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Oil biodegradation in seawater and impact of dispersants on oil biodegradation characteristics

D2.1

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Executive Summary

Biodegradation of petroleum compounds in marine environments is based on the ability of certain marine microorganisms to degrade petroleum compounds. Microorganisms capable of degrading petroleum compounds include bacteria, fungi and algae. The fate of oil in marine systems is dependent on a number of physical, chemical, and biological factors working independently or in consort with one another. In natural marine environments, biodegradation of crude oil involves a succession of microbial species present at the consortia operating in metabolic networks. Microbial degradation of oil hydrocarbons is an oxidative process in oxic water column, but in the absence of oxygen the degradation of hydrocarbons occurs in the presence of other electron acceptors such as Fe^{3+}, SO_4^{2-} and NO_3^-. Oil biodegradation efficiency and kinetic parameters of marine bacterial strains and microbial communities has been assessed in many studies that include different matrices (water, sediment, soil and sea-ice). Dispersants have been routinely used in the oil spill remediation, and they are believed to increase oil biodegradation efficiency. Despite this, the impact of dispersants on oil degrading microbial community structure and activity remains controversial, and the effectiveness of dispersants and their toxicity to marine microorganisms have been questioned. The main challenge is to compare the oil biodegradation rates between different studies due to the differences in experiment setups and the methods used for measuring the oil degradation rate. Most commonly used method is measuring the disappearance of the oil hydrocarbons during the test using GC/MS or GC-FID, the subsequent calculation of mass balance as an indication of the biodegradation percentage between start and end without any intermediate time points.

In the WP2 laboratory experiments, oil biodegradation kinetic parameters are explored in microcosm experiments using seawater and sediments. The aim of these experiments is to study the biodegradation potential of microbes taken from three geographically different locations: coastal area of the Baltic Sea (Tvärminne), open area of the Baltic Sea (RV Aranda) and Norwegian Sea (Narvik). In addition, the effect of dispersant on the biodegradation efficiency and microbial community abundance and structure will be assessed. The experiments are conducted using North Sea crude oil and dispersant Finasol 51 in Water Accomodated Fractions (WAF). So far the results have shown a 92 % degradation of crude oil without dispersant at 5°C at the open area of the Baltic Sea. Dispersion of crude oil was effective with Finasol 51, which resulted in 150 times higher concentration of oil. The concentration of dispersed oil is difficult to adjust to measurable levels in the biodegradation experiments as droplets of free phase compounds are behaving differently during dilution phases. The experimental work is still ongoing.

1. Oil biodegradation rate and kinetic parameters in marine environment

Crude oils are principally mixture of hydrocarbons– on average ~30% linear and branched alkanes, ~ 30% cyclic alkanes, ~ 30% aromatics (molecules that contain at least one aromatic ring, usually with cyclic and linear alkane substituents) and ~10% molecules with heteroatoms such as S, O, and N (Hazen et al. 2016). The intrusion of oil into a marine system provides a substantial surplus of carbon for microbial growth. However, low availability of nutrients such as nitrogen and phosphorus can limit microbial growth and thus oil degradation. Bioremediation of hydrocarbons is a promising mitigation strategy but challenges remain, particularly due to low microbial metabolic rates in cold, ice-covered seas. Most of the hydrocarbons in dispersed oil are degraded in aerobic marine waters with a half-life of days to months. Oil that becomes entrained in anaerobic sediments is also likely to have a long residence time, although it too will eventually be biodegraded. In contrast, oil that reaches shorelines is likely to be too concentrated, have lower levels of nutrients, and have a far longer residence time in the environment.

Biodegradation of petroleum compounds in marine environments is based on the ability of microorganisms to degrade petroleum compounds. Microorganisms capable of degrading petroleum compound include bacteria, fungi and algae (Xue et al. 2015). The most common microorganisms involved in the oil degradation is bacteria and many different species have been identified to degrade petroleum hydrocarbons e.g. *Acinetobacter, Pseudomonas, Rhodococcus* (Santini 2015). Although there are many different species capable of oil degradation, perhaps the most important species have been considered to be *Alcanivorax* genus (Yakimov et al. 2007).

