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Short communication

Biodegradation of oil spill dispersant surfactants in cold seawater

Authors: Odd Gunnar Brakstad^{A*}, Trond R. Størseth^A, Anders Brunsvik^B, Kristin Bonaunet^A, Liv-Guri Faksness^A

^ASINTEF Ocean AS, Dept. of Environment and New Resources, ^BSINTEF Industry, Dept. Biotechnology and Nanomedicine, N-7465 Trondheim

*Corresponding author Odd G. Brakstad; e-mail odd.brakstad@sintef.no; phone +47 98243447.

Key words: Oil spill dispersants; surfactants; biotransformation; seawater; low temperature

12 **ABSTRACT**

13 Dispersants are used as an oil spill response method in seawater to increase oil-degradation
14 rates. While biodegradation of chemically dispersed oil has been well documented, only a few
15 studies have focused on the degradation of the dispersant compounds themselves. The
16 objective of this study was to determine the biodegradation of dispersant surfactants in cold
17 seawater, relevant for deep sea or Arctic conditions. Biotransformation of the surfactants
18 dioctyl-sodium sulfosuccinate (DOSS), Tween 80, Tween 85, and α/β -
19 ethylhexylsulfosuccinate (EHSS, expected DOSS hydrolysis product) in the commercial
20 dispersants Corexit 9500, Dasic Slickgone NS and Finasol OSR52 were determined. The
21 biotransformation studies of the surfactants were performed in natural seawater at 5°C over a
22 period of 54 days with no oil present. The surfactants were tested at 1, 5, and 50 mg/L, the
23 lower concentration being as close as possible to expected field concentrations. Experiments
24 with dispersants concentrations of 1 mg/L resulted in rapid biotransformation of Tween 80
25 and Tween 85 with depletion after 8 days, while DOSS showed rapid biotransformation after
26 a lag period of 16 days. The degradation half-life of DOSS increased from 4.1 days to > 500
27 days as Corexit 9500 concentrations went from 1 mg/L to 50 mg/L, emphasizing the
28 importance of performing experiments at dispersant concentrations as close as possible to
29 environmentally relevant concentrations. EHSS showed limited degradation compared to
30 other surfactants. This study shows that the surfactants DOSS, Tween 80 and Tween 85 in the
31 three chemical dispersants studied are biodegradable in cold seawater, particularly in
32 environmentally relevant concentrations.

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36 1. Introduction

37 Application of chemical dispersants is an oil spill response (OSR) technology used to
38 remove oil slicks from the sea surface, but has also been used during subsurface oil spills.
39 Several studies have shown increased oil biodegradation when using dispersants on oil spills
40 because smaller, more bioavailable droplets are formed (Brakstad et al., 2014; Bælum et al.,
41 2012; Hazen et al., 2010; McFarlin et al., 2014; Prince et al., 2013; Siron et al., 1995;
42 Techtmann et al., 2017; Venosa and Holder, 2007), while few have investigated the
43 biodegradation of the dispersant compounds themselves. Commercial dispersants consist of a
44 mixture of solvents and surfactants. The solvent fraction often consists of petroleum
45 distillates, while the surfactant fraction includes dioctylsodiumsulfosuccinate (DOSS), Tween
46 80, Tween 85, Span 80, and the DOSS hydrolysis product α/β -ethylhexylsulfosuccinate
47 (EHSS)(Place et al., 2010; Place et al., 2016). Concern was raised on the potential persistence
48 of the surfactants after the Deepwater Horizon (DWH) oil spill (Kujawinski et al., 2011;
49 White et al., 2014).

50 Laboratory studies with enrichment cultures from the Gulf of Mexico showed that the
51 petroleum distillates were biodegradable at 5°C (Bælum et al., 2012), and that DOSS was
52 degraded faster at 25°C than 5°C (Campo et al., 2013). DOSS was also readily biodegradable
53 (> 60 % biodegradable) in standard aquatic ultimate (mineralization) biodegradability test at
54 20°C (García et al., 2009). DOSS degradation was also reported in seawater (8°C)
55 (Kleindienst, et al., 2015). After a dispersant treatment of an oil spill, the dispersed oil will
56 rapidly dilute (Lee et al., 2013). The dispersant concentration will then also be very low.
57 Measured DOSS concentrations after the Deepwater Horizon oil spill varied from 0.01 $\mu\text{g/L}$
58 to 13 $\mu\text{g/L}$ (Kujawinski et al., 2011; Place et al., 2016).

