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Quantitative Determination of Microbial Oil Degradation and Of Oil Absorption by a New Oil-Binding System in a Baltic Sea Mesocosm Experiment

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ABSTRACT 299731:

In this study, a novel oil-binding system for marine application was developed within the joint research project "BIOBIND" ("Airborne clean-up of oil pollution at sea with biogenic oil binders"). The system's components include oil-absorbing solids, made of biogenic and biodegradable wood-fiber, that can be dropped from an aircraft and subsequently recovered either at sea or along the coast. The binder-based system was tested together with oil-degrading microbial communities previously isolated from coastal water samples of the Baltic Sea. In a first attempt at a meso-scale setup, mesocosms containing different combinations of seawater, oil binders, crude oil, and oil-degrading bacteria were established. These experiments sought answers to the following questions: (1) How does the microbial community isolated from the Western Baltic Sea react to oil entries? (2) What happens to the crude oil? (3) How efficient is the oil absorption capacity of the developed binders? Microbial activity was monitored by measuring the oxygen, phosphate, and ammonia contents of the mesocosms. Weight loss of the whole crude was estimated using a gravimetric method. In one of the mesocosms, the selected inoculum degraded around 25 % of the added crude oil. In another, in which the absorption efficiency of the oil binders was examined, more than 98 % of the crude oil was absorbed. Further molecular details on the fate of the oil were obtained using gas chromatography with a flame ionization detector and mass spectrometry to quantify alkanes and polycyclic aromatic hydrocarbons, respectively; both were efficiently degraded by the selected inoculum. The oil absorption and oil-degrading capabilities of a system consisting of oil binders and oil-degrading microbial communities at the meso-scale was shown. These promising preliminary tests recommend its further development for use in responding to small- and medium-size oil spills in near-coastal shallow-water areas.

INTRODUCTION:

The Baltic Sea is one of the world's largest brackish water bodies and as such has a very special ecosystem. Nevertheless, the increasing traffic and volume of oil and other hazardous materials transported through the Baltic Sea imply a greater risk of incidents resulting in highly toxic spills. Forecasts for the near future predict a continuing rise in marine traffic and oil tankers in particular (HELCOM, 2010; Parkhomenko and Korzhonok, 2013), but the highest risk of contamination by oil and oil products comes from illegal oil releases (HELCOM, 2010). Indeed, in 2009, approximately 97 % of oil contamination was < 0.1 m³ (HELCOM, 2010); these contaminations comprised a total volume of 50 t. Current systems developed to respond to oil spills in the North Sea and Baltic Sea are ship-based and have a number of weaknesses. Because of vessel draft, for instance, technologies

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for use in near-coastal shallow-water areas cannot be employed. Additionally, commonly used marine pollution response systems are only focused on oil spills covering several hundred or thousand tons, but do not handle small areas or volumes (e.g., illegal oil entries). To overcome these limitations, the joint research project "BIOBIND" ("Airborne clean-up of oil pollution at sea with biogenic oil binders") was created in 2011. The "BIOBIND" system includes: (1) an aircraft-deployable technology, (2) a low-cost spectral method for the specification and monitoring of oil spills, (3) biodegradable oil binders made of natural products and designed to act in combination with oil-degrading microbial communities from the Baltic Sea, and (4) appropriate recovery techniques at sea and along the coast. With these specialized and new approaches, "BIOBIND" focuses on small oil spills (≤ 50 t), occurring in near-coastal shallow waters and environmentally-sensitive areas.

Individual species of the microbial community respond to contamination based on their ability to use oil as a carbon source (Leahy and Colwell, 1990; Yang et al., 2009; Edwards et al., 2011). This characteristic of oil-degrading bacteria acts as a self-help mechanism and underlies the approach of the joint research project "BIOBIND", in which oil spills are managed using an oil-degrading microbial consortium together with a biogenic, oil-binding material (Siewert et al., 2014). Since exogenous microbial species are likely to upset the ecosystem of the Baltic Sea, it was important to identify and isolate indigenous oil degrading bacteria (Safonova and Koenig, 2014). The requirements for the oil binders included their ability to withstand strong mechanical forces during storage, distribution, and recovery and to stay afloat even under rough marine conditions (Unbehaun et al., 2014). The biogenic oil-binding material consists of pine wood fibers that are processed, with a hydrophobizing additive (about 10 %, w/w), into small 50 mm x 50 mm square blocks with a thickness of 4 mm (Unbehaun et al., 2014). During the absorption process, the oil not only sticks to the surface of the binder, but can also penetrate into the interior of the block.