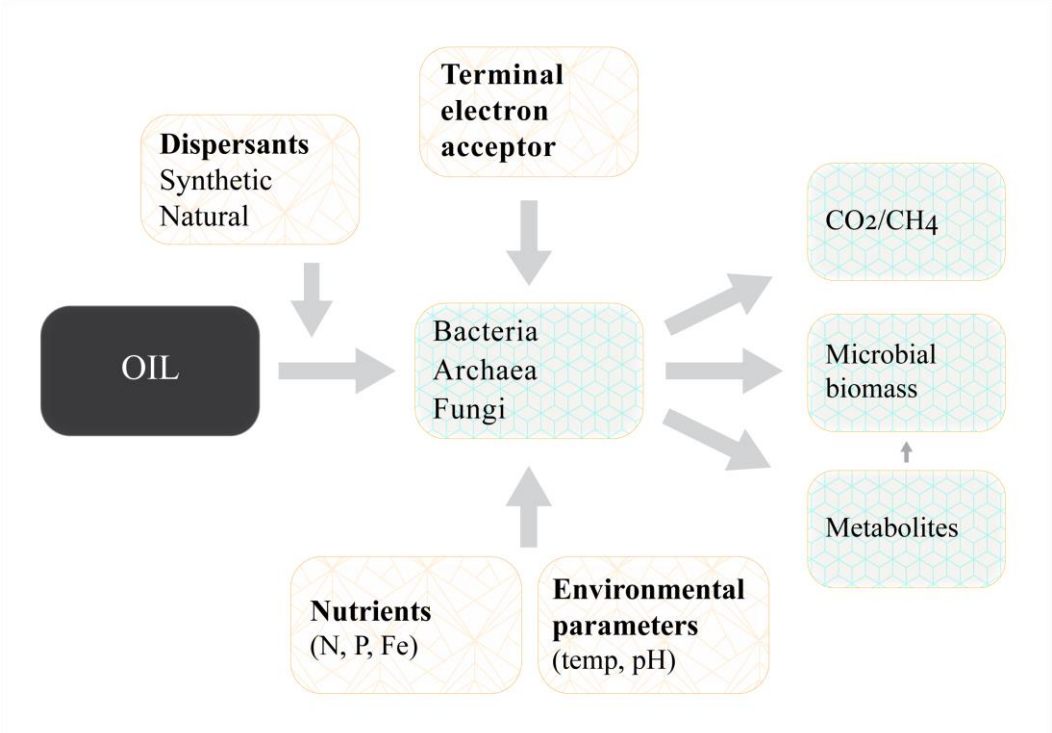

Figure 1. Schematic representation of the environmental factors affecting oil biodegradation in the marine environment. Marine oli-degrading microbes can use during oil degradation electron acceptors such as oxygen, Fe^{3+}, SO_4^{2-} and NO_3^-.

Microbial degradation of hydrocarbons is often an oxidative process where the initial attack is performed by oxygenases and peroxidases. Aerobic conditions are thus necessary for degradation to occur through these pathways. Surface waters are rarely oxygen limited, but oxygen concentrations can decrease throughout the water column and under some circumstances there can be anoxic conditions in bottom waters. Degradation of hydrocarbons in the absence of oxygen has been shown to occur in the presence of other electron acceptors such as Fe^{3+}, SO_4^{2-} and NO_3^-

(Foght 2008). In natural marine environments, biodegradation of crude oil involves a succession of microbial species present at the consortia (Alkatib et al. 2011). This is because a single species can metabolize only certain range of oil hydrocarbons and therefore many different bacterial species are usually involved in the oil biodegradation process (Röling et al. 2002).

Oil biodegradation in the marine environment is mediated by microbial consortia operating in metabolic networks, in which the product of one oxidation process fuels another. Although a particular microbial species is capable of degrading a certain crude oil component, crude oil degradation is more appropriately viewed as a cooperative biodegradation network. Oil-degrading microbial networks are regulated by bottom-up environmental factors (e.g., temperature and the availability of nutrients and electron acceptors) (Figure 1).

1.1. Oil biodegradation kinetic parameters of marine bacterial strains

Because oil biodegradation in marine environment usually involves multiple bacterial species, assessing the degradation of oil using only a single bacterial strain can be strongly biased. However, there are some studies conducted with isolated microbial strains on microbe plates. Microbial strains have also been tested to assess their viability for bioremediation purposes in the cleanup of polluted sites (e.g. Alkatib et al. 2011).

Oil biodegradation rates for single strains vary similarly as with microbial consortia from natural seawater samples. The biodegradation efficiency of *Pseudomonas aeruginosa* and *Escherichia fergusonii* has been examined by Pasumarthi & Chandrasekaran (2013). Biodegradation was assessed by microcosm experiments at room temperature using hydrocarbon contaminated sediment taken from Velsao Beach (India), and by inoculating it with bacteria isolated and enriched from the same sediment samples. Degradation was also measured in the separate microcosm experiment using n-hexadecane as a model hydrocarbon. Hydrocarbon degradation was measured by GC-MS. Bacteria present in the enrichment culture were identified by sequencing (16S rDNA) followed by DGGE. Based on the results alkanes ranging from C_{12} to C_{33} were highly degraded compared to n-C_{34}. Degradation rates for individual compounds C_{12}-C_{33} were 86.1-99.3 % and for C_{34}-C_{40} 76.8-60 %.

In a study by Santisi et al. (2015) bacterial isolates of three different strains (*Pseudomonas stutzeri*, *Rhodococcus erythpolis* and *Alcanivorax borkumensis*) and their mixed consortia were assessed for biodegradation efficiency. The aim of the study was to elucidate the cooperative actions and degradation rates of mixed microbial populations and compare it with individual strains. The study was conducted in microcosms using crude oil at 22°C temperature for the period of 15 days. Hydrocarbons were analyses using GC-FID and expressed as TERHC (Total Extracted and Resolved Hydrocarbons).

1.2. Oil biodegradation kinetic parameters of marine bacterial community (water column, sediments, shoreline)

Numerous studies can be found in literature on petroleum biodegradation rate parameters in different environmental compartments. However, comparing the biodegradation rates between different studies is problematic because of the differences in experiment setups and the methods used for measuring the degradation rate. In some studies, the degradation efficiency has been measured utilizing chemical biomarkers such as hopane or pristine/phytane ratios, half-lives of certain petroleum compounds and in some studies, it has been measured as degradation of all hydrocarbon compounds (or as TPH, Total Petroleum Hydrocarbons) or only certain hydrocarbon

compounds. There are also many differences in the experimental conditions affecting the degradation, for example, experiment type (e.g. microcosm, mesocosm or culture plate studies), oil concentration, oil type, test temperature, experiment duration and a possible addition of nutrients or dispersant). In many of the experiments oil has been applied directly to the experimental vessels or seawater, but in some cases, experiments have been conducted in WAF (Water Accommodate Fractions), and therefore the oil composition and concentrations may differ greatly between the different studies. Because of the many differences between different studies, giving accurate estimates for oil biodegradation rates is difficult.

Petroleum hydrocarbon biodegradation studies have been carried out in different matrices, including water, sediment, soil and sea-ice. The majority of the studies were conducted in seawater, followed by the soil, sediment and ice. When searching for the studies, emphases was put on finding studies conducted in the cold temperatures using medium or heavy oil types, however also studies with higher temperatures and lighter oil types have been included. Overview of the published degradation rates for different marine compartments is summarised in Table 1.

