



Sustainable microalgae-based technology for biotransformation of benzalkonium chloride in oil and gas produced water: A laboratory-scale study

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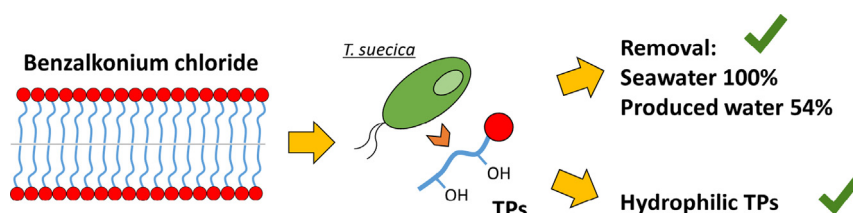
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HIGHLIGHTS

- BAC biotransformation was evaluated in microalgae experiments during 14 days.
- BAC was completely eliminated after *Tetraselmis suecica* experiments in seawater.
- BAC was eliminated at 54% after *Tetraselmis suecica* experiments in produced water.
- BAC was transformed into isomeric TPs from hydroxylation and dehydration.
- The TPs decreased on hydrophobicity and their tendency to adsorb into sediments.

GRAPHICAL ABSTRACT



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ABSTRACT

Many countries have implemented stringent regulatory standards for discharging produced water (PW) from the oil and gas extraction process. Among the different chemical pollutants occurring in PW, surfactants are widely applied in the oil and gas industry to provide a barrier from metal corrosion. However, the release of these substances from the shale formation can pose serious hazardous impacts on the aquatic environment. In this study, a low-cost and eco-friendly microalgae laboratory-scale technology has been tested for biotransformation of benzalkonium chloride (BAC_{C12} and BAC_{C14}) in seawater and PW during 14-days of treatment (spiked at 5 mg/L). From the eight microalgae strains selected, *Tetraselmis suecica* showed the highest removal rates of about 100% and 54% in seawater and PW, respectively. Suspect screening analysis using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) allowed the identification of 12 isomeric intermediates generated coming from biotransformation mechanisms. Among them, the intermediate [OH-BAC_{C12}] was found as the most intense compound generated from BAC_{C12}, while the intermediate [2OH-BAC_{C14}] was found as the most intense compound generated from BAC_{C14}. The suggested chemical structures demonstrated a high reduction on their amphiphilic properties, and thus, their tendency to be adsorbed into sediments after

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water discharge. In this study, *Tetraselmis suecica* was classified as the most successful specie to reduce the surfactant activity of benzalkonium chloride in treated effluents.

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1. Introduction

Over the last decade, the horizontal drilling and hydraulic fracturing techniques have been motivated by a rapid increase of conventional and unconventional energy production such as oil and gas extraction (Patterson et al., 2017). The amount of oilfield produced water (PW) accounts for up to 10–20 times the oil volume produced and roughly, it has been estimated to be >70 billion barrels per annum in the world in 2009 (Al-Ghouti et al., 2019). In some cases, re-injection of the PW back into the wells is turning into a very common practice for its re-utilization in oil and gas operations (Fakhru'l-Razi et al., 2009; Jiménez et al., 2018). However, operational discharges from the offshore industry have created public concern since they may represent a potential and continuous input of hazardous pollutants entering in the aquatic environment (Bakke et al., 2013).

Numerous pollutants such as organic compounds, heavy metals, salts and chemical additives such as biocides and corrosion inhibitors are used during drilling, fracturing and operating process of the well (Al-Ghouti et al., 2019; Jiménez et al., 2018). The Environmental Protection Agency (EPA) has identified over 1000 chemicals used in fracturing fluids, from which 27 chemicals are known or suspected carcinogenic, or listed as hazardous pollutants that may impact drinking water (EPA, 2012; Torres et al., 2016). Among them, benzalkonium chloride (BAC) is classified as a quaternary ammonium salt (based on C₁₂ and C₁₄ alkyl chain homologs, mainly) detected at a concentration of 19.5 mg/L in hydraulic fracturing fluids, and identified at a frequency of 54% in flowback and PWs (Chen and Carter, 2017; Ferrer and Furlong, 2001; Ferrer and Thurman, 2015). BAC is applied in the oil and gas industry as: i) a corrosion inhibitor for the protection of metallic materials by adsorption of surfactant on metals (Chen et al., 1998; Liu et al., 2017; Pinnock et al., 2018); and ii) as an emulsifier to destabilize water-in-oil emulsions and enhance the final crude-oil quality (He et al., 2015; Kedar and Bhagwat, 2019). The corrosion-protective activity of BAC mainly depends on the concentration and amphiphilic properties as well as the surface properties of metals (Zhu et al., 2017). These compounds tend to form a monolayer or bilayer micelle structure which provides a barrier that preserves deeper layers of the metal from further corrosion (Zhu et al., 2017). Up to now, quaternary ammonium surfactants account for about 10% of the surfactant market (Brycki et al., 2014; Wu et al., 2019), and about 75% of the employed amount is released into water bodies (Zhang et al., 2011). Once these compounds enter the ecosystem, their strong ability to be adsorbed into sediments and organic matter makes biodegradation a complex and demanding process (Brooks et al., 2018; Ferrer and Furlong, 2002; Khan et al., 2015; Tezel et al., 2006). Therefore, advanced water treatments should be developed for the removal of these pollutants prior to wastewater discharge and/or water reuse (Fakhru'l-Razi et al., 2009; Nasiri et al., 2017).

The treatment of PW has been performed through different treatment methods including physical (e.g. membrane filtration, adsorption), chemical (e.g. precipitation, oxidation), and biological (e.g. activated sludge, biological aerated filters), among others (Al-Ghouti et al., 2019; Chang et al., 2019; Jiménez et al., 2018; Lester et al., 2015). Even so, most of the physical-chemical technologies studied have unveiled disadvantages, mainly due to their acquisition and exploitation costs. The development of low-cost and eco-friendly technologies may provide a potential alternative to decrease the load of hazardous chemicals while minimizing the energy consumption (Gupta and Bux, 2019; Jaén-Gil et al., 2019b; Muñoz and Guieysse, 2006; Xiong et al., 2017). In this regard, several authors have

demonstrated that specific photosynthetic microorganisms can remove pollutants from wastewater (Hom-Díaz et al., 2017; Kabra et al., 2014; Xiong et al., 2017). Among them, microalgae have proven to be also effective for the removal of organic substances through biodegradation, photo-degradation and sorption processes (Escudero-Oñate and Ferrando-Climent, 2019; Hom-Díaz et al., 2017). However, despite numerous studies have addressed the removal of organic pollutants using microalgae treatment (Ferrando and Matamoros, 2020), less attention has been paid to the time-course profile of transformation products (TPs) generated from the parent compounds (Jaén-Gil et al., 2018). The presence of these unknown chemicals can play an important role since they might be more persistent and/or toxic than the parent compound (Picó and Barceló, 2015). One of the main drawbacks in their identification is the lack of analytical standards and rapid analytical methods for confirmation of their presence in treated effluents (Schymanski et al., 2009). In this context, further advances on the analytical methods for characterizing the potential environmental impacts of unconventional oil and gas development have been recently performed (Liden et al., 2019; Santos et al., 2019; Sitterley et al., 2020).

The main objective of this study is to explore the capabilities of microalgae as a cost-effective and environmentally friendly solution for treating polluted seawater and PW from oil and gas extraction processes. A case study for the degradation of BAC is presented here. The identification of the isomeric intermediates generated has been performed to evaluate the environmental implication of these unknown pollutants in treated effluents (as surface-active agents). This study demonstrated that microalgae water treatment can be successfully applied for water decontamination and water reuse.

2. Materials and methods

2.1. Chemicals, microalgae and water collection

Benzalkonium chloride (BAC) reference standard was provided from Sigma-Aldrich (Steinheim, Germany) at a high purity grade (>95%) containing a mixture of alkylbenzyltrimethylammonium chlorides with different alkyl chain lengths (C₁₂ and C₁₄, mainly). Microalgae strains *Rhodomonas salina*, *Nannochloropsis oculata*, *Emiliania huxleyi*, *Dunaliella tertiolecta*, *Isochrysis galbana*, *Tetraselmis suecica*, *Dunaliella salina*, and *Phaeodactylum tricornutum* were purchased from the Norwegian Culture Collection of Algae (NORCCA). The culture media used for microalgae growth and measured strain characteristics are provided in Supplementary Material, S1-S2. Seawater (salinity of 35 g/L and pH of 7.8) was collected in 1 L polyethylene terephthalate (PET) bottles from the Bygdøy peninsula located in the west of Oslo city (Norway). Produced water (salinity of 68 g/L and pH of 6.9) had been collected in 1 L PET bottles from an offshore oil and gas extraction well located in the North continental shelf (further details of the composition and exact location remain confidential).