After various laboratory-scale determinations, in this study the "BIOBIND" system was tested in a meso-scale experiment under near-natural conditions, which produced the challenge of representative sampling. Classical techniques of oil analytics were used to quantify oil absorption capacities of the binders, the oil-degrading rates of the bacterial consortium, and the losses of crude oil due to evaporation and adsorption. Degradation of the total crude oil was determined gravimetrically, as this comparatively simple method provides a rapid overview of processes involving crude oil as a whole, not only its component substances (Dutta and Harayama, 2000; Delille et al., 2009; Gertler et al., 2009). To obtain more detailed information on the degradation process, the concentrations of linear alkanes and polyaromatic hydrocarbons (PAH) were determined before and after treatment. These two groups of organic contaminants are typical target analytes and their analysis facilitates comparisons with the levels reported in other studies (Foght et al., 1999; Dutta and Harayama, 2000; Cho and Oh, 2012; Kadali et al., 2012). A gas chromatograph (GC) with a flame ionization detector (FID) or coupled with a mass spectrometer (GC-MS) was used for measurements of alkanes and PAH, respectively.

METHODS:

Mesocosm experimental set-up:

The mesocosm experiments were started on board the FS *Elisabeth Mann Borgese* in the beginning of July 2013. Seven tanks made of residue-free polyethylene were filled with approximately 290 L of seawater from the Bay of Mecklenburg (pore size of the filter: 63 µm) filtered so as to preserve the natural community of microbes and plankton, but hold

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back particles and impurities. This volume of seawater corresponded to a filling height of 40 cm and a surface area of 0.72 m². The salinity was 10, as was the pH. To ensure minimal circulation of the water mass, pumps (18V) were inserted in the tanks. Appropriate amounts of NH_4Cl (10 µmol/L) and Na_3PO_4 (1 µmol/L) were added to the seawater in the mesocosms to reproduce the relatively low nutrient levels in the Baltic Sea in summer (Nehring and Matthäus, 1991) and thus to mimic natural conditions (Nausch and Nehring, 1996; Nausch et al., 2011). Tank 1 contained only seawater and represented the uncontaminated and unaffected system. Control values of nutrients were measured in samples taken from this tank to provide reference values after interpretation of the nutrient data. A microbial inoculum, consisting of a combination of different bacterial cultures originally isolated from the Baltic Sea (Safonova and Koenig, 2014), was added to tank 3. The initial criterion for bacterial selection was tolerance of varying levels of: salinity, crude oil, phenol, and phenanthrene. In further steps, the efficiency of oil degradation was determined and guided the final selection of the components of the inoculum (Rubarth et al., 2013).

After the seawater, nutrients, and inoculum (tank 3 only) were mixed for 1 h, 184 g of crude oil (Russian Export Blended, not weathered) was added to selected tanks (Table 1), so as to form a thin (0.3 mm) film in the respective mesocosms. After another hour, the oil binders were added. To achieve a surface coverage of 0.11 m²/m² (area of the binders/surface area of the tank), 32 oil binders were added to tanks 4, 5, 6, and 7. In tank 4, the binders were dipped in a solution of the microbial inoculum for a few seconds directly before use (short incubation). The binders used in tank 7 were incubated with a solution of the microbial inoculum for 24 h and then dried thereafter at room temperature (long incubation). These tanks (4 and 7) contained a very complex system (seawater, crude oil, binders, and the microbial inoculum). The experiments performed in these tanks were designed to investigate oil absorption capacity and microbial oil degradation in the combined system. Desorption effects related to the oil binders were evaluated in tank 5 (seawater and binders), which therefore provided both a reference and the blank values in determinations of oil absorption capacity. The mixture in tank 6 (seawater, crude oil, binders) yielded fundamental information about the oil absorption capacity of the binders.

To keep the ammonia and phosphate concentrations at about 10 µmol/L and 1 µmol/L, respectively, nutrients were added as needed during the experiment. The oxygen supply was monitored and regulated by intensifying the pump power, as needed. To ensure comparability between the tanks, all pumps were regulated by a central control, such that the effects caused by intensifying the pump power (e.g., higher evaporation) equally influenced all of the tanks. However, increased evaporation of the crude oil due to regulation of the pump was unlikely as the pumps consistently operated in a comparatively low power range. Additionally, the temperature was not regulated in any of the tanks, although it was continuously recorded in tank 1. After one month, the mesocosm experiment was stopped when the binders were removed.