Table 1. Overview of different oil biodegradation studies conducted at different environmental compartments

Compartment	Temperature	Oil type	Number of studies/Source	Experiment durations	Degradation ranges
Sea-water	<15 °C	Heavy Fuel	2 (Venosa et al. 2007 & Brown et al. 2016)	7-46 d	65-71 %, 9.9 d half-lives (alkanes)
		Light/Medium Fuel	11 (Deppe et al. 2004, Brakstad and Bonaunet 2006, Lin et al. 2009, Hazen et al. 2010, Reunamo et al. 2013, Reunamo et al. 2016, Kristensen et al. 2015, Crisafi et al. 2016, Garneu et al. 2016, Mcfarlin et al. 2016)	15-77 d	18-90 %, 2.2-3.5 d half-live (alkanes)
	>15 °C	Heavy Fuel	5 (Hozumi et al. 2000, Medina-Bellver et al. 2005, Gertler et al. 2009, Vila et al. 2010, Germano de Almeida et al. 2017)	21-42 d	Heavy: 30-93.5 %
		Light/Medium Fuel	1 (Kadali et al. 2013)	28 d	28 %
Sediment	<15 °C	Heavy Fuel	2 (Hua et al. 2006, Jimenez et al. 2007	70-220 d	16-96 %
		Light/Medium Fuel:	1 (Reunamo et al. 2016)	77-119 d	51-59 % anoxic sediment, Fe-Mn concretions: 35-80% oxic: 1-18 mg kg-1 d-1, anoxic: 1-9 mg kg^{-1} d^{-1}
	>15 °C	Heavy fuel	3 (Gallego et al. 2006, Genovese et al. 2014, Fernandes-Alvares et al. 2006)	90-730 d	35-100 %
Soil	<15 °C	Light/Medium Fuel	6 (Margesin et al. 1997, Coulon et al. 2004, Salminen et al. 2004, Björklöf et al. 2008, Delille & Coulon 2008, Wang et al. 2015)	42-660 d	30-80 % oxic: 2-23 mg TPH kg^{-1} d^{-1} anoxic: 1.12 mg TPH kg^{-1} d^{-1}
	>15 °C	Heavy Fuel	1 (Cai et al. 2016)	90 d	20 % (TPH) to 95 % (PAH, hopane normalised)
Sea-ice	<15 °C	Light/Medium Fuel	9* (Gerdes et al. 2005, 2006a, 2006b, 2006c, 2006d, Brakstadt et al. 2008, Helmke et al. 2013)	30 d to >2 yr	1.7-5.4 % (hexadecane)

Most of the published biodegradation studies have been carried out as small-scale laboratory (microcosm) experiments, although soil, sediment and ice-water experiment also included mesocosm or field studies. Some of the studies were conducted using weathered contaminated samples, and some using samples spiked with petroleum hydrocarbons. Most experiments were carried out in oxic conditions, but some sediment and soil experiments were carried out also in anoxic conditions.

1.2.1. Chemical analysis

The most commonly used method for chemical analysis of petroleum hydrocarbons is to use gas chromatography combined with mass spectrophotometry (GC-MS) and/or flame ionization detector (GC-FID). With GC-FID hydrocarbons are commonly measured as total petroleum hydrocarbons, which include all hydrocarbon fractions, or separately as different fractions, for example, middle

fractions C10-21, heavy fractions C21-C40. For detection of individual compounds, such as specific polycyclic aromatic hydrocarbons (PAHs) or phytane and hopane, GC-MS is required.

1.2.2. Measurement of oil biodegradation

There is high variability in the methods used for measuring biodegradation rate of hydrocarbons. Most commonly used method is measuring the disappearance of hydrocarbons during the test using GC/MS or GC-FID, the subsequent calculation of mass balance as an indication of the biodegradation percentage between start and end without any intermediate time points. In some cases, biodegradation percentages are reported for individual hydrocarbon fractions, but more often as a range of different fractions (e.g. Björklöf et al. 2008, Kristensen et al. 2015), or including all fractions (total petroleum hydrocarbons) (e.g. Cai et al. 2016, Yu et al. 2013). The biodegradation rate can be calculated by taking the duration of the experiment and the concentration of petroleum hydrocarbon in the environmental compartment is question into account. For estimation of true kinetic parameters of oil biodegradation sampling at several time points are needed. The biodegradation may also be reported as half-lives, or as ratios between the HC fractions and chemical biomarkers, such as phytane (Gerdes et al. 2006) or hopane (e.g. Gallego et al. 2006, Fernandes-Alvares et al. 2006).

1.2.3. Oil biodegradation in seawater

From the studies selected for this review, 7 studies were conducted with heavy fuel oil, rest of the studies (12) were conducted with medium or lighter fuels.

Studies with heavy fuels were carried out in lower temperatures ranging between 0-5 °C (Venosa 2007, Brown 2016) (study durations 7-46 d) and in higher temperatures 17-25 °C (study durations 21-42 d). The degradation rates were between 65-71% and 9.9 d (half-lives) for studies with temperatures <15 °C and 30-93.5% for studies with temperature >15 °C.

Biodegradation studies with lighter fractions were carried out in temperatures between -1.7 to 15 °C. They showed overall higher degradation percentages than heavier fractions. However, the methods used in the experiments for measuring the biodegradation rates, as well as experiment durations, had great variability, making it difficult to the compare the biodegradation results between different studies. The degradation percentages for all hydrocarbon fractions ranged from 0.1% (Kristensen et al. 2015) up to 90% (Lin et al. 2009) for experiments with duration <28 d, for longer studies (duration >28 d) the degradations were between 0.85% (Sharma & Schiewer 2016) to 100 % (Brakstad and Bonaunet 2006).