2.2. Microalgae BAC experiments in spiked seawater

Microalgae strains were incubated in seawater for 7 days in sterile seawater with the addition of the growth media presented in Supplementary Material, S1. All microalgae experiments were carried out separately in 100-mL glass containers, which were filled with 60 mL of sterile seawater, over a 14-days' time span (in triplicate). Firstly, microalgae experiments were performed in light for each microalga selected by spiking the parent compounds simultaneously at an initial

concentration of 5 mg/L (light biomass experiments). To evaluate the inhibition growth effect of microalgae in presence of BAC, control experiments were carried out in the presence of light without spiking the parent compounds (light control experiments). The relative growth percentages are calculated using Eq. (1):

$$\text{Relative growth (\%)} = \left(\frac{N^\circ \text{ cells at given time}}{N^\circ \text{ cells at initial time}} \right) 100 \quad (1)$$

In addition, a straightforward photo-degradation study was performed by spiking the compounds in light at 5 mg/L without the presence of the microalgae strains selected (light abiotic experiments). Hydrolysis mechanisms were also evaluated by spiking the parent compounds at 5 mg/L in darkness and without the presence of the microalgae strains selected (dark abiotic experiments). All batch experiments were introduced in an own-designed photobioreactor equipped with a continuous cold-white light-emitting diodes (LED) lamp (Fig. S1). The light intensity in the photobioreactor was 5000 lm and the photoperiod, 24 h. Stirring was provided by an orbital shaker set at 340 rpm at a constant temperature of 20 ± 2 °C. In the case of the dark abiotic experiments, vials were covered with aluminum foil to inhibit photo-degradation mechanisms from taking place. Samples were collected at 0, 6, 24, 48, 72, 144, 192, 240, and 336 h of treatment. A total volume of 3 mL of liquid samples was withdrawn from the containing vessel, transferred to plastic vials, and centrifuged at 2000 rpm for 15 min. The supernatants were collected, transferred to amber glass vials and stored at -20 °C till analysis by liquid chromatography coupled to mass spectrometry (LC-MS/MS).

2.3. Sample analysis and data processing

The collected samples from light biomass (all microalgae), light abiotic and dark abiotic experiments were analyzed in a liquid-chromatography system coupled to a high-resolution mass spectrometer LC-Orbitrap-QExactive™ (Thermo Fisher Scientific). In addition, light control experiments (without spiking the parent compounds) were also analyzed to avoid false-positives features coming from microalgae metabolism. Analysis of samples was performed using an automated suspect screening methodology based on software prediction tools for compound identification. Briefly, 20 µL of samples were injected in a SM-C18 column (150 mm × 2.0 mm, 5 µm; Sherzo, lmtakt) at room temperature of 20 °C. The mobile phases used were (A) 10 mM ammonium formate in water at pH 3.0 and (B) acetonitrile. The optimized chromatographic gradient was performed at a constant flow rate of 0.5 mL/min as follows: initial mobile phase composition (95% A) held for 1 min, followed by a decrease in composition A to 5% within 9 min, then to 0% in 3 min, held for 2 min, and up to 95% in 1 min and held for 1 min. The high-resolution mass spectrometer Orbitrap-QExactive™ was equipped with a heated electrospray ionization source (HESI-II). The analyses were performed in positive and negative ionization modes. As no results were found for negative mode, further data processing was performed in positive mode only. Samples were recorded in full-scan mode within a mass-to-charge (m/z) range of 50 to 700 m/z at a resolving power of 70,000 FWHM (MS). Ion fragmentation was performed in data-dependent acquisition (DDA) mode for the three most intense ions (TOP 3) at a resolving power of 35,000 FWHM (MS/MS). The mass spectrometry conditions were designed as follows: spray voltage at, 3.5 kV; source heater temperature, 300 °C; capillary temperature, 350 °C; sheath gas flow, 40 arb; auxiliary gas flow, 20 arb; collision energy, 55 eV in higher-energy collisional dissociation (HCD); dynamic exclusion, 10 s; and isolation window, 2 Da. The entire system was controlled via Xcalibur 3.0 software.

Computational data files containing chromatograms and mass spectra files from light biomass (all microalgae), light abiotic and dark abiotic experiments were processed through Compound Discoverer 2.1 software (Thermo Fisher Scientific). This software was connected to

Mass Frontier 7.0 software (Thermo Fisher Scientific) and was applied using an adapted suspect screening methodology previously reported (Jaén-Gil et al., 2018). Further information on the software parameters selected is presented in Table S2. Prior to automatic software data processing, input files were loaded into the software together with the chemical structures of the parent compounds (BAC_{C12} and BAC_{C14}) and potential chemical transformations for the prediction of suspected intermediates. With this information, a simulated list of tentative TP exact masses was automatically created to be further detected in sample data files after starting automatic software data processing.

Automatic software data processing started by filtering MS data between 50 and 700 Da and retention times between 0.5 and 12 min at a signal-to-noise ratio of three. To compensate for the small differences in retention times, chromatographic alignment was performed by using a mass tolerance error of ± 5 ppm and a maximum retention time shift of ± 0.3 min. Immediately after, the list of predicted TP exact masses previously created was matched with the filtered experimental data for the detection of tentative features in samples. In addition, peaks detected in blanks (seawater) were extracted from the background at a maximum sample/blank ratio of three. For confirmation purposes, MS/MS spectra collected was automatically elucidated by fragment structure prediction comprising a mass tolerance of ± 5 ppm and a signal-to-noise ratio of three. After software data processing, a principal component analysis (PCA) was automatically generated by using the same software (Compound Discoverer 2.1) to evaluate the significant differences between samples collected along treatments (light biomass, light abiotic and dark abiotic) in terms of new and unknown features generated (evaluated in chromatographic area). This automated statistical analysis allowed to graphically point out the best microalgae specie for the transformation of BAC_{C12} and BAC_{C14} in seawater experiments.

Finally, semi-quantification of the elimination and transformation of the parent compounds in treated samples was performed for the most efficient microalga in terms of microalgae growth and compound transformation (*T. suecica*). Relative percentages were expressed as: the chromatographic area of each parent compound (or intermediate) at a given experimental time regarding the chromatographic area of the parent compound at the initial time.

2.4. Elucidation of isomeric transformation products (TPs)

The presence of multiple chromatographic peaks for each intermediate identified pointed out the presence of isomeric compounds in *T. suecica* light biomass experiments. Thus, tentative evaluation of the isomer chemical structures for each intermediate generated was performed using a more advanced suspect screening methodology. The analysis was carried out in a liquid-chromatography system coupled to a high-resolution mass spectrometer LC-QTOF (Agilent Technologies, Santa Clara). Briefly, 20 µL of each sample was injected in a ZORBAX Eclipse XDB-C8 column (150 mm × 4.6 mm, 3.5 µm; Agilent Technologies) at a constant room temperature of 20 °C. The mobile phases selected were: (A) 0.1% formic acid in water and (B) acetonitrile. The optimized chromatographic gradient was performed at a flow rate of 0.6 mL/min as follows: initial mobile phase composition (90% A) held for 5 min, followed by a decrease in composition A to 0% in 30 min, then 10 min post run time. The high-resolution mass spectrometer was equipped with a heated electrospray ionization source (ESI) coupled to a Jet Stream source. Sample analysis was performed using in-source ion fragmentation in positive ionization mode. Parent and precursor ions were recorded in full-scan MS from m/z 40 to 1000 range at a resolving power of 30,000 FWHM. Further structural elucidation of isomers identified was performed by fragmentation of precursor ions using *target-MS/MS* mode, as well as their fragment ions imitating *pseudo-MS³* instrumental conditions (Ferrer et al., 2018). Mass spectrometry conditions were selected as follows: capillary voltage, 3.5 kV;

in-source fragmentation voltage, 175–190 eV; sheath gas temperature, 350 °C; drying gas temperature, 250 °C; sheath gas flow, 11 L/min; nebulizer pressure, 45 psi; drying gas: nitrogen at 10 L/min; collision energy, between 20 and 40 eV. For confirmation purposes, manual checking of MS/MS spectra was performed comprising a mass tolerance of ± 5 ppm.

Finally, the tentatively identified isomeric compounds were evaluated in terms of surface-active agents using the estimated hydrophobicity as a quantitative structure-activity relationship parameter. For that purpose, Chemicalize from ChemAxon (www.chemaxon.com) was used for the estimation of $\log D$ values (at a pH value of 7.5) of the intermediates identified. This parameter expresses the partition of a chemical compound between the lipid and aqueous phases.

2.5. Microalgae BAC experiments in spiked produced water (PW)

The own-designed photobioreactor was used to evaluate the removal efficiency of BAC_{C12} and BAC_{C14} in spiked PW after the experiments performed using *T. suecica* (Fig. S1). This microalga was selected since it provided the best performance on pollutant removal in seawater experiments. Real PW experiments were performed following the experimental procedure described in Section 2.2 in duplicate. Briefly, experiments were carried out in 100 mL glass containers, which were filled with 60 mL of spiked PW over 14 days of treatment. Microalgae experiments were spiked at an initial concentration of 5 mg/L of the parent compounds BAC_{C12} and BAC_{C14} . Moreover, non-spiked experiments were also performed in PW to evaluate the inhibition effect of this matrix on microalgae growth. Samples were collected at the beginning and the end of the experiment at 0 h and 336 h, respectively. The analysis of samples was performed as described in Section 2.3. The relative presence (%) of the parent compounds and intermediates generated were evaluated as: the measured chromatographic area at a given time relative to the chromatographic area of the parent compound at the initial time.