Due to the large size of the tanks, replication were impractical and only individual samples were taken (except binder extraction, n = 3), limiting statistical evaluation of the water sampling data. However, as several samples were measured over a period of one month, extreme outliers could be detected. Additionally, the system had been previously tested on a laboratory scale, so its behavior was not completely unknown. Furthermore, all of the methods used in this study were previously validated. The results of the described experiment are therefore discussed and interpreted in light of these considerations. To

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confirm the results, two more mesocosm experiments started in September 2013 and February 2014.

Table 1: Microcosm experimental set-up.

	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7
Seawater	x	x	x	x	x	x	x
Crude oil		x	x	x		x	x
Inoculum			x				
Binder				x	x	x	x
Inoculum (long incubation)							x
Inoculum (short incubation)				x			

Detailed information about the behavior (e.g., adsorption, evaporation, degradation) of crude oil in a natural system was obtained from tank 2 (seawater and crude oil). Moreover, the effects of oil adsorption on the tank wall and evaporation could be considered, which improved the accuracy of the data in the remaining tanks. Although the natural community was present in tank 2, the respective samples served as an "abiotic" control as no inoculum was added.

Sampling and Analyses:

Sampling:

Two different methods of water sampling were used, depending on the further processing of the samples. For measuring nutrients and oxygen, approximately 600 mL of seawater was taken from an outlet valve near the bottom of the mesocosm. Sampling was performed immediately after filling the mesocosms and after the first addition of nutrients. During the first three days of the experiment, samples were taken twice daily; later sampling was conducted weekly. For gravimetric or GC analysis of the oil, a teflon cylinder with a diameter of 4 cm was used to sample the whole water column. The bottom and top of the cylinder were sealed with a stainless steel plug screwed onto a thin stainless steel bar and a teflon plug, respectively. The sampled water was transferred as quickly as possible to a graduated separatory funnel. During the experiment, the sample volume was between 360 mL and 380 mL. The exact volume was noted and taken into account in the final calculations. Both the cylinder and the stainless steel bar were rinsed with methylene chloride and hexane to transfer all traces of oil to the funnel. A separate sampling cylinder was used for each tank to avoid cross-contamination. The first sample was collected 2 h after crude oil addition (i.e.,

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1 h after the addition of the binders to the respective mesocosms). Within the first three days, water samples for oil analyses were obtained daily. Further sampling was performed on days 7, 14, and 21 of the experiment. One month (28 days) after starting the experiments, three binders each were removed from tanks 4, 5, 6, and 7 and stored in residue-free plastic boxes at 5 °C for one week.

Oil Analyses:

All solvents (Walter-CMP), reagents, and standards were of high commercial quality and purity. The water samples taken for oil analyses were extracted twice with 20 mL of methylene chloride and then once with 20 mL of hexane. The oil-filled binders were crushed with a spatula and extracted five times with 50 mL of methylene chloride. The respective combined extracts were dried over sodium sulfate and evaporated to 6 mL and 8 mL on a rotary evaporator (30 °C), respectively. Aliquots of 4 mL and 5 mL were taken, respectively, and evaporated at room temperature (20 °C) for precision-scale gravimetric analysis. Prior to GC measurements, 20 µL of the extract was purified on a conditioned coupled column filled with deactivated silica gel and alumina and rinsed with heptane (15 mL) and heptane:toluene (2:1, 30 mL). At this step of sample preparation, 400 µL of a mixture consisting of deuterated PAH (PAH-Mix 9 deuterated (Dr. Ehrenstorfer, Augsburg, Germany), 1-methylnaphthalene D10 (LGC Standards, Wesel, Germany), and 5-α-androstane (Merck, Darmstadt, Germany)) were added as internal standards. For quantification, a standard mix containing all target analytes (PAH-Mix 9 (Dr. Ehrenstorfer), several alkylated PAH (LGC Standards), and TRPH Standard New Jersey (LGC Standards)) was measured twice every six to eight samples.

Instrumental Analyses:

A GC-FID (Thermo Scientific, Trace GC Ultra) was used for alkane analyses (C_{20}-C_{40}). The column was 30 m x 0,25 mm, 0.25 µm film thickness (DB-5MS, Agilent). The program was 40 °C (2 min), 8 °C/min, 320 °C (10 min). Hydrogen was used as the carrier gas (1.0 ml/min gas flow) and the split/splitless injection was applied with an injection volume of 1 µL. PAH were measured by GC/MS (Thermo Trace GC coupled to a Thermo Automass III MS). The system was operated at 70 eV in positive ion mode. Chromatography was performed with a capillary column (DB-5MS, 60 m × 0.25 mm, 0.25 µm film thickness, Agilent) with helium (5.0) as the carrier gas (flow rate: 1.5 mL/min) and PTV solvent split injection, with an injection volume of 2 µL. The oven temperature was 50 °C for the first 3.5 min., increasing to 190 °C at 12 °C/min then to 300 °C at 5 °C/min, holding at this temperature for 25 min. The MS was operated using selected ion mode.

