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Biotransformation and dissolution of petroleum hydrocarbons in natural flowing seawater at low temperature

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Abstract

The objective of this study was to establish methods for controlled studies of hydrocarbon depletion from thin oil films in cold natural seawater, and to determine biotransformation in relation to other essential depletion processes. Mineral oil was immobilized on the surface of hydrophobic Fluortex fabrics and used for studies of microbial biodegradation in an experimental seawater flow-through system at low temperatures (5.9–7.4 °C) during a test period of 42 days. The seawater was collected from a depth of 90 m, and microbial characterization by epifluorescence microscopy, fluorescence *in situ* hybridization, and most-probable number analysis showed relatively larger fractions of archaea and oil-degrading microbes than in the corresponding surface water. Chemical analysis of hydrocarbons attached to the fabrics during the test period showed that *n*-alkanes (C_{10}–C_{36}) were decreased by 98% after 21 days, while naphthalenes were depleted by 99–100% during the same period. At the end of the period 4–5 ring polyaromatic hydrocarbon (PAH) compounds were removed by 82% from the fabrics. Analysis of the recalcitrant pentacyclic triterpane $C_{30}17\alpha$(H),21β(H)-hopane showed that the oil remained adsorbed to the fabrics during the test period.

Comparison of depletion analysis with calculation of hydrocarbon dissolution in a flow-through system indicated that naphthalenes and smaller PAH compounds (alkylated 2-ring and 3-ring compounds) were removed from the fabrics by dissolution. The data further implied that depletion of *n*-alkanes and 4–5 ring PAH hydrocarbons were the result of biotransformation processes. PCR amplification of bacterial 16S rRNA genes from microbes adhering on the immobilized oil surfaces showed the dominance of a few bands when analysed in denaturing gradient gel electrophoresis (DGGE). Sequence analysis of DGGE bands revealed phylogenetic affiliation to the α- and γ-subdivisions of proteobacteria and to the *Chloroflexus–Flavobacterium–Bacteroides group*.

Introduction

Biodegradation of hydrocarbons (HCs) is an essential weathering process after oil discharges to the marine water column. HC degradation rates in the marine seawater column generally follow the order *n*-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes (Perry 1984). The process is usually aerobic, requiring terminal or sub-terminal oxidation of the alkanes (Harayama et al. 1999), while aromatic HC ring structures are broken through hydroxylation and carboxylation processes (Cerniglia 1992). HC biodegradation in the water column is associated both with water-soluble or -miscible compounds, and with the oil–water interphases, mainly on droplets and thin oil films with high surface–volume ratio (Bartha & Atlas 1987; Button et al. 1992; Floodgate 1984). A significant number of studies have shown that it is difficult to predict

the extent and rates of HC degradation processes in marine environment, due to the many factors involved in the process (Atlas & Bartha 1992; Leahy & Colwell 1990; Margesin & Schinner 1999).

Oil discharges generate a dynamic situation on the oil–water interphases, caused by a variety of physical and chemical processes, including wind, currents, oil weathering, film generation, and oil dispersion. Thus, immiscible oil surfaces are constantly washed with seawater, and new microbes are continuously contacting the oil film or droplet surfaces. Simulation of these dynamic conditions may be achieved rather in flow-through systems than by closed static experiments. Various controlled flow-through systems have been described which simulate biodegradation under marine conditions, including microcosms (e.g. MacNaughton et al. 2003; Röling et al. 2002; Swannell & Daniel 1999; Xu et al. 2003), and large tank-based mesocosms (e.g. Santas & Santas 2000; Siron et al. 1995; Wade & Quinn 1979; Yamada et al. 2003). However, since dispersed oil was used in most of these systems it was impossible to differentiate between HC processes in the oil and water phases.

We here describe a simple seawater flow-through system in which the oleophilic HC of crude oil was immobilized to hydrophobic Fluortex fabrics. This system simulated the dynamics on the immiscible oil and water interphases and enabled the studies of essential HC depletion processes, including biotransformation and dissolution. Microbial community dynamics were investigated on the immobilized oil surfaces during the biodegradation processes.

