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Ultimate and Primary Biodegradation of a Range of Nonpolymeric and Polymeric Surfactants in Seawater

Odd G. Brakstad,[a,*] Antonio Sarno,[a] Roy Geerts,[b] James Dawick,[c] Abel Machado,[d] and Philipp Hopp[e]

[a]Department of Climate and Environment, SINTEF Ocean, Trondheim, Norway
[b]Nouryon Specialty Chemicals B.V., Deventer, The Netherlands
[c]Innospec, Cheshire, UK
[d]Procter & Gamble, Strombeek Bever, Belgium
[e]BASF Personal Care and Nutrition, Monheim, Germany

Abstract: Surfactants are chemicals commonly used in a wide range of domestic and industrial products. In the present study, ultimate biodegradation of 18 surfactants representing different classes (including several polymeric alcohol ethoxylates [AEs]) was determined in seawater at 20 °C by a Closed Bottle test method. After 28 days of incubation, 12 surfactants reached 60% biodegradation and were considered to be readily biodegradable in seawater. The results for the six additional surfactants indicated that the 60% pass level may be reached by extended incubation time, or that reduced biodegradation could be associated with toxicity of the chemicals. All these six surfactants were biodegraded >20% after 28 days, indicative of primary biodegradation in seawater. Polymeric ethoxylates with high numbers of ethylene oxide (EO) groups (40–50 EO groups) were more slowly biodegraded than polyethoxylates with 4 to 23 EO groups. Biodegradation experiments of the AE C12 EO9 (3 to 18 EO groups) in a carousel system at 20 °C with natural seawater and a surfactant concentration of 500 µg/L showed rapid primary biodegradation by targeted analyses of the AE, with >99% primary biodegradation after 2 days of incubation. The surfactant depletion coincided with temporary formation of polyethylene glycols, suggesting that central fission is an important degradation step in seawater. A primary biodegradation experiment in the carousel system with C12 EO9 was conducted in the presence of suspended particulate materials (SPMs; marine phytoplankton and clay particles), showing that the presence of SPMs did not hamper the primary biodegradation of the surfactant. Separation of fractions in 20-µm steel filters indicated some particle association of the surfactant. *Environ Toxicol Chem* 2023;42:1472–1484. © 2023 SETAC

Keywords: Biodegradation; Marine particles; Seawater; Surfactants

INTRODUCTION

There is an increasing concern related to the oceans as a sink for pollutants (Willis et al., 2022). Industrial and domestic chemicals may enter marine waters, either by direct discharges or indirectly via sewage runoffs. Persistent chemicals may lead to continuously increasing contamination and the potential risk of long-term adverse effects on biota (Cousins et al., 2019). Persistence is therefore regarded as a keystone for current and future chemical risk assessments (Matthies et al., 2016; Whale et al., 2021). For instance, under the European Union chemicals legislation Registration, Evaluation, Authorization and Restriction of Chemicals (EU REACH, Regulation No. 1907/2006, [European Council, 2022]), a persistence assessment is required as part of the persistent, bioaccumulative, toxic/very persistent and very bioaccumulative (PBT/vPvB) and persistent, mobile, toxic/very persistent and very mobile (PMT/vPvM) assessment. Substances classified as PBT/vPvB or PMT/vPvM are regarded as of high environmental concern under REACH. (Arp & Hale, 2019; European Chemicals Agency [ECHA], 2017a; Neumann et al., 2015).

For EU REACH, the criteria for persistent or very persistent chemicals in marine environments are defined by half-lives of >60 days in seawater or >180 days in marine sediments (ECHA, 2017a). While EU REACH registration is mandatory for chemicals produced or imported at annual amounts higher than 1 ton, polymeric substances are still exempt from registration; however, the European Commission has proposed to include certain polymers within their scope of registration (Hafer, 2021). For the North Sea and North Atlantic offshore industry, use and discharges of chemicals to the marine environment are regulated according to rules set out by the OSPAR

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* Address correspondence to odd.g.brakstad@sintef.no
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Commission (named after the Oslo and Paris Conventions). The OSPAR commission also aims at identifying PBT chemicals (OSPAR, 2006), although the specific cut-off criteria are slightly different from those used for EU REACH (OSPAR, 2019). In the OSPAR regulation, assessment of biodegradability or persistence is also important as one of several key criteria for identifying chemicals used in offshore oil and gas exploration, and production activities as candidates for priority action and substitution (OSPAR, 2013).

Screening tests for ready biodegradability in freshwater and seawater are described by the Organisation for Economic Co-operation and Development (OECD), with freshwater tests according to the OECD test guidelines 301 and 310 (OECD,1992a, 2014) and biodegradability in seawater according to OECD test guideline 306 (OECD, 1992b). These tests play a central role in understanding the ultimate biodegradation potential of chemicals. In freshwater tests the pass levels for ready biodegradability classification are 70% removal of dissolved organic carbon (DOC) or 60% removal indicated by theoretical oxygen demand (ThOD) or theoretical carbon dioxide ($ThCO_2$) within 28 days of incubation. Substances reaching these levels in the seawater test are deemed as "likely to fulfil the criteria for ready biodegradability" (ECHA, 2017b). If the pass levels are reached within 60 days of incubation, substances are still considered as nonpersistent under EU REACH (ECHA, 2017a). Biodegradation below the pass levels after 60 days suggests only partial degradation and potential persistency of a substance; however, this may be further assessed by higher-tier biodegradation simulation tests in the most relevant environmental compartment/media (ECHA, 2017a). Results from the seawater test are further deemed as indicative of primary biodegradation if ThOD or DOC removal is >20% (OECD, 2006).

Surfactants are used in large quantities in domestic and industrial products serving a wide array of use applications in different market sectors. This group of chemicals contain a hydrophilic head and a hydrophobic tail, and they are used for lowering surface or interfacial tensions between immiscible liquids, between gases and liquids, or between liquids and solids. Surfactants are separated into four classes, depending on the properties of the hydrophilic head: anionic, cationic, nonionic, and amphoteric surfactants (Ketola, 2016). The global use of surfactants in industrial and domestic sectors has grown from 1.7 million tons in 1984 to 15.9 million tons in 2014, and with expected use of 34 million tons in 2022 (Aboulhassan et al., 2006; Palmer & Hatley, 2018). The major quantities of these products, which often have a "down the drain" route of disposal, are handled and removed in wastewater treatment plant processes with typical efficiencies of 90%–99%, partly depending on class of surfactants (González et al., 2007; Mungray & Kumar, 2008). Despite efficient wastewater treatments, small fractions of surfactants may reach freshwater and eventually end up in the marine environment. Surfactants used in the offshore oil and gas industry, for instance as corrosion inhibitors, biocides, demulsifiers, and as chemicals in enhanced oil recovery (cEOR) or oil spill dispersants, also contribute to the input of these chemicals to marine water.

Many surfactants have been tested for ready biodegradability according to standard OECD screening test guidelines (e.g., HERA, 2009; Madsen et al., 2001). A review of marine biodegradation of 65 surfactants showed mineralization half-lives of <1–50 days (Jackson et al., 2016). Most of the tests ($n = 37$) were performed on linear alkylbenzene sulfonates, and there is still a lack of systematic biodegradation data across various types of surfactants in the marine environment. Only a few studies have been performed to investigate seawater biodegradability of polymeric surfactants, such as the nonionic ethoxylated and/or propoxylated aliphatic alcohols (and their anionic sulfated derivatives like alkyl ether sulfates; Jackson et al., 2016; Pérez-Carrera et al., 2010; Traverso-Soto et al., 2013; Vashon & Schwab, 1982).

Different from nonpolymeric surfactants with short defined hydrophilic head groups, the polymeric surfactants also contain hydrophilic entities of repetitive units (e.g., ethylene oxide), resulting in homologues of variable chain lengths (Matsuoka, 2015). The biodegradation pathways of alcohol ethoxylates (AEs) under aerobic conditions in freshwater have been reported to proceed by three mechanisms: (a) step-wise exo-cleavage of ether linkages and sequential removal of ethylene glycol (C_2) units from linear AEs, (b) cleavage of the alkyl-ether bond (central fission) with separation of the hydrophobic alkyl and hydrophilic polyethylene glycol (PEG) moieties, and (c) ω/β-oxidation with generation of a terminal alcohol and a carboxylic acid in the alkyl-chain (ω-oxidation), followed by β-oxidation and formation of acetyl CoA (Scott & Jones, 2000; White et al., 1996; Wu et al., 2019). The central fission mechanism is important for the subsequent biodegradation process because both PEG and aliphatic alcohol biodegradation are reported to occur rapidly (Bernhard et al., 2008; Brakstad, Farooq, et al., 2018), but has so far not been reported in seawater.

Different sorption processes, including adsorption, absorption, and other solute-aggregate interactions to marine particles, may be important for the fate of chemicals. Surfactants are known to adsorb fast to surfaces of silica and clay materials (Atkin et al., 2000; Sánchez-Martín et al., 2008). Surfactant-particle associations may involve complex processes and differ between surfactant groups (Sánchez-Martín et al., 2008). Sorption mechanisms may include both hydrophobic properties and polar interactions with the particle surfaces, and saturated surfaces may result in bilayers of adsorbed and dissolved surfactants (Droge et al., 2009). Compared with freshwater, seawater ions alter repulsive and attraction forces between particles and molecules by neutralizing surface charges (Gan & Liu, 2008), which may aid the attachment of certain surfactants to marine particles. In a study of AE sorption to marine sediments, adsorption and bilayer formation to mineral surfaces dominated the sorption behavior of most AE homologues (Droge et al., 2009). Particle sorption processes may result in seabed sedimentation and burial processes, and subsequent reduction of depletion processes like biodegradation. Assessing and investigating the biodegradation behavior of chemical sorption to suspended particulate materials (SPMs) in the marine water column is therefore important

to understand the ultimate fate and potential for seabed sedimentation.