Nutrient Analyses:

Ammonia nitrogen was determined photometrically as indophenol blue according to the Berthelot reaction (Searle, 1984). Soluble reactive phosphate was measured using a photometric method and molybdenum blue (Johnson and Pilson, 1972). The dissolved oxygen content was determined using the Winkler method (Carpenter, 1965).

RESULTS/DISCUSSION:

In this work, the oil absorption capacity of the tested binders and the efficiency of oil degradation by a bacterial consortium were analyzed in seven mesocosms. All results were normally distributed (David test, P = 0.01). In the following, nutrient concentrations are discussed for all tanks used to examine oil degradation by the inoculum (tanks 3, 4, and 7). The concentrations of alkanes and PAH are reported, as are gravimetric data derived from the

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water column samples taken from these tanks and the oil absorption capacity of the binders from tanks 4, 6, and 7.

Nutrients:

Within the first ten days of the experiment, the oxygen concentration diminished continuously in all seven tanks. In tank 1, representing the unaffected natural system, the oxygen concentration decreased by around 15 %, from 6 mL/L to 5 mL/L. In tank 2, the oxygen concentration decreased by around 35 %, and in tanks 3, 4, and 7 by around 50 % (Figure 1). This larger decrease in the latter three tanks was indicative of greater microbial activity (Leahy and Colwell, 1990). After ten days, the pump power was regulated in all tanks to guarantee oxic conditions (c_{oxygen} > 2 mL/L) and the concentration stabilized at 3 to 4 mL/L (Figure 1).

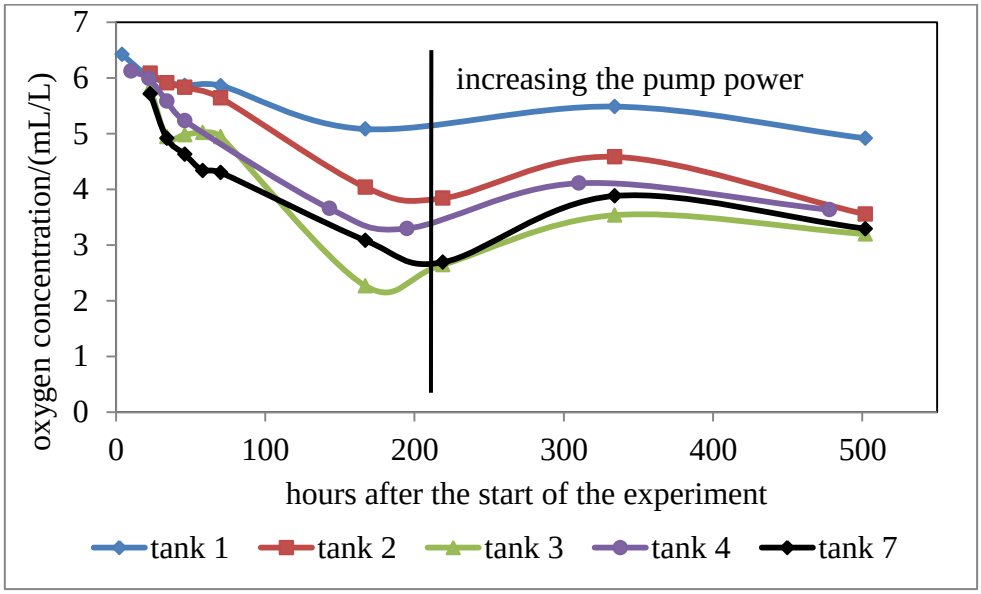

Figure 1: Oxygen concentration (in mL/L seawater) in tanks 1, 2, 3, 4, and 7.

