

Contradictory Conclusions Surrounding the Effects of Chemical Dispersants on Oil Biodegradation

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ABSTRACT

Dispersed oil has now been shown to have a ‘half-life’ of 10-30 days in numerous lab and field-based experiments. On the other hand, the biodegradation of floating oil slicks is much slower, and unless dispersed by heavy weather or the addition of chemical dispersants, spilled oil will likely strand on a shoreline where it may persist for years. Dispersants are designed to mix into the oil to lower the interfacial tension between the oil and the seawater, allowing even minor turbulence to generate small droplets that have essentially neutral buoyancy. Whether droplets are generated by breaking waves in the absence of added dispersants, or by much less turbulence after dispersant application, the enormous volume of seawater available for dilution impedes the coalescence of the droplets. Droplet formation stimulates biodegradation by dramatically increasing the oil surface area for microbial colonization. Even though the majority of peer-reviewed literature strongly indicates that chemical dispersants have minimal effects once oil is dispersed, a sub-set of publications report negative effects of dispersants on microorganisms and oil biodegradation. It is likely that differences in experimental design and expectations have led to different conclusions regarding the effects of dispersants. As interest in oil spill response grows throughout the scientific community, it is important to understand how similar scientific questions have led to varying conclusions. Here we highlight the importance of experimental

design and how the use of specific methods can produce apparently contradictory results.

Various methods from numerous publications involving the fate of undispersed oil and dispersed oil will be compared and contrasted. We will focus on specific details of experimental design that impact the results and conclusions of various oil biodegradation studies, such as temperature, concentration, media storage, and substrate handling. An emphasis will be placed on experimental relevance and challenges associated with replicating real world phenomena in the laboratory.

INTRODUCTION

The frequency and volume of oil spills from tankers has decreased significantly in the past 40 years (ITOPF, 2018). Knowledge gained from the 1989 *Exxon Valdez* oil spill has resulted in critical advances in both mechanical safety standards (e.g. double hulled tankers) and science-based oil spill response technology. Even though decades of research supports the overall environmental benefit of applying dispersants to surface oil spills, concerns surrounding the use of chemical dispersants persist as researchers are tasked with summarizing the knowledge gained from the Deepwater Horizon oil spill. During that tragedy, more than 500 million liters of light crude oil (Barbier, 2015) were discharged at the well head over 84 days in 1500 m of seawater 77 km offshore. The Deepwater Horizon oil spill response included mechanical recovery, aerial delivery of chemical dispersants and *in situ* burning. In a novel development, subsea dispersant injection (SSDI) was implemented to disperse the escaping oil at depth, hindering re-coalescence into larger droplets and reducing volatile oil from surfacing near vessels and emergency response personnel (Rorick et al., 2012).

While dispersants were initially aimed at reducing bird mortality and minimizing ‘black tides’ arriving at shorelines (Canevari, 1974; Hayes, 1999; Lessard and DeMarco, 2000), it is

now apparent that the dramatic dilution associated with dispersion and the radically increased surface area available for microbes to degrade the oil are major benefits. Nevertheless, even though dispersants were proven to be quite effective during the Deepwater Horizon spill (Bejarano et al., 2013), some members of the scientific community remain unconvinced of the benefits of dispersants (Kleindienst et al., 2015a).

Dispersants are carefully crafted mixtures of surfactant compounds and a hydrocarbon carrier solvent (Canevari, 1974; Word et al., 2015). When dispersants are mixed with oil they reduce the interfacial tension between the oil and the water, allowing the oil to be mixed into the water as tiny droplets with much less energy than would be required in the absence of dispersants. Because the effect is to ‘make the oil disappear’, there has been concern that dispersants mask a problem rather than deal with it (Bradshaw, 2014). Transforming an oil slick into tiny droplets in the water column is indeed the main function of dispersants, but this also has the important effect of increasing the surface area of the oil available to oil-degrading microorganisms and thus increasing its biodegradation (Brakstad et al., 2015). A typical dispersant application aims for one part dispersant for every 20 parts of oil, a dispersant to oil ratio (DOR) of 1:20.

‘Oil spill dispersants’ were first used on a large scale following the *Torrey Canyon* spill off the southwest coast of England in 1967, and there were anecdotal reports that they had significant adverse environmental impacts (Southward and Southward, 1978). But extrapolating environmental impacts from the reported response activities is difficult, at best. First, the measured effects were seen on shorelines that may or may not have received treatment with chemicals and/or flame throwers (Cowan, 1968). Second, the products used were principally commercial degreasers, designed to be used in factories and not the open environment.

Nevertheless, the apparent negative effects ignited public concern surrounding the use of dispersants and motivated scientists to formulate environmentally safe yet effective products that are intended to be applied some distance from shorelines.

