference according to
543 Post Hoc test ($P \geq 0.05$) (uppercase letters for culture duration and lowercase letters for tested
544 concentrations).

545 **Fig. 4.** LC-MS/MS ion chromatograms showing the degradation of 100 mg L⁻¹ initial DCF
546 concentration by *Picocystis* sp.: (a) DCF without microalgae, (b) microalgae without DCF and
547 (c) DCF in presence of *Picocystis* sp.

548 **Fig. 5.** ECI-MS2 spectra of DCF degradation products obtained in presence of *Picocystis* sp.:
549 (a) DP1 and (b) DP2.

550

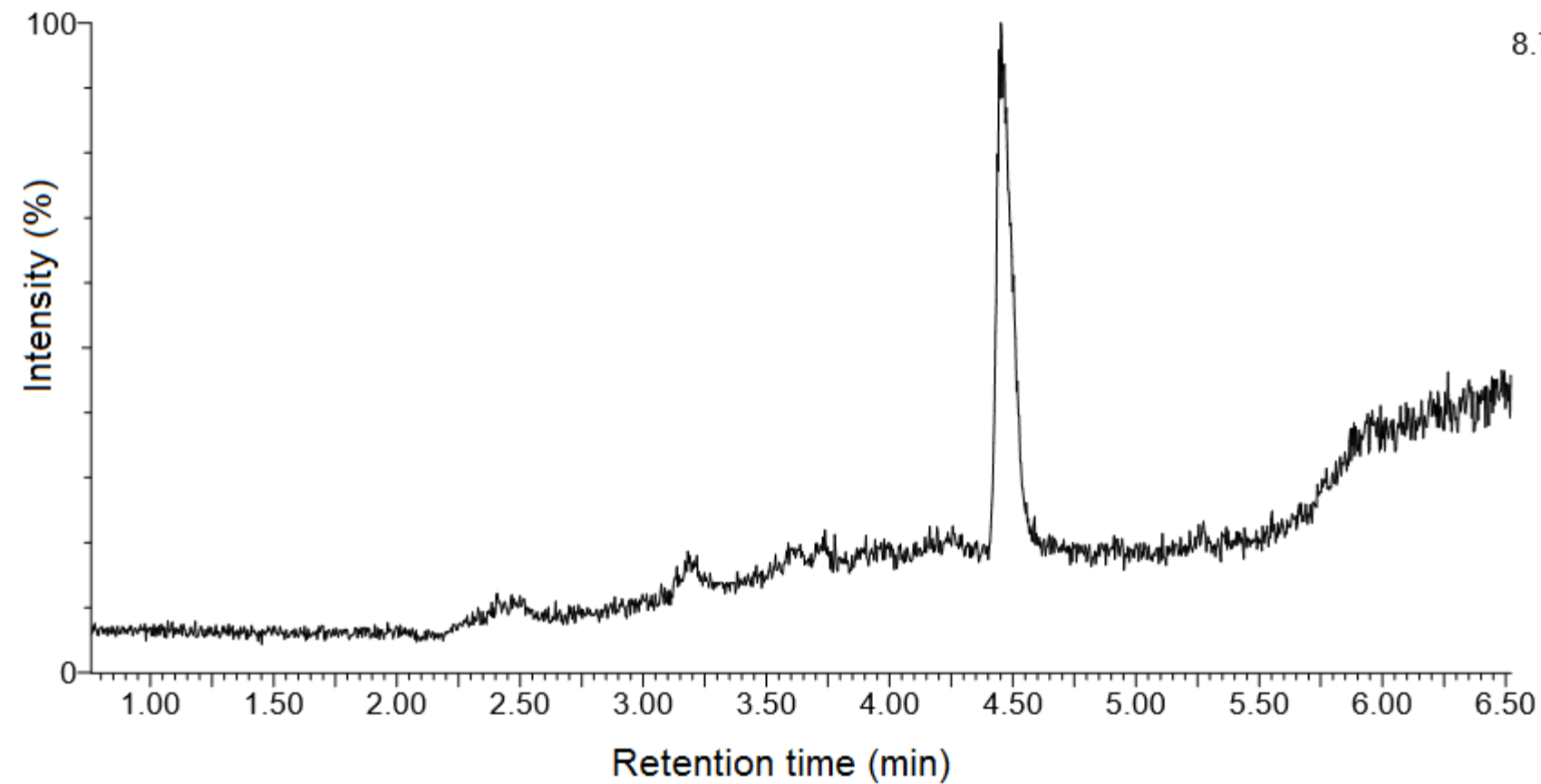
551 **Tables caption**

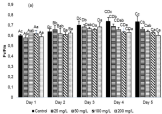
552 **Table 1.** Growth inhibition percentage of *Picocystis* sp. and *Graesiella* sp., calculated from
553 the area under the growth curves, as a function of DCF concentrations after 1, 2, 3, 4 and 5