1.2.4. Oil biodegradation in the sediment compartment

Studies using heavier fuel oil included experiments conducted in sediments (Garrett et al. 2003) and beach/sediment samples containing weathered oil from the Prestige oil spill (Gallego et al. 2006, Fernadez-Alvares et al. 2006, Jimenes et al. 2007). These studies were generally long, from 90 to 220 days. Because studies were conducted at different scales (field and mesocosm), the temperatures during the experiments varied. The biodegradation efficiency in the studies conducted in cold (3.8-6 °C) varied from 16 % (total hydrocarbons) (Hua et al. 2006) to 38-96 % (compared to hopane) (Jimenez et al. 2007). At 20 °C a crude oil biodegradation percentage of 20 % was obtained and it could be enhanced up to 97% removal using aeration of the sediment (Genovese et al. 2014), however, the oil was analyzed by TERHC (total extracted and resolved hydrocarbons) fraction.

1.2.5. Oil biodegradation in the soil compartment

Petroleum hydrocarbon degradation in soil is carried out at various scales including microcosm, mesocosm and pilot-scale studies. Research on removal of heavy petroleum products in soil is mostly carried out at temperatures above (ca. 20 °C) the Arctic average, and there is a clear gap of

knowledge in heavy petroleum biodegradation at a low temperatures. Durations for soil experiments varied between 3 and 12 months.

The biodegradation percentages for heavy fuels commonly observed in literature are between 20 and 54 % (Cai et al. 2016) for the studies that reported the results as TPH. At a lower temperatures, 30-78% biodegradation percentages are often observed (Björklöf et al. 2008, Wang et al. 2015), but the time frame is considerably longer (60-365 d). Degradation was also observed in anoxic conditions (Björklöf et al 2008, Salminen et al. 2004) with rates of 1-12 mg TPH kg^{-1} d^{-1} which was around 50 % compared to rates under oxic conditions of 2-34 mg TPH kg^{-1} d^{-1}.

1.2.6. Oil biodegradation in the ice compartment

Biodegradation studies on sea-ice have been conducted using medium and lighter fuel oils. Two of the studies were conducted as microcosms, but in total most of the studies were conducted as either mesocosm or field studies. Some of the experiments were carried out using melted water from ice cores. In many studies describing sea-ice, hydrocarbon degradation is described qualitatively, and no numeric estimations of the degradation were given. Experiment durations were very long, ranging from 3 months to over 3 years due to the low degradation in the ice.

1.3. Oil biodegradation laboratory experiments in GRACE WP2

In the WP2 laboratory experiments, kinetic parameters of oil biodegradation were investigated by microcosm experiments using seawater and sediment. The aim of the experimental work in WP2 is to investigate the biodegradation potential of microbes from three geographically different locations: coastal area of the Baltic Sea (Tvärminne), open sea of the Gulf of Bothnia (RV Aranda, Fig. 2) and Norwegian Sea (Narvik). In addition, the effect of dispersant on the biodegradation efficiency and microbial abundance and community structure is investigated. The experiments were conducted using North Sea naphthenic crude oil and dispersant Finasol 51 in Water Accomodated Fractions (WAF).

The seawater microcosm experiments are conducted in 1L bottles at 4°C in the dark in shakers (approximately 100 rpm). There are 4 sampling points (0 hours, 24 hours, 48 hours and 12 days) and three different treatments: Control (no oil), WAF (only crude oil) and CE-WAF (oil + dispersant). From each time point, separate bottles were taken for molecular biology and chemical analyses.

WAF and CE-WAF were prepared using guidelines provided by WP3 with some modifications. WAF was prepared by adding 5g oil to 1L water, shaking for 50 hours and settling for 2 hours before use. CEWAF is prepared by making dispersant/oil mix in 1:10 ration and adding the this mixture 5g to 1L water, stirring 40 hours and settling for 2 hours. Both treatments were prepared at 10 °C temperature.

Molecular biology analyses include quantification of functional genes related to petroleum hydrocarbon degradation by quantitative real-time PCR (qPCR), 16S rDNA amplicon-based sequencing of bacterial communities and shotgun metagenomics. In chemical analysis total petroleum hydrocarbons (C_{10}-C_{40}) are analysed by gas chromatography-mass spectrometry (GC-MS) to estimate the crude oil degradation rates.

Figure 2. Water canisters and buckets used for the sampling of seawater and sediment on-board RV Aranda.