Current chemical dispersants on the United States (US) EPA National Contingency Plan Subpart J list (US EPA, 2020) have undergone substantial laboratory testing to ensure their environmental safety. Although these dispersants are pre-approved for use, they are used quite sparingly: NOAA has only 27 records of dispersant use in U.S.A. waters since 1968 (NOAA, 2019). There are 19 dispersants on the National Contingency Plan Product List (US EPA, 2020), but to date the most widely used in the USA is Corexit[®], with Corexit[®] 9527 and 9500 being used in the response to the Deepwater Horizon blowout. The formulations of Corexit[®] 9527 and Corexit[®] 9500 are quite similar, as they both contain the ionic surfactant *bis*-(2-ethyl-hexyl)sulfosuccinate (DOSS), the non-ionic surfactants sorbitan monooleate (Span 80), sorbitan monooleatepolyethoxylate (Tween 80), and sorbitan trioleatepolyethoxylate (Tween 85), with hydrotreated light petroleum distillate and dipropylene glycol *n*-butyl ether (Corexit[®] 9500) or 2-butoxyethanol (Corexit[®] 9527) as solvents. All these components are Generally Regarded as Safe (GRAS) by the Food and Drug Administration when used as a food additive (Burdock and Carabin, 2004). The intrinsic toxicities of the Corexit[®] formulations have been extensively studied and Corexit[®] 9500 falls into the moderately toxic category for mysid shrimp and silverside fish (US EPA, 2010; Gardiner et al., 2013). Surfactants are the predominant ingredients in many household products as well as dispersants, and the acute toxicity of Corexit[®] 9500 to marine aquatic organisms is either similar to or less toxic than common household cleaning agents (Word et al., 2015). Even though these dispersants have high dispersant effectiveness with minimal associated toxicity (Hemmer et al., 2011; Gardiner et al., 2013;

Stroski et al., 2019), there is still concern from the public about the environmental impact of dispersants (Franklin and Warner, 2011).

Many laboratory and field studies have reported the fate and effects of oil, dispersed oil, and Corexit® alone. Unfortunately, a subset of publications have fueled concern against dispersants by incorporating environmentally unrealistic methods that are known to produce questionable results and reporting unsupported conclusions. These publications have become a source of confusion among spill responders. In 2015, a study by Kleindienst et al. gained notoriety for claiming that dispersants exert a negative effect on microbial hydrocarbon degradation rates, but the article actually presented data showing rapid biodegradation in the presence of dispersant that would not have been seen with undispersed oil. Nonetheless, this finding was obscured by that unsubstantiated claim and Kleindienst et al. (2015b) is still widely cited despite more than a dozen papers disputing its conclusion (Prince et al., 2016a; Techtmann et al., 2017; McFarlin et al., 2018; Gofstein et al., 2020; Brakstad et al. 2018).

The objective of this paper is to identify common experimental variables in biodegradation experiments that are known to influence the results and impact the conclusions surrounding the use of dispersants. This review can assist oil spill response decision makers in determining if a study is likely to mimic the fate of oil during a real spill event. We aim to highlight the biodegradation rates of oil in different marine environments while explaining that contradictory conclusions often depend on what is being compared. We believe that when the biodegradation of dispersed oil is compared to the biodegradation of undispersed oil it is clear that effective dispersion dramatically decreases the lifetime of spilled oil in the environment.

Tables 1 and 2 include half-lives of total detectable hydrocarbons, and total alkanes, respectively, from studies that attempted to mimic environmental conditions during a surface oil

spill. Studies that (1) utilized indigenous microorganisms as the inoculum, (2) were conducted at temperatures and with nutrient concentrations representative of the inoculum's environment, and (3) contained concentrations of oil or dispersant that represented those measured during an oil spill are listed in Tables 1 and 2. For comparison, Olson et al. (2017) and Zhuang et al. (2016) are included in the table as they were conducted with high oil and nutrient concentrations, with the later also including an artificial inoculum.

DISCUSSION

The rate at which oil degrades depends upon environmental conditions as well as physical attributes of the oil, such as the amount and type of oil. The main environmental variables that influence the fate of oil include its concentration, nutrient concentrations, oxygen concentration, temperature, microbial abundance and the presence of oil degrading genes in the indigenous microbiota. The following section outlines how these physical and environmental variables can be manipulated in laboratory studies to influence the fate of oil.

Dissolved or dispersed oil

Depending upon the environment of interest, the method of introducing oil or dispersant into experimental incubations can impact bioavailability and thus biodegradation. Only a few compounds in a spilled oil have significant solubility (Prince et al., 2017), and these are lost from oil droplets quite near the source (Murray et al., 2019). Nevertheless, Kleindienst et al. (2015b) performed biodegradation incubations using a Chemically-Enhanced Water Accommodated Fraction (CE-WAF), a method originating from aquatic toxicity standard operating protocols (Singer et al., 2000). A CE-WAF is typically made with filtered seawater, oil (0.01 – 25 g/L) with a DOR of 1:20 (using Corexit[®] 9500 or 9527), moderate mixing energy for 18 hours

followed by a 6 hour settling time. CE-WAFs are the preferred systems for aquatic toxicity tests because the long settling time is a defined technique to minimize droplets of oil in the aspirated sample (since oil droplets rise to the surface). This subsequently limits the physical effects of droplets on the test species and allows the results to indicate chemical effects as intended for aquatic toxicity tests. Nevertheless, CE-WAFs were not designed for use in biodegradation experiments of whole oil because the defined removal of oil droplets creates an unrealistic system that limits the opportunity for certain oil-degraders to attach to the surface of droplets and biodegrade particulate oil. In fact most of the oil (typically >90%) added to the vessels to generate WAF and CE-WAF floats to the surface during the quiescent settling time, so any experiments on the fate of the accommodated fraction are ignoring the substantial majority of the oil. Furthermore, by removing most droplets, insoluble hydrocarbons are removed from the CE-WAF sample. This not only creates a test system that neglects the influence of the majority of hydrocarbons within the oil, it also prevents the use of conserved internal markers, such as hopane, as these compounds remain with the particulate oil. Hopane is very resistant to biodegradation and serves as a conserved internal standard within the oil for assessing the biodegradation of oil compounds (Prince et al., 1994). This means that when using CE-WAFs, estimates of biodegradation have to rely on absolute amounts of measured analytes rather than on the ratio of biodegradable to non-biodegraded hydrocarbons, which in turn substantially increases the noise in the experimental data.