The present study aimed to assess the ultimate biodegradability in seawater across four different classes of surfactants using the OECD 306 methodology. Several of the tested surfactants represented polymeric AEs, for which there is limited information on biodegradation in seawater. Our study further aimed to verify if aerobic biodegradation mechanisms in seawater are similar to reported mechanisms in freshwater for a polymeric AE, and if surfactant biodegradation behavior in seawater was affected by the presence of suspended marine particles.

METHODOLOGY

Surfactants

A total of 18 chemicals were included in the present study, including amphoteric ($n = 1$), anionic ($n = 5$), nonionic ($n = 9$), and cationic ($n = 3$) surfactants (Table 1). Of these, 11 surfactants were polymeric AEs with ≥2.5 moles of EO groups (Table 1). The surfactants were supplied by Innospec, BASF, Sasol Germany, Shell Chemical, or were purchased from Sigma-Aldrich (Table 1). Some of the substances contained impurities, including cocoamidopropyl betaine (1.5% free fatty acids, 0.7% sodium glycolate, 20 ppm sodium monochloroacetate), C8 EO5 C (5%–10% water, 1.5% ethoxylated octan-1-ol), C12 EO11 C (10-15% ethoxylated C10-C16 alcohols), and C10 EO8 (<6 ppm EO monomer). The surfactants were stored as recommended by the suppliers, either at room temperature or at 4–5 °C.

Seawater

Natural seawater used as inoculum was provided as subsurface seawater from 80 m depth from a local Norwegian fjord (Trondheimsfjord), outside the harbor of Trondheim (63.445°N, 10.379°E). The subsurface seawater was supplied to the laboratories of SINTEF Ocean through a pipeline system passing a sand filter for removal of coarse particles. The pipeline inlet well is below the thermocline, securing a stable temperature independent of season. The subsurface seawater held an average salinity of 34 PSU, was 100% saturated with oxygen (~10 mg/ dissolved oxygen) and had a temperature of 6-8°C. In addition, an experiment with a surfactant and marine particles was performed with natural surface seawater. The surface seawater was collected from the harbor area in Trondheim, in an area not considered to be affected by effluents from the local river (Nidelva; 63.444°N, 10.416°E). Mineral nutrient analyses of subsurface seawater and surface seawater are shown in Supporting Information, Table S1.

Ultimate biodegradation screening tests

Ultimate biodegradation screening tests were performed in subsurface seawater using the Closed Bottle test method in accordance with OECD guideline 306 (OECD, 1992b). All surfactants described in Table 1 were tested. Subsurface seawater used for the screening tests were collected in 10-L polypropylene screw-capped carboys and stored in the dark at 20 °C for 5–7 days. Measured concentrations of dissolved oxygen were never reduced by more than 15% during aging (results not shown), and additional aeration was therefore not included during the acclimation period. At the end of the acclimation, the subsurface seawater was aerated for 20–30 min by blowing sterile filtered (0.22 µm) air through the seawater. Aerated subsurface seawater was supplemented with 1 ml/L seawater of each of four different mineral nutrient solutions, as described in OECD guideline 306 (OECD, 1992b). After aeration and nutrient amendment, the average concentrations of dissolved oxygen in the subsurface seawater for the different tests were measured to be 7.2 ± 0.1 mg/L in the seawater blanks. Concentrations of colony-forming units (CFUs) were determined in the subsurface seawater before and after acclimation by inoculating 10 µl subsurface seawater on the surfaces of Difco Marine Agar 2216 (BD Biosciences) dishes (triplicate). The agar dishes were incubated at 20 ± 2 °C for 6–7 days before surface colonies were counted.

Test substances (prepared in stock solutions of 1000 mg/L of active ingredient [a.i.] in deionized water) were applied in final concentrations of 2 mg a.i./L in aged, aerated, and nutrient-amended subsurface seawater (OECD, 1992b). The test solutions were then distributed into 275-ml capped flasks without remaining headspace or air bubbles for biological oxygen demand (BOD) determination. The flasks were incubated static at 20 ± 2 °C for up to 64 or 75 days. Flasks were sacrificed (triplicate) for analyses at days 7, 14, 21, 28, and 64/75 days of the incubation period, while one replicate was used for day 0 analyses. Sodium benzoate (CAS no. 532-32-1) was used as positive control reference substance (2 mg/L in aged, aerated, and nutrient-amended subsurface seawater). Blank solutions of aged, aerated, and nutrient-amended subsurface seawater without test or reference substances were included for analyses of dissolved oxygen. Some flasks with test solutions were poisoned with a biocide (50 mg/L $HgCl_2$) and incubated to determine the abiotic degradation of test substances. Inhibition tests (toxicity control) were performed in which the reference substance (sodium benzoate) was mixed with test substance (2 mg/L sodium benzoate + 2 mg/L of each test substance) in aged, aerated, and nutrient-amended subsurface seawater and incubated as described above.

Dissolved oxygen analyses were performed with a dissolved oxygen probe (YSI BOD probe; YSI) connected to a dissolved oxygen meter (YSI). BOD and ThOD were determined, as described in the OECD guideline 306 (OECD, 1992b). For all surfacants, $ThOD_{NH3}$ was determined. Because oxygen consumption by nitrification may be assumed for nitrogen (N)-containing compounds (Hurwitz et al., 1947), $ThOD_{NO3}$ was also determined for the N-containing betaines and quaternary ammonium compounds (QAC). Structural formulas and molecular weights of ethoxylated surfactants were calculated using the mean average mol EO values, provided by the suppliers (Table 1 and Supporting Information, Table S2).

TABLE 1: Surfactants included in the present study

#	Class	Subclass	CAS no.	Name[a]	C-chain	EO	Salt	a.i. (%)	Supplier
1	Amphoterics	Betaines	61789-40-0	Cocoamidopropyl betaine	12-14	–	Sodium	30-32[b]	Innospec
2	Anionic	Alkyl sulfates	151-21-3	Sodium dodecyl sulfate	12	–	Sodium	100	Sigma-Aldrich
3	Anionic	Alkylether sulfates	68891-38-3	Sodium salt of alkyl ether sulfate C12-14 with EO (C12-14 EO4 S)	12–14	4	Sodium	70	BASF
4	Anionic	Alkylether carboxylates	53563-70-5	2-(2-(octyloxy)ethoxy)-acetic acid (C8 EO5 C)	8	5	Acid	60-100	Innospec
5	Anionic	Alkylether carboxylates	27306-90-7	2-(2-(dodecyloxy)ethoxy)-acetic acid (C12 EO5 C)	12	5	Acid	60-100	Innospec
6	Anionic	Alkylether carboxylates	27306-90-7	2-(2-(dodecyloxy)ethoxy)-acetic acid (C12 EO11 C)	12	11	Acid	60-100	Innospec
7	Nonionic	Ethoxylates	9002-92-0	Dodecan-1-ol, ethoxylated (≥2.5moles EO; C12 EO5)	12	5	–	90-100	Sasol
8	Nonionic	Ethoxylates	9002-92-0	Dodecan-1-ol, ethoxylated (≥2.5 moles EO; C12 EO9)	12	9	–	90-100	Sasol
9	Nonionics	Ethoxylates	9002-92-0	Poly(oxy-1,2-ethanediyl), α-dodecyl-ω-hydroxy- (C12 EO23)	12	23	–	100	Sigma-Aldrich
10	Nonionics	Ethoxylates	9002-92-0	Dodecanol, ethoxylated (>20 EO; C12 EO40)	12	40	–	100	Sasol
11	Nonionics	Ethoxylates	68439-46-3	C9-C11 Alcohol ethoxylate (C10 EO8)	10	8	–	100	Shell
12	Nonionic	Ethoxylates	69011-36-5	Poly(oxy-1,2-ethanediyl), alpha.-tridecyl-.omega.-hydroxy-.branched (C13 EO18)	13	18	–	100	BASF
13	Nonionic	Ethoxylates	68439-49-6	Alcohols, C16-18, ethoxylated (C16-18 EO50)	16-18	50	–	100	BASF
14	Nonionic	Alkyl polyglucosides	59122-55-3	n-Dodecyl β-d-glucopyranoside	12	–	–	100	Sigma-Aldrich
15	Nonionic	Alkyl polyglucosides	69227-93-6	n-Dodecyl β-d-maltoside	12	–	–	100	Sigma-Aldrich
16	Cationic	QAC[c], trimethyl	112-00-5	Dodecyltrimethylammonium chloride	12	–	–	100	Sigma-Aldrich
17	Cationic	QAC[c], trimethyl	112-02-7	Hexadecyltrimethylammonium chloride	16	–	–	100	Sigma-Aldrich
18	Cationic	QAC[c]	68391-01-5	*N*-Benzyl-*N*,*N*-dimethyltetradecan-1-aminium chloride (BAC)[d]	14	–	–	100	Sigma-Aldrich

[a]Abbreviations of ethoxylates used in the study are shown.
[b]Diluted in water.
[c]Quaternary ammonium compound.
[d]Abbreviation used in the study.
The surfactants were classified in classes and subclasses. The numbers of carbons in the alkyl chains (C-chain), average numbers of EO (EO), and the salts are included as information provided by the suppliers. The percentage active ingredient (a.i.) in the products is shown. Several of the surfactants have been given abbreviations in brackets for simplicity.