Materials and methods

Seawater source

Seawater was collected from a depth of 90 m in a non-polluted Norwegian fjord (Trondheimsfjord) through a continuous pipeline supply system. The seawater passes a sand filter to remove coarse particles, but no other water treatment is subjected. Surface water from Trondheim harbour was collected on sterile bottles (500 ml) for quantitative microbial analysis.

Oil immobilization

A fresh paraffinic oil (Statfjord batch no. 97-0264; specific density 0.850 g/cm^3) was used in this study. The oil (50 μL) was applied to 500 ml sterile seawater (oil loading 1:10,000) in a beaker (i.d. 8.5 cm) at room temperature to generate a thin homogeneous oil film. Hydrophobic Fluortex fabrics (Sefar Inc., Thal, Switzerland; production reference 09-150-s36) were pre-cut in squares of 1 cm^2, washed in dichloromethane (DCM), then in sterile seawater, and subsequently applied on the surface of the oil film for 60 min. The fabrics were then carefully rinsed in two baths of sterile seawater to remove excess oil. Thin fishing lines (diameter 0.030 mm) with knots in the end (pre-washed in DCM and rinsed in sterile seawater) were carefully forced through each fabric.

Flow-through systems

Biodegradation experiments in flow-through systems were conducted in polyethylene (PE) boxes (36 × 17 cm). Natural seawater was continuously supplied from the seawater pipeline system as a laminar flow, generated by a diffuser system, with a velocity of 3 cm/min (constant water level of 10 cm). The experiments were conducted in darkness at a constant air temperature of 4 °C. Dissolved oxygen and temperature were monitored during the experiment (Figure 1), showing that oxygen varied between 7.5 and 8.6 mg/l (mean 7.9 mg/l; ±0.09 °C 95% confidence interval), and seawater temperature between 5.9 and 7.4 °C (mean 7.2 °C; ±0.25 °C 95% confidence interval).

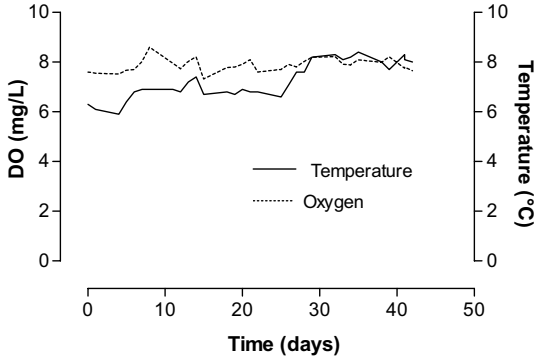

Figure 1. Dissolved oxygen (DO) and temperature in flow-through box.

Fabrics with immobilized oil were tread through Pasteur pipettes with disposable pipette tips in the upper end to lock the lines. The Pasteur pipettes with the fabrics were mounted in the flow system with clips on a bar across the box. A number of 20 fabrics were mounted in each box.

Fabrics (quadruplicate) were removed from the system at the test start, and after 7, 14, 21, 28, and 42 days in the flowing seawater.

Chemical analysis

Each fabric was extracted with 50 ml dichlormethane (DCM). The solvent was dried (Na_2SO_4), filtered (glass wool), and concentrated to 0.5–1.0 ml in 2 ml GC vials on a TurboVap 500 closed cell concentrator (Zymark Co., Hopkinton, MA). A surrogate recovery standard of *o*-terphenyl (100 μl) was added to a final concentration of 20 μg/ml.

Determination of total extractable organic compounds (TEOC; C_{10}–C_{36}) in DCM extracts was performed by GC-FID analysis (Hewlett Packard Model HP5890II gas chromatograph with a flame ionisation detector) using 0.05–15 μg/l Statfjord crude oil for generating external calibration curves. As internal standards 100 μl of 5-α-androstane (SIS-THC) and 100 μl of d10-fluorene (RIS-PAH) were added. The isoprenoids pristane and phytane were also determined by GC-FID analysis. Aromatic pseudo-compound groups were determined by GC-MS analysis. These were grouped as Naph-1, Naph-2, PAH-1, and PAH-2; the compound composition within each group is described in Table 1. GC-MS analysis also included quantification of the pentacyclic triterpane $C_{30}17\alpha$(H),21β(H)-hopane.