Conclusion: biodegradation incubations based on water-accommodated fractions neglect the impact of particulate oil droplets, which are actually the major fraction of dispersed oil in the sea. Experiments aimed at understanding the biodegradation of spilled oil should not use ‘water-accommodated fractions’ because these only represent a small fraction of the whole oil.

Microorganisms

The inoculum (i.e. the quantity and diversity of microbe(s) added to the incubation) in biodegradation experiments can vary drastically and the type of inoculum has a substantial effect on the rate of oil biodegradation. Different research groups have used an abundance of a single microbial species, a selection of different species, an enriched natural community of microorganisms selected from a specific environment, or a fresh sample from a natural seawater. Diverse microbial communities are known to support biodegradation across the widest range of hydrocarbons, and soils are known to have a higher abundance of microorganisms than fresh water and seawater, sequentially. Of course experiments with freshly collected seawater are generally more environmentally relevant than those with laboratory-made seawater (e.g. Instant Ocean); however, lab-made test media allow control over environmental variables, such as nutrient concentrations and microbial structure.

Conclusion: Experiments should use natural fresh seawater from as close to the potentially impacted site as possible, or use water from a similar marine environment.

Nutrients

Crude oil is an unusual substrate for microbial growth in that while it provides a rich source of carbon and energy, it lacks the other elements essential for microbial growth, such as biologically available nitrogen, phosphorus and iron. Fortunately for oil-degrading microbes, these nutrients are available at low levels in seawater (e.g. Levitus, 1993), and as dispersed oil dilutes in the hours and days after dispersion, the microbes degrading the oil are provided the nutrients they need at appropriate levels.

If oil gets to a shoreline it becomes more concentrated as a coating on beach solids. Biodegradation can then become limited by available nutrients, even though they are naturally provided by tidal exchange in seawater environments. In such situations, biodegradation could be increased by the careful application of fertilizers, as demonstrated during the *Exxon Valdez* oil spill response (Bragg et al., 1994). But it would not be advisable to add nutrients to open water environments, such as lakes, rivers, or oceans, where many trophic levels (i.e. algae, fish and marine mammals) are sensitive to and negatively impacted by high nutrient concentrations.

When nutrients are added to oil biodegradation experiments with high oil concentrations (> 20 ppm), microorganisms are no longer limited by indigenous nutrient concentrations and are thus able to biodegrade oil at faster rates than incubations without added nutrients. When determining the environmental relevance of experimental biodegradation results, it is important to know the concentration of added nutrients and how this concentration reflects the environment of interest. As expected, the fastest biodegradation rates in Table 1 are from experiments with added nutrients. Both Olson et al. (2017) and Zhuang et al. (2016) incubated high oil concentrations, 67 and 700 ppm, respectively, with high nutrient concentrations and reported half-lives of ~ 3 days for total alkanes (Table 2). Such unrealistically high nutrient concentrations are commonly added to biodegradation experiments to support the biodegradation of high concentrations of oil, but it is important to recognize that such high concentrations of oil only occur on shorelines, and have little relevance to open water, where substantial dilution occurs (Lee et al., 2013).

Conclusion: Oil added to experimental incubations should be added at concentrations as environmentally realistic as possible – a few ppm or less for open sea environments, to ensure indigenous nutrient concentrations will sustain the full extent of biodegradation and prevent

nutrient limitation. If laboratory experiments are incubated for long periods of time (e.g. > 28 days) in a closed system, it may be necessary to add a small amount of nutrients to maintain indigenous nutrient concentrations, as natural nutrient renewal is prevented in sealed containers. For soil and shoreline biodegradation experiments, the advantages of adding nutrients are well documented (Bragg et al., 1994) but concentrations should be monitored as high nutrient concentrations can be toxic to some microorganisms (Chaineau et al., 2005).

Temperature

The widespread use of refrigeration to preserve meats (Daughtry et al., 1997) has led to the general expectation that low temperatures dramatically slow microbial growth, with the corollary that oil biodegradation will be slow in cold water. But in fact it is now clear that the indigenous microbes of any particular environment are adapted to the temperatures of that environment, and various community members possess reasonably similar growth rates at their natural temperatures. Furthermore, all show similar dependence around their natural conditions – they slow a little if it gets colder, and speed up if it gets a little warmer (Ratkowsky et al., 1982).

The key finding is that the rate of metabolism (i.e. growth and biodegradation) at the ‘normal’ temperature of growth for a microbial community is remarkably similar in Arctic and temperate climates (Rivkin et al., 1996). For example, McFarlin et al. (2014), Brakstad et al. (2015), and Prince et al. (2013) conducted experiments at -1 °C, 5 °C and 8 °C and reported half-lives of 37, 26, and 14 days, respectively, for total measurable oil (Table 1). Alkanes which make up approximately 3 - 10% of a crude oil, were shown to degrade at an estimated rate of 0.23 day⁻¹ (half-life = 3) at -1.7°C in microcosms containing sea ice and seawater collected from Canada’s Northwest Passage and in the absence of added nutrients (Garneau et al., 2016). The Arctic and sub-Arctic experiments mimicked by McFarlin et al. (2014) and Brakstad et al. (2015) produced

slightly longer half-lives compared to the temperate experiment mimicked by Prince et al. (2013), but the difference likely includes temperature effects on the viscosity of oil. Similarly, Rubicic et al. (2018) measured biodegradation rates of dispersed oil over 64 days at different temperatures and reported that total alkanes had a slight decrease in half-lives at 13 °C (5-7 days) compared to 5 °C (10-17 days) (Table 1), but again the overall biodegradation rate of total dispersed oil was quite similar at 5 °C and 13 °C as shown by the residual chemical profile at day 64. This indicates extrapolates from one environment at its natural temperature to another by using a relationship such as Q_{10} (the change in rate over a 10 °C range) may be misleading (Bagi et al., 2013).