The $ThOD_{NH3}$ of selected AEs were also calculated from the fractional distribution of homologues, obtained by chemical analyses, according to the following formula:

$$ThOD_{mix} = 1/\sum\left(\frac{f_i}{ThODi}\right) \quad (1)$$

where f_i is the fractional distribution homologue i in the mixture and *ThODi* is the $ThOD_{NH3}$ of homologue i.

Abiotic degradation of the test substances was identified as the percentage of OD reduction in poisoned seawater from day 0 to days 64/75. Toxicity was assessed as the percentage of reduction in BOD by the mixture of the reference and test substances compared with the sum of the BOD of the separate solutions of the two substances.

Primary biodegradation experiments

Primary biodegradation of the nonionic polymeric AE C12 EO9 (surfactant #8, Table 1) was experimentally determined by target-specific chemical analyses. This chemical represented "an average" of the polymeric ethoxylated surfactants with respect to the numbers of aliphatic carbons and EO groups. The experiment was performed in a continuously rotating carousel test system, as previously described for oil biodegradation experiments (Brakstad et al., 2015). The system consisted of 2-L flasks (Schott) mounted on wheels which were provided with a gear motor (SEW Eurodrive) for rotation around the carousel axis (see Supporting Information, Figure S1). Each carousel contained two wheels with a total of 16 flasks. The flasks were baked (450 °C, 3 h), thoroughly rinsed with Milli-Q water and autoclaved (121 °C, 20 min) before use. The flasks were rinsed with a mixture of methanol and isopropanol (50% of each) to prevent surfactants from attaching to the glass walls, as previously reported (Brakstad, Størseth, et al., 2018; Place et al., 2016). The solvent was evaporated before surfactant solutions were applied.

C12 EO9 was applied in subsurface seawater to the flasks at a final nominal concentration of 500 µg/L in triplicate. No nutrient amendment or aging of the subsurface seawater was performed. Sterilized controls were prepared by adding 100 mg/L $HgCl_2$ (triplicate), while blank solutions (duplicate) contained subsurface seawater only. The flasks were mounted on carousels with a headspace of 300 ml to secure turbulence in the systems and were incubated at 20 °C with slow rotation (0.75 rpm) for 14 days. Samples (50 ml) were collected from all flasks after 0, 2, 4, 7, and 14 days for LC-MS/MS analyses. In addition, a primary biodegradation experiment was performed with the high-molecular AE C16-18 EO50 (surfactant #13, Table 1) at the same conditions as for C12 EO9.

An experiment with the surfactant C12 EO9 was also performed with surface seawater in the presence of algae and/or mineral particles. The marine diatom *Skeletonema pseudocostatum* NIVA-BAC (NIVA Culture Collection), with reported cell length of 2–61 µm and diameter of 2–21 µm (http://nordicmicroalgae.org), was used in the experiment. The algal culture was grown at 20 °C for 10 days in a seawater-based medium (International Organisation for Standardisation, 2016). Algal growth was monitored by cell counting in a Bürker haemocytometer, using light microscopy at 400× magnification. The haemocytometer results were used to inoculate the phytoplankton strain at a final concentration of 1×10^4 diatom cells/ml in the experiment. Kaolin ($Al_2Si_2O_5(OH)_4$; Sigma-Aldrich) was used as mineral particles with a reported median size of 18.9 (±15.3) µm, and a density of 2.65 g/cm^3, according to information from the supplier. The mineral particles were used in a final concentration of 5 mg/L. The surfactant was applied at a final concentration of 500 µg/L to surface seawater in 2-L flasks (washed and treated with solvents as described above) with (i) phytoplankton, (ii) mineral particle, (iii) mixtures of phytoplankton and mineral particle, or (iv) only surface seawater. The different treatments were also tested with surface seawater sterilized with $HgCl_2$ (50 mg/L final concentration). Blanks included the different treatments without surfactant. All flasks were mounted on carousels with a headspace (300 ml) and incubated at 20 °C for 64 days. Samples of 100 ml were collected for analyses after 0, 7, 14, 28, and 64 days. Each sample was filtered through a steel filter with mesh size of 20 µm (Teichhansel Teichshop/Siebgewebeshop) to separate water (filtrates) and particles with sizes >20 µm. Both water and filter samples were stored at −20 °C until extraction and analyses.

Extraction and chemical analyses

Liquid–liquid extraction of seawater samples was performed by the solvent acetate. The steel filters were freeze-dried, weighed, and placed in 5-ml extraction solutions of methanol/ethyl acetate/water (78/20/2). The solutions with the filter were then sonicated in an ultrasound bath at 40 kHz frequency at room temperature (Bransonic Ultrasonic Cleaner, model 1210E-MT) for 5 min, placed in a new extraction solution (5 ml), and sonicated again. The filters were then removed from the solution, freeze-dried, and weighed. The tubes with desorbed material were sonicated (15 min) in the ultrasound bath and pelleted by drying with N_2 at room temperature. The sample was then resuspended in 500 µl of acetonitrile (50%), and the material from the same sample was pooled and analyzed.

Targeted LC-MS/MS analyses of C12 EO9 and C16-18 EO50, and their PEGylated biodegradation products, were performed with an Agilent 1290 HPLC coupled to a 4670 triple quadrupole mass spectrometer equipped with an electrospray ion source. High performance liquid chromatography separation for all analyses was achieved with a fused core Ascentis Express AQ-C18 column (2.1 × 150 mm, 2.7 µm particle size; Supelco) kept at 30 °C using 10-µl injection volume and a flow rate of 300 µl/min. The mobile phase consisted of 10 mM ammonium formate containing 0.1% formic acid (A) and 90% acetonitrile 10% water in 10 mM ammonium formate containing 0.1% formic acid (B). To avoid contaminants from entering the MS ion source, the first 5 minutes of each run were diverted to waste. The mass spectrometry analyses were performed using positive ionization in multiple reaction monitoring

mode. The electrospray source parameters were as follows for all compounds: curtain gas temperature 250 °C, curtain gas glow 4 L/min, nebulizer pressure 60 psi, sheath gas temperature 200 °C, sheath gas flow 6 L/min, capillary voltage 2000 V, and nozzle voltage 500 V. For C12 EO9, the $[M+NH_4]^+$ precursor ions used were 336.3, 380.3, 424.4, 468.4, 512.5, 556.5, 600.6, 644.6, 688.6, 732.6, 776.7, 820.7, 864.7, 908.8, 952.8, and 996.8 *m/z* for EO3 to EO18, respectively. The same product ions were used for all precursor ions: 89.1 (CE 25) and 133.1 (CE 20). For C16-18 EO50, $[M+NH_4]^{4+}$ precursor ions were used with the same product ions as for C12 EO9. For PEG degradation products, $[M+H^+]^+$ and $[M+NH_4^+]^+$ to $[M+NH_4^+]^{3+}$ precursor ions were used. The same product ions were used for all precursor ions: 177.1 (CE 25) and 133.1 (CE 20). External calibration curves of C12 EO9 and PEG 400 (0.5–1000 µg/L) were established for quantifications of surfactant and PEG metabolites, respectively.

A recovery test was performed by direct spiking of C12 EO9 samples (100 ng/ml), filtering the samples, and treating as described above. The recovery was 103.7% of the expected concentration of C12 EO9.

Statistical analyses

Statistical analyses were performed as paired or unpaired *t*-tests using the "Analysis" module of GraphPad Prism version 9.2.0 (GraphPad Software). Variances were determined from standard deviations (SD^2), calculated as the average of squared deviations from the mean.

RESULTS AND DISCUSSION

Structural variations of AEs

The ethoxylate surfactants tested in the present study are complex mixtures composed of homologues with structural variations (different EO numbers). Composition profiles and homologue distributions were demonstrated with two nonionic AEs by LC-MS/MS analyses (Supporting Information, Figure S2). A chromatogram of the AE surfactant C12 EO9 (surfactant #8; Table 1) with a reported mean average ethoxylate distribution profile of 9 mol EO showed detectable homologues with three to 18 EO groups and eight to nine EO groups with the highest normalized signal intensities (Supporting Information, Figure S2A). The AE surfactant C16-18 EO50 (surfactant #13; Table 1), with a reported mean average ethoxylate distribution of 50 mol EO, showed presence of homologues with 47–65 EO groups, and C16 to C18 alkyl chains (Supporting Information, Figure S2B). This variation in structures and molecular weights could have implications when interpreting calculations of ThODs. Calculations of ThODs showed small variations between the homologues. Based on their fractional distributions (Equation 1) determined by LC-MS/MS analyses (Supporting Information, Figure S2), the average ThOD (±SD) of C12-EO9 was calculated to be 2.27 ± 0.14 mg O_2/mg test substance (variance $0.022 \pm 1.0\%$ of the average) and 1.94 ± 0.4 mg O_2/mg test substance (variance $0.0004 \pm 0.02\%$ of the average) for C16-18-EO50. These ThOD values therefore represented the fractionally abundant homologues of these surfactants. The calculated variances showed low dispersions of homologue ThODs. The ThOD values for C12 EO9 and C16-18 EO50 calculated from information given by the suppliers were 2.23 mg O_2/mg test substance and 1.95 mg O2/mg test substance, respectively (Supporting Information, Table S2). These data deviated from the values determined from fractional distributions by only 1.7% (C12 EO9) and 0.5% (C16-18 EO50). The structural variations of the ethoxylated homologues could therefore be neglected for ThOD calculations, and ThODs used to calculate the biodegradation percentages were determined from the information of average EO groups given by the suppliers of the chemicals (Supporting Information, Table S2).