59 In this study we determined if surfactants in common commercial dispersants biodegrade
60 in cold natural seawater, and at as close as practical to expected environmental concentrations
61 considering analytical limitations. This is of relevance for the use of dispersants as an OSR
62 method in the Arctic, as well as for subsurface oil spills.

63

64 **2. Material and methods**

65 *2.1 Dispersants*

66 Three commercial dispersants were used in this study, Corexit 9500A (Nalco
67 Environmental Solutions LLC, Tx, USA), Dasic Slickgone NS (Dasic International Ltd., UK),
68 and Finasol OSR-52 (Total Special Fluids, France).

69

70 *2.2 Seawater*

71 Seawater (SW) was collected from a depth of 80 m (below thermocline) in a non-
72 polluted Norwegian fjord (Trondheimsfjord; 63°26'N, 10°23'E), supplied by a pipeline system
73 from the source to the SINTEF laboratories (salinity of 34 ‰, temperature of 6-8°C, and
74 dissolved oxygen (DO) of 8.5 mg/L when reaching the laboratory). The SW was acclimated
75 to 5°C (7 days before start of the experiments).

76

77 *2.3 Experimental setup*

78 A biodegradation experiment was performed in 250 mL flasks (Schott). The SW was
79 supplied with solutions of mineral nutrients, according to OECD Guideline 306 (OECD,
80 1992). SW for sterilized controls was sterile-filtered (0.22 µm) and supplied with a biocide

81 (HgCl₂; 100 mg/L final concentration). The biodegradation experiment was performed with
82 final concentrations of all dispersants at 1 mg/L in natural SW, and without oil. In addition,
83 Corexit was tested at concentrations of 5 and 50 mg/L. Sterilized controls were prepared with
84 1 mg/L Corexit. Solutions (150 mL) were transferred to the 250 mL flasks and incubated for
85 up to 54 days on a shaking table at 5°C. Samples (triplicate) were collected after 0, 4, 8, 16,
86 32 and 54 days and frozen (-20°C) until analysis.

87

88 *2.4 Analyses – LC-MS*

89 The surfactants DOSS, Tween 80, Tween 85, Span 80 and EHSS were analysed by
90 liquid chromatography triple quadrupole mass spectrometry (LC-QQQ-MS), using a
91 modification of the method described by Place et al. (2016). Frozen samples were thawed and
92 transferred to new flasks (250 mL), pre-washed with 5 mL MeOH/IPA (methanol:isopropanol
93 1:1), to avoid surfactant glass wall attachment. Each original test flask was then rinsed three
94 times each with 15 mL MeOH/IPA, and rinse solutions pooled with the SW sample in the new
95 flask (total solution of 75% sample and 25% solvent). Injections (500 µL) of the SW/solvent
96 mixture were separated on an Agilent Zorbax SB-C18 column (5 µm, 2.1 x 150 mm) using a
97 gradient from 5-60% of acetonitrile in deionized water containing 0.5 mM sodium acetate.
98 For the MS detection, multiple reaction monitoring transitions were set up (Place et al. 2016).
99 A standard curve was prepared in the range from 1-500 ng/mL for DOSS, Span 80, Tween 80
100 and Tween 85. For EHSS no standard existed, and data for it was therefore given as relative
101 response values of the peak area.

102

103 *2.5 Calculations*

104 Depletion of quantified surfactants were determined as the percentage of start
105 concentrations (C_0) at the different sampling times (% C of C_0). If biotransformation was to
106 be corrected for depletion in sterilized controls, this was calculated as $(C/C_0)/(C_{ster}/C_{0-ster})$.

107 First-order biotransformation rates and half-lives were determined by non-linear regression
108 analyses as previously described (Brakstad et al., 2004). The rate coefficients (k_1) and half-
109 lives ($t_{1/2}$) were determined by the option "one-phase decay" in GraphPad Prism vs. 6.0
110 (GraphPad Software Inc., La Jolla, CA, USA). If non-responsive lag-periods were observed,
111 lag-periods, rate coefficients and half-lives were determined by "plateau followed by one-
112 phase decay" in GraphPad Prism.