Dissolution

The rate of change in the concentration of a HC in the oil phase is expressed by the following equation:

$$\frac{dc_i}{dt} = -kS(K_i c_i - c_i^{\infty}).$$

In this equation, c_i and c_i^{∞} (kg/m^3) are concentrations of a hydrocarbon compound '*i*' in the oil and the water phase, k (m/s) is the mass transfer coefficient between the oil and water phase and S (m^{-1}) is the specific surface area (exposed area/oil volume). K_i is the partitioning coefficient for the compound '*i*', defined as

$$K_i = \frac{Ms_i}{M_i \rho},$$

where M_i (kg/kmol) and s_i (kg/m^3) are molar weight and solubility of the hydrocarbon compound '*i*', while M (kg/kmol) and ρ(kg/m^3) and are the molar weight and density of the oil phase.

In an open (flow-through) system, the concentration in the water phase may be neglected, and the dissolution equation can be simplified into

$$\frac{dc_i}{d\tau} = -K_i c_i,$$

where $\tau = kSt$ is a non-dimensional time variable.

Depletion and dissolution rates

The rates of depletion and dissolution were calculated by non-linear regression analysis, according to first-order reaction kinetics, using the GraphPad Prism version 3.0 (GraphPad Software Inc., San Diego, CA):

$$y = C_0 e^{-kt},$$

where y is HC concentration (μg/cm^2) after time t (days), C_0 initial concentration, e is Log to the base e, k is the rate constant for the reaction per days of exposure, and t is the time (days).

Table 1. Naphthalenes and PAH included in oil pseudo-groups

Abbreviation	Compounds
Naph-1	C_0- to C_1-naphthalenes
Naph-2	C_2- to C_3-naphthalenes
PAH-1	C_4-naphthalenes, biphenyl, acenaphthylene, acenaphthene, dibezofurane, C_0- to C_1-fluorenes, C_0- to C_1-phenanthrenes/anthracenes, C_0- to C_1-dibenzothiophenes
PAH-2	C_2- to C_3-fluorenes, C_2- to C_4-phenanthrenes/anthracenes, C_2- to C_4-dibenzothiophenes, Fluoranthrene, pyrene, C_1- to C_3-fluoranthrenes/pyrenes, benz(a)anthracene, C_0- to C_4-crysenes, benzo(b,k)fluoranthene, benzo(e,a)pyrene, perylene, dibenzo(a,h)anthracene, benzo (g,h,i)perylene, indeno(1,2,3-c,d)pyrene

Statistical analysis

Parametric two-tailed *t*-test was performed by GraphPad Prism 4.0 (GraphPad Software).

Quantitative microbiological analysis

Total microbial cell concentrations in the seawater were determined by epifluorescence microscopy (DAPI), while distribution of bacteria and archaea was determined by fluorescence *in situ* hybridization (FISH) as previously described (Glöckner et al. 1999). Most probable number (MPN) calculations of heterotrophic microbes were determined after seawater inoculation in Marine Broth 2216 (Difco Labs., Detroit, MI) at 10 °C for 7 days. Oil-degrading microbes were measured in marine Bushnell–Haas broth (Brown & Braddock 1990) with 0.1% (vol/vol) of a crude oil (Statfjord) as carbon source and incubation at 10 °C for 14 days. After incubation fluorescein diacetate (FDA; 0.1 mg/ml final concentration) was added to the Bushnell–Haas media for 60 min to detect viable cultures before the results were recorded. FDA fluorescence signals were caused by esterase hydrolysis (Chrzanowski et al. 1984).

PCR, denaturing gradient gel electrophoresis (DGGE), cloning and sequencing

Nucleic acids were extracted from fabrics with immobilized oil by hot phenol–chloroform–isoamylalcohol according to standard procedures (Sambrook & Russel 2001). Recovered nucleic acids were quantified by ethidium bromide (Sambrook & Russel 2001) and stored at −20 °C until analysis.