Ultimate biodegradation of the surfactants

The ultimate biodegradation testing of the various surfactants using OECD guideline 306 consisted of four separate tests, performed from January 2020 to January 2021 (see Supporting Information, Table S2). One of the four subsurface seawater samples used for testing was incubated for 6 days for determination of bacterial concentrations, resulting in 167 ± 58 CFU/ml. Bacterial concentrations in the three other subsurface seawater samples were incubated for 7 days, resulting in concentrations ranging from 433 ± 115 to 2100 ± 265 CFU/ml. Thus, the concentration of viable heterotrophic bacteria in the collected subsurface seawater was relatively low and could also have been affected by incubation time. In comparison, data from a recent interlaboratory ring-test of the OECD 306 test showed CFU concentrations in the raw seawaters used by the participating laboratories ranging between 480 and 82 000 CFU/ml (Ott, Martin, Acharya, et al., 2020). Seawater acclimation at 20 °C (7 days) resulted in CFU concentrations ranging from 2400 ± 10 to 9333 ± 569 CFU/ml. Comparison of CFU concentrations in the original and acclimated subsurface seawater samples showed 1.4-fold (April 2020) to 56-fold (June 2020) increases during the acclimation period. In the OECD 306 ring-test counts, CFU changes in seawater during the acclimation periods ranged between 0.2- (CFU reductions) to 142-fold increases for the different participating laboratories (Ott, Martin, Acharya, et al., 2020). In a recent study, changes of the bacterial concentrations in seawater during temperature acclimation for the OECD 306 test were related both to seawater depths and season (Wennberg et al., 2022).

The OECD 306 test has been criticized because of the low concentrations of competent bacteria compared with the freshwater ready biodegradability tests, which use activated sludge or sewage effluents as inoculum (OECD, 1992a, 1992b, 2014; Ott, Martin, Snape, et al., 2020; Wennberg et al., 2022). Higher inoculum concentrations achieved by tangential flow filtration resulted in reduced lag-periods and shorter half-lives of three reference substances (aniline, 4-fluorophenol, and 4-dichlorophenol) when compared with the standard OECD 306 test (Ott, Martin, Snape, et al., 2020). The inoculum effect on

biodegradation of two surfactants (C12 EO9 and BAC; see Table 1) was compared in our study by filtering subsurface seawater through aquarium filters, obtaining 13 ± 7 times higher inoculum after acclimation (Supporting Information, Figure S3). When BOD results with "low" and "high" inocula (Supporting Information, Figure S3) were pairwise compared for C12 EO9 after 28 and 64 days of incubation by paired *t*-test, the BOD results were comparable ($p = 0.2349$). No significant inoculum effect was therefore determined for these two surfactants.

The dissolved oxygen depletions in the aged and nutrient-amended subsurface seawater (blanks without test substance) after 28 days of incubation ranged between 3.9% and 11.1%, and between 10.5% and 16.2% after 64 and 75 days of incubation, respectively. The subsurface seawater blank respiration and the biodegradation of the reference substance were within the validity criteria of the OECD 306 test (<30% seawater blank respiration after 28 days, 60.9%–77.3% biodegradation of reference substance sodium benzoate [as average of three replicates in each test; OECD, 1992b]). Abiotic dissolved oxygen depletions were <1.0% in all the sterilized controls poisoned with $HgCl_2$ (Table 2).

The results from the ultimate biodegradation testing of the 18 surfactants after 28 days and at the termination of the tests are shown in Table 2. Biodegradation by time is shown in Supporting Information, Figure S4. Twelve of the tested surfactants showed biodegradation above 60% after 28 days, including the betaine (both by $ThOD_{NH3}$ and $ThOD_{NO3}$ determinations), the alkyl sulfate, three of four tested alkyl ether sulfates, five of the seven tested AEs, one of two tested alkyl polyglucosides, and one of three tested QACs (both by $ThOD_{NH3}$ and $ThOD_{NO3}$ determinations). We therefore considered these 12 surfactants to be readily biodegradable in seawater (ECHA, 2017b). Six surfactants showed <60% biodegradation after 28 days (Table 2), including one alkylether carboxylate, two high molecular weight AEs, one alkyl polyglucoside, and two QACs.

Three ethoxylated surfactants did not reach 60% biodegradation after 28 days, the high-molecular AEs C12 EO40 and C16-18 EO50, and the alkylether carboxylate C12 EO11 C (Table 2 and Supporting Information, Figure S4), although >60% biodegradation was reached for C12 EO40 after 64 days of incubation. Biodegradation of >60% demonstrate ultimate biodegradation in seawater and may also be reached for C16-18 EO50 and C12 EO11 C with incubation times extending the 64 days test period, because biodegradation of these surfactants reached 56.8 ± 1.9% (C16-18 EO50) and 57.7 ± 0.2% (C12 EO11 C) at the end of the test (Table 2 and Supporting Information, Figure S4), which is close to the 60% threshold level. None of these surfactants were associated with reference substance inhibition (Table 2). Mineralization of both C16-18 EO50 and C12 EO11 C is also concluded based on the expected biodegradation pathways, which do not form persistent degradation products. The proposed pathways for AEs in freshwater described in the introduction are also applicable for the C12 EO11 C. Mineralization of C12 EO11 C, however, also involves the removal of the carboxylate end group, which is removed in a step that removes one glycol unit together with the carboxylate end group. Glyoxylic acid and glycol are subsequently liberated by cleavage of the ether bond (Van Ginkel, 1996).

TABLE 2: Percentage ultimate biodegradation ($ThOD_{NH3}$ or $ThOD_{NO3}$) of the surfactants after 28 days of incubation, and at the termination of the tests (64 or 75 days)

#	Name	$ThOD_{NH3}$ (%)		$ThOD_{NO3}$ (%)		Biodegradability	Abiotic test[a] (%)	Inhibition test[b] (%)
		28 days	64/75 days	28 days	64/75 days			
1	Cocoamidopropyl betaine	72.3 ± 0.8	107.0 ± 2.9	62.6 ± 0.7	92.6 ± 2.5	RB	<1.0	<1.0
2	Sodium dodecyl sulfate	68.1 ± 4.0	73.3 ± 2.5			RB	<1.0	<1.0
3	C12-14 EO4 S	65.1 ± 0.2	69.1 ± 1.8			RB	<1.0	7.4
4	C8 EO5 C	65.1 ± 0.7	68.4 ± 1.8			RB	<1.0	<1.0
5	C12 EO5 C	64.1 ± 2.7	68.0 ± 1.4			RB	<1.0	<1.0
6	C12 EO11 C	55.4[c]	57.7 ± 0.2				<1.0	<1.0
7	C12 EO5	69.1 ± 3.2	87.7 ± 4.3			RB	<1.0	<1.0
8	C12 EO9	69.7[c]	65.4[c]			RB	<1.0	<1.0
9	C12 EO23	63.6 ± 0.8	67.8 ± 0.8			RB	<1.0	<1.0
10	C12 EO40	23.2[c]	65.9 ± 2.2				<1.0	<1.0
11	C10 EO8	65.9 ± 0.2	64.0 ± 1.3			RB	<1.0	<1.0
12	C13 EO18	61.2 ± 1.5	70.5 ± 4.0			RB	<1.0	<1.0
13	C16-18 EO50	26.1 ± 2.9	56.8 ± 1.9				<1.0	<1.0
14	n-Dodecyl β-d-glucopyranoside	54.6 ± 1.4	52.2 ± 1.6				<1.0	11.0
15	n-Dodecyl β-D-maltoside	73.6 ± 2.1	64.2 ± 3.4			RB	<1.0	<1.0
16	Dodecyltrimethylammonium chloride	73.1 ± 0.1	64.5 ± 1.7	67.8 ± 0.1	59.9 ± 1.6	RB	<1.0	<1.0
17	Hexadecyltrimethylammonium chloride	45.6 ± 2.8	48.4 ± 0.3	43.0 ± 2.6	45.7 ± 7.8		<1.0	14.0
18	BAC	27.7[c]	34.0[c]	26.4[c]	32.3[c]		<1.0	31.6

[a]Data for abiotic tests show degradation in seawater sterilized by $HgCl_2$.
[b]Inhibition of the reference substrate (sodium benzoate) caused by the test substance.
[c]Two replicates included.
The results are shown as mean ± SD. $ThOD_{NO3}$ was determined only for the N-containing substances. Surfactants showing >60% biodegradation within 28 days in the subsurface seawater are described as "likely to be readily biodegradable in seawater" (RB). Results of abiotic tests and inhibition tests are also shown. For full names of surfactants with abbreviations, see Table 1. The results are shown as mean ± SD of three replicates, except when noted.

The temporal relations between mean average numbers of EO groups and biodegradation of polyethoxylated surfactants, including both alkylether carboxylates and AEs, are shown in Figure 1. Comparison of biodegradation between surfactant groups with four to 23 EO groups and the high-molecular groups with 40 and to 50 EO groups showed larger differences in biodegradation after 7–28 days than after 64 days (Supporting Information, Figure S5). The differences in biodegradation therefore decreased at the end of the tests, confirming that degradation was only delayed for the high-molecular weight polyethoxylated AEs, although 60% biodegradation was still not reached after 64 days for C16-18 EO50. The average molecular weights (MWs) of the polyethoxylated compounds with delayed biodegradation in our study (C12 EO40 and C16-18 EO50) were 1948 and 2445 Da (Supporting Information, Table S2). Reduced biodegradation rates for substrates with increasing MWs between 2000 and ≥4500 Da were reported by Bernard et al. (2008), when studying polyethoxylated compounds with different MWs (250–58 000 Da) in artificial seawater as DOC removal. Biodegradation rates were therefore reduced in relation to increased numbers of AEs in their study (Bernhard et al., 2008). In large AEs, the molecules may be inaccessible for the central fission mechanism reported to result in fast biodegradation (Bernhard et al., 2008), and these molecules may more likely be biodegraded by the slower and stepwise terminal degradation processes (White et al., 1996).