554 days of exposure. Data are represented as mean \pm SD (n=3). Same letters indicate no
555 significant difference according to Post Hoc test ($P \geq 0.05$) (uppercase letters for culture
556 duration and lowercase letters for tested concentrations).

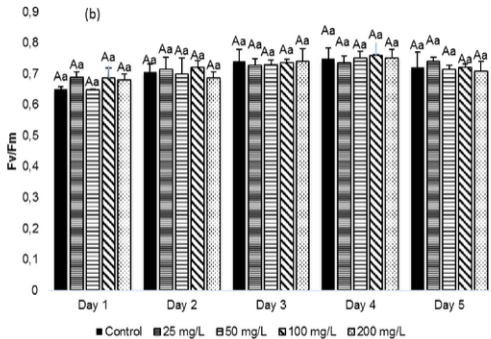
557 **Table 2.** Total (Tr) and abiotic removal (Ab), accumulated/adsorbed amount (Ac/Ad),
558 biodegradation percentage (Bp) and specific removal (RS) of DCF by *Picocystis* sp. and
559 *Graesiella* sp. cells exposed during five days to initial concentrations of 25, 50 and 100 mg L⁻¹
560 DCF. Data are represented as mean \pm SD (n=3). Different letters within the same column
561 indicate significant difference according to Duncan test ($P \geq 0.05$).