PCR amplification of bacterial 16S rDNA was performed with the domain-specific primers Bac341f (5′-CCT ACG GGA GGC AGC AG-3′) and Bac907r (5′-CCC CGT CAA TTC CTT TGA GTT-3′). For DGGE a 40-mer GC-clamp (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G-3′) was added to the 5′-end of the Bac341f primer, yielding a PCR fragment of 590 bp (Muyzer et al. 1993). The amplification was conducted as a 'touchdown' PCR to reduce formation of spurious by-products (Don et al. 1991), as previously described (Teske et al. 1996). Annealing temperature was initially set at 65 °C, then decreased by 1 °C every second cycle until 55 °C, at which point 25 additional cycles were carried out.

DGGE was performed with a continuous gradient of 20–70% of the denaturing agents urea and formamide (100% denaturants corresponded to 7 M urea and 40% deionised formamide), essentially as described by Teske et al. (1996). Each well contained 0.5–1.0 μg DNA. DGGE was run at 60 °C in a DCode Universal Mutation Detection System (Bio-Rad, Hercules, Ca) at 150 V constant voltage for 4.5 h. Gels were stained for 20–30 min with SYBR Gold (Molecular Probes, Leiden, The Netherlands), and stained gels were scanned in a GelDoc system (Bio-Rad).

Selected DGGE bands were carefully cut out from the gel with sterile scalpels, eluted overnight at 4 °C in 50 μl sterile water, PCR re-amplified, and cloned with the Qiagen PCR Cloning^{Plus} Kit (Qiagen GmbH, Hilden, Germany), as described by the manufacturer. Cells were spread on LB agar plates with ampicillin (100 μg/ml agar), IPTG (50 μM) and X-Gal (80 μg/ml), and 5–10 putative positive clones from each DGGE band were transferred to LB broth with ampicillin. Plasmids were prepared by GeneElute Plasmid Miniprep kit (Sigma, St. Louis, Ma), and plasmids stored at −20 °C.

PCR product inserts were amplified using primers for the M13 sites on the plasmid. These primers target sequences flanking the inserts on the plasmids (Sambrook & Russel 2001). M13 PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis with *Hae*III and *Rsa*I (Massana et al. 1997), and amplicons representing dominant RFLP types from each DGGE band were submitted for partial sequence analysis (Eurogentec, Ivoz-Ramet, Belgium). Sequence alignments were performed by the BLAST program of the National Centre for Biotechnology Information (Altschul et al. 1997).

Results

Seawater – microbial characterization

The seawater was collected at a depth of 90 m depth, close to the seabed. Measurements over several years of salinity, NO_3–NO_2, *o*-PO_4, dissolved organic carbon (DOC) and heterotrophic microbes have shown only small variations from year to year and with season. The concentrations

Table 2. Concentrations of microbial cells (DAPI), bacteria (FISH-EUB338), archaea, (FISH-ARCH915), viable heterotrophic (MPN-HB) and oil-degrading (MPN-ODB) microbes in surface and bottom seawater at the start of the experiments. The results were recorded with 95% confidence interval (CI)

Parameter	Surface water (cells/ml ± 95% CI)	Bottom water (cells/ml ± 95% CI)
DAPI	$7.2 \times 10^5 \pm 5.4 \times 10^4$	$3.3 \times 10^5 \pm 2.1 \times 10^4$
FISH-EUB338	$5.8 \times 10^5 \pm 2.0 \times 10^4$	$2.3 \times 10^5 \pm 8.7 \times 10^3$
FISH-ARCH915	$1.5 \times 10^5 \pm 2.3 \times 10^4$	$1.5 \times 10^5 \pm 2.0 \times 10^3$
MPN-HB	$1.4 \times 10^4 \pm 1.8 \times 10^3$	$3.5 \times 10^3 \pm 2.1 \times 10^2$
MPN-ODB	$6.7 \times 10^2 \pm 7.0 \times 10^1$	$6.7 \times 10^2 \pm 7.0 \times 10^1$

of microbial cells in surface and bottom seawater were determined as total counts by epifluorescence microscopy with DAPI stain, while bacteria and archaea were differentiated by FISH, using domain-specific Cy3-labelled DNA probes. The results of Table 2 show that total and bacterial concentrations were higher in surface than in bottom seawater. Archaeal concentrations in surface and bottom seawater were similar, indicating that the archaea constituted a larger fraction of the microbial population in the bottom than the surface water. MPN-counts showed 20 and 5 times higher concentrations of heterotrophes than of oil-degrading bacteria in surface water and bottom water, respectively (Table 2). The MPN counts of heterotrophs were approximately 1–2% of DAPI counts.