3. Results and discussion

3.1. Microalgae survival and growth in seawater BAC experiments

Microalgae strains were incubated in seawater for 7 days in sterile seawater with the addition of the growth media presented in Supplementary Material, S1. After microalgae growth, microscopic characterization was carried out for the selected microalgae strains in terms of cell concentration, cell length and growth cell rates (Supplementary Material, S2). Then, microalgae experiments were performed during 14 days' treatment in the own-designed photobioreactor as presented in Section 2.2.

Non-spiked light control experiments showed an increase on cell concentration for most of the microalgae selected up to 155% in *R. salina*, 103% in *E. huxleyi*, 169% in *T. suecica*, 76% in *D. salina*, and 16% in *P. tricornutum* after 14 days of treatment (Fig. 1a). These results indicated the suitability of these microalgae to grow in sterile seawater medium. In contrast, while cell concentration remained constant along the time-course experiments using *D. tertiolecta*, the percentage values decreased up to 27% and 18% in *N. oculata* and *I. galbana*, respectively. The growth inhibition of these last strains in non-spiked seawater highlighted their low applicability for the removal of pollutants in spiked seawater experiments.

Light biomass experiments demonstrated strong growth inhibition in the presence of the parent compounds BAC_{C12} and BAC_{C14} (Fig. 1b). For most of the microalgae selected, cell concentration values decreased by up to 33% in *R. salina*, 40% in *N. oculata*, 19% in *E. huxleyi*, 21% in *D. tertiolecta*, 36% in *I. galbana*, 86% in *D. salina*, and 55% in *P. tricornutum* after 14 days of treatment. These results evidenced the potentially hazardous effects of these substances in the aquatic environment and the need to attain their complete removal. In this context, *T. suecica* was suggested as the most promising microalgae strain for the removal of BAC_{C12} and BAC_{C14} since the growth percentage increased up to 108% after 14 days of treatment. To ensure the efficiency

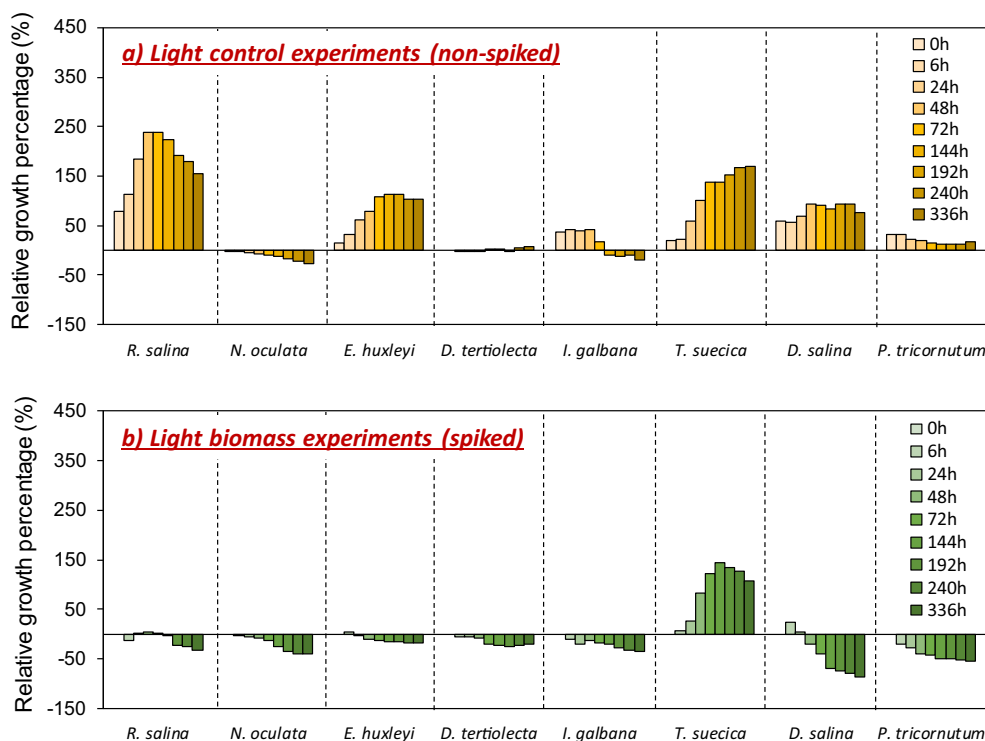


Fig. 1. Microalgae growth in seawater experiments along 14 days of treatment: a) light control experiments (non-spiked) and; b) light biomass experiments (spiked with BAC_{C12} and BAC_{C14}). Calculations are performed using Eq. (1).

of microalgae in terms of biodegradation, elucidation of the mechanisms involved in their removal was further performed.

3.2. Evaluation of degradation mechanisms in microalgae seawater experiments

Samples collected from all microalgae experiments performed in light biomass, dark abiotic, and light abiotic conditions were analyzed in LC-Orbitrap-QExactive™. The data collected was filtered (applying the parameters presented in Table S2) and automatically processed using a PCA statistical approach in Compound Discoverer 2.1 software (Fig. 2). The goal of this statistical tool was to evaluate the significant differences in terms of the generation of new and unknown features during the different experimental conditions tested (expressed in chromatographic area), regarding initial experimental time. After data filtering, the total number of tentative exact masses detected in samples from BAC_{C12} and BAC_{C14} degradation (and used for statistical analysis) were 2412 and 2241 features, respectively.

Dark abiotic and light abiotic experiments showed no statistical differences in terms of total detected features (over the 14 days of treatment) in comparison with the samples collected at the initial experimental time (Fig. 2). These values demonstrated the negligible contribution of hydrolysis and photo-degradation mechanisms on pollutant degradation. Indeed, the transformation of BAC_{C12} and BAC_{C14} was mainly attributed to a biological mechanism only: while no statistical differences were observed in light biomass experiments using *R. salina*, *N. oculata*, *E. huxleyi*, *I. galbana*, *D. salina* and *P. tricornutum* microalgae strains, significant differences were found using *D. tertiolecta* and *T. suecica*. The tentative detection of new and unknown features after BAC_{C12} and BAC_{C14} degradation using *D. tertiolecta* was inconsistent with its growth inhibition found up to 21% (Fig. 1b). As a consequence, it can be assumed that the cell inhibition effects might not be attributed to the presence of the parent compounds only but also to the presence of the generated TPs at the end of the experiments, which may pose higher toxic effects to this microalgae strain. Likewise, *T. suecica* also demonstrated statistical differences in the presence of new and

unknown features generated along light biomass experiments. In comparison with *D. tertiolecta*, this microalgae was much more resistant to the presence of the generated intermediates since cell concentration increased up to 108% (Fig. 1b). As a result, *T. suecica* was classified the most successful microalgae strain tested for biodegradation and biotransformation of BAC_{C12} and BAC_{C14} in seawater experiments. Further assessment of removal efficiencies and identification of TPs was performed for this microalgae strain only.

3.3. Removal and biotransformation of BAC in *T. suecica* seawater experiments

The removal percentages of the spiked parent compounds (BAC_{C12} and BAC_{C14}) in *T. suecica* light biomass experiments over the 14 days of treatment are presented in Fig. 3a. As observed, BAC_{C12} was practically eliminated after 72 h of treatment (3 days). Likewise, the parent compound containing the longer alkyl chain length (BAC_{C14}) was similarly resistant to biodegradation but achieving complete removal at 144 h of treatment (6 days). In both cases, partial removal through microalgae sorption of the parent compounds into microalgae biomass cannot be discarded. After elucidation of the intermediates generated, their transformation through multiple hydroxylation reactions followed by a dehydration step was suggested as the most relevant chemical reactions involved in degradation pathways (Fig. 3b). More extended information on the identification of the transformation products by accurate mass can be found in Supplementary Material, S3.

Single hydroxylation of BAC_{C12} into [OH-BAC_{C12}] was found at a relative presence of 40% after 24 h of treatment (Fig. 3c). This intermediate was rapidly transformed into the secondary hydroxyl intermediate [2OH-BAC_{C12}] up to 18% after 244 h of treatment, while further dehydration into [(OH-BAC_{C12})-H₂O] was found at a very low percentage value <1%. This fact indicates that the secondary hydroxylation reaction was favored instead of chemical reduction though the dehydration step. In contrast, the secondary hydroxyl intermediate generated [2OH-BAC_{C12}] was mainly transformed into the dehydrated intermediate [(2OH-BAC_{C12})-H₂O] up to a percentage of 9% after 244 h of treatment,