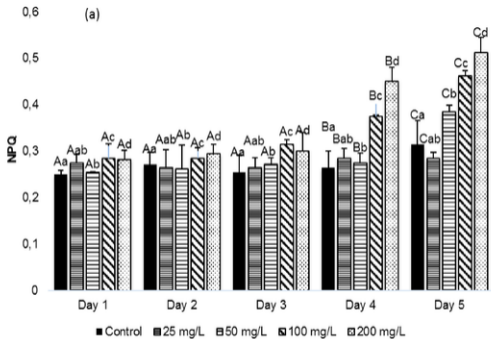
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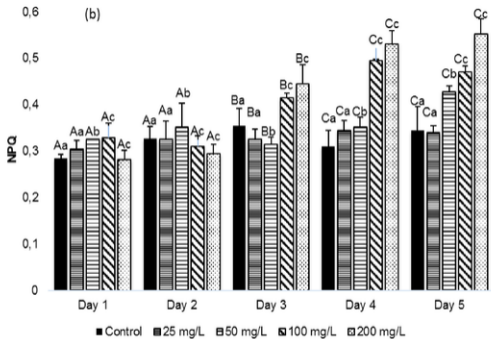
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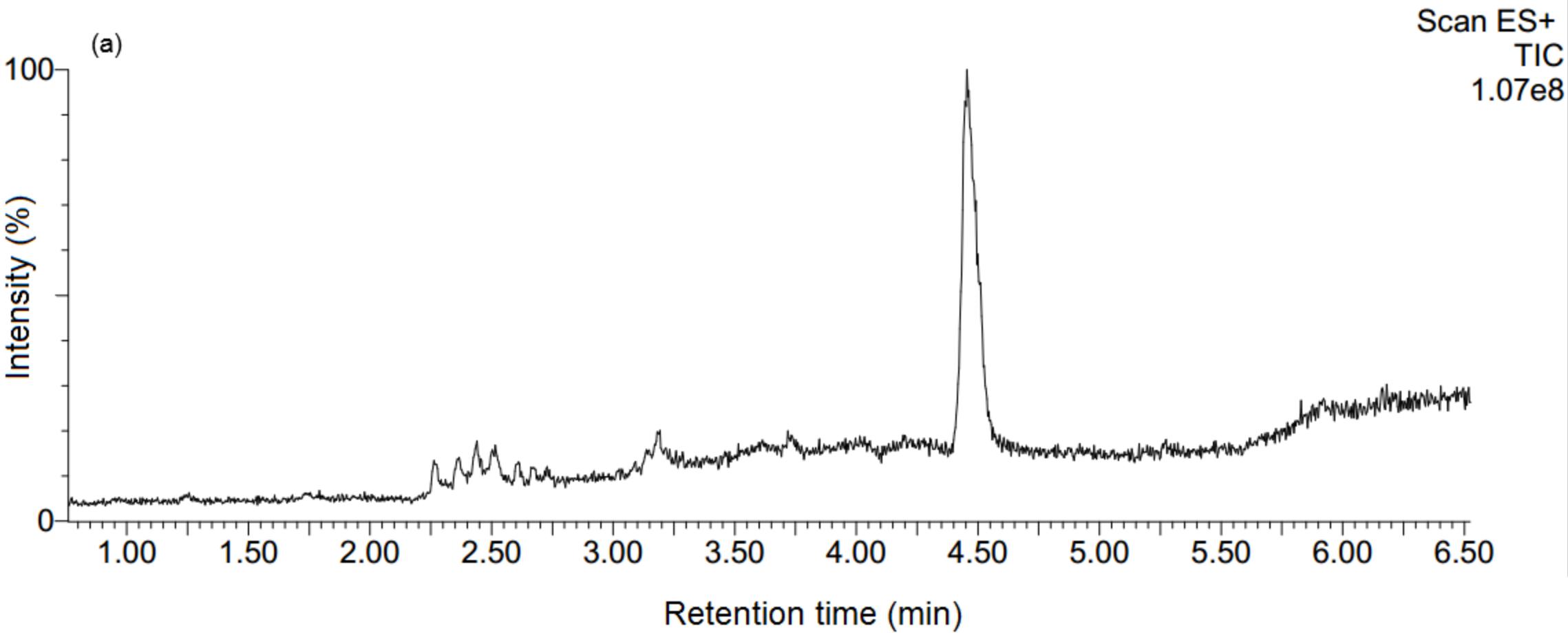