Alas what may limit biodegradation is the physical properties of the spilled oil, in particular the pour point; waxy crudes rich in larger alkanes gel at temperatures <0 °C, and pose a challenge to both mechanical recovery and dispersion (Strøm-Kristiansen et al., 1997). Most oils, however, remain liquid while floating at sea and are amenable to dispersion with the aid of dispersants during the window of opportunity (Lessard and DeMarco, 2000). Once dispersed, even at polar temperatures, crude oils are likely to undergo significant biodegradation (McFarlin et al., 2014; Garneau et al., 2016).

Conclusion: Extrapolating rates from one temperature to another is unlikely to be accurate – far better to do experiments at the temperature of interest.

Oil concentration

The type of crude oil and its concentration has a significant effect on the rate of biodegradation of both total hydrocarbons and individual hydrocarbons. It is well known that light crude oils biodegrade faster than heavier crudes, as heavier crudes contain a relatively

larger quantity of higher molecular weight hydrocarbons, resins and asphaltenes that are known to degrade more slowly (Prince et al. 2013). Oils that have been biodegraded in their initial reservoir are not likely to degrade promptly if spilled at sea (King et al., 2014).

As Prince and Butler (2014) pointed out, to determine the effects of dispersant application on the biodegradation of a surface oil spill, one should compare the dispersed oil plume to a contiguous surface oil slick, not to a physically-dispersed oil. Prince and Butler (2014) utilized floating glass booms to hold oil as a slick during an incubation experiment. Their treatment with Corexit dispersed the oil immediately into the flask and 84% of the oil was lost in 40 days, while the treatment with oil-alone stayed as a slick and only 33% of the oil was lost in 40 days. This emphasizes that if spill responders choose not to apply a dispersant, they must recognize that it is likely that the oil will remain a surface slick of concentrated oil that would impact surface dwelling organisms and continue to float until it hits a shoreline. Therefore, if researchers aim to determine the effects of dispersant application on the fate of a surface oil spill, a control treatment mimicking a surface oil slick should be included in experimental designs.

Various methods have been utilized to add oil to biodegradation experiments, with the majority directly adding oil and dispersant to the water's surface. Methods for creating the dispersed treatments are more variable with some incorporating direct addition of oil and dispersant separately, premixing oil and dispersant together, or utilizing a droplet generator. Unfortunately, many experiments have utilized 'water accommodated fractions', without (WAF) or with dispersants (Chemically Enhanced Water Accommodated Fraction – CEWAF). These follow the methodology of Singer et al. (2000), which was aimed at reproducible methods for determining the toxicity of dispersed oil. Physical fouling with oil droplets complicates efforts to determine the worst-case 'toxicity' of oil to macrobiota (small fish and shrimp) rather than its

general lethality, due to challenges separating physical effects from chemical effects. Singer et al. (2000) developed methods that minimized the formation of droplets while encouraging the dissolution of soluble components, but in allowing most of the oil to float on the surface of their initial bottles, and drawing the ‘accommodated fractions’ from the bottom of those vessels, they typically ignored the vast majority, typically >90%, of the oil. Experiments that add low concentrations of oil directly to experimental systems more closely model marine oil spills.

Dispersant application during an oil spill response is not always necessary, especially if low volumes of oil are spilled. If enough wave action (or other mixing energy) is present, low oil concentrations disperse naturally, without the aid of a chemical dispersant. McFarlin et al. (2014) conducted incubations with natural seawater and 2.5 ppm of crude oil with and without a small amount of dispersant (0.167 ppm), and showed that physically dispersed oil biodegraded at a similar rate as chemically dispersed oil at low oil concentrations (Table 1). Prince et al., 2016b found no significant differences between three different dispersants, and Brakstad et al. (2018) showed that droplet size has a greater impact on biodegradation rate than the type and amount of dispersant applied. In fact oil biodegradation is most strongly influenced by the surface area of oil, with faster rates observed for smaller oil droplets regardless of the presence of dispersant (Brakstad et al., 2015).

Concentrations of dispersed oil under successfully dispersed slicks or in subsurface ‘plumes’ are very low (<1 ppm within hours; Lee et al., 2013; Bejarano et al., 2013), yet most experiments are conducted with oil at concentrations more than a magnitude higher because of the detection limits of analytical instruments. High oil concentrations in static biodegradation experiments decrease oil biodegradation rates to unrealistically low levels by creating a scenario that is never encountered in the real world. The higher the oil concentration in a closed

experimental system, the higher the likelihood that oil will be in larger droplets, with smaller surface to volume ratios, and that nutrients will limit biodegradation. Microbes in experiments with lower oil concentrations are able to rely on the indigenous nutrients in the seawater for metabolic functions. Higher oil concentrations in dispersed oil incubations also increases the frequency that oil droplets encounter each other, promoting agglomeration to form larger droplets that eventually coalesce and float or adhere to the glass walls of incubation containers. Larger droplets (and the floating oil slick) likely hinder the diffusion of soluble aromatics to the droplet-water interface. Prince et al. (2017) showed that these effects began to be significant at 100 ppm oil in New Jersey seawater, and therefore recommended doing experiments at lower concentrations than that.