113

114

115 **3. Results and discussion**

116 *3.1 Comparison of abiotic and biotic conditions*

117 We report data for DOSS and its hydrolysis product EHSS as well as the non-ion
118 surfactants Tween 80 and Tween 85 at different dispersant concentrations. However, Span 80
119 was not included in the analyses, due to inconsistent analytical stability in our samples.
120 Corexit surfactant analyses in sterilized SW at low concentrations (1 mg/L) showed 16%
121 DOSS depletion after 54 days, and 2 % EHSS depletion, while concentrations of Tween 80
122 and Tween 85 were higher (136-181%) after 54 days, compared to the start of the experiment
123 (Fig. S1, Supplementary Information (SI)). There was little difference between depletion
124 calculated in the biotic samples (C/C_0) to depletion calculations that accounted for loss in the
125 sterilized controls $(C/C_0)/(C_{ster}/C_{0-ster})$, as shown in Fig. S2, SI. These results were also
126 confirmed in sterilized SW at high Corexit concentrations (50 mg/L), showing 4% DOSS
127 depletion at the end of the experiment, while concentrations of Tween 80, Tween 85 and

128 EHSS ranged between 103% to 132% of the start concentrations (results not shown).
129 Surfactant depletion in the abiotic controls were therefore neglected, and biodegradation was
130 therefore the main depletion mechanism.

131

132 *3.2 Biotransformation at different dispersant concentrations*

133 Surfactant concentrations at the start of a biotransformation experiment with three
134 Corexit concentrations (1, 5 and 50 mg/L) showed that distribution of DOSS, Tween 80 and
135 Tween 85 was similar for all concentrations at the start of the experiment (Table S1, SI). All
136 Corexit concentrations (1 mg/L, 5 mg/L and 50 mg/L) had a Tween80:Tween85:DOSS ratio
137 of 0.3:1:1. These results were in the range of the results reported by Place et. al (2016). The
138 reported biodegradation results were treated as percentages of the starting concentration,
139 which allowed for a comparison of the biodegradation rates. Differences between biotic and
140 sterilized samples showed that the depletion in the biotic samples were caused by
141 biodegradation (Fig. S1). The SW was amended with a standard solution of mineral nutrients
142 (OECD, 1992), since previous mineral nutrient analyses (Brakstad et al., 2015) indicated a
143 risk of nutrient deficiency with Corexit concentrations of 50 mg/L.

144 The non-ionic surfactants were biotransformed by >96 % after 4 days of incubation at
145 all concentrations (Fig. 1), in agreement with previous data (Kleindienst et al., 2015).
146 Biotransformation of the anionic surfactant (DOSS), however, did depend on concentration
147 and showed typical lag-periods (16 days) at dispersant concentrations of 1 mg/L and 5 mg/L.
148 After the lag-period, DOSS was rapidly biotransformed (Fig. 1), with half-lives of 4 to 6 days
149 (Table S2). At 50 mg/L, DOSS biotransformation was <10% at the end of the test period (Fig.
150 1). Possible explanations for the poor DOSS biotransformation at the high dispersant
151 concentration could be dissolved oxygen (DO) limitations and/or toxic responses of high

152 dispersant concentrations to the microbial communities in the SW, although the headspace in
153 the test flasks (250 ml flasks with 150 ml dispersant in SW) should secure DO exchange
154 between headspace and SW. In addition, rapid biotransformation of the non-ionic surfactants
155 at all dispersant concentrations did not indicate DO depletion or toxic responses during
156 biotransformation. Recent studies of DOSS biotransformation at high surfactant
157 concentrations (~6 mg/L surfactant) with bacterial cultures from GoM, also showed slow
158 biotransformation at 5°C (Bælum et al., 2012; Campo et al., 2013). Interestingly, DOSS was
159 shown to be biotransformed even faster in the presence of oil than without oil, using oil-
160 degrading bacterial enrichment cultures incubated at 25°C (Techtmann et al., 2017).
161 However, oil-degrading enrichment cultures enriched at 5°C did not degrade DOSS in their
162 study to the same extent as shown in our study. Dispersants applied to an oil discharge will
163 rapidly dilute, and the biotransformation data at the lower concentrations used in this study
164 are therefore more realistic for real spill situations. The results from this study also emphasize
165 the importance if not using unrealistically high concentrations of surfactants in biodegradation
166 experiments. The data also showed that EHSS responses at 1 and 5 mg/L Corexit
167 concentrations did not change significantly, but increased at 50 mg/L (Fig. 1). EHSS has been
168 reported to be a biotransformation product from microbial ester hydrolyses of DOSS (Hales,
169 1993; Campo et al., 2013). EHSS was present in the dispersant before the start of the
170 biodegradation study, probably as it is an intermediate in the synthesis of DOSS (Place et al.,
171 2016). Further, no indications of EHSS as a biodegradation product of DOSS were found in
172 our studies. EHSS accumulation at high concentration (50 mg/L Corexit) was not related to
173 any DOSS depletion, while DOSS depletion at the lower concentrations (1 mg/L and 5 mg/L
174 Corexit) did not result in any clear correlations with the EHSS concentrations.