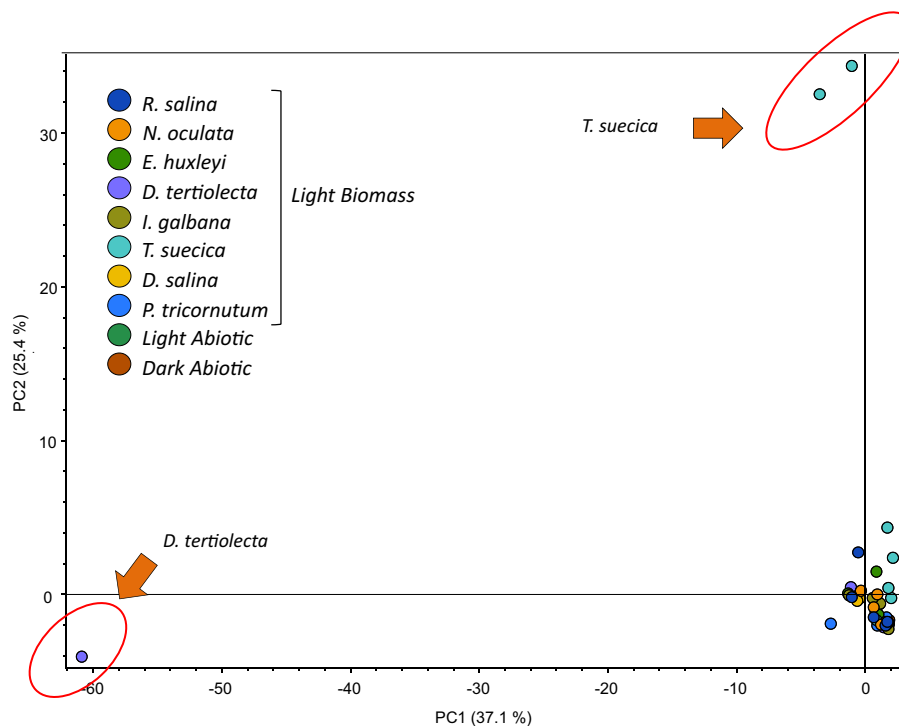


Fig. 2. PCA loadings for the evaluation of the presence of new and unknown TPs generated in light biomass (*R. salina*, *N. oculata*, *E. huxleyi*, *D. tertiolecta*, *I. galbana*, *T. suecica*, *D. salina*, and *P. tricornutum*), light abiotic and dark abiotic experiments.

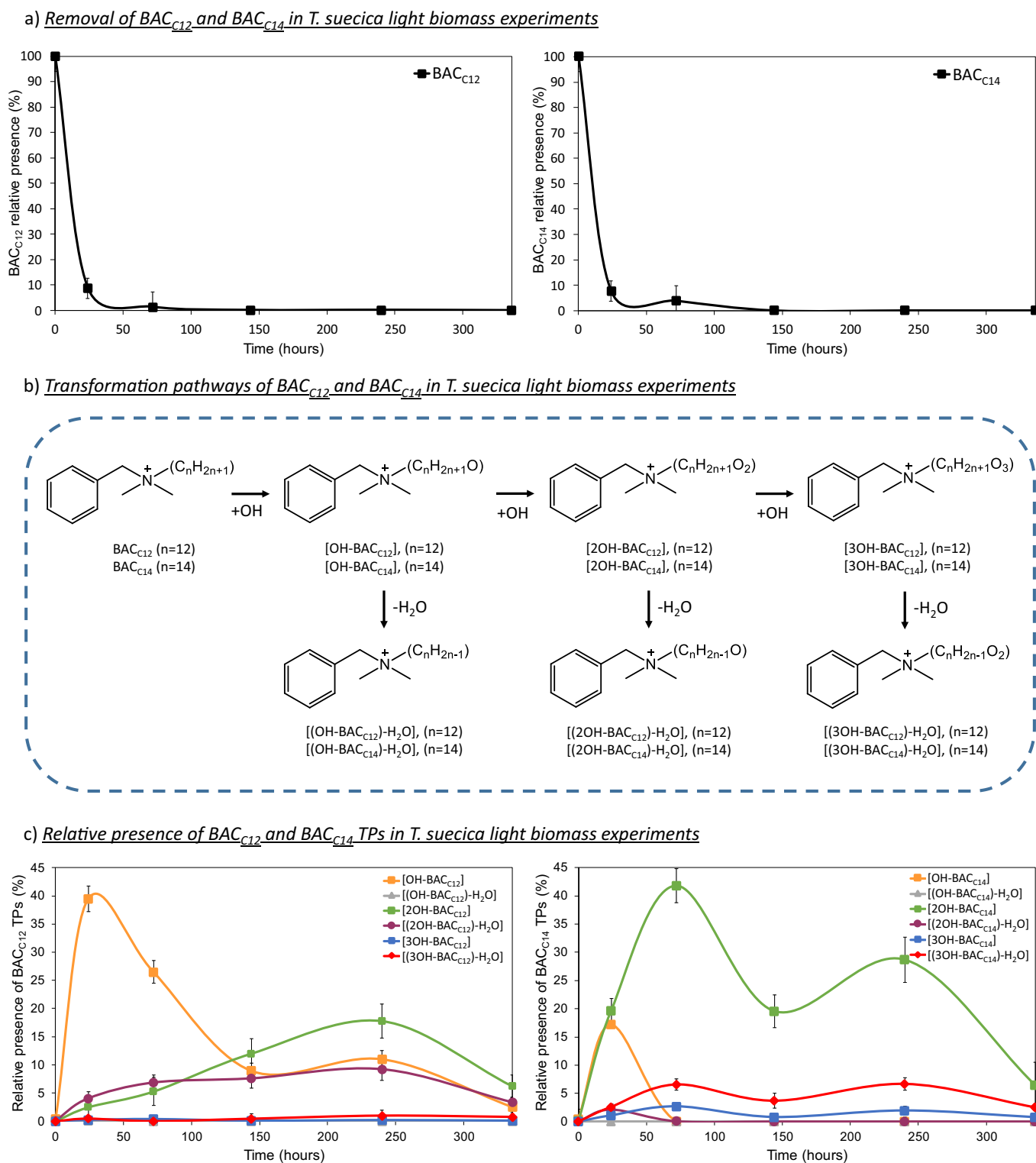


Fig. 3. a) Removal of BAC_{C12} and BAC_{C14}, b) transformation pathways, and c) relative presence of the intermediates identified along *T. suecica* light biomass experiments in seawater.

while the hydroxylation intermediate [3OH-BAC_{C12}] was detected at a relatively low presence of <1%. On the contrary to the previous case, the dehydration reaction was favored instead of the hydroxylation step. This can be explained by the increase of the steric effects on the alkyl chain moiety when increasing the number of hydroxylation steps, and thus, promote a dehydration step for the successive intermediates generated (Wu et al., 2019).

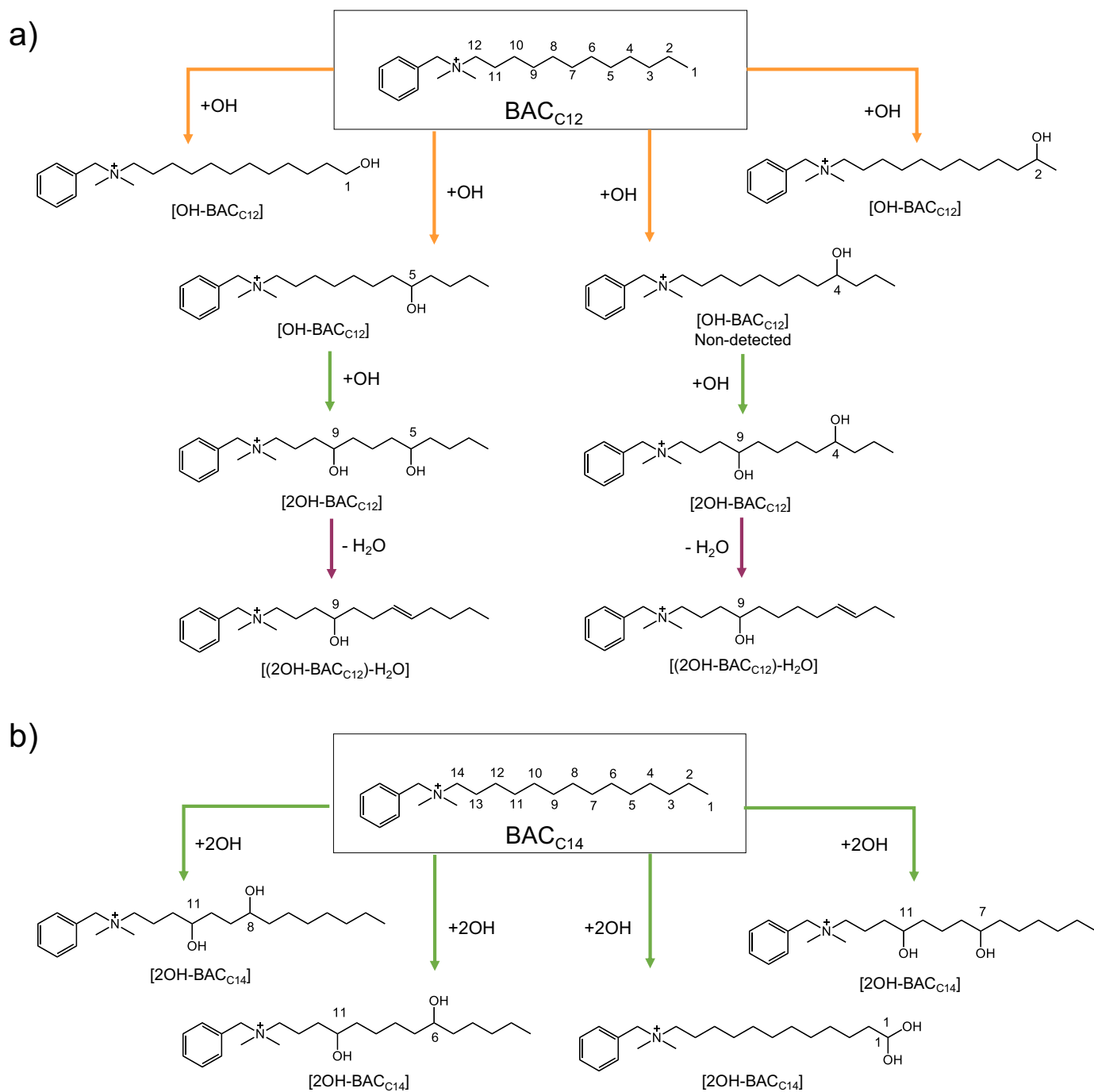
In comparison to BAC_{C12} transformation, single hydroxylation of BAC_{C14} into [OH-BAC_{C14}] was detected at a relative lower percentage of 17% after 24 h of treatment (Fig. 3c). Indeed, it was completely degraded and transformed into the secondary hydroxyl intermediate [2OH-BAC_{C14}] at a high relative presence of 42% after 72 h of treatment (3 days). In contrast to BAC_{C12} transformation, a lower relative presence of [(2OH-BAC_{C14})-H₂O] was observed generated from