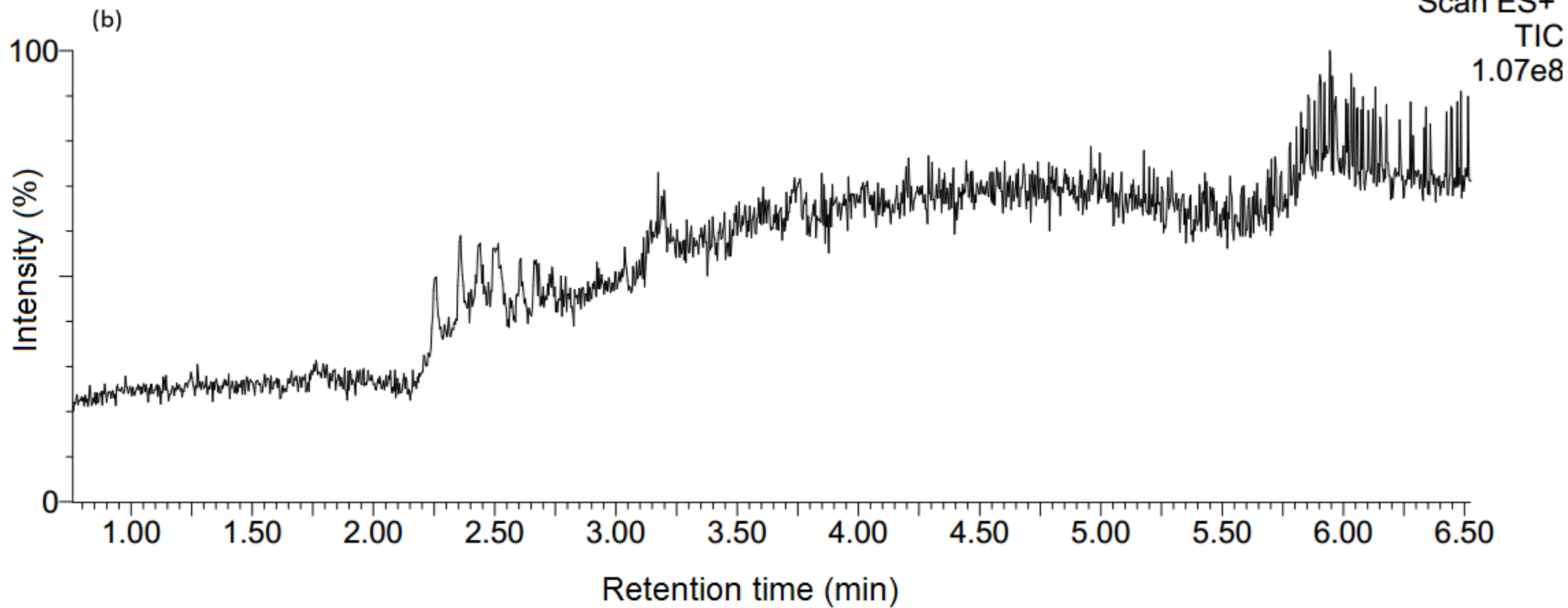


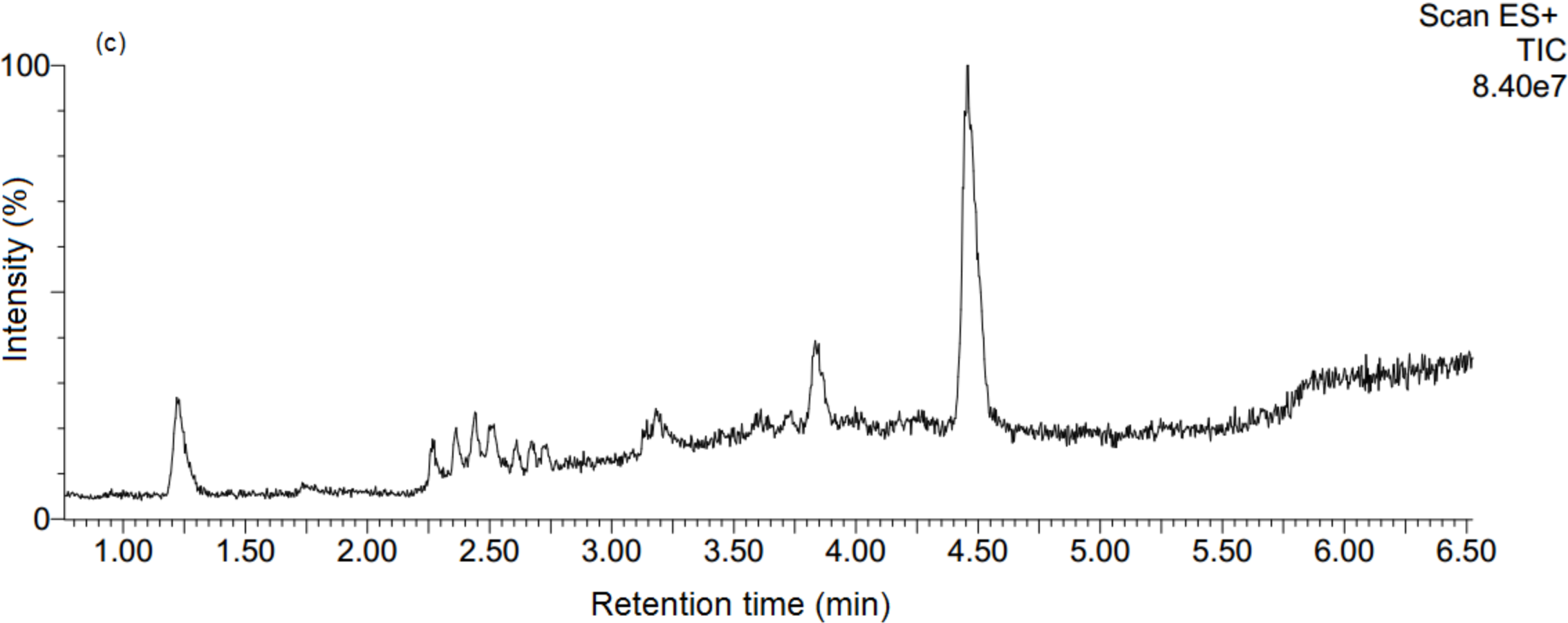