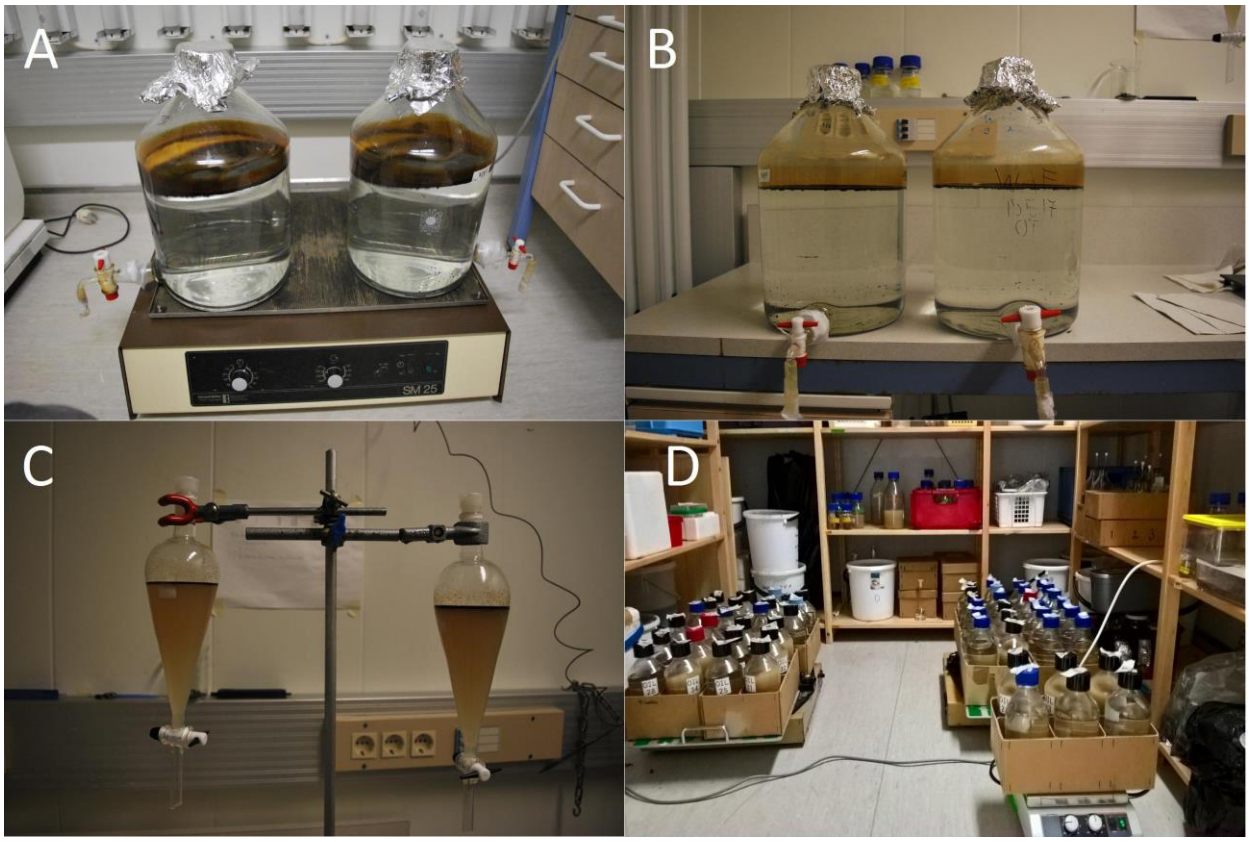

Figure 3. Different steps of WAF (crude oil) and CE-WAF (crude oil and dispersant) preparations for the microcosm experiments. Picture A: shaking of WAF bottles. Pictures B and C: settling of WAF and CE-WAF before bottling. Picture D: filled microcosm bottles in shakers.

As of July 2017, two seawater experiments have already been conducted. The laboratory analyses are still in progress, but the preliminary results from petroleum hydrocarbon analysis are presented below.

1.3.1. First microcosm experiment with Tvärminne seawater

The first experiment was conducted with seawater from Tvärminne, which is located in the coast of Baltic Sea. The sea water samples were taken on 14th of May from the surface, approx. 0.5 meter depth at the Tvärminne Zoological station. In the first experiment the WAF and CE-WAF were used without dilution. Experiment was conducted in triplicates at 4 °C temperature; altogether the experiment consisted from 72 bottles. The results of the petroleum hydrocarbon analysis are shown in Figure 4.

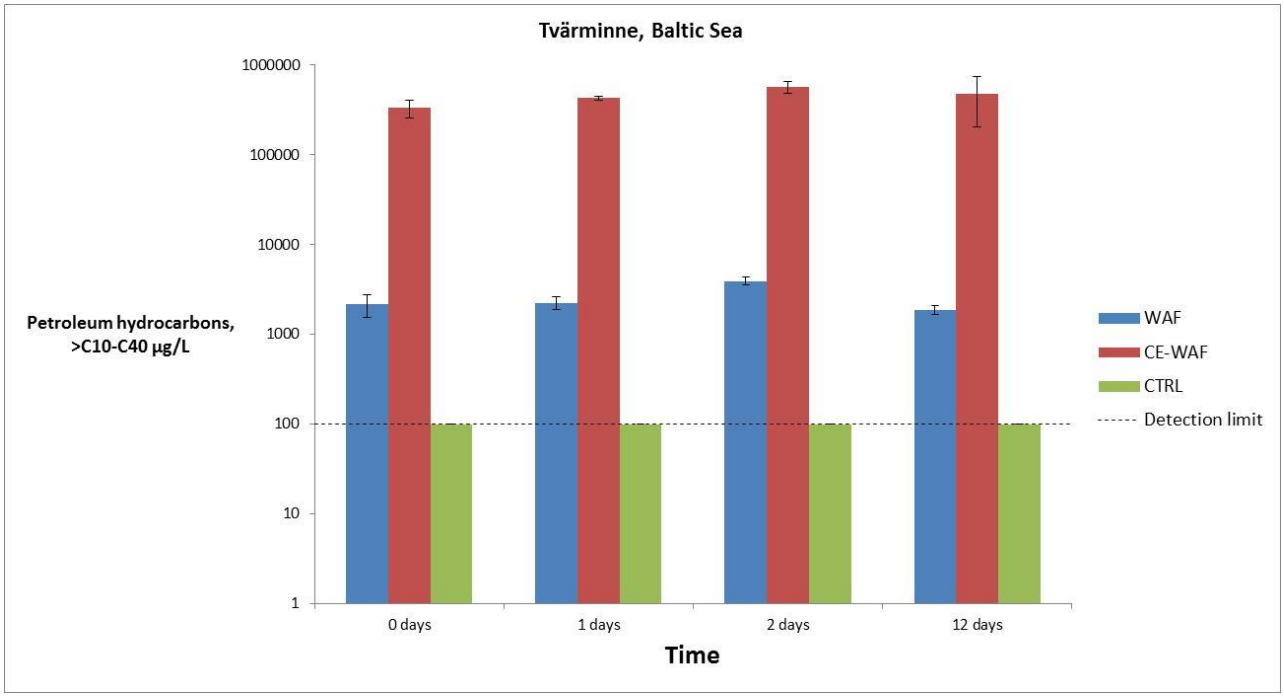

Figure 4. Petroleum hydrocarbon analysis results from microcosm experiment with seawater from Tvärminne amended with North Sea naphthenic crude oil and dispersant Finasol 51. Concentrations are averages calculated from three replicates, notice that the concentrations in the graph are in logarithmic scale. Error bars are standard deviations from three replicates. Abbreviations: WAF - Water Accomodated Fractions, Control (no oil), and CE-WAF - oil + dispersant.