the dehydration of the intermediate [2OH-BAC_{C14}]. In this particular case, a third hydroxylation of the parent compound was prioritized [3OH-BAC_{C14}] attaining values of 3% after 72 h of treatment, while its successive dehydration [(3OH-BAC_{C14})-H₂O] was found at a relatively high presence of 7%. This last transformation pathway is in agreement with those indicating that longer alkyl chain length allows increasing the extent of chemical biodegradation for surfactants (Wu et al., 2019).

These transformations have been previously described in the removal of organic pollutants through the intracellular enzymes present in microalgae involving cytochrome P450 system (Pflugmacher and Sandermann, 1998; Song et al., 2020; Sutherland and Ralph, 2019; Thies et al., 1996). Enzymatic degradation is promoted by monooxygenase

enzymes which catalyze the insertion of the oxygen atom(s) into an organic substrate using molecular oxygen (O₂) as an oxygen donor (Kiki et al., 2020; Torres Pazmiño et al., 2010). One of the key roles of these enzymes is to transform a contaminant into a more hydrophilic compound through the addition of hydroxyl groups in their chemical structure (Sutherland and Ralph, 2019). Otherwise, no evidence on the direct correlation between this enzymatic system and their further dehydration reaction step was found, although it was previously described to also occur in microalgae water treatments (Song et al., 2020). In this sense, other abiotic or biological mechanisms cannot be discarded.

All these results demonstrated that microalgae-based treatment allowed to rapidly transform the persistent pollutants BAC_{C12} and



BAC_{C14}. Nonetheless, it is important to mention that the biotransformation of the parent compounds into the hydroxyl intermediates allowed to generate a set of new compounds more susceptible to further biodegradation. The presence of these unknown chemicals can also play an important role in the overall toxicity of treated effluents, since they might be more persistent and/or toxic than the parent compound (Picó and Barceló, 2015). Even so, structural elucidation using the automatic suspect screening approach evidenced the presence of several co-eluting isomers (with different relative position of the hydroxyl group in their chemical structures) for most of the intermediates identified. The application of more advanced screening approaches was required to discern among their chemical structures, and properly evaluate their hazardous effects in the aquatic environment.

3.4. Elucidation of isomeric intermediates in *T. suecica* seawater experiments

The samples collected from *T. suecica* light biomass experiments were analyzed in a LC-QTOF to elucidate the major isomer structures generated from the most intense intermediates identified (Fig. 4): [OH-BAC_{C12}], [2OH-BAC_{C12}], [(2OH-BAC_{C12})-H₂O] and [2OH-BAC_{C14}]. For that purpose, in-source ion fragmentation was carried out in order to generate MS data files containing the ionized precursor ion as well as their fragment ions. Then, Target-MS/MS of ionized parent ions as well as MS/MS of their fragment ions (pseudo-MS³) were investigated for the elucidation of isomeric chemical structures (Ferrer et al., 2018). Accurate mass was essential for the determination of unequivocal degradation products.

Structural elucidation allowed the identification of eight different isomeric compounds from BAC_{C12} degradation presented in Fig. 4a and Supplementary Material, S4. Among them, four isomers were suggested for the intermediate [OH-BAC_{C12}], two for the intermediate [2OH-BAC_{C12}], and two for the intermediate [(2OH-BAC_{C12})-H₂O]. Nonetheless, although hydroxylation in position 4 for [OH-BAC_{C12}] was suggested in the degradation pathway, it was not detected in samples (Fig. 4a). On the other hand, structural elucidation allowed the identification of four isomeric compounds generated for the intermediate [2OH-BAC_{C14}] (Fig. 4b).

An example of isomeric identification for [2OH-BAC_{C14}] is presented in Fig. 5. As observed, the presence of the fragment ion m/z 91.0543 in Target-MS/MS spectra of the parent ion [2OH-BAC_{C14}] confirmed the position of the two hydroxyl groups along the alkyl chain moiety instead of being attached to the phenyl moiety (Fig. 5b). To evaluate the exact position of these two hydroxyl groups along the alkyl chain moiety, MS/MS of the fragment ion at m/z 272.2591 was performed (emulating pseudo-MS³ conditions) and presented in Fig. 5c. As observed, the ion at m/z 254.2479 was generated after the loss of a water molecule, so it means that one of the hydroxyl groups is fragmented. The second hydroxyl position was suggested at position 1 due to the presence of the fragment ion m/z 224.2369 generated at one bond removed from the first radical site. Otherwise, the first water loss was also observed at positions 6, 7 and 8 due to the presence of the fragment ions m/z 184.1693, m/z 170.1535 and m/z 156.1382, respectively. Likewise, the second hydroxyl position was suggested at position 11 due to the presence of a high-intensity fragment ion at m/z 84.0805, also generated at one bond removed from the secondary radical site. Since the different TPs may pose differentiated effects on the aquatic environment, the estimation of their surfactant activities was further performed.

3.5. Environmental impacts of the isomers generated in *T. suecica* seawater experiments

The surfactant activity of the intermediates identified was evaluated according to the hydrophobicity of their tentatively chemical structures. The correlation of their calculated $LogD$ values with their retention times in chromatographic separation was performed and presented in

Fig. 6. The reduction in $LogD$ values of the intermediates generated from the parent compounds (BAC_{C12} and BAC_{C14}) indicates an increase in hydrophilicity. On the other hand, since a reverse-phase chromatography was performed, hydrophilic intermediates are expected to undergo less affinity for the stationary phase and elute earlier.

Results reveal a higher hydrophobicity for the parent compound BAC_{C14} ($LogD = 3.52$ at 23.4 min) than for BAC_{C12} ($LogD = 2.63$ at 21.7 min) due to the presence of a higher number of hydrophobic carbons in BAC_{C14} alkyl chain moiety (Fig. 6). Both parent compounds decreased in $LogD$ and chromatographic retention times after their transformation into the successive hydroxyl intermediates from BAC_{C12} degradation: [OH-BAC_{C12}] ($LogD = 1.17$ at 16.9 min) > [2OH-BAC_{C12}] ($LogD = -0.14$ at 13.6 min) > [3OH-BAC_{C12}] ($LogD = -1.53$ at 11.5 min). The higher retention time observed for [(2OH-BAC_{C12})-H₂O] is explained by the addition of an instauration in its chemical structure from [2OH-BAC_{C12}]. Likewise, similar results were observed for those intermediates generated from BAC_{C14} degradation according to Fig. 6: [2OH-BAC_{C14}] ($LogD = 0.75$ at 14.7 min) > [3OH-BAC_{C14}] ($LogD = -0.64$ at 12.7 min). These values demonstrated that *T. suecica* may generate more hydrophilic compounds through the addition of a hydroxyl group on their chemical structures (Sutherland and Ralph, 2019). Since corrosion-protective activity mainly depends on amphiphilic properties, it is concluded that *T. suecica* may reduce the tendency of surfactants to be adsorbed into sediments after water discharge. Moreover, the generation of more hydrophobic intermediates after dehydration of hydroxyl intermediates into [(2OH-BAC_{C12})-H₂O] ($LogD = 0.88$ at 17.6 min) and [(3OH-BAC_{C14})-H₂O] ($LogD = 0.56$ at 15.6 min) also demonstrated higher hydrophilicity than the parent compounds, decreasing $LogD$ values and retention times in both cases (Fig. 6).