HC depletion from fabrics

HC depletion from oil immobilized on the fabrics were determined by GC-FID (C_{10}–C_{36} *n*-alkanes) and GCMS (Naph-1, Naph-2, PAH-1, and PAH-2) analysis during the flow-through experiment (Figure 2). Amounts of *n*-alkanes on the fabrics were decreased by 80, 92, and 98% after 7, 14, and 21 days of the experiment, respectively. Detectable Naph-1 had disappeared from the fabrics after 14 days, while only 2% residue was left after 7 days. Naph-2 was reduced by 88–99% after 7–21 days of the flow-through period, while corresponding decrease for PAH-1 was 71–95% for PAH-1. Immobilized PAH-2 was reduced by 59% after 21 days, and by 82% after 42 days.

Oil desorption

HC depletion from the fabrics may be the result of three processes, oil desorbtion, dissolution, and biodegradation. Oil desorption was determined by measurement of the recalcitrant isoprenoids pristine and phytane, and the pentacyclic triterpane $C_{30}17\alpha(H),21\beta(H)$-hopane (Figure 3). Negligible decreases of immobilized hopane were measured

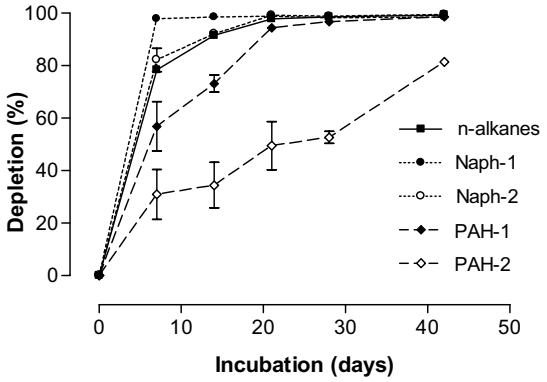

Figure 2. Depletion of *n*-alkanes (C_{12}–C_{36}), naphthalenes and PAH from Fluortex fabrics, presented as the percentage decreases of HCs immobilized at the start of the experiment.

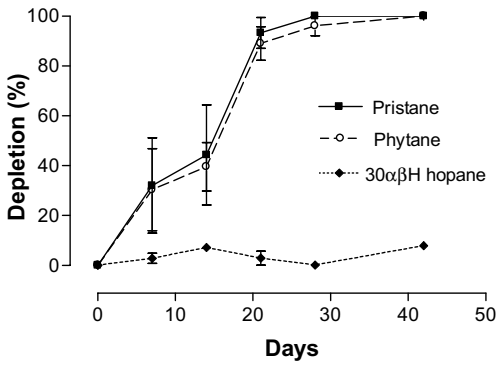

Figure 3. Depletion of pristane, phytane, and $30\alpha\beta$H hopane from Fluortex fabrics, presented as the percentage decreases of the compounds immobilized at the start of the experiment.

during the experiments ($k_{hopane} < 0.0001$), while pristine or phytane were reduced below detection limits after 3–4 weeks ($k_{pristane} = 0.05004$; $k_{phytane} = 0.04341$). The results for the hopane compound indicated that the oil remained adsorbed on the fabrics during the experiments, and there was no significant oil washout. However, the isoprenoids were of limited value as oil biomarkers in the study.

HC dissolution

HC dissolution in the flow-through experiment was calculated based on the partition characteristics of the HC pseudo-compound in a flow-through system, thus excluding concentrations of HCs in the water phase, due to the continuous removal by the water current. Computed dissolution from the fabrics of the *n*-alkanes and the aromatic HC is shown in Figure 4. Calculated dissolution was negligible for *n*-alkanes ($k = 0.00060$) and PAH-2 ($k = 0.00089$), but was rapid for Naph-1 ($k = 1.166$), Naph-2 ($k = 0.2071$), and PAH-1 ($k = 0.1304$). When data set for depletion and dissolution were compared by parametric *t*-test (Figures 2 and 4), significant differences were determined for *n*-alkanes ($p = 0.0045$) and PAH-2 ($p = 0.0121$), but not for Naph-1 ($p = 0.993$), Naph-2 ($p = 0.7821$), and PAH-1 ($p = 0.2329$), indicating that dissolution was the main depletion process for the latter pseudo-compound groups.