To ensure sufficient nutrient availability throughout the experiment, phosphate and ammonia were monitored and added, as needed. In tank 1, additional nutrients were first required more than two weeks after the start of the experiment. In all other tanks, additional nutrients were required starting on day 8 and weekly thereafter. Within the first three days, the ammonia concentration increased slightly in tanks 3 and 7. This was attributed to degradation of phytoplankton (Collos et al., 1992). Subsequent reductions of phosphate and ammonia were likely due to a high level of microbial activity that could not be traced to oil degradation alone, as it occurred in all tanks. Nevertheless, microbial activity was at least in part related to the combination of the crude oil, binders, and microbial inoculum because the activity in tank 1 was much lower than the mean value in all other tanks (Student's t-test, $P = 0.02$). Unfortunately, the water temperature in the tanks increased up to 27 °C ± 2 °C (on average) in response to the high summer outdoor temperatures. As both the average temperature and the temperature variation in the tanks were higher than in the Baltic Sea (surface water, average temperature approx. 20 °C, daily variation approx. 1 °C), the experimental conditions might have had an additional impact on the microbial and biological activity. To determine this temperature effect, two more mesocosm experiments were established in September 2013 and February 2014. Along with the crude oil, the additives in

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the binders might have served as a carbon source (Mobaiyen et al., 2013). The activity of nitrifying bacteria could be excluded because the nitrate concentrations in samples from day 8 of the experiment were very low.

Oil analyses:

Gravimetric analyses:

The gravimetric data of tank 1 (seawater) did not differ significantly from those for tank 5 (seawater and binders), indicating that the binders did not release much organic matter. The percentage loss of crude oil in tank 2 (seawater and crude oil) over the one-month experiment was 60 %, attributable to processes such as evaporation (and even the possible increase in evaporation due to pump regulation on day 10), adsorption onto the tank walls, the pump, etc., and degradation by the natural consortium since losses of this order of magnitude are typical for crude oil (Mackay and McAuliffe, 1988). The loss of crude oil in tank 3 (seawater, crude oil, inoculum) was 85 %, 25 % higher than in the control (Figure 2). This difference suggested successful oil degradation by the added inoculum beginning on day 8.

In tank 6 (seawater, crude oil, binders), the decrease in the amount of crude oil was likely related to the addition of the binders, which already after one hour had retained 47 % of the oil (Figure 3). On the second day of the experiment, only 6 % of the crude oil remained in the water column, and from the third day on less than 2 %. The absorbed oil was retained by the binders over the course of the experiment, as determined from measurements of the water column.

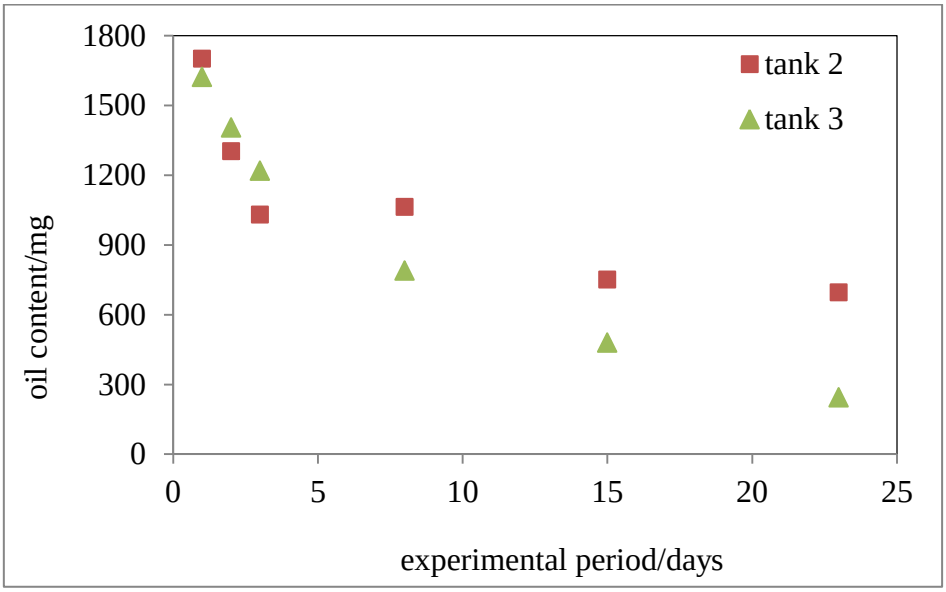

Figure 2: Loss of crude oil (in mg) in tanks 2 and 3, determined by gravimetrica analyses.