Conclusion: Dispersed oil is present at very low concentrations in the real world, so experiments aimed at understanding its fate must do experiments at reasonably similar concentrations. Experiments aimed at understanding the effects of dispersants on oil degradation must include a control where the oil will not disperse.

CONCLUSIONS

If conclusions from oil biodegradation experiments are to inform spill responders on the fate of oil and the efficiency of dispersants, then those experiments must include experimental variables (e.g. inoculum, temperature, and concentration of nutrients, oil, and dispersant) that mimic those of the environment of interest. In particular, experimenters must bear in mind that dispersants aid the dispersion of oil slicks, but have the potential to leave the dispersed droplets due to their water solubility. Furthermore, components of Corexit are degraded at similar rates to that of dispersed oil (McFarlin et al., 2018). In summary, dispersants have no significant impact on the biodegradation of physically dispersed oil (Tables 1 and 2), but that dispersed oil,

however generated, is degraded much more rapidly than when it is in a slick. Field samples of spilled oil have shown that floating oil slicks experience minimal biodegradation at sea (Aeppli et al., 2014).

Biodegradation experiments that aim to inform the fate of oil in the environment should carefully consider the experimental design as some environmental variables can profoundly impact the results and conclusions of laboratory experiments. For example, it is important to include a treatment mimicking a surface oil slick if the objective of the experiment is to determine the effect of dispersant application on a surface spill. Furthermore, it is important for researchers to acknowledge the limitations of experiments conducted with ‘water-accommodated fractions’. Initial oil concentrations in biodegradation experiments should be as environmentally realistic as possible to ensure that natural nutrient concentrations can sustain biodegradation and prevent nutrient limitation. In conclusion, experimental design should account for natural dilution and be conducted at the temperature of interest and with indigenous microorganisms from the environment of interest. Experimental results should not be used to support environmental predictions if the experiment does not mimic the environment of interest.

Oil Type	Half-life	Inoculum	Temp.	Dispersant Type	Citation
Alaska North Slope crude oil	14 days	NJ seawater, USA	8°C	None	Prince et al., 2013
Alaska North Slope crude oil	11 days	NJ seawater, USA	8°C	Corexit® 9500 at DOR 1:20	Prince et al., 2013
Alaska North Slope crude oil	7 days	NJ seawater, USA	21°C	Corexit® 9500 at DOR 1:20	Prince and Butler. 2014
Alaska North Slope crude oil	33 days	Barrow seawater, USA	-1°C	None	McFarlin et al, 2014
Alaska North Slope crude oil	25 days	Barrow seawater, USA	-1°C	Corexit® 9500 at DOR 1:20	McFarlin et al, 2014
Macondo crude oil	26 days	Trondheimsfjord seawater, Norway	5°C	Corexit® 9500 at DOR 1:100	Brakstad et al., 2015
Macondo crude oil	11 days	Gulf of Mexico seawater, USA	5°C	Corexit® 9500 at DOR 1:100	Wang et al., 2016
Alaska North Slope crude oil	7 days	NJ seawater, USA	20°C	Corexit® 9500 at DOR 1:20	Prince et al., 2016b
Alaska North Slope crude oil	7 days	NJ seawater, USA	20°C	Dasic Slickgone NS at DOR 1:20	Prince et al., 2016b
Alaska North Slope crude oil	7 days	NJ seawater, USA	20°C	Finasol OSR52 at DOR 1:20	Prince et al., 2016b
European crude oil	13 days	Logy Bay seawater, Canada	5°C	Corexit® 9500 at DOR 1:15	Prince et al., 2016c
European crude oil	10 days	NJ seawater, USA	21°C	Corexit® 9500 at DOR 1:15	Prince et al., 2017
Bintulu crude oil	28 days	Penang seawater, Malaysia	26°C	none	Zahed et al., 2010
Bintulu crude oil	15 days	Penang seawater, Malaysia	26°C	Corexit® 9500 at DOR 1:20	Zahed et al., 2010

Table 1. Summary of half-lives of total detectable hydrocarbons from oil biodegradation experiments that have attempted to mimic environmental conditions during an oil spill. The type of inoculum, temperature (temp.), and the type of dispersant used (including its dispersant to oil ratio, DOR) is described for each measured half-life. We note that ‘Total detectable hydrocarbon’ (as measured by Gas Chromatography) typically measures 50-70% of an oil, and not the ‘missing’ components of higher molecular weight hydrocarbons, resins and asphaltenes, which do not enter standard gas chromatography systems.