175

176 *3.2 Biotransformation of surfactants in different dispersants*

177 Surfactant biotransformation was determined in Corexit, Dasic and Finasol at low
178 concentrations (1 mg/L). Relative DOSS and Tween 80 distributions were lower in Dasic than
179 the two other dispersants, while Tween 85 was higher (Table S1). Biotransformation of the
180 non-ionic surfactants was fast in all dispersants (Fig. 2). Tween 85 was completely depleted in
181 all dispersants after 4 days. Tween 80 in Dasic showed a short lag-period (4 days), but was
182 completely transformed after 8 days of incubation. Also, DOSS biotransformation was
183 comparable in all dispersants, although Dasic showed a shorter lag-period and a less steep
184 depletion slope than Corexit and Finasol. However, the sum of the lag-periods and half-lives
185 of DOSS were comparable in all dispersants (20 days, 28 days and 24 days in Corexit, Dasic
186 and Finasol, respectively) (Table S2). The biotransformation of EHSS was faster in Finasol
187 than in Corexit and Dasic.

188

189 4. Conclusions

190 Biotransformation of surfactants in the commercial dispersants Corexit 9500, Dasic
191 Slickgone NS and Finasol OSR52, and at different Corexit concentrations, was investigated in
192 cold natural SW at 5°C. Biotransformation of Tween 80, and Tween 85 occurred particularly
193 fast, with reductions below analytical detection limits after 4 to 8 days. DOSS
194 biotransformation exhibited typical lag-periods when low dispersant concentrations were
195 used, but was then rapidly depleted. This study shows that the surfactants DOSS, Tween 80
196 and Tween 85 in the dispersants are biodegradable in cold SW, in particular at low
197 environmentally relevant concentrations. The results showed the importance of not using
198 unrealistically high surfactant concentrations, compared to relevant environmental
199 concentrations after oil spill operations. It is therefore These data may have implications for

200 the use of dispersants as an oil spill response method in cold environments such as the Arctic
201 and for subsurface dispersant injection during deep sea oil spills.

202

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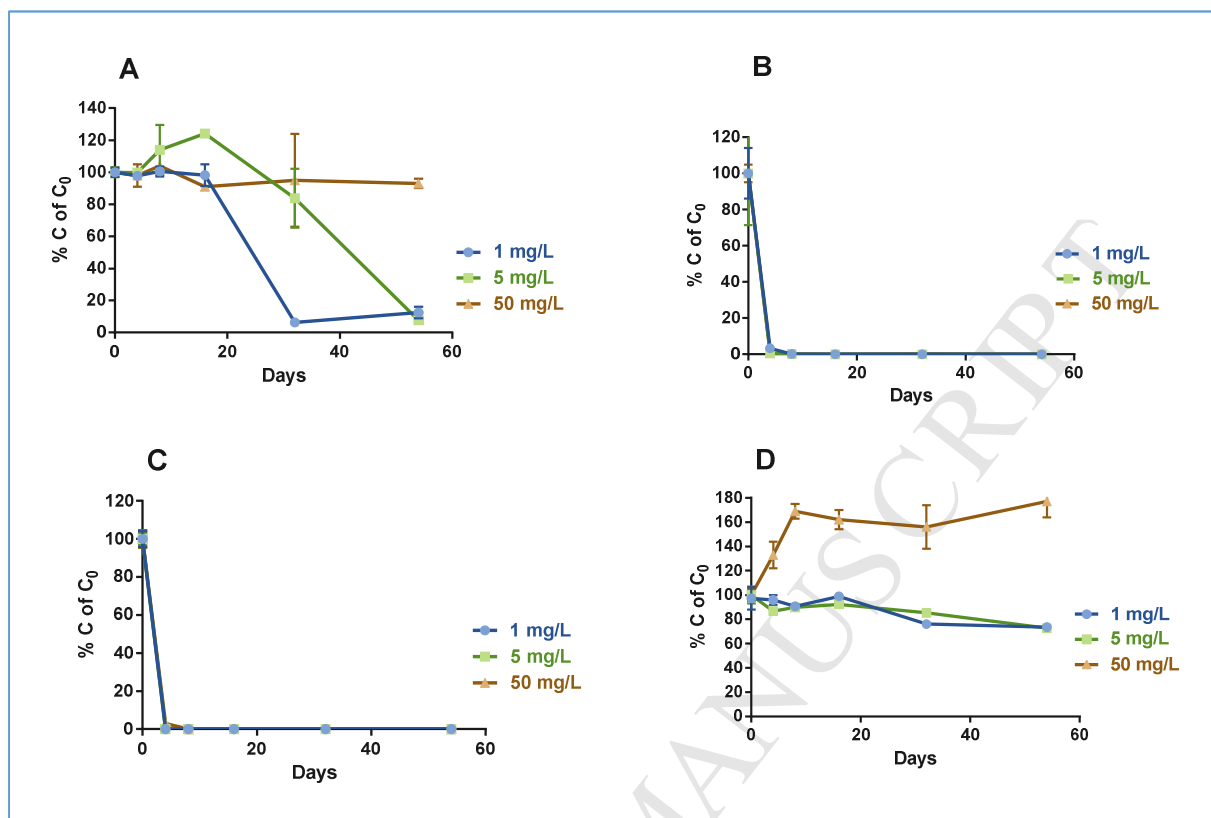
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Figures