Biotransformation

In Figure 5 the distribution between dissolution and transformation in the flow-through system is visualized during the study period. We assumed that biotransformation represented the differences between total depletion and dissolution of HCs. The results from the figure showed that 98% of the *n*-alkane and 76% of PAH-2 removal from the fabrics were the result of biotransformation at the end of the experiments (42 days). For Naph-1, Naph-2 and PAH-1 the depletion was caused exclusively by dissolution (99–100% of depletion) at the end of the test period.

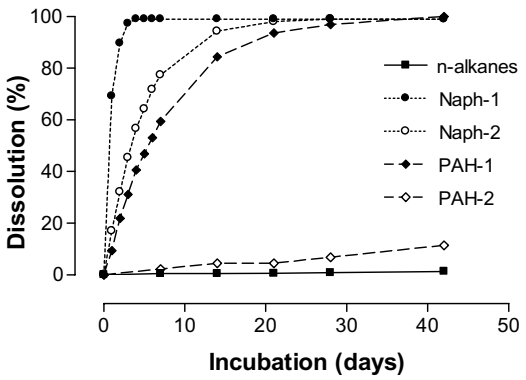

Figure 4. Computed dissolution (%) of *n*-alkanes (C_{12}–C_{36}), naphthalenes and PAH from an oil film to seawater under temperature and current conditions relevant for the flow-through experiment.

Microbes on immobilized oil

Bacterial communities adhering to the immobilized oil on the fabrics were examined by DGGE analysis of PCR-amplified 16S rRNA gene fragments. The results of Figure 6 show changes in the community profiles during the flow-through period. After 14 and 28 days a few strong DGGE

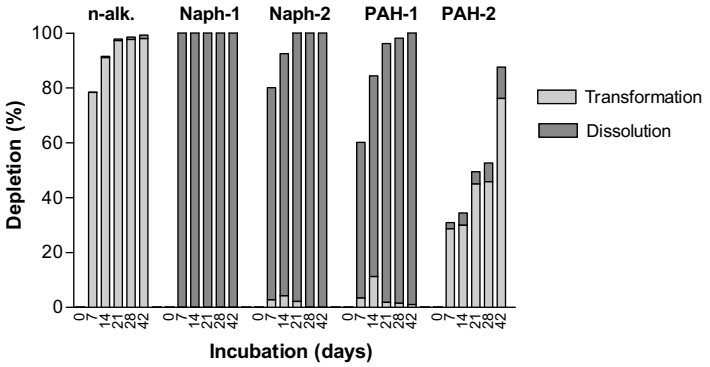

Figure 5. Depletion of *n*-alkanes, naphthalenes, and PAH, calculated as the distribution between dissolution and biotransformation.

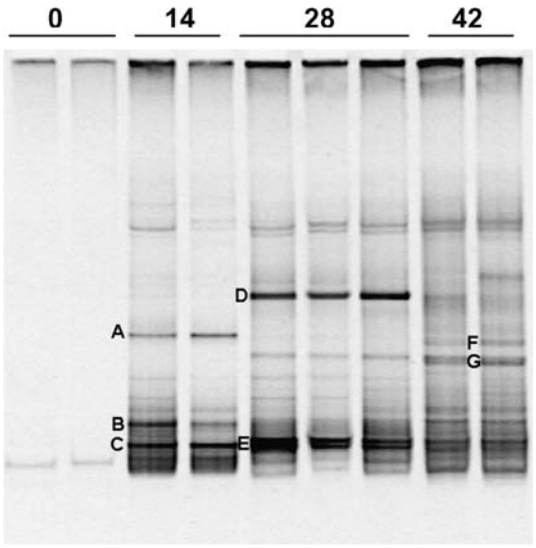

Figure 6. PCR-DGGE analysis of bacterial 16S rRNA genes adhering to fabrics with immobilized oil. Fabrics were removed as replicates from the flow system at the start (0), and after 14, 28, and 42 days of the test period. Bands removed for cloning and sequencing are marked with letters to the left.