The polyglucoside n-dodecyl-β-d-glucopyranoside did not reach 60% ultimate biodegradation after 64 days (Table 2 and Supporting Information, Figure S4), although biodegradation after 28 days (54.6 ± 1.4%) nearly reached this pass level for readily biodegradability. The closely related compound dodecyl-β-d-maltoside was considered readily biodegradable in seawater in these studies (Table 2), and polyglucosides are in general reported to be readily biodegradable in freshwater screening tests (Jurado et al., 2011, 2012; Madsen et al., 2001). The toxicity test also indicated some inhibition of the reference substance (sodium benzoate) caused by dodecyl-β-d-glucopyranoside (Table 2), and ultimate biodegradation may therefore be achieved at lower test concentrations than used in our study (2 mg/L). Based on data from this and other studies of ultimate biodegradation, polyglucosides may therefore be expected to be biodegradable and not persistent in seawater (Willing et al., 2004).

While the C12-alkylated QAC dodecyltrimethylammonium chloride could be considered as readily biodegradable, the C16-alkylated hexadecyltrimethylammonium chloride and the benzylated BAC showed <60% biodegradation at the end of the tests, and without increased degradation between day 28 and test termination (Table 2 and Supporting Information, Figure S4).

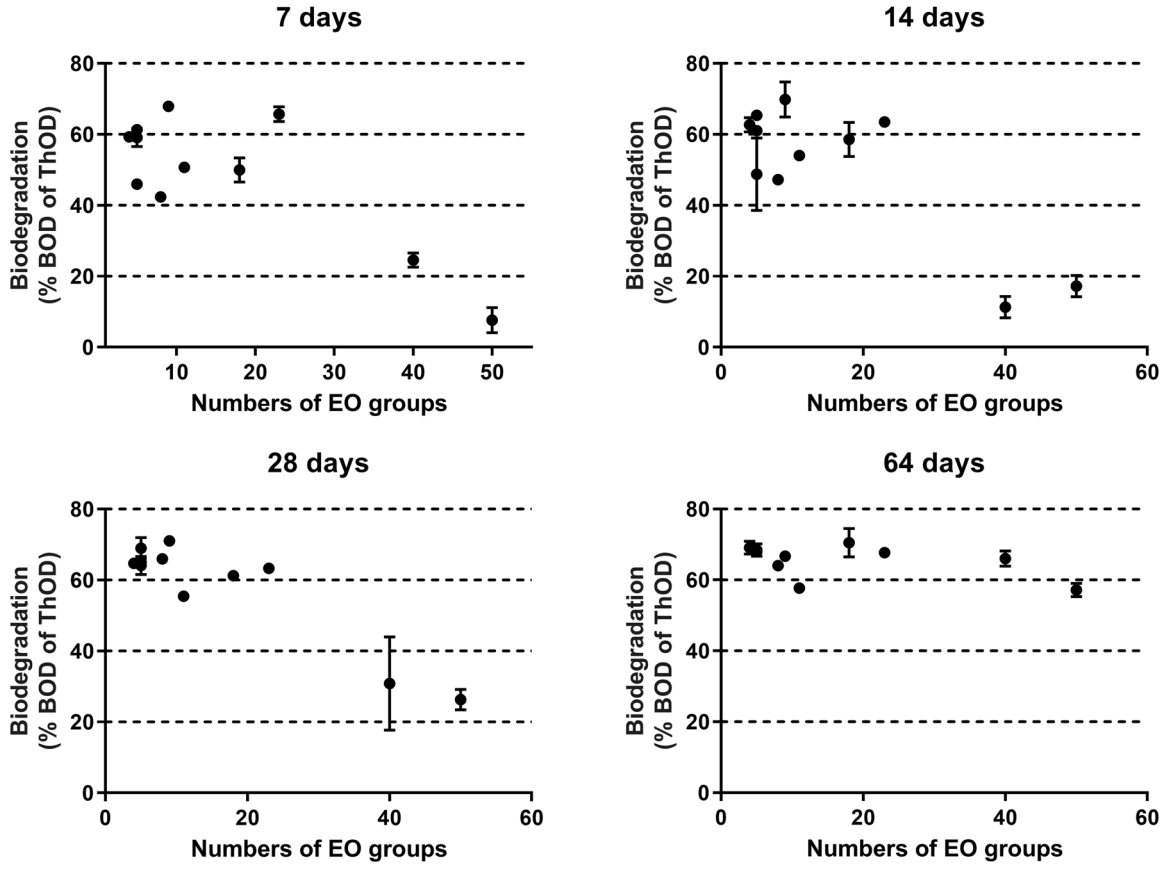

FIGURE 1: Biodegradation of polymeric surfactants related to numbers of ethylene oxide (EO) groups as provided by the suppliers of the chemicals. The results (average ± SD) are shown after incubation for 7, 14, 28, and 64 days. BOD = biological oxygen demand.

Toxicity tests further showed reference substance inhibition associated with hexadecyltrimethylammonium chloride and BAC, but not with dodecyltrimethylammonium chloride (Table 2). Biodegradation of QACs have shown variable results (Madsen et al., 2001), and these substances are often used as biocides. Thus, toxicity might hamper the biodegradation by reducing the bacterial activities in the test vessels. Studies with the QAC cetylpyridinium chloride showed inhibition of the OECD 310 headspace biodegradation test at 1 mg/L (Timmer et al., 2019). The reduced biodegradation of the two QACs in the OECD 306 test may therefore be associated with inhibition of bacterial activities. Silica particles have been used in freshwater screening tests to reduce toxic substance concentrations of cationic surfactants in the water phase, thereby improving degradation properties and obtaining ready biodegradability results for these substances (Timmer et al., 2019; Van Ginkel et al., 2008). In addition to toxicity, alkyl chain lengths have also been reported to affect biodegradation. In a study with effluents from a sewage plant as microbial inoculum, complete DOC removal was measured with a C14 benzyl dimethyl ammonium chloride within 10 days of incubation, while DOC removal with a C16 benzyl dimethyl ammonium chloride compound was negligible after 21 days of incubation in the same inoculum (Garcıa et al., 2001). Nevertheless, the two QACs C16-alkylated hexadecyltrimethylammonium chloride and benzylated BAC showed biodegradation of >20% in seawater, which indicate primary biodegradation (OECD, 2006). Substances with inconclusive biodegradability results from screening tests may be further tested in simulation tests at low test substance concentrations before classified as persistent or not (ECHA, 2017a, 2017b).

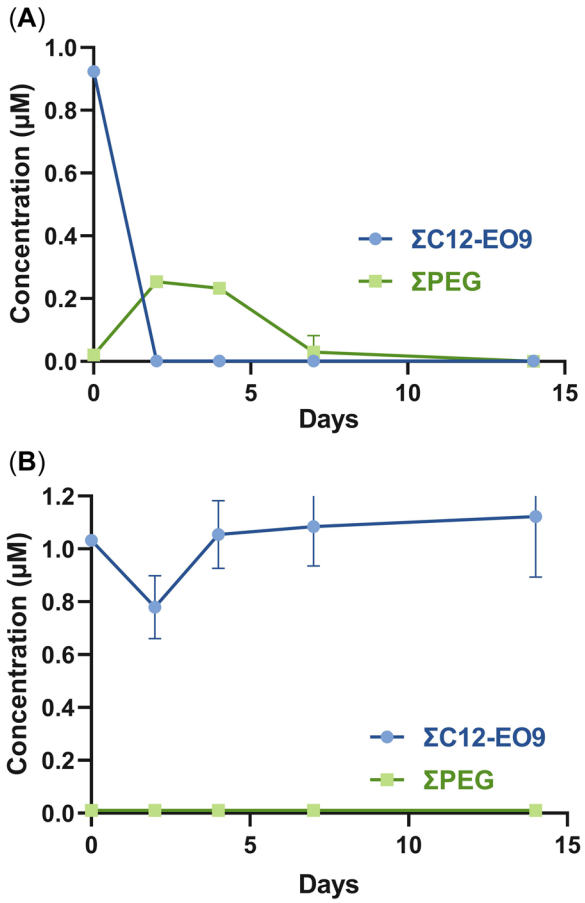

FIGURE 2: Concentrations of ∑C12 EO9 (C12 EO3-C12 EO18) and ∑PEG (EO3 EO18) in natural (**A**) and sterilized (**B**) subsurface seawater. The results are shown as mean ± SD of three replicates. EO = ethylene oxide; PEG = polyethylene glycol.