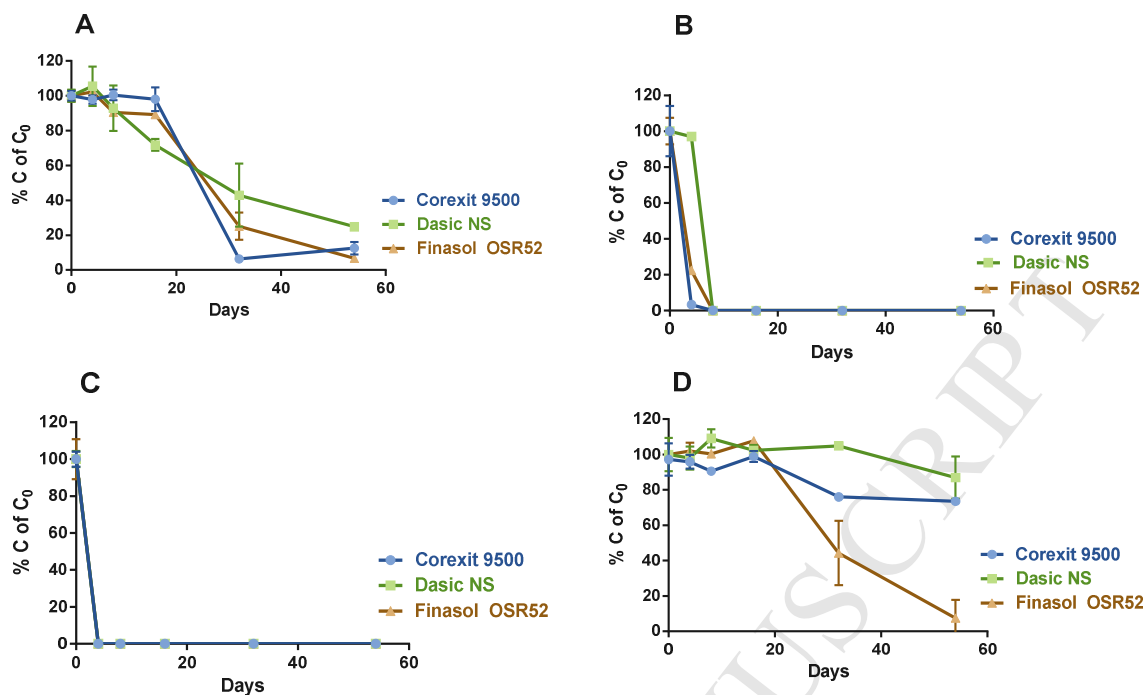


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270 Fig. 1. Biotransformation of DOSS (A), Tween 80 (B), Tween 85 (C) and EHSS (D) in
271 Corexit 9500A at three different dispersant concentrations, 1 mg/L, 5 mg/L and 50 mg/L. The
272 results were determined as % depletion of the concentration at the start of the experiment
273 (C₀).

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277 Fig. 2 Biotransformation of DOSS (A), Tween 80 (B), Tween 85 (C) and EHSS (D) in
278 Corexit 9500A, Dasic NS and Finasol OSR52. The results were determined as % depletion of
279 the concentration at the start of the experiment (C₀). The experiment was performed with low
280 dispersant concentrations (1 mg/L).

Highlights

- Surfactants in commercial dispersants were biotransformed in natural seawater at 5°C
- Non-ionic surfactants transformed faster than the anionic surfactant DOSS
- DOSS biotransformation increased by reduced concentrations
- Surfactant will be rapidly biodegrade at environmentally relevant concentrations

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