Oil Type	Half-life	Inoculum	Temp.	Dispersant Type	Citation
Macondo crude oil	~3 days	Gulf of Mexico seawater, USA+ high oil and nutrients	25°C	none	Olson et al., 2017
Macondo crude oil	~3 days	Gulf of Mexico seawater, USA + high oil and nutrients	25°C	Corexit [®] 9500 at DOR 1:20	Olson et al., 2017
Statfjord C crude oil	9 days	Trondheimsfjord seawater, Norway	5°C	none	Brakstad et al., 2018
Statfjord C crude oil	9 days	Trondheimsfjord seawater, Norway	5°C	Slickgone NS at DOR 1:100	Brakstad et al., 2018
Statfjord C crude oil	12 days	Trondheimsfjord seawater, Norway	5°C	Slickgone NS at DOR 1:25	Brakstad et al., 2018
Statfjord C crude oil	10 days	Trondheimsfjord seawater, Norway	5°C	Slickgone NS at DOR 1:10	Brakstad et al., 2018
Grane crude oil	10 days	Trondheimsfjord seawater, Norway	5°C	Corexit [®] 9500 at DOR 1:100	Ribicic et al., 2018
Grane crude oil	5 days	Trondheimsfjord seawater, Norway	13°C	Corexit [®] 9500 at DOR 1:100	Ribicic et al., 2018
Troll crude oil	17 days	Trondheimsfjord seawater, Norway	5°C	Corexit [®] 9500 at DOR 1:100	Ribicic et al., 2018
Troll crude oil	7 days	Trondheimsfjord seawater, Norway	13°C	Corexit [®] 9500 at DOR 1:100	Ribicic et al., 2018
South Louisiana crude oil	25 days	Artificial + enrichment culture from Gulf of Mexico seawater + high oil and nutrients	5°C	none	Zhuang et al., 2016
South Louisiana crude oil	9 days	Artificial + enrichment culture from Gulf of Mexico seawater + high oil and nutrients	5°C	JD-2000 at DOR 1:25	Zhuang et al., 2016
South Louisiana crude oil	3 days	Artificial + enrichment culture from Gulf of Mexico seawater + high oil and nutrients	25°C	none	Zhuang et al., 2016
South Louisiana crude oil	3 days	Artificial + enrichment culture from Gulf of Mexico seawater + high oil and nutrients	25°C	JD-2000 at DOR 1:25	Zhuang et al., 2016
Arabian Light	3 days	Under ice, Resolute Passage Nunavut, Canada	-1.7°C	none	Garneau et al., 2016

Table 2. Summary of half-lives of total alkanes. The table includes oil biodegradation experiments that have attempted to mimic environmental conditions during an oil spill, with exception to Olson et al. (2017) and Zhuang et al. (2016). The type of inoculum, temperature (temp.), and the type of dispersant used (including its dispersant to oil ratio, DOR) is described for each measured half-life. We note that total alkanes only account for 3-10% of most oils.

REFERENCES

- Aeppli, C., Carmichael, C.A., Nelson, R.K., Lemkau, K.L., Graham, W.M., Redmond, M.C., Valentine, D.L., Reddy, C.M. 2012. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. *Environmental Science and Technology*. 46: 8799-8807. DOI: 10.1021/es3015138
- Bagi, A., Pampanin, D.M., Brakstad, O.G., Kommedal, R. 2013. Estimation of hydrocarbon biodegradation rates in marine environments: a critical review of the Q10 approach. *Marine Environmental Research*. 89: 83–90. 10.1016/j.marenvres.2013.05.005
- Barbier, C.J. 2015. Oil Spill by the oil rig “Deepwater Horizon” in the Gulf of Mexico on April 20, 2010. Case 2:10-md-02179-CJB-SS, Document 14021, MDL 2179, Section J. <http://www.laed.uscourts.gov/sites/default/files/OilSpill/Orders/1152015FindingsPhaseTwo.pdf> (last accessed January 2021).
- Bejarano A.C., Levine, E., Mearns, A.J. 2013. Effectiveness and potential ecological effects of offshore surface dispersant use during the Deepwater Horizon oil spill: a retrospective analysis of monitoring data. *Environmental Monitoring and Assessment*. 185: 10281-1095. DOI: 10.1007/s10661-013-3332-y
- Bradshaw, E.A. 2014. State-corporate environmental cover-up: The response to the 2010 Gulf of Mexico oil spill. *State Crime Journal*. 3: 163-81. DOI: 10.13169/statecrime.3.2.0163
- Bragg, J.R., Prince, R.C., Harner, E.J., Atlas, R.M. 1994. Effectiveness of bioremediation for the *Exxon Valdez* oil spill. *Nature*. 368: 413-418. DOI: 10.1038/368413a0

- Brakstad, O.G., Ribicic, D., Winkler, A., Netzer, R. 2018. Biodegradation of dispersed oil in seawater is not inhibited by a commercial oil spill dispersant. *Marine Pollution Bulletin*. 129: 555-561. DOI: 10.1016/j.marpolbul.2017.10.030
- 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. DOI: 10.1016/j.marpolbul.2015.02.006
- Burdock, G.A., and Carabin, I.G. 2004. Generally recognized as safe (GRAS): history and description. *Toxicology Letters*. 150: 3-18. DOI: 10.1016/j.toxlet.2003.07.004
- Canevari, G. 1974. Oil slick dispersant and method. United States patent. US 3,793,218.
- Chaineau, C.H., Rougeux, G., Yepremian, C., Oudot, J. 2005. Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biology and Biochemistry*. 37: 1490-1497. DOI: 10.1016/j.soilbio.2005.01.012
- Cowan, E. 1968. Oil and water: the Torrey Canyon Disaster. Lippincott.
- Daughtry, B.J., Davey, K.R., King, K.D. 1997. Temperature dependence of growth kinetics of food bacteria. *Food Microbiology*. 14: 21-30. DOI: 10.1006/fmic.1996.0064
- Franklin, C.L. and Warner, L.J. 2011. Fighting chemicals with chemicals: the role and regulation of dispersants in oil spill response. *Natural Resources and Environment*. 26: 7-10.
- Garneau, M.È., Michel, C., Meisterhans, G., Fortin, N., King, T.L., Greer, C.W., Lee, K. 2016. Hydrocarbon biodegradation by Arctic sea-ice and sub-ice microbial communities during microcosm experiments, Northwest Passage (Nunavut, Canada). *FEMS Microbiology Ecology*. 92: fiw130. 10.1093/femsec/fiw130