Based on the results, the oil concentrations (picture shows fractions >C_{10}-C_{40}) were very high in the WAF and CE-WAF treatments. The CE-WAF concentrations were 330 000 µg/L in the beginning and 470 000 µg/L at the end of the experiment, showing no degradation during the experiment. In WAF treatment the average initial concentrations was ca. 2200 µg/L and increased during the experiment to 3900 µg/L before decreasing to1800 µg/L at the end. These results indicated that the oil concentrations using the raw WAF and CE-WAF were most likely too high for the biodegradation experiments and probably inhibited microbial activity. The high oil concentration was also observed during the test as many WAF and especially CE-WAF bottles had very noticeably oil layers at surface and side of the bottles (see Figure 5).

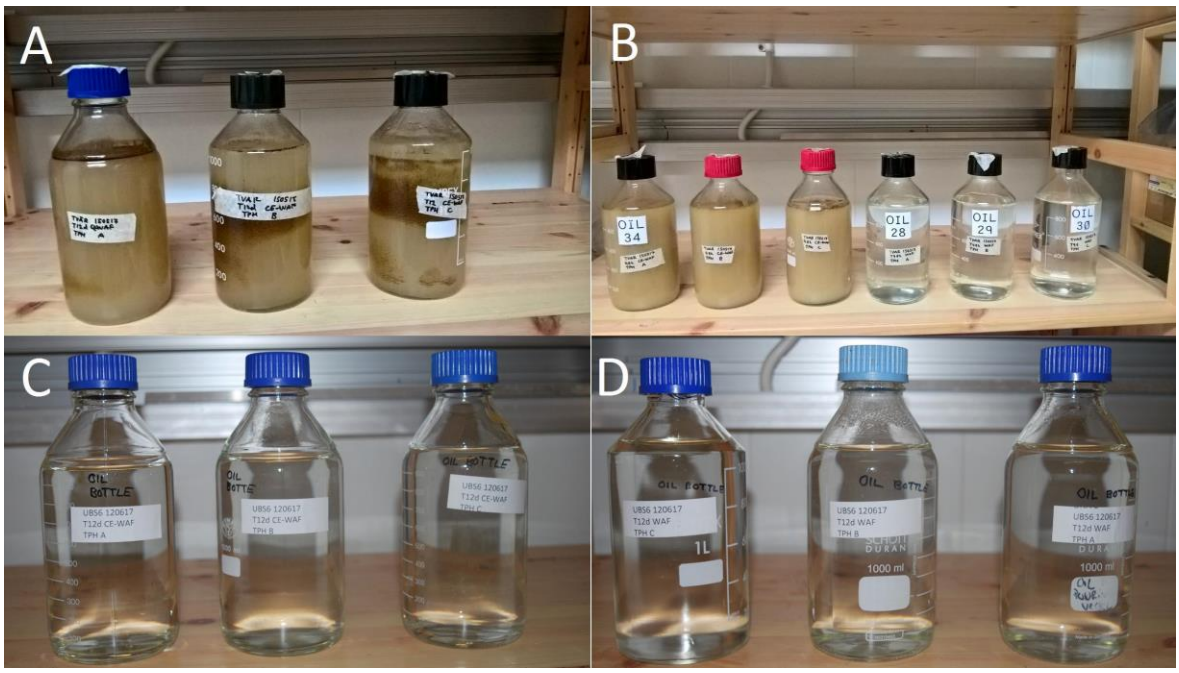

Figure 5. Microcosm bottles with different treatments from the two experiments. Pictures A and B are from the first experiment with undiluted WAF (only oil) and CE-WAF (oil and dispersant), bottles in picture A are from CE-WAF treatment and in picture B from CE-WAF and WAF treatments. Pictures C and D are from the second experiment, bottles in picture C are from CE-WAF treatment (diluted 1:1000) and in picture D from WAF (diluted 1:1) treatment.

1.3.2. Second microcosm experiment (Seawater from Gulf of Bothnia)

The second microcosm experiment used seawater from open sea of Gulf of Bothnia. Seawater samples were taken on board SV Aranda on 8th of June 2017 during COMBINE 2 cruise from depth of approx. 0.5 meter. Seawater was stored at 4 °C before use.

Based on the high concentrations observed in the first experiment, WAF and CE-WAF were diluted for the second experiment. WAF was diluted 1:1 and CE-WAF 1:1000. Experiment was conducted in triplicates at 4 °C temperature; altogether the experiment consisted from 72 bottles. The results of the petroleum hydrocarbon analysis are shown in Figure 6.