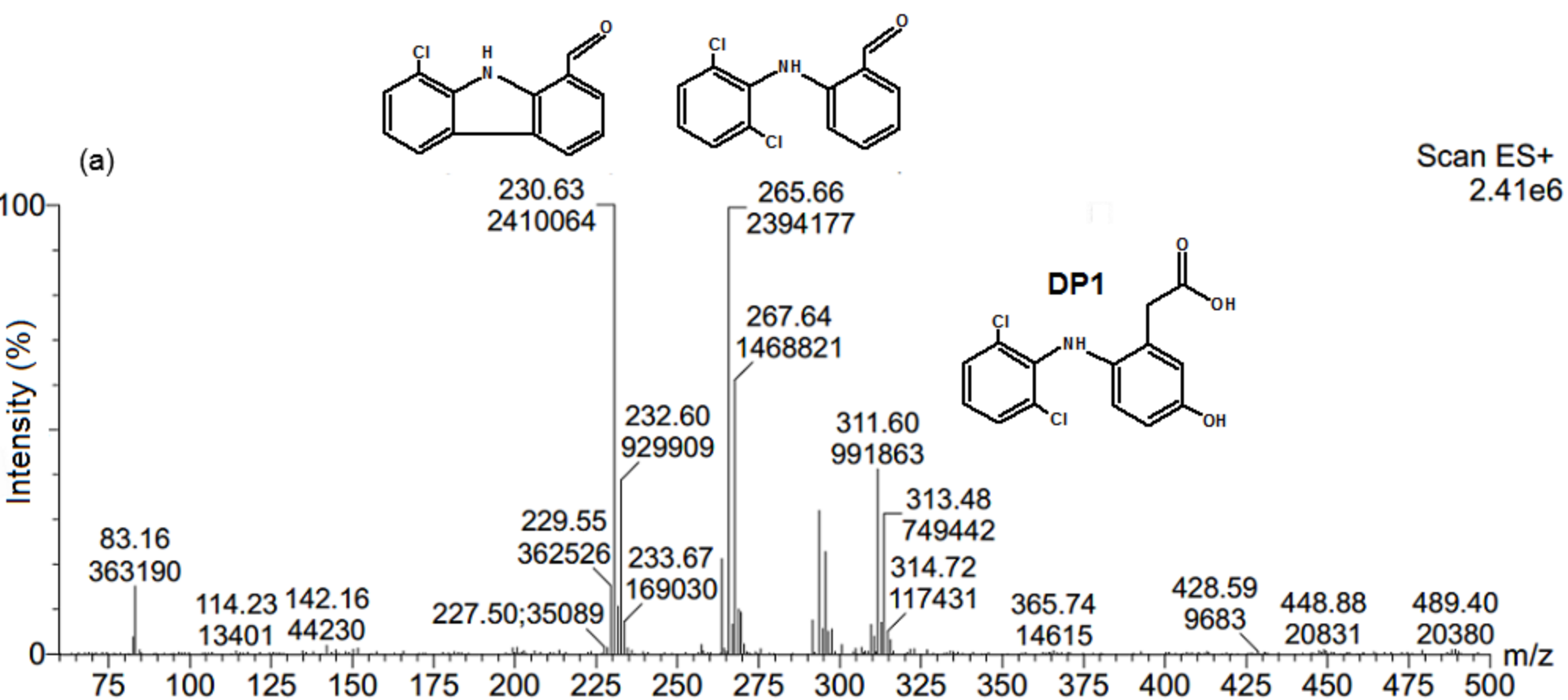