Primary biodegradation of an ethoxylated surfactant and PEG formation

Central fission has been reported to be an important biodegradation mechanism of polymeric ethoxylated surfactants in freshwater (Sparham et al., 2008), and this mechanism will result in PEG degradation products which are rapidly biodegraded (Bernhard et al., 2008; White et al., 1996). We therefore analyzed if the same degradation process occurred also in natural seawater in relation to primary biodegradation of the polymeric AE C12 EO9. A low nominal concentration (500 µg/L) of the surfactant C12 EO9, containing 3 to 18 EO groups (Supporting Information, Figure S2), was applied to fresh subsurface seawater without any nutrient amendment and incubated in the carousel system at 20 °C. When samples of the surfactant were analyzed by LC-MS/MS, rapid surfactant depletion was measured in natural subsurface seawater (Figure 2A), in contrast to sterilized subsurface seawater (Figure 2B), substantiating that depletion was caused by primary biodegradation. The depletion was fast, and >99% biodegradation was achieved after 2 days of incubation. The surfactant depletion in natural subsurface seawater coincided with temporary formation of PEGs with 5 to 18 EO groups (Figure 2A). PEG concentrations reached a peak of 0.253 ± 0.012 µM after 2 days of incubation, 12-fold higher than the PEG concentration in the surfactant at the start of the test (day 0). However, PEG concentrations were rapidly reduced and reached background levels after 7 days of incubation. PEG concentrations in the sterilized subsurface seawater did not rise above a background level (Figure 2B). These data further suggested that central fission is an important degradation step of this AE also in seawater, as previously shown in freshwater environments (Budnik et al., 2016; Marcomini et al., 2000; Sparham et al., 2008; Steber & Wierich, 1985). Closer examination of the C12 EO9 surfactant and its PEG components with different EO groups during the biodegradation period revealed complete or near-to complete depletion across the EO3 to EO18 compounds after 2 days of incubation (99.6%–100.4% degradation; Figure 3A). Analyzed PEGs with different EO groups showed rapid accumulation from day 0 to day 2 of the incubation for all homologues analyzed (EO5 to EO18), increasing by factors of 16.3 ± 0.5 (EO18) to 53.2 ± 1.0 (EO9), with an average of 34.0 ± 0.8 (Figure 3B). After 7 days of incubation, concentrations of all PEG components were reduced to the same low levels as at the start of the test (<0.002 µM) or below analytical detection limits. The formation and degradation patterns

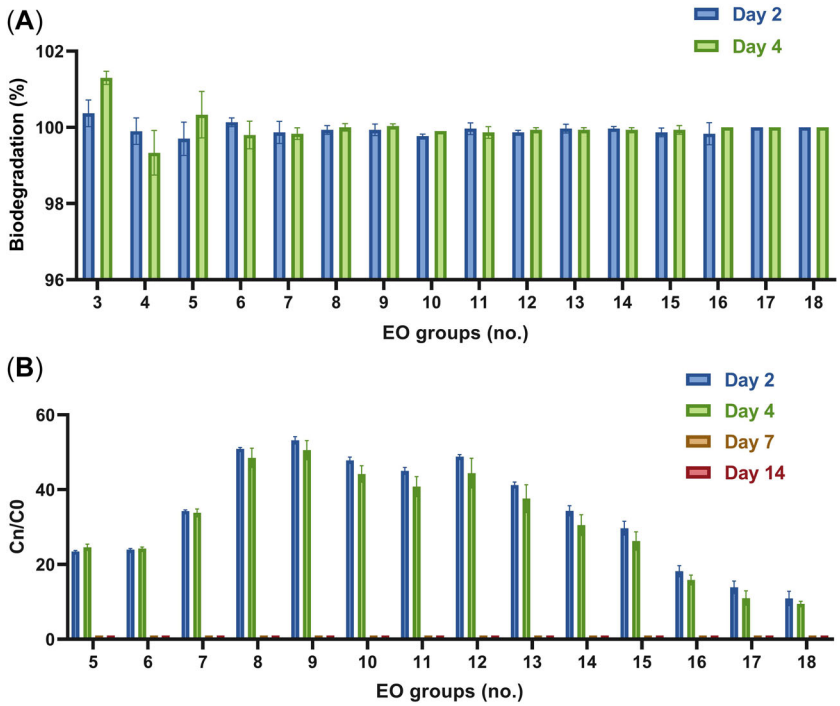

FIGURE 3: Percentage primary biodegradation in natural subsurface seawater of EO3 to EO18 compounds of C12 EO9 after 2 and 4 days of incubation (**A**), and ratios between concentrations of polyethylene glycols with different ethylene oxide (EO) numbers after incubations for 2–14 days (Cn) compared with their concentrations and at the start (C0) of the experiment (**B**). The results are shown as mean ± SD of three replicates.

were therefore similar for all measured PEG homologues. Further degradation of PEGs is expected to occur by splitting of C_2 units off the chain, which has been shown to result in temporary formation of short-chain PEG homologues (Bernhard et al., 2008). However, no temporary accumulations of short-chain PEG homologues were measured in our study.

In contrast to the C12 EO9 surfactant, no PEG formation of the high-molecular AE C16-18 EO50 was measured when incubated at the same conditions as C12 EO9 (Supporting Information, Figure S6). This substance may therefore not be degraded through the central fission mechanisms, but rather through other mechanisms described to occur under freshwater conditions, for instance by stepwise terminal degradation processes (White et al., 1996).

Primary biodegradation in the presence of marine particles

Because surfactants may easily adsorb to surfaces like clay and silica (Atkin et al., 2000; Sánchez-Martín et al., 2008), adsorption processes could theoretically hamper degradation in seawater. Thus, we investigated whether primary biodegradation was affected by particles in the seawater. The AE C12 EO9 was incubated in natural seawater in the presence of SPM in the form of the diatom *S. pseudocostatum*, clay material (kaolin), or a mixture of the two. The concentrations of phytoplankton (10^4 cells/ml) represented typical North Sea algal blooms (Gieskes & Kraay, 1986), while the kaolin concentrations (5 mg/L) described a compromise between typical coastal and oceanic SPM concentrations (Fettweis et al., 2007; Sheldon et al., 1972). After incubation (20 °C) of SPMs and surfactant (500 µg/L), samples were separated (20 µm steel filters) because SPMs may typically result in particle aggregation (Alldredge & Silver, 1988; de La Rocha & Passow, 2007). Particle aggregates were observed visually in all samples with SPM, both with and without surfactant (not shown). The total depletion of the surfactant measured in filtrates and filter-trapped fractions after 7 days of incubation at 20 °C was near-complete in all treatments with natural seawater (≥95.5%), while no depletion (<1%) was measured in sterilized fractions (Supporting Information, Figure S7). When concentrations in C12 EO9 in fractions of filtrates and filter-trapped materials of each sample were measured after 7 days of incubation and determined as the percentages of the total surfactant concentration in the sample at the start of the experiment, 0.4 ± 0.1% of the initial concentrations was left in the surface seawater samples (sum of fractions in filtrates and filter-trapped materials) without added SPMs, while 0.2 ± 0.1% surfactant was left in surface seawater with added SPMs (Figure 4). The primary biodegradation of the surfactant therefore occurred rapidly in the presence of the SPMs and was not negatively affected by the presence of the particles.

Particle association was also indicated from the measurements because fractions of the surfactants were measured in the filter-trapped materials (Figure 4). Comparison of weight-based concentrations of residual surfactant concentration showed preferential presence of C12 EO9 in the filter-trapped materials, when compared with the filtrates (Supporting Information, Table S3). Visible aggregates were also observed in

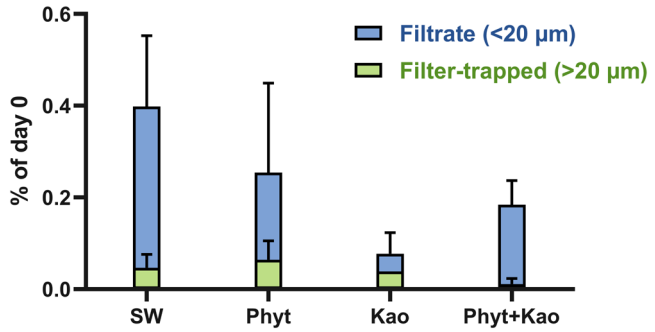

FIGURE 4: Primary biodegradation in natural subsurface seawater of EO3 to EO18 compounds of C12 EO9 (A) and ratios between concentrations at days 2–4 (Cn) to concentrations at the start of the experiment (d0) of polyethylene glycols with different ethylene oxide (EO) numbers (B). The results are shown as mean ± SD of three replicates. SW = seawater; Phyt = phytoplankton; Kao = kaolin.

seawater without phytoplankton or mineral particles in the experiment, probably due to natural background levels of SPMs. The concentrations of SPMs used in our studies may be low compared with concentrations in typical coastal and estuarine environments. SPM concentrations in coastal North Sea regions have been reported in the range of 25–100 mg/L (Fettweis et al., 2007), compared with 0.02–1 mg/L in oceanic seawater (Sheldon et al., 1972). Although these variable SPM concentrations will affect the relative surfactant interactions with SPM, our data indicate that surfactant biodegradation may not be hampered by marine particles. Studies of AE sorption to clay particles have also shown higher adsorption coefficients in relation to increased ethoxylate and alkyl chain lengths (Droge et al., 2009). Particle-sorbed surfactants may also stimulate bacterial attachment to the particles, and in a study with anionic surfactants and river sediments, attachment of indigenous bacteria to the sediments was stimulated by biodegradable surfactants, while recalcitrant surfactants and nonsurface-active carbon sources were ineffective in promoting attachment (Marchesi et al., 1997). The nature of the marine particles may also be important for biodegradation, and investigations of hydrocarbons integrated in oil-related marine snow aggregates have, for instance, shown that the aggregates may become hot spots for hydrocarbon biodegradation (Henry et al., 2020; Wirth et al., 2018). The complexities of sediment–surfactant interactions are further illustrated by the reduction of the toxicity of QACs and increase of biodegradation by different clay materials, although bioavailabilities may be limited depending on surfactant and clay material (Timmer et al., 2020).