3.6. Removal and transformation of BAC in *T. suecica* produced water experiments

Evaluation of the applicability of *T. suecica* treatment for the removal of BAC_{C12} and BAC_{C14} in spiked PW was performed over 14 days of treatment. The same experimental procedure described in Section 2.2 was carried out. In this particular case, the growth of *T. suecica* decreased to 62% compared to the growth rates attained in sterile seawater experiments (108%). Likewise, the elimination of the parent compounds BAC_{C12} and BAC_{C14} attained lower removal rates up to 56% and 52%, respectively. These values can be explained by their difference on pH values and salinity reported, but also to the presence of other hazardous contaminants, which may also inhibit microalgae growth such as BETX (benzene, ethylbenzene, toluene, xylene) and PAHs (polycyclic aromatic hydrocarbons) (Jia et al., 2019). This can affect the stability of the parent compounds but also the total biomass produced at the initial point of the experiments performed. Despite this, results reveal the presence of [OH-BAC_{C12}] after 14-day treatment at a relative percentage value of 17%, while the successive major intermediates identified [2OH-BAC_{C12}] and [(2OH-BAC_{C12})-H₂O] were not detected. In the case of BAC_{C14} transformation, results showed the presence of [2OH-BAC_{C14}] after 14-day treatment at a relative percentage value of 22%, while the successive major intermediates identified [3OH-BAC_{C14}] and [(3OH-BAC_{C14})-H₂O] were not observed. Despite this, it was previously reported that changes in matrix composition may lead to different presence and transformation of TPs (Jaén-Gil et al., 2019a), and thus, changes in degradation pathways from those identified in seawater cannot be discarded. Besides, some of the other tested microalgae might also be more resistant to PW than *T. suecica*, and therefore, capable of achieving greater attenuation of BAC in real PW. In conclusion, a lower extent of the removal of BAC and the intermediates generated were observed treating real PW. Nevertheless, the presence of BAC_{C12} and BAC_{C14} intermediates, which were identified in seawater experiments and reduced their tendency to adsorb into sediments, were also detected in PW.

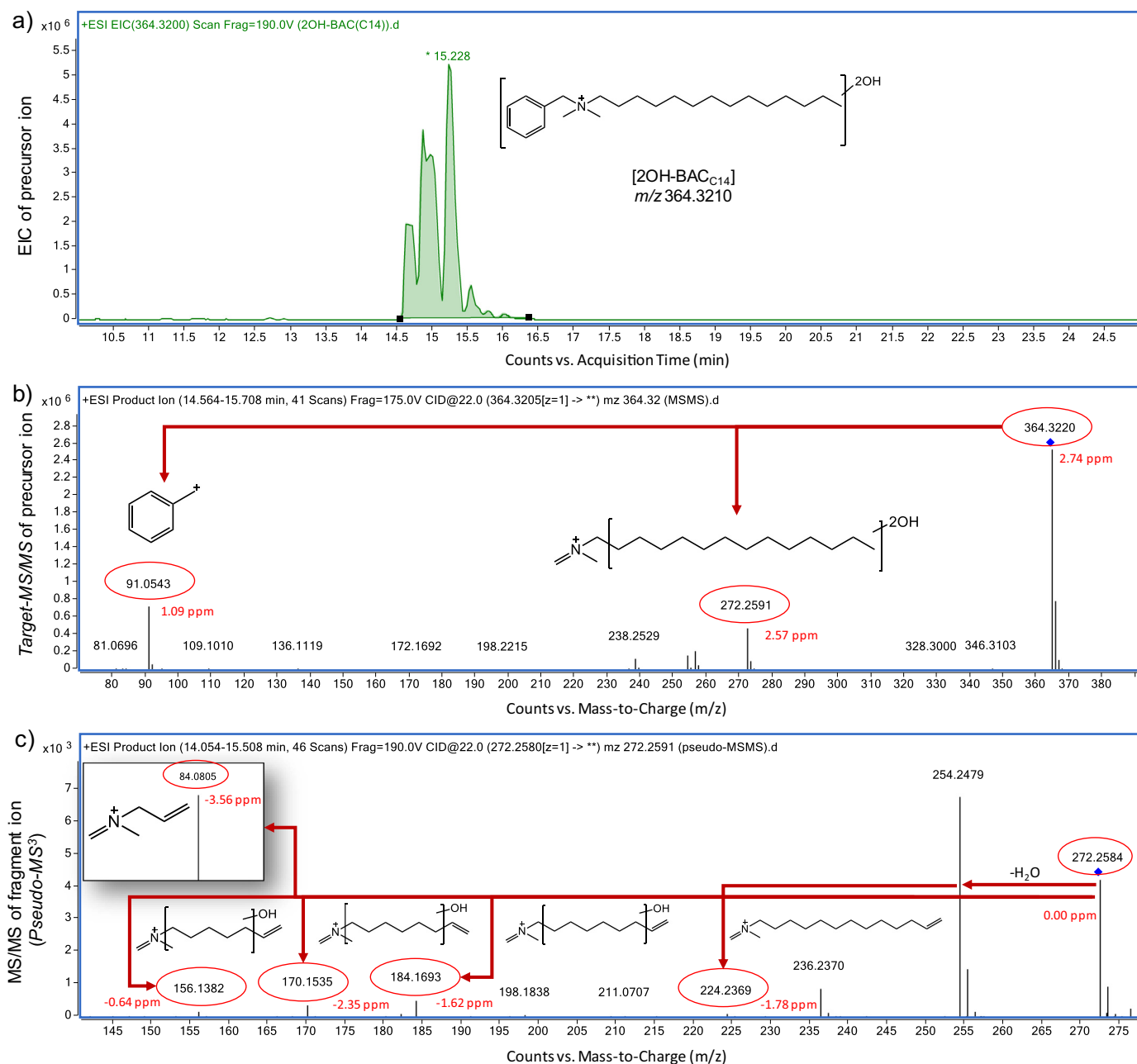


Fig. 5. Elucidation of the isomeric chemical structures for the intermediate [2OH-BAC_{C14}] generated in *T. suecica* light biomass experiments: a) extracted-ion chromatogram (EIC) of the precursor ion, b) target-MS/MS of the precursor ion, and c) pseudo-MS³ of the fragment ion m/z 272.25. In all cases, the calculated mass errors are always within ± 5 ppm.

3.7. Limitations and application of the microalgae-based technology

Important constraints to the deployment of the microalgae approach assessed here would be the lack of enough radiation and lack of nutrients in the PW. Even though in our manuscript we have employed energy-efficient LED sources, the upscaling of this technology would not be economically feasible and therefore, natural sunlight capable of providing sufficient radiation to support the microalgae growth would be required. The treatment approach presented here would be, however, perfectly suitable to exploitation fields onshore with large availability of land in the surroundings and located in regions with high intensity of solar irradiation (such as the Middle East and the oilfields in Texas). On the other hand, regarding the lack of nutrients that could provoke a collapse of the microalgae photobioreactor, PW generally contains a sufficient concentration of nitrogen and phosphorus.

Most of the physicochemical technologies employed for PW treatment (membrane filtration, adsorption, precipitation and/or oxidation) have so far unveiled disadvantages, mainly due to their large acquisition and exploitation costs as well as their inefficiency towards the removal of special hazardous pollutants such as the scale and corrosion inhibitors. A potential upscaling of this biotreatment would involve seeding the photobioreactor with a concentrated inoculum of the appropriate microalgae to ensure enough concentration of biomass and guarantee its successful growth. An appropriate reactor configuration would be i.e., a raceway pond, that would receive the PW extracted from the site and where the homogeneous contact of the microalgae with the fluid would be provided by a regular rotary paddle system. Such a treatment scheme would be very low energy-consuming compared to others such as membranes and would not add other chemicals to the water such as the powerful oxidants employed in advanced oxidation