Gardiner, W.W., Word, J.Q., Word, J.D., Perkins, R.A., McFarlin, K.M., Hester, B.W., Word, L.S., Ray, C.M. 2013. The acute toxicity of chemically and physically dispersed crude oil to key arctic species under arctic conditions during the open water season. *Environmental Toxicology and Chemistry*. 32: 2284-2300. DOI: 10.1002/etc.2307

Gofstein, T.R., Perkins, M., Field, J., Leigh, M.B. 2020. The interactive effects of crude oil and Corexit 9500 on their biodegradation in Arctic seawater. *Applied and Environmental Microbiology*. 86(21). DOI: 10.1128/AEM.01194-20

Hayes, M.O. 1999. *Black Tides*. University of Texas Press.

Hemmer, M.J., Barron, M.G., Greene, R.M. 2011. Comparative toxicity of eight oil dispersants, Louisiana sweet crude oil (LSC), and chemically dispersed LSC to two aquatic test species. *Environmental Toxicology and Chemistry*. 30: 2244-52. DOI: 10.1002/etc.619

ITOPF, 2018. *ITOPH Handbook 50 Years Promoting Effective Spill Response 1968-2018*.

https://www.itopf.org/fileadmin/data/Documents/Company_Lit/ITOPF_Handbook_2018.pdf

(last accessed January 2021).

King, T.L., Robinson, B., Boufadel, M., Lee, K. 2014. Flume tank studies to elucidate the fate and behavior of diluted bitumen spilled at sea. *Marine Pollution Bulletin*. 83: 32-37. DOI: 10.1016/j.marpolbul.2014.04.042

Kleindienst, S., Paul, J.H., Joye, S.B. 2015a. Using dispersants after oil spills: impacts on the composition and activity of microbial communities. *Nature Reviews Microbiology*. 13: 388-396. DOI: 10.1038/nrmicro3452.

- Kleindienst, S., Seidel, M., Ziervogel, K., Grim, S., Loftis, K., Harrison, S., Malkin, S. Y., Perkins, M.J., Field, J., Sogin, M.L., Dittmar, T. 2015b. Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proceedings of the National Academy of Sciences*. 112: 14900-14905. DOI: 10.1073/pnas.1507380112
- Lee, K., Nedwed, T., Prince, R.C., Palandro, D. 2013. Lab tests on the biodegradation of chemically dispersed oil should consider the rapid dilution that occurs at sea. *Marine Pollution Bulletin*. 73: 314-318. DOI: 10.1016/j.marpolbul.2013.06.005
- Lessard, R.R. and DeMarco, G. 2000. The significance of oil spill dispersants. *Spill Science and Technology Bulletin*. 6: 59-68. DOI 10.1016/S1353-2561(99)00061-4
- Levitus, S., Conkright, M.E., Reid, J.L., Najjar, R.G., Mantyla, A. 1993. Distribution of nitrate, phosphate and silicate in the world oceans. *Progress in Oceanography*. 31: 245-73. DOI: 10.1016/0079-6611(93)90003-V
- McFarlin, K.M., Perkins, M., Field, J.A., Leigh, M.B. 2018. Biodegradation of crude oil and Corexit 9500 in Arctic seawater. *Frontiers in Microbiology*. 9:1788. DOI: 10.3389/fmicb.2018.01788
- McFarlin, K.M., Prince, R.C., Perkins, R., Leigh, M.B. 2014. Biodegradation of dispersed oil in Arctic seawater at -1°C. *PloS one*. 9:e84297. DOI: 10.1371/journal.pone.0084297
- Murray, K.J., Boehm, P.D., Prince, R.C. 2019. The importance of understanding transport and degradation of oil and gasses from deep-sea. In: Murawski, S.A., Ainsworth, C.H., Gilbert, S., Hollander, D.J., Paris, C.B., Schlüter, M., Wetzel, D.L. (Eds.), *Deep Oil Spills, Facts Fate and Effects*. Springer, Cham. 86-106. DOI: 10.1007/978-3-030-11605-7_6

NOAA, 2019. Map of the Month: Dispersant Use in or near US Waters.

<https://response.restoration.noaa.gov/map-month-dispersant-use-or-near-us-waters> (last accessed January 2021).

Olson, G.M., Gao, H., Meyer, B.M., Miles, M.S., Overton, E.B. 2017. Effect of Corexit 9500A on Mississippi Canyon crude oil weathering patterns using artificial and natural seawater.

Heliyon. 3:e00269. DOI: 10.1016/j.heliyon.2017.e00269

Prince, R.C., Butler, J.D., Redman, A.D. 2017. The rate of crude oil biodegradation in the sea.

Environmental Science and Technology. 51: 1278-1284. DOI: 10.1021/acs.est.6b03207

Prince, R.C., Coolbaugh, T.S., Parkerton, T.F. 2016a. Oil dispersants do facilitate biodegradation of spilled oil. Proceedings of the National Academy of Sciences. 113: E1421-E1421. DOI:

10.1073/pnas.1525333113

Prince, R.C., Kelley, B.A., Butler, J.D. 2016b. Three widely-available dispersants substantially increase the biodegradation of otherwise undispersed oil. Journal of Marine Science: Research and Development. 6: 183. DOI: 10.4172/2155-9910.1000183.

Prince, R.C., Nash, G.W., Hill, S.J. 2016c. The biodegradation of crude oil in the deep ocean.