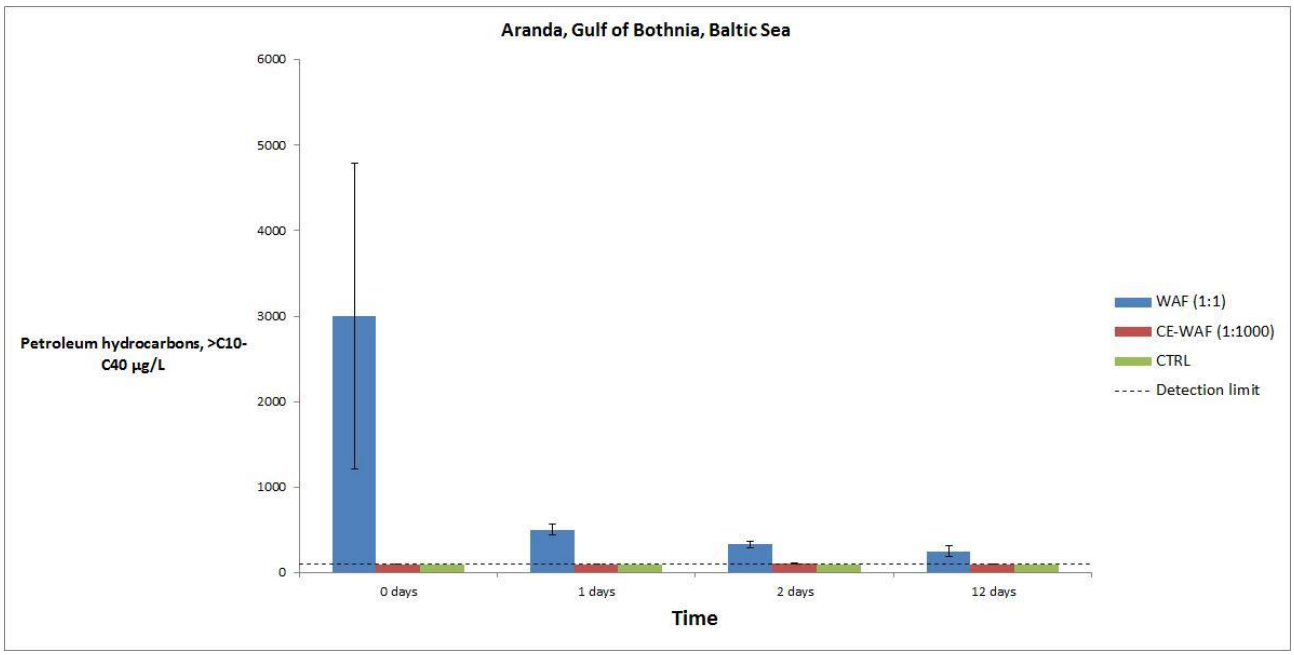

Figure 6. Petroleum hydrocarbon results from microcosm experiment with seawater from Gulf of Bothnia amended with North Sea naphthenic crude oil and dispersant Finasol 51. Concentrations are averages calculated from three replicates. Error bars are standard deviations from three replicates.

According to the analysis results, CE-WAF concentrations for >C_{10}-C_{40} fractions were under the detection limit (>100 µg/L) during the whole experiment. Only the CE-WAF measurements for medium petroleum fractions (>C_{10}-C_{21}) from day 1 had concentrations slightly over the detection limit (concentrations between 55-60 µg/L). These results indicated that the dilution used for CE-WAF in the second experiment was too high and should be changed for the future experiments.

In the beginning of the experiment, the average WAF concentration (3000 µg/L) was actually higher than in the first experiment, however there were big differences between the replicates (concentrations were 1100 µg/L, 2500 µg/L and 5400 µg/L). Despite the high average initial concentration, WAF concentrations decreased constantly over the time and were at the end of the experiment ca. 250 µg/L. Therefore, compared to the first experiments, the WAF concentration was not too high and didn't inhibit the biodegradation process. A degradation value of 92 % was obtained in seawater with WAF at 5 °C. This is a significant amount compared to earlier reported values at 5 °C.

Dispersion of crude oil was effective with Finasol 51 with a 150 times higher concentration of oil in the first experiment. The concentration of dispersed oil is, however, difficult to adjust to measurable levels in the experiments as droplets of free phase compounds are behaving differently during dilution phases. In real conditions in situ dispersed oil will rapidly dilute, and for that reason diluted WAF and CE-WAF are more relevant to study (Lee et al. 2013). Additionl experiments will be needed in order to assess the degradation rate of dispersed crude oil.

2. Marine oil-degrading microbial community structure

Crude oil has been part of the marine environment for millions of years, and microbes that use its rich source of energy and carbon are found in seawater, sediments, and shorelines from the tropics to the Polar regions. Some 175 prokaryotic genera in seven phyla of Bacteria and Archaea, and a similar number of fungal genera, can use hydrocarbons as their sole or major carbon source (Hazen et al., 2016). Among the known aerobic oil degraders are members of the *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria*, and *Deltaproteobacteria* and the *Firmicutes* and fungi (Head et al. 2006). Many aerobic microorganisms use saturated hydrocarbons exclusively as their carbon source (e.g., *Alcanivorax* spp., *Marinobacter* spp.,

Oleiphilus spp., *Oleispira* spp., or *Planomicrobium spp.),* whereas others *(e.g., Cycloclasticus spp., Neptumonas spp.)* use PAHs exclusively (Head et al. 2006).
Aerobic hydrocarbon degrading microbes are found throughout the marine water column and in oxic sediments even in deep waters whereas anaerobic hydrocarbon degraders are primarily found in anoxic sediments and within hydrocarbon seeps. The complex composition of the crude oil is matched by a complex community of microbes that degrade it, and this community changes as biodegradation proceeds.

Data from multiple deep-sea hydrocarbon basins around the world (Eastern Mediterranean sea, the Great Australian Bight, and the Caspian Sea) have shown that oil-degrading microbes are always present, and relatives of the *Oceanospirilalles*, *Colwellia* and *Cycloclasticus* routinely bloom when deep-water from these locations is amended with oil (Hazen et al. 2016). These genera appear to be cosmopolitan cold-adapted oil degraders in polar surface waters and the deep ocean around the world. Generally, lower temperature reduces oil degrading bacterial richness and catabolic diversity in the marine environment (Meng et al. 2016).

Sediments influenced by hydrocarbon discharge have characteristic features: They are often oil stained, highly reducing, and support high rates of microbial activity. Whilst dynamics in the bacterial communities in the aerobic zones of coastal sediments are well characterized and the key players in hydrocarbon biodegradation have been identified, the subtidal ecology of the anaerobic community is still not well understood (Acosta-González and Marqués, 2016). Main alkane degraders in polluted sediments of a subantarctic coastal environment belong to *Bacteroidetes* and *Proteobacteria* phyla (Guibert et al. 2016). Bacterial taxa correlating with hydrocarbon pollution in sediment include families of anaerobic or facultative anaerobic lifestyle, such as *Desulfuromonadaceae, Geobacteraceae, and Rhodocyclaceae* (Espinola et al. 2017).

3. Impact of dispersants on marine microbial community structure and oil biodegradation activity

The fate of oil in marine environment is dependent on a number of physical, chemical, and biological factors working independently or in consort with one another. The most important factor appears to be the physical state of the oil. This state can be modified by adding dispersants– mixtures of surfactants designed to lower the interfacial tension of oil and water so that tiny oil droplets are formed. The smaller oil droplets would enhance biodegradation because the surface to volume ratio would favour attachment and subsequent oil biodegradation.