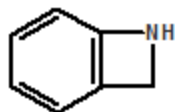
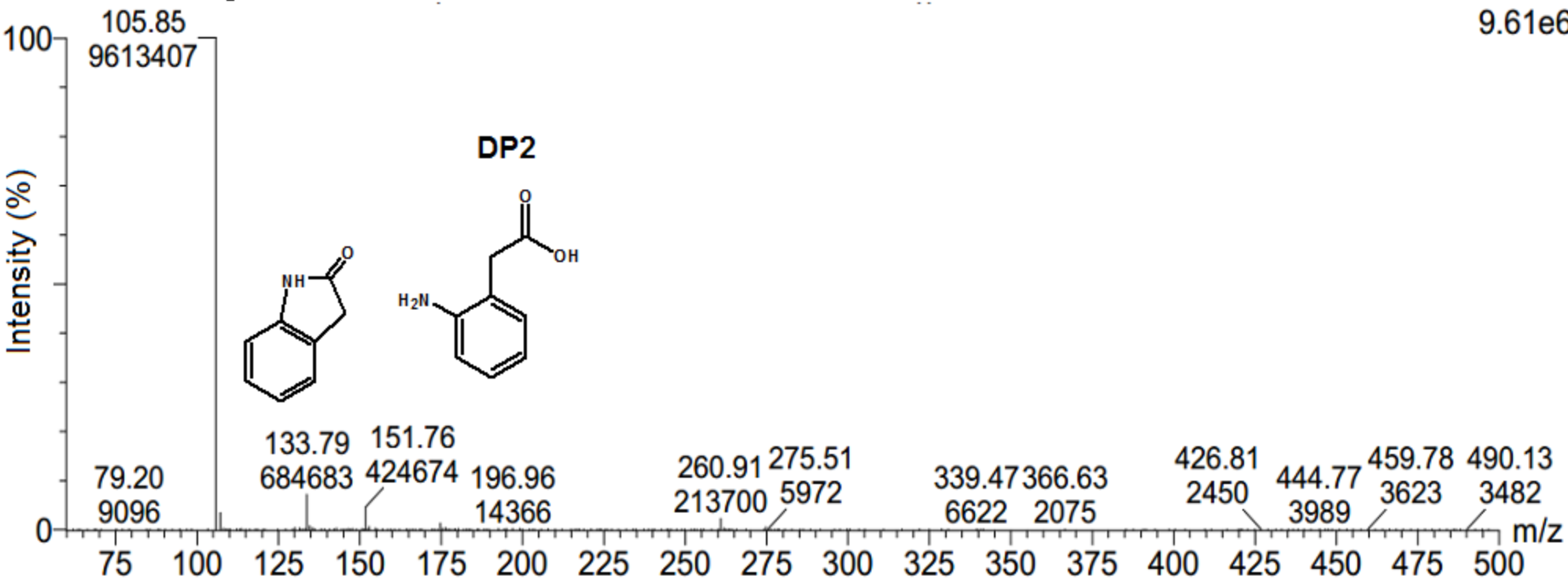