CONCLUSIONS

In the present study, ultimate biodegradation was determined for 18 surfactants of the subclasses betaines, alkylsulfates, alkylether sulfates, alkylether carboxylates, AEs, alkyl polyglycosides, and QACs. Most of the surfactants ($n = 12$) were shown to be readily biodegradable in seawater (>60% biodegraded in 28 days), including eight of the 11 polyethoxylates. All 18 surfactants were biodegraded >20%, which is indicative of primary biodegradation in seawater (OECD, 2006). In addition, four of the six surfactants, including three polyethoxylates, with <60% biodegradation in 28 days, showed increased biodegradation close to (>50% biodegradation) or above the 60% threshold level by extended incubation times (65 to 75 days), which is considered as evidence for nonpersistence in the marine environment, according to EU REACH criteria (ECHA, 2017a). The degradation of the polymeric ethoxylated surfactants was partly related to the numbers of EO groups. The longer EO chains of 40 and 50 EOs resulted in slower degradation than those with 23 or fewer EO groups, probably due to different biodegradation mechanism. Rapid primary degradation by central fission with formation of PEGs homologues was determined with the AE C12 EO9, but not with C16-18 EO50, and other mechanisms resulting in slower degradation may therefore occur in the high-molecular polyethoxylates. The PEG homologues formed from polyethoxylated biodegradation were rapidly biodegraded, demonstrating the importance of the central fission mechanism for rapid biodegradation of polyethoxylates also in seawater. For the QACs, reduced biodegradation was related to the toxicity of these compounds, as determined by the reference substance inhibition test, and improved biodegradation may therefore be achieved by reduced substrate concentrations or by other test modifications to reduce toxicity.

It has been suggested that for assessing the biodegradation potential of substances in seawater, the respective screening test (OECD 306) may be improved by increasing the inoculum concentration and extending the incubation period (Ott et al., 2019). Our study with different surfactant types indicated that increased inoculum concentrations did not substantially impact the outcome of the screening tests; however, a prolonged incubation time turned out to be valuable to assess the biodegradation potential of polymeric surfactants in seawater.

In conclusion, the biodegradation tests performed indicate that most tested surfactants possess a high biodegradation potential in seawater and will be quickly degraded if directly discharged from ships or offshore installations, or if entering the marine compartment via sewage runoffs. However, chemicals may be introduced to the marine environments in wastewater with high solid concentrations and/or will come in contact with SPMs, particularly in coastal and estuarine areas. Although our studies indicated some surfactant association with particle aggregates, the presence of SPMs in the form of suspended phytoplankton and clay particles did not show to affect primary biodegradation of the AE C12 EO9 in seawater when compared with the seawater without SPMs. The conditions used in the present study were chosen to be representative for typical pelagic marine conditions but did not reflect solid-rich wastewater or marine compartments with high particle concentrations, such as coastal areas or estuaries. Thus, further investigations are needed to unveil the environmental fate of surfactants under these specific conditions.

Supporting Information—The Supporting Information is available on the Wiley Online Library at https://doi.org/10.1002/etc.5632.

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Author Contributions Statement—**Odd G. Brakstad**: Conceptualization; Data curation; Formal analyses; Investigation; Methodology; Project administration; Supervision; Visualization; Writing—original draft; Writing—review & editing. **Antonio Sarno**: Data curation; Formal analyses; Investigation; Methodology; Project administration; Supervision; Visualization; Writing—original draft; Writing—review & editing. **Roy Geerts**: Conceptualization; Funding acquisition; Resources; Validation; Writing—review & editing. **James Dawick**: Funding acquisition; Resources; Validation; Writing—review & editing. **Abel Machado**: Conceptualization; Funding acquisition; Resources; Validation; Writing—review & editing. **Philipp Hopp**: Conceptualization; Funding acquisition; Project administration; Resources; Validation; Writing—review & editing.

Data Availability Statement—Raw data and calculations are available on the web-page https://figshare.com/account/home under the item "Ultimate and primary biodegradation of a range of nonpolymeric and polymeric surfactants in seawater."

REFERENCES

Aboulhassan, M., Souabi, S., Yaacoubi, A., & Baudu, M. (2006). Removal of surfactant from industrial wastewaters by coagulation flocculation process. *International Journal of Environmental Science and Technology*, *3*, 327–332.

Alldredge, A. L., & Silver, M. W. (1988). Characteristics, dynamics and significance of marine snow. *Progress in Oceanography*, *20*, 41–82.

Arp, H. P. H., & Hale, S. E. (2019). REACH: Improvement of guidance and methods for the identification and assessment of PMT/vPvM substances (Report No. FB000142/ENG). Umweltbundesamt, Dessau-Rosslau. http://www.umweltbundesamt.de/publikationen

Atkin, R., Craig, V. S., & Biggs, S. (2000). Adsorption kinetics and structural arrangements of cationic surfactants on silica surfaces. *Langmuir*, *16*, 9374–9380.

Bernhard, M., Eubeler, J. P., Zok, S., & Knepper, T. P. (2008). Aerobic biodegradation of polyethylene glycols of different molecular weights in wastewater and seawater. *Water Research*, *42*, 4791–4801.

Brakstad, O. G., Farooq, U., Ribicic, D., & Netzer, R. (2018). Dispersibility and biotransformation of oils with different properties in seawater. *Chemosphere*, *191*, 44–53.

Brakstad, O. G., Nordtug, T., & Throne-Holst, M. (2015). Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Marine Pollution Bulletin*, *93*, 144–152.

Brakstad, O. G., Størseth, T. R., Brunsvik, A., Bonaunet, K., & Faksness, L.-G. (2018). Biodegradation of oil spill dispersant surfactants in cold seawater. *Chemosphere*, *204*, 290–293.

Budnik, I., Zembrzuska, J., & Lukaszewski, Z. (2016). Bacterial strains isolated from river water having the ability to split alcohol ethoxylates by central fission. *Environmental Science and Pollution Research*, *23*, 14231–14239.

Cousins, I. T., Ng, C. A., Wang, Z., & Scheringer, M. (2019). Why is high persistence alone a major cause of concern? *Environmental Science: Processes & Impacts*, *21*, 781–792.

Droge, S. T. J., Yarza-Irusta, L., & Hermens, J. L. M. (2009). Modeling nonlinear sorption of alcohol ethoxylates to sediment: The influence of molecular structure and sediment properties. *Environmental Science & Technology*, *43*, 5712–5718.

European Chemicals Agency. (2017a). *Guidance on Information Requirements and Chemical Safety Assessment Part C: PBT/vPvB assessment. Version 3.0*.

European Chemicals Agency. (2017b). *Guidance on Information Requirements and Chemical Safety Assessment. Chapter R. 7b: Endpoint Specific Guidance*.

European Council. (2022). Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC (Text with EEA relevance)Text with EEA relevance. http://data.europa.eu/eli/reg/2006/1907/2022-12-17

Fettweis, M., Nechad, B., & Van den Eynde, D. (2007). An estimate of the suspended particulate matter (SPM) transport in the southern North Sea using SeaWiFS images, in situ measurements and numerical model results. *Continental Shelf Research*, *27*, 1568–1583.

Gan, W., & Liu, Q. (2008). Coagulation of bitumen with kaolinite in aqueous solutions containing Ca^{2+}, Mg^{2+} and Fe^{3+}: Effect of citric acid. *Journal of Colloid and Interface Science*, *324*, 85–91.

Garcıa, M., Ribosa, I., Guindulain, T., Sanchez-Leal, J., & Vives-Rego, J. (2001). Fate and effect of monoalkyl quaternary ammonium surfactants in the aquatic environment. *Environmental Pollution*, *111*, 169–175.

Gieskes, W. W., & Kraay, G. W. (1986). Analysis of phytoplankton pigments by HPLC before, during and after mass occurrence of the microflagellate *Corymbellus aureus* during the spring bloom in the open northern North Sea in 1983. *Marine Biology International Journal of Life Oceans and Coastal Waters*, *92*, 45–52.

Van Ginkel, C. G. (1996). Complete degradation of xenobiotic surfactants by consortia of aerobic microorganisms. *Biodegradation*, *7*, 151–164.

Van Ginkel, C. G., Gancet, C., Hirschen, M., Galobardes, M., Lemaire, P., & Rosenblom, J. (2008). Improving ready biodegradability testing of fatty amine derivatives. *Chemosphere*, *73*, 506–510.

González, S., Petrovic, M., & Barceló, D. (2007). Removal of a broad range of surfactants from municipal wastewater–comparison between membrane bioreactor and conventional activated sludge treatment. *Chemosphere*, *67*, 335–343.

Hafer, J. (2021). REACH registration of polymers: Identifying polymers of low concern. *International Chemical Regulatory and Law Review*, 4, 21-25.

Henry, I. A., Netzer, R., Davies, E. J., & Brakstad, O. G. (2020). Formation and fate of oil-related aggregates (ORAs) in seawater at different temperatures. *Marine Pollution Bulletin*, *159*, 111483.

HERA. (2009). *Human and environmental risk assessment on ingredients of European household cleaning products*. Alcohol ethoxylates. Version 2.0 https://heraproject.com/files/34-f-09%20hera%20ae%20report%20version%202%20-%203%20sep%2009.pdf

Hurwitz, E., Barnett, G., Beaudoin, R., & Kramer, H. (1947). Nitrification and BOD. *Sewage Works Journal*, *19*, 995–999.