Marine Pollution Bulletin. 111: 354-357. DOI: 10.1016/j.marpolbul.2016.06.087

Marine Pollution Bulletin. 111: 354-357. DOI: 10.1016/j.marpolbul.2016.06.087

Prince, R.C. and Butler, J.D. 2014. A protocol for assessing the effectiveness of oil spill

dispersants in stimulating the biodegradation of oil. Environmental Science and Pollution

Research. 21: 9506-9510. DOI: 10.1007/s11356-013-2053-7

Prince, R.C., McFarlin, K.M., Butler, J.D., Febbo, E.J., Wang, F.C., Nedwed, T.J. 2013. The primary biodegradation of dispersed crude oil in the sea. *Chemosphere*. 90: 521-526. DOI: 10.1016/j.chemosphere.2012.08.020

Prince, R.C., Elmendorf, D.L., Lute, J.R., Hsu, C.S., Haith, C.E., Senius, J.D., Dechert, G.J., Douglas, G.S., Butler, E.L. 1994. 17 α (H),21(H)-hopane as a conserved internal marker for estimating the biodegradation of crude oil. *Environmental Science and Technology*. 28:142-145. DOI: 10.1021/es00050a019

Ratkowsky, D.A., Olley, J., McMeekin, T.A., Ball, A. 1982. Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology*. 149: 1-5.

Ribicic, D., McFarlin, K.M., Netzer, R., Brakstad, O.G., Winkler, A., Throne-Holst, M., Størseth, T.R. 2018. Oil type and temperature dependent biodegradation dynamics-Combining chemical and microbial community data through multivariate analysis. *BMC Microbiology*. 18: 83. DOI: 10.1186/s12866-018-1221-9

Rivkin, R.B., Anderson, M.R., Lajzerowicz, C. 1996. Microbial processes in cold oceans. I. Relationship between temperature and bacterial growth rate. *Aquatic Microbial Ecology*. 10: 243-254. DOI: 10.3354/ame010243

Rorick, R., Nedwed, T., DeMarco, G., Cooper, C. 2012. Comment on "A Tale of Two Spills: Novel Science and Policy Implications of an Emerging New Oil Spill Model". *Bioscience*. 62: 1009. DOI: 10.1525/bio.2012.62.12.16

Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerdema, R.S. 2000. Standardization of the preparation and quantitation of water-accommodated fractions

of petroleum for toxicity testing. *Marine Pollution Bulletin*. 40: 1007-1016. DOI:

10.1016/S0025-326X(00)00045-X

Southward, A.J. and Southward, E.C. 1978. Recolonization of rocky shores in Cornwall after use of toxic dispersants to clean up the *Torrey Canyon* spill. *Journal of the Fisheries Board of Canada*. 35: 682-706. DOI: 10.1139/f78-120

Stroski, K.M., Tomy, G., Palace, V. 2019. The current state of knowledge for toxicity of Corexit EC9500A dispersant: a review. *Critical Reviews in Environmental Science and Technology*. 49: 81-103. DOI: 10.1080/10643389.2018.1532256

Strøm-Kristiansen, T., Lewis, A., Daling, P.S., Hokstad, J.N., Singaas, I. 1997. Weathering and dispersion of naphthenic, asphaltenic, and waxy crude oils. In: *International Oil Spill Conference Proceedings*. 1997(1): 631-636.

Techtmann, S.M., Zhuang, M., Campo, P., Holder, E., Elk, M., Hazen, T.C., Conmy, R., Santo Domingo, J.W. 2017. Corexit 9500 enhances oil biodegradation and changes active bacterial community structure of oil-enriched microcosms. *Applied and Environmental Microbiology*. 83: e03462-16. DOI: 10.1128/AEM.03462-16.

US EPA, 2020. Product Categories under the National Contingency Plan Subpart J.

<https://www.epa.gov/emergency-response/alphabetical-list-ncp-product-schedule-products-available-use-during-oil-spill> (last accessed January 2021).

US EPA, 2010. Comparative Toxicity of Louisiana Sweet Crude Oil (LSC) and Chemically Dispersed LSC to Two Gulf of Mexico Aquatic Test Species. Washington, DC: U.S. Environmental Protection Agency.

Wang, J., Sandoval, K., Ding, Y., Stoeckel, D., Minard-Smith, A., Andersen, G., Dubinsky, E.A., Atlas, R., Gardinali, P. 2016. Biodegradation of dispersed Macondo crude oil by indigenous Gulf of Mexico microbial communities. *Science of the Total Environment*. 557: 453-468. DOI: 10.1016/j.scitotenv.2016.03.015

Word, J.Q., Clark, J.R., Word, L.S. 2015. Comparison of the acute toxicity of Corexit 9500 and household cleaning products. *Human and Ecological Risk Assessment: An International Journal*. 21: 707-725. DOI: 10.1080/10807039.2014.920227

Zahed, M.A., Aziz, H.A., Isa, M.H., Mohajeri, L. 2010. Effect of initial oil concentration and dispersant on crude oil biodegradation in contaminated seawater. *Bulletin of Environmental Contamination and Toxicology*. 84: 438-442. DOI: 10.1007/s00128-010-9954-7

Zhuang, M., Abulikemu, G., Campo, P., Platten, W.E., Suidan, M.T., Venosa, A.D., Conmy, R.N. 2016. Effect of dispersants on the biodegradation of South Louisiana crude oil at 5 and 25°C. *Chemosphere*. 144: 767-774. DOI: 10.1016/j.chemosphere.2015.08.040