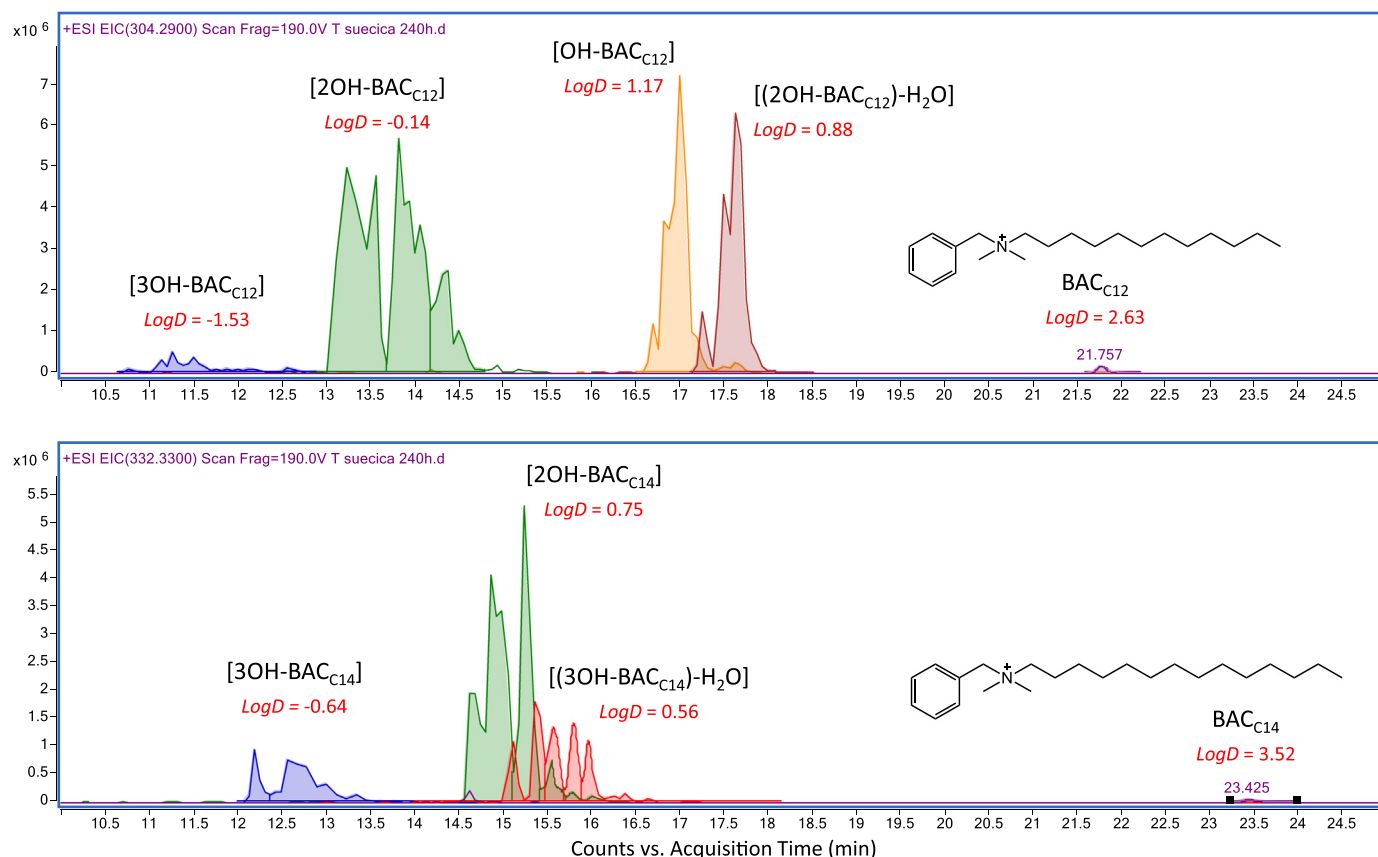


Fig. 6. LogD values for the parent compounds (BAC_{C12} and BAC_{C14}) and the major intermediates identified during *T. suecica* light biomass experiments. In addition, LogD values are correlated with their chromatographic retention times.

processes. This microalgae-based approach would provide an economically viable method to reuse PW, i.e., for industrial re-use or agriculture. The biomass purged out from the reactor would require being dewatered and afterward the biosolids could be, i.e., delivered to an incineration plant for further recovery of their energy content.

4. Conclusions

Low-cost and eco-friendly microalgae technologies have been tested to evaluate the degradation of benzalkonium chloride (BAC_{C12} and BAC_{C14}) in seawater and produced water using eight microalgae strains. Among them, *Tetraselmis suecica* provided the best performance in terms of growth and transformation. While complete elimination was obtained in seawater experiments after 14 days of treatment, lower removal rates were attained in produced water (54%). Among the degradation mechanisms involved, biotransformation was the major removal mechanism identified while no relevant contribution from abiotic mechanisms such as photo-degradation and hydrolysis was observed. Suspect screening analysis using LC-HRMS allowed the elucidation of several isomeric intermediates and suggest their transformation pathways. Results reveal a high reduction on their amphiphilic properties in comparison with the parent compound, and thus, their tendency to be adsorbed into sediments after water discharge. As a conclusion, *Tetraselmis suecica* was classified as the most successful microalgae strain to reduce the surfactant activity of benzalkonium chloride in seawater and produced water.

CRedit authorship contribution statement

Adrián Jaén-Gil: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft,

Writing - review & editing, Visualization, Project administration. **Laura Ferrando-Climent:** Conceptualization, Formal analysis, Writing - original draft, Supervision, Funding acquisition, Resources, Writing - review & editing, Project administration. **Imma Ferrer:** Conceptualization, Supervision, Funding acquisition, Resources, Writing - review & editing. **E. Michael Thurman:** Conceptualization, Supervision, Funding acquisition, Resources, Writing - review & editing. **Sara Rodríguez-Mozaz:** Writing - review & editing. **Damià Barceló:** Writing - review & editing. **Carlos Escudero-Oñate:** Conceptualization, Writing - original draft, Supervision, Funding acquisition, Resources, Writing - review & editing, Project administration.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2020.141526>.