International Organisation for Standardisation. (2016). ISO 10253:2016. *Water quality—Marine algal growth inhibition test with Skeletonema sp. and Phaeodactylum tricornutum* (p. 18).

Jackson, M., Eadsforth, C., Schowanek, D., Delfosse, T., Riddle, A., & Budgen, N. (2016). Comprehensive review of several surfactants in marine environments: Fate and ecotoxicity. *Environmental Toxicology and Chemistry*, *35*, 1077–1086.

Jurado, E., Fernández-Serrano, M., Lechuga, M., & Ríos, F. (2012). Environmental impact of ether carboxylic derivative surfactants. *Journal of Surfactants and Detergents*, *15*, 1–7.

Jurado, E., Fernández-Serrano, M., Núñez-Olea, J., Lechuga, M., Jiménez, J., & Ríos, F. (2011). Effect of concentration on the primary and ultimate biodegradation of alkylpolyglucosides in aerobic biodegradation tests. *Water Environment Research*, *83*, 154–161.

Ketola, A. (2016). *Determination of surfactants in industrial waters of paper- and board mills*. Master's Thesis, University of Jyväskylä.

Madsen, T., Boyd, H. B., Nylén, D., Pedersen, A. R., Petersen, G. I., & Simonsen, F. (2001). Environmental and health assessment of substances in household detergents and cosmetic detergent products. *Environmental Project*, *615*, 221.

Marchesi, J. R., White, G. F., Russel, N. J., & House, W. A. (1997). Effect of river sediment on the biodegradation kinetics of surfactant and non-surfactant compounds. *FEMS Microbiology Ecology*, *23*, 55–63.

Marcomini, A., Zanette, M., Pojana, G., & Suter, M. J. F. (2000). Behavior of aliphatic alcohol polyethoxylates and their metabolites under standardized aerobic biodegradation conditions. *Environmental Toxicology and Chemistry*, *19*, 549–554.

Matsuoka, H. (2015). Polymer surfactant. In S. Kobayashi & K. Müllen, (Eds.), *Encyclopedia of polymeric nanomaterials* (pp. 1917–1922). Springer.

Matthies, M., Solomon, K., Vighi, M., Gilman, A., & Tarazona, J. V. (2016). The origin and evolution of assessment criteria for persistent, bioaccumulative and toxic (PBT) chemicals and persistent organic pollutants (POPs). *Environmental Science Processes & Impacts*, *18*, 1114–1128.

Mungray, A. K., & Kumar, P. (2008). Anionic surfactants in treated sewage and sludges: Risk assessment to aquatic and terrestrial environments. *Bioresource Technology*, *99*, 2919–2929.

Neumann, M., Schwarz, M., Sättler, D., Oltmanns, J., Vierke, L., & Kalberlah, F. (2015). *A proposal for a chemical assessment concept for the protection of raw water resources under REACH*. Extended Abstract for the Oral Presentation at the 25th SETAC Annual Meeting.

Organisation for Co-operation and Development. (1992a). *Test No. 301: Ready Biodegradability. OECD Guidelines for the Testing of Chemicals, Section 3*. OECD Publishing.

Organisation for Co-operation and Development. (1992b). *Test No. 306: Biodegradability in Seawater. OECD Guidelines for the Testing of Chemicals, Section 3*. OECD Publishing.

Organisation for Co-operation and Development. (2006). *Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3. Part 1: Principles and strategies related to the testing of degradation of organic chemicals. OECD Guidelines for the Testing of Chemicals, Section 3*. OECD Publishing.

Organisation for Co-operation and Development. (2014). *Test No. 310: Ready Biodegradability—CO_2 in sealed vessels (Headspace Test). OECD Guidelines for the Testing of Chemicals, Section 3*.OECD Publishing.

OSPAR. (2006). *Dynamic selection and prioritisation mechanism for hazardous substances (new DYNAMEC manual)*. OSPAR Commission.

OSPAR. (2013). *Implementation of OSPAR Recommendation 2006/3 on environmental goals for the discharge by the offshore industry of chemicals that are, or which contain substances identified as candidates for substitution* (p. 50). OSPAR Commission.

OSPAR. (2019). *The OSPAR list of chemicals for priority action suggestions for future actions* (p. 81). OSPAR Commission.

Ott, A., Martin, T. J., Acharya, K., Lyon, D. Y., Robinson, N., Rowles, B., Snape, J. R., Still, I., Whale, G. F., & Albright, III., V. C. (2020). Multi-laboratory validation of a new marine biodegradation screening test for chemical persistence assessment. *Environmental Science and Technology*, *54*, 4210–4220.

Ott, A., Martin, T. J., Snape, J. R., & Davenport, R. J. (2020). Increased cell numbers improve marine biodegradation tests for persistence assessment. *Science of the Total Environment*, *706*, 135621.

Palmer, M., & Hatley, H. (2018). The role of surfactants in wastewater treatment: Impact, removal and future techniques: A critical review. *Water Research*, *147*, 60–72.

Pérez-Carrera, E., León, V. M., Lara-Martín, P. A., & González-Mazo, E. (2010). Influence of the hydrophilic moiety of anionic and nonionic surfactants on their aerobic biodegradation in seawater. *Science of The Total Environment*, *408*, 922–930.

Place, B. J., Perkins, M. J., Sinclair, E., Barsamian, A. L., Blakemore, P. R., & Field, J. A. (2016). Trace analysis of surfactants in Corexit oil dispersant formulations and seawater. *Deep Sea Res. Part II: Topical Studies in Oceanography*, *129*, 273–281.

de La Rocha, C., & Passow, U. (2007). Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep Sea Research Part II: Topical Studies in Oceanography*, *54*, 639–658.

Sánchez-Martín, M., Dorado, M., Del Hoyo, C., & Rodríguez-Cruz, M. (2008). Influence of clay mineral structure and surfactant nature on the adsorption capacity of surfactants by clays. *Journal of Hazardous Materials*, *150*, 115–123.

Scott, M. J., & Jones, M. N. (2000). The biodegradation of surfactants in the environment. *Biochimica et Biophysica Acta*, *1508*, 235–251.

Sheldon, R., Prakash, A., & Sutcliffe, Jr., W. (1972). The size distribution of particles in the ocean 1. *Limnology and Oceanography*, *17*, 327–340.

Sparham, C., Rehman, N., Melling, J., Van Duynhoven, J., & Marshall, S. (2008). Biodegradability of highly ethoxylated nonionic surfactants: Determination of intermediates and pathways of biodegradation. *Environmental Toxicology a Chemistry*, *27*, 1069–1076.

Steber, J., & Wierich, P. (1985). Metabolites and biodegradation pathways of fatty alcohol ethoxylates in microbial biocenoses of sewage treatment plants. *Applied and Environmental Microbiology*, *49*, 530–537.

Timmer, N., Gore, D., Sanders, D., Gouin, T., & Droge, S. T. (2019). Toxicity mitigation and bioaccessibility of the cationic surfactant cetyltrimethylammonium bromide in a sorbent-modified biodegradation study. *Chemosphere*, *222*, 461–468.

Timmer, N., Gore, D., Sanders, D., Gouin, T., & Droge, S. T. (2020). Application of seven different clay types in sorbent-modified biodegradability studies with cationic biocides. *Chemosphere*, *245*, 125643.

Traverso-Soto, J. M., Lara-Martín, P. A., León, V. M., & González-Mazo, E. (2013). Analysis of alcohol polyethoxylates and polyethylene glycols in marine sediments. *Talanta*, *110*, 171–179.

Vashon, R. D., & Schwab, B. S. (1982). Mineralization of linear alcohol ethoxylates and linear alcohol ethoxy sulfates at trace concentrations in estuarine water. *Environmental Science and Technology*, *16*, 433–436.

Wennberg, A. C., Meland, S., Grung, M., & Lillicrap, A. (2022). Unravelling reasons for variability in the OECD 306 marine biodegradation test. *Chemosphere*, *300*, 134476.

Whale, G., Parsons, J., van Ginkel, K., Davenport, R., Vaiopoulou, E., Fenner, K., & Schaeffer, A. (2021). Improving our understanding of the environmental persistence of chemicals. *Integrated Environmental Assessment and Management*, *17*, 1123–1135.

White, G. F., Russel, N. J., & Tidswell, E. C. (1996). Bacterial scission of ether bonds. *Microbiological Reviews*, *60*, 216–232.

Willing, A., Messinger, H., & Aulman, W. (2004). Ecology and toxicology of alkyl polyglycosides. In U. Zoller (Ed.), *Handbook of detergents. Part B: Environmental impact* (pp. 487–521). Marcel Dekker.

Willis, K. A., Serra-Goncalves, C., Richardson, K., Schuyler, Q. A., Pedersen, H., Anderson, K., Stark, J. S., Vince, J., Hardesty, B. D., & Wilcox, C. (2022). Cleaner seas: Reducing marine pollution. *Reviews in Fish Biology and Fisheries*, *32*, 145–160.

Wirth, M. A., Passow, U., Jeschek, J., Hand, I., & Schulz-Bull, D. E. (2018). Partitioning of oil compounds into marine oil snow: Insights into prevailing mechanisms and dispersant effects. *Marine Chemistry*, *206*, 62–73.

Wu, Q., Zhao, L., Song, R., & Ma, A. (2019). Research progress on surfactant biodegradation. *IOP Conference Series: Earth and Environmental Science*, *227*(5), 052023. https://doi.org/10.1088/1755-1315/227/5/052023