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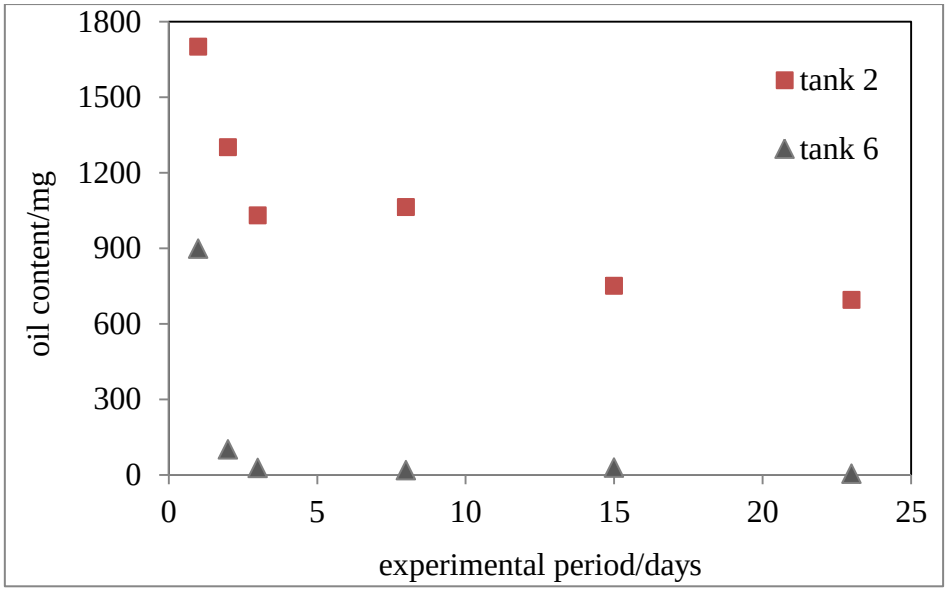

Figure 3: Loss of crude oil (in mg) in tanks 2 and 6, determined by gravimetric analyses.

Additionally, after 28 days, the oil from the binders was extracted and the retained amount measured gravimetrically. Each binder from tank 6 contained 4.00 g ± 0.13 g (n = 3) of crude oil; thus the 32 binders in the tank would have retained ~ 128 g of crude oil, 70 % of the added amount. The difference between the retained crude oil and the amount detected in the water column (from day 3, < 2 %) can be explained by evaporation, adsorption onto the tank walls, etc. In tanks 4 and 7, the effects of short and long incubations of the inoculum with the binders were assessed. Under either condition, the oil absorption capacity of the binders was clearly reduced. In tanks 4 and 7, the binders retained 1.00 g ± 0.04 g (n = 3) and 0.90 g ± 0.04 g (n = 3), respectively, or scaled up 32 g and 29 g of crude oil for the 32 binders in each tank (17 % and 16 % of the added amount of oil). The reduction in oil absorption capacity suggested the absorption of water during the incubation period. Water uptake by the binders prior to their application to a water-oil-system enhances their hydrophilic properties and thus causes a preferential increase in water absorption while decreasing oil absorption (results based on preliminary unpublished experiments). Another reason for the reduced oil absorption capacity may have been the production of surfactants by the microbial inoculum and the adherence of these compounds to the surface of the binders. These surfactants could have increased the hydrophilic properties of the binders (Rambeloarisoa et al., 1984).

Alkanes and PAH:

No alkanes were detected in tanks 1 (seawater) and 5 (seawater and binders). Naphthalene was present in these two tanks as the only PAH and the concentration ranges were the same. They were therefore averaged and the resulting mean value was used as the blank value, subtracted in determinations of the naphthalene concentration in all other tanks. The following discussion of alkane and PAH concentrations is based on samples from tanks 2, 3, 4, and 7.

Measurements from the first sampling day showed that C_{20}-C_{40} alkane concentrations significantly differed between tanks with (tanks 4 and 7: 25.2 mg and 25.4 mg, respectively) and without (tanks 2 and 3: 40 mg each) binders (Figure 4). According to gravimetric analyses, oil retention by the binders within the first hour was 36 %-37 % in tanks 4 and 7.

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This result is in excellent agreement with the alkane concentration, which was 36 %-37 % lower in tanks 4 and 7 than in tanks 2 and 3.