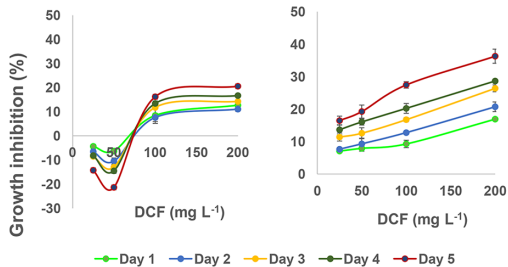
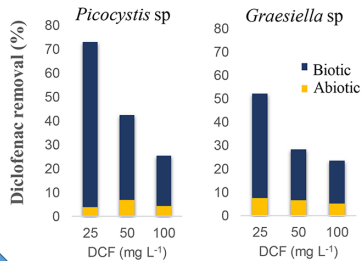
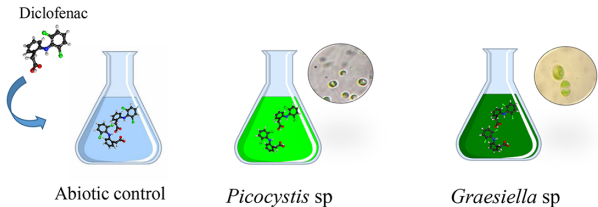
(b)

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Growth inhibition percentage (%)

	Day 1	Day 2	Day 3	Day 4	Day 5
<i>Picozystis</i>					
25 mg L⁻¹	-4.37 ± 0.85Cb	-6.55 ± 0.91Ab	-8.54 ± 0.82ABb	-8.18 ± 0.62BCb	-14.27 ± 2.62Ab
50 mg L⁻¹	-6.32 ± 1.02Ca	-10.48 ± 1.32Aa	-12.95 ± 0.41Aba	-14.51 ± 1.03BCa	-21.36 ± 2.03Aa
100 mg L⁻¹	8.41 ± 3.24Cc	7.61 ± 1.69Ac	11.77 ± 0.61Abc	13.42 ± 1.06BCc	16.20 ± 1.06Ac
200 mg L⁻¹	12.67 ± 1.53Cd	11.05 ± 0.96Ad	14.24 ± 0.52Abd	16.68 ± 0.56BCd	20.56 ± 3.56Ad
<i>Graesiella</i>					
25 mg L⁻¹	7.10 ± 0.24Aa	7.65 ± 0.50Ba	11.41 ± 1.24Ca	13.67 ± 1.53Da	16.48 ± 1.38
50 mg L⁻¹	7.95 ± 0.90Ab	9.31 ± 1.76Bb	12.61 ± 1.69Cb	16.05 ± 0.96Db	19.27 ± 2.02
100 mg L⁻¹	9.30 ± 1.15Ac	12.76 ± 0.26Bc	16.77 ± 0.61Cc	20.24 ± 1.52Dc	27.48 ± 0.99
200 mg L⁻¹	16.93 ± 0.32Ad	20.74 ± 1.49Bd	26.42 ± 1.06Cd	28.68 ± 0.56Db	36.33 ± 2.18

	Tr (%)	Ab (%)	Ac/Ad (%)	Bp (%)	SR (mg g⁻¹)
<i>Picocystis</i>					
25 mg L⁻¹	73.04 ± 3.82a	3.83± 1.05a	0.04± 0.01a	69.17± 4.88a	13.22 ± 1.27a
50 mg L⁻¹	42.46 ± 2.08b	6.81± 2.22b	0.12± 0.02b	35.53± 4.32b	12.39 ± 1.66a
100 mg L⁻¹	24.81± 1.58c	4.28 ± 1.32ab	0.17± 0.04b	20.36± 2.94c	18.96±3.55a
<i>Graesiella</i>					
25 mg L⁻¹	52.21 ± 3.22a	7.53± 1.14a	0.21± 0.06a	44.47± 4.42a	6.65± 0.95a
50 mg L⁻¹	28.34± 1.17b	6.52± 1.27a	0.44± 0.04a	21.38± 2.48b	7.31± 1.19a
100 mg L⁻¹	23.5± 1.82c	5.15± 1.53a	0.31± 0.03a	18.04± 3.38b	12.23± 3.24b



Biodegradation products