References

- Al-Ghouti, M.A., Al-Kaabi, M.A., Ashfaq, M.Y., Da'na, D.A., 2019. Produced water characteristics, treatment and reuse: a review. *J. Water Process Eng.* 28, 222–239. <https://doi.org/10.1016/j.jwpe.2019.02.001>.
- Bakke, T., Klungsøyr, J., Sanni, S., 2013. Environmental impacts of produced water and drilling waste discharges from the Norwegian offshore petroleum industry. *Mar. Environ. Res.* 92, 154–169. <https://doi.org/10.1016/j.marenvres.2013.09.012>.
- Brooks, S.J., Escudero-Oñate, C., Gomes, T., Ferrando-Climent, L., 2018. An integrative biological effects assessment of a mine discharge into a Norwegian fjord using field transplanted mussels. *Sci. Total Environ.* 644, 1056–1069. <https://doi.org/10.1016/j.scitotenv.2018.07.058>.
- Brycki, B., Waligórska, M., Szulc, A., 2014. The biodegradation of monomeric and dimeric alkylammonium surfactants. *J. Hazard. Mater.* 280, 797–815. <https://doi.org/10.1016/j.jhazmat.2014.08.021>.
- Chang, H., Li, T., Liu, B., Vidic, R.D., Elimelech, M., Crittenden, J.C., 2019. Potential and implemented membrane-based technologies for the treatment and reuse of flowback and produced water from shale gas and oil plays: a review. *Desalination* 455, 34–57. <https://doi.org/10.1016/j.desal.2019.01.001>.
- Chen, H., Carter, K.E., 2017. Characterization of the chemicals used in hydraulic fracturing fluids for wells located in the Marcellus Shale Play. *J. Environ. Manag.* 200, 312–324. <https://doi.org/10.1016/j.jenvman.2017.05.069>.
- Chen, L.J., Lin, S.Y., Huang, C.C., 1998. Effect of hydrophobic chain length of surfactants on enthalpy-entropy compensation of micellization. *J. Phys. Chem. B* 102, 4350–4356. <https://doi.org/10.1021/jp9804345>.
- EPA, 2012. Study of the Potential Impacts of Hydraulic Fracturing on Drinking Water Resources Progress Report. WWW Document. Environ. Prot. Agency URL. <http://www2.epa.gov/sites/production/files/documents/hf-report20121214.pdf>. (Accessed 1 June 2019).
- Escudero-Oñate, C., Ferrando-Climent, L., 2019. Microalgae for Biodiesel Production and Pharmaceutical Removal from Water. , pp. 1–28 https://doi.org/10.1007/978-3-319-97922-9_1.
- Fakhru'l-Razi, A., Pendashteh, A., Abdullah, L.C., Biak, D.R.A., Madaeni, S.S., Abidin, Z.Z., 2009. Review of technologies for oil and gas produced water treatment. *J. Hazard. Mater.* 170, 530–551. <https://doi.org/10.1016/j.jhazmat.2009.05.044>.
- Ferrando, L., Matamoros, V., 2020. Attenuation of nitrates, antibiotics and pesticides from groundwater using immobilised microalgae-based systems. *Sci. Total Environ.* 703, 134740. <https://doi.org/10.1016/j.scitotenv.2019.134740>.
- Ferrer, I., Furlong, E.T., 2001. Identification of alkyl dimethylbenzylammonium surfactants in water samples by solid-phase extraction followed by ion trap LC/MS and LC/MS/MS. *Environ. Sci. Technol.* 35, 2583–2588. <https://doi.org/10.1021/es001742v>.
- Ferrer, I., Furlong, E.T., 2002. Accelerated solvent extraction followed by on-line solid-phase extraction coupled to ion trap LC/MS/MS for analysis of benzalkonium chlorides in sediment samples. *Anal. Chem.* 74, 1275–1280. <https://doi.org/10.1021/ac010969l>.
- Ferrer, I., Thurman, E.M., 2015. Analysis of hydraulic fracturing additives by LC/Q-TOF-MS. *Anal. Bioanal. Chem.* 407, 6417–6428. <https://doi.org/10.1007/s00216-015-8780-5>.
- Ferrer, I., Thurman, E.M., Zweigenbaum, J.A., 2018. *Auto MS / MS and Identification of Unknowns in Water Samples 5994–0322EN*.
- Gupta, S. kumar, Bux, F., 2019. Application of Microalgae in Wastewater Treatment Volume 1: Domestic and Industrial Wastewater Treatment, Application of Microalgae in Wastewater Treatment. <https://doi.org/10.1007/978-3-030-13909-4>.
- He, L., Lin, F., Li, X., Sui, H., Xu, Z., 2015. Interfacial sciences in unconventional petroleum production: from fundamentals to applications. *Chem. Soc. Rev.*, 5446–5494 <https://doi.org/10.1039/C5CS00102A>.
- Hom-Díaz, A., Jaén-Gil, A., Bello-Laserna, I., Rodríguez-Mozaz, S., Vicent, T., Barceló, D., Blánquez, P., 2017. Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting. *Sci. Total Environ.* 592, 1–11. <https://doi.org/10.1016/j.scitotenv.2017.02.024>.
- Jaén-Gil, A., Hom-Díaz, A., Llorca, M., Vicent, T., Blánquez, P., Barceló, D., Rodríguez-Mozaz, S., 2018. An automated on-line turbulent flow liquid-chromatography technology coupled to a high resolution mass spectrometer LTQ-Orbitrap for suspect screening of antibiotic transformation products during microalgae wastewater treatment. *J. Chromatogr. A* 1568, 57–68. <https://doi.org/10.1016/j.chroma.2018.06.027>.
- Jaén-Gil, A., Buttiglieri, G., Benito, A., Gonzalez-Olmos, R., Barceló, D., Rodríguez-Mozaz, S., 2019a. Metoprolol and metoprolol acid degradation in UV/H2O2 treated wastewaters: an integrated screening approach for the identification of hazardous transformation products. *J. Hazard. Mater.*, 120851 <https://doi.org/10.1016/j.jhazmat.2019.120851>.
- Jaén-Gil, A., Castellet-Rovira, F., Llorca, M., Villagrasa, M., Sarrà, M., Rodríguez-Mozaz, S., Barceló, D., 2019b. Fungal treatment of metoprolol and its recalcitrant metabolite metoprolol acid in hospital wastewater: biotransformation, sorption and ecotoxicological impact. *Water Res.* 152, 171–180. <https://doi.org/10.1016/j.watres.2018.12.054>.
- Jia, X., Jin, D., Li, C., Lu, W., 2019. Characterization and analysis of petrochemical wastewater through particle size distribution, biodegradability, and chemical composition. *Chinese J. Chem. Eng.* 27, 444–451. <https://doi.org/10.1016/j.cjche.2018.04.030>.
- Jiménez, S., Micó, M.M., Arnaldos, M., Medina, F., Contreras, S., 2018. State of the art of produced water treatment. *Chemosphere* 192, 186–208. <https://doi.org/10.1016/j.chemosphere.2017.10.139>.
- Kabra, A.N., Ji, M.K., Choi, J., Kim, J.R., Govindwar, S.P., Jeon, B.H., 2014. Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*. *Environ. Sci. Pollut. Res.*, 12270–12278 <https://doi.org/10.1007/s11356-014-3157-4>.
- Kedar, V., Bhagwat, S.S., 2019. Demulsification of crude oil emulsion by capacitive sensor system measurement: introduction to apparatus and methodology. *J. Dispers. Sci. Technol.* 40, 1777–1784. <https://doi.org/10.1080/01932691.2018.1541415>.
- Khan, A.H., Topp, E., Scott, A., Sumarah, M., Macfie, S.M., Ray, M.B., 2015. Biodegradation of benzalkonium chlorides singly and in mixtures by a *Pseudomonas* sp. isolated from returned activated sludge. *J. Hazard. Mater.* 299, 595–602. <https://doi.org/10.1016/j.jhazmat.2015.07.073>.
- Kiki, C., Rashid, A., Wang, Y., Li, Y., Zeng, Q., Yu, C.P., Sun, Q., 2020. Dissipation of antibiotics by microalgae: kinetics, identification of transformation products and pathways. *J. Hazard. Mater.* 387, 121985. <https://doi.org/10.1016/j.jhazmat.2019.121985>.
- Lester, Y., Ferrer, I., Thurman, E.M., Sitterley, K.A., Korak, J.A., Aiken, G., Linden, K.G., 2015. Characterization of hydraulic fracturing flowback water in Colorado: implications for water treatment. *Sci. Total Environ.* 512–513, 637–644. <https://doi.org/10.1016/j.scitotenv.2015.01.043>.
- Liden, T., Santos, I.C., Hildenbrand, Z.L., Schug, K.A., 2019. Analytical methods for the comprehensive characterization of produced water. *Sep. Sci. Technol. (New York)* 11, 199–217. <https://doi.org/10.1016/B978-0-12-815730-5.00009-0>.
- Liu, Hongwei, Gu, T., Lv, Y., Asif, M., Xiong, F., Zhang, G., Liu, Hongfang, 2017. Corrosion inhibition and anti-bacterial efficacy of benzalkonium chloride in artificial CO₂-saturated oilfield produced water. *Corros. Sci.* 117, 24–34. <https://doi.org/10.1016/j.corsci.2017.01.006>.
- Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Res.* 40, 2799–2815. <https://doi.org/10.1016/j.watres.2006.06.011>.
- Nasiri, M., Jafari, I., Parniankhouy, B., 2017. Oil and gas produced water management: a review of treatment technologies, challenges, and opportunities. *Chem. Eng. Commun.* 204, 990–1005. <https://doi.org/10.1080/00986445.2017.1330747>.
- Patterson, L.A., Kunschik, K.E., Wiseman, H., Fargione, J., Maloney, K.O., Kiesecker, J., Nicot, J.P., Baruch-Mordo, S., Entekin, S., Trainor, A., Sakers, J.E., 2017. Unconventional oil and gas spills: risks, mitigation priorities, and state reporting requirements. *Environ. Sci. Technol.* 51, 2563–2573. <https://doi.org/10.1021/acs.est.6b05749>.
- Pflugmacher, S., Sandermann, H., 1998. Cytochrome P450 monooxygenases for fatty acids and xenobiotics in marine macroalgae. *Plant Physiol.* 117, 123–128. <https://doi.org/10.1104/pp.117.1.123>.
- Picó, Y., Barceló, D., 2015. Transformation products of emerging contaminants in the environment and high-resolution mass spectrometry: a new horizon. *Anal. Bioanal. Chem.* 407, 6257–6273. <https://doi.org/10.1007/s00216-015-8739-6>.
- Pinnock, T., Voordouw, J., Voordouw, G., 2018. Use of carbon steel ball bearings to determine the effect of biocides and corrosion inhibitors on microbiologically influenced corrosion under flow conditions. *Appl. Microbiol. Biotechnol.* 102, 5741–5751. <https://doi.org/10.1007/s00253-018-8974-9>.
- Santos, I.C., Hildenbrand, Z.L., Schug, K.A., 2019. A review of analytical methods for characterizing the potential environmental impacts of unconventional oil and gas development. *Anal. Chem.* 91, 689–703. <https://doi.org/10.1021/acs.analchem.8b04750>.
- Schymanski, E.L., Bataineh, M., Goss, K.U., Brack, W., 2009. Integrated analytical and computer tools for structure elucidation in effect-directed analysis. *TRAC - Trends Anal. Chem.* 28, 550–561. <https://doi.org/10.1016/j.trac.2009.03.001>.
- Sitterley, K.A., Linden, K.G., Ferrer, I., Thurman, E.M., 2020. Desalting and concentration of common hydraulic fracturing fluid additives and their metabolites with solid-phase extraction. *J. Chromatogr. A* 1622, 461094. <https://doi.org/10.1016/j.chroma.2020.461094>.
- Song, C., Wei, Y., Sun, J., Song, Y., Li, S., Kitamura, Y., 2020. Biodegradation and metabolic fate of thiamphenicol via *Chlorella* sp. UTEX1602 and L38. *Bioresour. Technol.* 296, 122320. <https://doi.org/10.1016/j.biortech.2019.122320>.
- Sutherland, D.L., Ralph, P.J., 2019. Microalgal bioremediation of emerging contaminants - opportunities and challenges. *Water Res.* 164, 114921. <https://doi.org/10.1016/j.watres.2019.114921>.
- Tezel, U., Pierson, J.A., Pavlostathis, S.G., 2006. Fate and effect of quaternary ammonium compounds on a mixed methanogenic culture. *Water Res.* 40, 3660–3668. <https://doi.org/10.1016/j.watres.2006.06.019>.
- Thies, F., Backhaus, T., Bossmann, B., Crimme, H., 1996. Xenobiotic biotransformation in unicellular green algae. *Plant Physiol.* 112, 361–370. <https://doi.org/10.1104/pp.112.1.361>.
- Torres Pazmiño, D.E., Winkler, M., Glieder, A., Fraaije, M.W., 2010. Monooxygenases as biocatalysts: classification, mechanistic aspects and biotechnological applications. *J. Biotechnol.* 146, 9–24. <https://doi.org/10.1016/j.jbiotec.2010.01.021>.
- Torres, L., Yadav, O.P., Khan, E., 2016. A review on risk assessment techniques for hydraulic fracturing water and produced water management implemented in onshore

- unconventional oil and gas production. *Sci. Total Environ.* 539, 478–493. <https://doi.org/10.1016/j.scitotenv.2015.09.030>.
- Wu, Q., Zhao, L., Song, R., Ma, A., 2019. Research progress of surfactant biodegradation. *IOP Conf. Ser. Earth Environ. Sci.* 227. <https://doi.org/10.1088/1755-1315/227/5/052023>.
- Xiong, J.Q., Kurade, M.B., Jeon, B.H., 2017. Biodegradation of levofloxacin by an acclimated freshwater microalga, *Chlorella vulgaris*. *Chem. Eng. J.* 313, 1251–1257. <https://doi.org/10.1016/j.cej.2016.11.017>.
- Zhang, C., Tezel, U., Li, K., Liu, D., Ren, R., Du, J., Pavlostathis, S.G., 2011. Evaluation and modeling of benzalkonium chloride inhibition and biodegradation in activated sludge. *Water Res.* 45, 1238–1246. <https://doi.org/10.1016/j.watres.2010.09.037>.
- Zhu, Y., Free, M.L., Woollam, R., Durnie, W., 2017. A review of surfactants as corrosion inhibitors and associated modeling. *Prog. Mater. Sci.* 90, 159–223. <https://doi.org/10.1016/j.pmatsci.2017.07.006>.