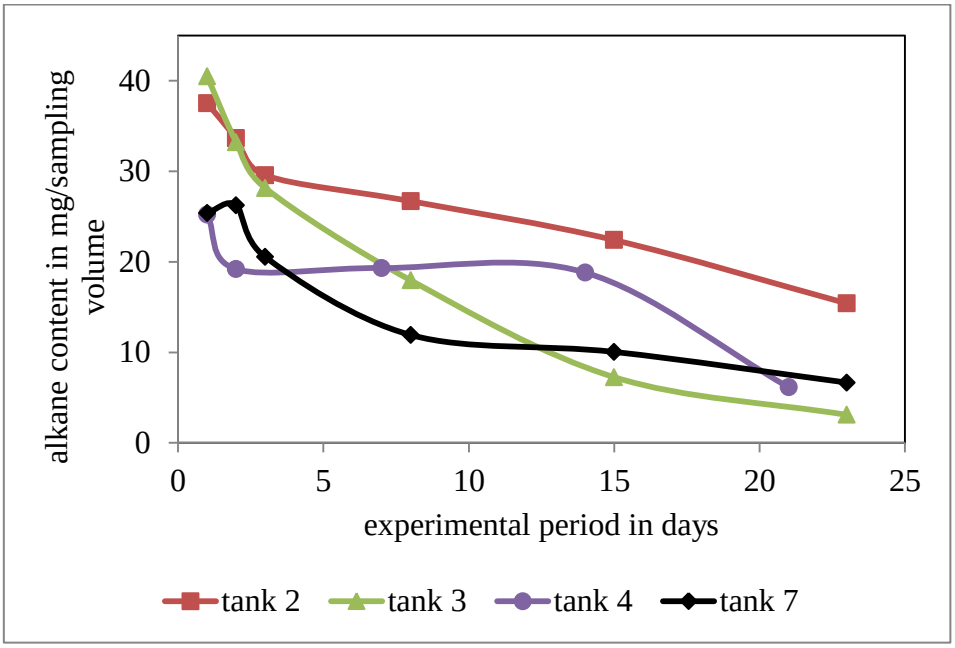

Figure 4: Alkane concentration (in mg per sampling volume) in tanks 2, 3, 4, and 7.

Throughout the experiment, the results of the alkane measurements were similar to those of the gravimetric analysis, a conclusion corroborated by the Bravais-Pearson correlation coefficients, which in all cases were > 95 % (Table 2).

Table 2: Bravais-Pearson correlation coefficients of oil content, determined by gravimetric analyses, and summed alkane and PAH concentrations for tanks 2, 3, 4, and 7.

Bravais-Pearson correlation coefficient	Tank 2	Tank 3	Tank 4	Tank 7
Alkanes	0.95	0.99	0.96	0.99
PAH	0.95	0.97	0.95	0.99

Figure 5 shows the percentage losses of selected oil-related compounds during the experiment. The loss of alkanes in tank 2, containing only seawater and oil, reflected both evaporation and adsorption onto the tank walls (50 % for C_{20}, and 10 % for C_{40}). Compounds with longer chain lengths remained in the mesocosms likely because of their lower volatility (Leahy and Colwell, 1990). Biodegradability decreases with increasing chain length (Leahy and Colwell, 1990). The greater loss observed in tanks 4 and 7 was partly due to oil being absorbed by the binders and possibly degraded by the inoculum. In ongoing studies, the efficiency of the microbial degradation of oil retained in the binders and the unassisted transition of the microorganisms from the binders to the water phase are being investigated. In tank 3, C_{20} was removed with the highest efficiency (93 %). The higher level of alkane losses (70 %-93 %) in this tank was attributed to microbial oil degradation by the inoculum.

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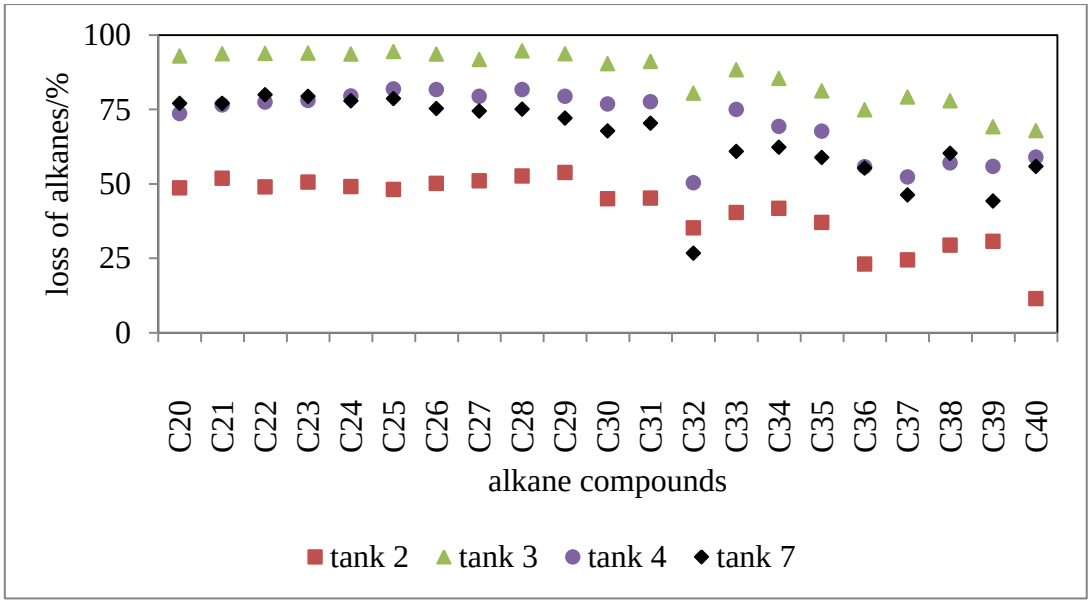

Figure 5: Loss of selected alkane compounds (in %) in tanks 2, 3, 4, and 7.