Dispersants are chemicals used as an emergency response to marine oil spills. Dispersants enhance the dissolution of oil into the water by breaking it into smaller droplets, decreasing the oil accumulation at the water surface and reducing the amount of oil disrupting the shoreline ecosystems. Dispersants have been used since the 1970s in several oil spills (e.g. Sea Empress, etc.), but the widest application of dispersants occurred during Deepwater Horizon accident in 2010. Dispersants could be divided in two broad categories: synthetic dispersants and natural surfactants (biosurfactants). Biosurfactants may contain lipopeptides and glycolipids or high molecular-mass polymers of plant or microbial origin. Biosurfactants possess several advantages over chemical surfactants, such as environmental compatibility, low toxicity, biodegradability, and maintained activity under extreme conditions of temperatures, salinity and pH values (De Almeida et al. 2016).

In laboratory studies dispersants have shown to increase oil biodegradation, for example, experiments conducted with isolates of *Colwellia* showed increased oil biodegradation with dispersant Corexit 9500 (Hazen et al. 2010), and the similar increase in oil biodegradation with *Colwellia* isolates treated with dispersants was observed by Baelum et al. (2012). The study of Macondo oil premixed with Corexit 9500 using coastal Norwegian seawater at a temperature 4–5 °C showed the importance of oil droplet size for biodegradation. Biotransformation of volatile and semivolatile hydrocarbons and oil compound groups was generally faster in the 10 µm than in the

30 µm dispersions (Brakstad et al. 2015). Biodegradation of dispersed oil is prompt and extensive when oil is present at the ppm levels expected from a successful application of dispersants - more than 80% of the hydrocarbons of lightly weathered Alaska North Slope crude oil was degraded in 60 d at 8 °C in unamended New Jersey (USA) seawater when the oil was present at 2.5 ppm by volume. The apparent half-life of the biodegradation of the hydrocarbons was 13.8 d in the absence of dispersant, and 11 d in the presence of Corexit 9500 (Prince et al 2013).

Although dispersants have been routinely used in the oil spill remediation and they are believed to increase oil biodegradation efficiency, the effectiveness of dispersants and their toxicity to marine microorganisms have been questioned. For example, in some laboratory studies dispersants did not increase oil biodegradation or even inhibited microbes (Lindstom and Braddock 2002, Couto et al. 2016, Kleinstadt et al. 2015). Lindström and Braddock (2002) observed that the use of dispersant inhibited hexadecane and phenanthrene mineralization but did not affect dodecane and 2-methyl-naphthalene mineralization. In a microcosm study by Couto et al. 2016 the addition of dispersant Ultraperse II didn't increase the biodegradation rates and reduced the microbial activity compared to biosurfactants. Microcosm study by Kleindienst (2015), conducted in WAF and using dispersant Corexit 9500, showed dispersants did not increase biodegradation but decreased microbiological activity. The presence of dispersant significantly altered the microbial community composition through selection for potential dispersant-degrading *Colwellia*, which also bloomed in situ in Gulf deep waters during the discharge. In contrast, oil addition to Deepwater samples in the absence of dispersant stimulated the growth of natural hydrocarbon-degrading *Marinobacter*. It has been proposed that at least some of the contradictions and differences in the biodegradation rates with dispersants are related to the composition of the oil, absolute and relative concentrations of oil and the characteristics of microbial population in the experimental work (Rahsepar 2016).

Dispersants have been shown to affect the microbial community activity and structure; however, as with the oil biodegradation rates the results have been mixed. For example, in a mesocosm study by Meng et al. (2016), the addition of oil increased the abundance of phyla *Nitrospirae*, class *Clostridia*, *Lachnospiraceae, Peptostreptococcaceae* and *Xanthomonadacease* family. The addition of dispersant did not affect negatively the microbial community composition, however abundance of *Cholorflexi*, *TM6*, *OP8*, Cyanobacteria and *Gemmatimonadetes* phyla increased. The study was conducted using crude oil from Shengli oilfield and dispersant GM-2. However, in another mesocosm study (Ortmann & Lu 2015) conducted using Corexit 9500 dispersant and Macondo 252 oil it was showed that the dispersants didn't decrease the microbial community richness. Dispersant Corexit 9500 has also been shown to reduce abundance of certain bacterial groups (*Marinobacteria* and *Acinetobacter* genera) (Kleinstadt et al. 2015).

Hydrocarbon-degrading bacterial species demonstrate a unique response to dispersed oil compared to their response to crude oil, with potentially opposing effects on toxicity (Overholt et al., 2016). While some bacterial species have the potential to enhance the toxicity of crude oil by producing biosurfactants, the same bacteria may reduce the toxicity associated with dispersed oil through degradation or sequestration. Techtmann and co-workers (2016) showed that several bacterial groups were inhibited by the addition of Corexit. Conversely, a number of some microbial species was stimulated by the addition of the dispersant, many of which were identified as known hydrocarbon-degrading bacteria.

Under certain conditions, at least part of the dispersed oil may reach the sediment, particularly if the dispersant is applied in coastal waters. Experimental results indicate that once the oil has penetrated the sediment, no significant differences exist between oil that contains dispersant and oil without dispersant (Macías-Zamora et al 2014). There are also some published studies on the effects of dispersants on the microbial communities in sediments. In a study by Ferguson et al. (2017) the use of dispersant (Superdispersant 25) did not show statistical difference in bacterial community at the depths of 500 or 1000 meters. Oil biodegradation rate was significantly increased in sediments collected from 1000 meter depth, but not with sediments from 500 m depth. Sediments treated with oil hydrocarbons showed increase in the abundance of *Gammaproteobacteria* and especially *Pseudoalteromonas, Pseudomonas, Halomonas* and *Colbetia*.

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