bands predominated, but the mobilities of these differed with sampling time. After 42 days the number of bands increased, with a decrease in the intensity of individual bands. Thus, the DGGE results indicated that a few bacterial genotypes were abundant on the immobilized oil films during the first 4 weeks, while the diversity increased towards the end of the biodegradation period.

A number of seven predominant DGGE bands (Figure 6) were eluted from the gel and re-amplified. The amplicons were then cloned and 16S rRNA gene fragments sequenced (Table 3). The sequenced clones were distributed within the α- and γ-subgroups of proteobacteria (5 DGGE bands) or the *Cytophaga–Flavobacteria–Bacteroides* group (2 DGGE bands). With the exception of an α-proteobacterium (matching with *Ruegeria atlantica*) different microbes were abundant on the oil films during the flow-through period.

Discussion

The Fluortex fabrics included in these studies are recommended as cleanup adsorbents for thin oil films (Greimann et al. 1995). In a recent study immobilisation of C_{12}–C_{40} alkanes to the fabrics was documented. In a static system with sterile seawater adsorbed alkanes showed insignificant desorption from the fabrics to seawater in sterile static systems, while aromatic compounds showed variable desorption, related to the oil–water partition coefficients for the compounds (Brakstad et al. 2002). These data concorded well with the results of the dissolution calculations presented here.

For field biodegradation studies the ratios between easily biodegradable and recalcitrant compounds (e.g. the isoprenoids pristine and phytane, or the pentacyclic triterpane compound $C_{30}17\alpha$(H), 21β(H)-hopane) have been determined to separate degradation processes from physical oil removal. However, the biological persistence of some of these compounds have been debated, and several studies have shown that the isoprenoids are degraded in the environment, although at slower rates than the *n*-alkane analogues (e.g. Douglas et al. 1996). In our studies both isoprenoid compounds were depleted from the immobilised oil within 14 days after test start. Also, degradation of

Table 3. Alignment of bacterial 16S rRNA genes originating from DGGE bands during biodegradation

DGGE band[a]	Closest match	Phylum or class	Similarity (%)
A	*Methylophaga thallasica*	γ-proteobacteria	98
B	Uncultured *Roseobacter* NAC11-7	α-proteobacteria	96
C	*Ruegeria atlantica*	α-proteobacteria	97
D	*Flexibacter aggregans*	CFB[b]	98
E	*Ruegeria atlantica*	α-proteobacteria	99
F	*Cytophaga* sp.	CFB[b]	93
G	*Rhodobium orientis*	α-proteobacteria	96

[a] See Figure 6.
[b] *Cytophaga–Flavobacteria–Bacteroides* group.

hopane compounds have been reported (Wang et al. 2000), but no depletion of the immobilised triterpane was measured here, showing that oil remained adsorbed to the fabrics during the experiments. The removal of the isoprenoids from the fabrics was therefore most probably the results of biodegradation rather than dissolution, due to the low polarities and water solubilities of these compounds. Thus depletion of immobilised oil compounds was the result of dissolution and biodegradation processes.

The immobilised oil is a non-aqueous-phase liquid (NAPL) for the HCs with different dissolution characteristics. In a static system the rate of change of a HC in the oil phase due to dissolution is a function of the mass transfer coefficient, the oil/water surface area, and the concentrations of the HC in the oil and water phases (Efroymson et al. 1995). As biotransformation proceeds in the water phase more HC is depleted from the oil phase (Ramaswami & Luthy 1997). In a flow-through system the HC concentrations in the water phase can be neglected due to the constant removal, and dissolution is thus increased. This implies that the mass transfer coefficient and the specific surface area will determine the time scale of the dissolution process. When this model was used for the calculation of the dissolution rates of the HC pseudo-compound groups, based on the known mass transfer coefficients for individual compound groups, computed and measured dissolution corresponded well for Naph-1, Naph-2 and PAH-1. These data strongly indicated that the depletion of the Naph-1, Naph-2 and PAH-1 compound groups was nearly entirely the result of dissolution processes. The combined sets of data made us conclude that the rapid depletion of the *n*-alkanes was exclusively the result of biotransformation. For the PAH-2 group a slow dissolution rate was measured, while the depletion rate was significantly higher. Thus, both processes contributed, but biotransformation was by far the most important of these.