Of the 16 PAH recognized as priority pollutants by the US Environmental Protection Agency (EPA) and 8 alkylated PAH frequently targeted for environmental analysis, 14 (10 PAH, 4 alkylated PAH) were detected and quantified. This result is in accordance with prior laboratory studies, in which the same compounds were identified (data not published). In the control samples from tank 2 (seawater and oil), after one month there were no two- and three-ring compounds (naphthalene to fluorene) whereas larger molecules (four and five rings) were still present (Table 3). This was mainly due to evaporation effects, as smaller molecules (two and three rings) are more volatile than larger ones (four and five rings). Similar results were recorded for samples from tank 7 (seawater, oil, and binders, long incubation), where there were also no two- and three-ring compounds. The remaining concentrations of larger compounds (phenanthrene to benz(a)pyrene) were up to 10 % lower in this tank than in tank 2. For two- and three-ring-PAH, their biodegradation by the added inoculum cannot be assumed because after 28 days they were also no longer present in the control samples (tank 2). Thus, it seems that any biodegradation by the added inoculum was overshadowed by losses due to evaporation. In tank 3 (seawater, oil, and inoculum) and tank 4 (seawater, oil, and binders, short incubation), no PAH remained at the end of the experiment. By contrast, the added inoculum (tank 3) was apparently able to degrade four- and five-ring PAH. In tank 4, the transfer of microorganisms from the binders, which were briefly incubated with the inoculum, to the water phase, where they were able to degrade the oil compounds, was likely. These results are consistent with the literature (Cho and Oh, 2012). After a long incubation time of the inoculum with the binders, the microorganisms were apparently unable to migrate into the water phase, which accounted for the absence of PAH degradation in tank 7. The behavior of microorganisms in the binder-water-system is currently being studied in our laboratory.

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Table 3: Loss of detected PAH (in %) in tanks 2, 3, 4, and 7 within duration of the mesocosm experiment (28 days).

	Loss of compounds/(%)			
	Tank 2	Tank 3	Tank 4	Tank 7
Naphthalene	100	100	100	100
2-Methylnaphthalene	100	100	100	100
1-Methylnaphthalene	100	100	100	100
1,7-Dimethylnaphthalene	100	100	100	100
Acenaphthylene	100	100	100	100
Acenaphthene	100	100	100	100
Fluorene	100	100	100	100
Phenanthrene	98.8	100	100	97.8
Fluoranthene	56.0	100	100	61.2
Pyrene	51.7	100	100	65.3
1-Methylpyrene	55.2	100	100	63.4
Chrysene	52.2	100	100	63.5
Benz(b)fluoranthene	48.7	100	100	55.4
Benz(a)pyrene	49.3	100	100	56.7

The PAH data pattern well agreed with the results of the gravimetric analyses. Again, the Bravais-Pearson correlation coefficients were in all cases > 0.95 (Table 2). The degradation efficiency of the inoculum was apparently higher for four- and five-ring PAH than for long-chained alkanes. Most bacterial strains are known which degrade a wide range of PAH, but not the alkanes (Lepo et al., 2003). Further studies with the selected inoculum will be done to confirm these results.

CONCLUSIONS:

The mesocosm experiments provided important information about the efficacy of "BIOBIND". For the first time, this novel oil-binding system was used outside the laboratory, in a meso-scale approach. The recently developed binders (Unbehaun et al., 2014) showed an impressive oil absorption capacity (approx. 70 %) in brackish water conditions similar to those in the Baltic Sea. Oil absorption was nearly complete one day after the addition of the binders. The best results were achieved using the binders alone (i.e., without the microbial inoculum). These binders do not impose an environmental burden as they are made of natural wooden fibers and release little organic matter (tank 5). Up to 25 % of the crude oil was degraded by the microbial inoculum (tank 3). The results obtained with a combination of

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microorganisms and binders (tank 4) were also promising and indicated the transfer of the microorganisms from the binders to the water phase and subsequent biodegradation of the crude oil. While further efforts are needed to optimize the "BIOBIND" system, the mesocosm experiments demonstrate its potential in responding to small- and medium-scale oil spills in near-coastal shallow waters.

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