HC dissolved in the seawater (e.g. compounds in the Naph-1, Naph-2 and PAH-1 pseudo-groups) will be rapidly biotransformed. Recent results from a mesocosm study run at 16–24 °C with dissolved HC showed that low-molecular weight (LMW) PAH (less than 3 aromatic rings) were depleted within 2 days (Yamada et al. 2003). Studies with water-soluble HC fractions in our lab showed that LMW PAH were biotransformed within 4–5 days in seawater at 13 °C (Brakstad et al. 1999).

The results of our studies are difficult to compare to most other reported biodegradation studies performed under simulated marine conditions. Most of these studies have been performed with dispersed oil, and the results from these will not separate between transformation of HC on oil droplets and in the water phase. Further, several of the studies have described biodegradation as the ratio between biodegradable and recalcitrant compounds (e.g. MacNaughton et al. 2003; Marty & Marty 1996; Siron et al. 1995; Swannell & Daniel 1999).

The experiments described here were performed at low seawater temperatures, between 6 and 8 °C. Lowering temperatures results in increased oil viscosity and reduced volatilization of short-chain alkanes (Margesin & Schinner 1999). While some studies of marine oil biodegradation in temperate climates have shown slower biotransformation in winter than in summer periods (Minas 1986), others have shown opposite results (Colwell et al. 1978). These differences may be related to the temperature-related changes in physical characteristics of the oils included in the studies rather than to microbial activities.

The microbial inoculum used in this study was a continuous supply of natural seawater from 90 m depth in a Norwegian fjord. Determination of total microbes, bacteria, and heterotrophic microbes showed lower concentrations in bottom than in surface seawater. However, determination of archaea and oil-degrading microbes did not show any decline in the bottom water, indicating increased contribution of archaea and oil-degrading microbes towards the bottom. This is in agreement with the findings from other studies (DeLong et al. 1999; Pernthaler et al. 2002).

During biodegradation of dispersed oil, or thin oil films, microbes adhere to the oil–water interphase. Cell wall hydrophobicity is associated with microbial oil surface adherence, and this hydrophobicity has been related to the synthesis of mycolic acids (Bendinger et al. 1993). Attached microbes may further change the oil surfaces through the production of biosurfactants (Desai & Banat 1997). Electron microscopic studies have revealed oil–bacteria-surfactant interphases, and bacterial adsorption to the oil is associated with a

highly amphiphatic bacterial surfactant interphases (Southam et al. 2001).

The bacterial communities adhering to the fabrics showed unambiguous changes in DGGE profiles. Continuous changes seemed to occur during the biodegradation period, with a few bands dominating early in the period, with a subsequent increase in band numbering at the end of the period. This is in agreement with recent results from studies of microbial diversities in North Sea waters during crude oil biodegradation (Brakstad & Lødeng, in press). The excised bands, constituting predominant bands during biodegradation, contained bacterial members affiliating mainly to the α-proteobacteria or *Chloroflexus–Flavobacterium–Bacteroides* groups. Several studies have shown that these groups are associated with oil biodegradation in marine environments (Chang et al. 2000; MacNaughton et al. 1999; Röhling et al. 2002).

In conclusion, studies with immobilized oil in flow-through systems may give valuable contributions to the determination and understanding of various processes after oil discharges to aquatic environments. For instance, HC dissolution and biodegradation from oil films, and the potential impacts in the water column, may be predicted after an oil spill in combination with remote sensing of oil films thickness (Brown & Fingas 2003). Further, if depletion from films and droplets are comparable, the system described here may be used for gaining new insights into dissolution, biodegradation and microbiological processes of dispersed oil. This may also have implications for studies of oil spill treatment with chemical dispersants.

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