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UKOOA phase II Task 3: Aerobic and anaerobic degradation of drill cuttings – results from small scale laboratory experiments

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Executive summary

The main goal of the small-scale biodegradation experiment series was to obtain biodegradation rates of THC in drill cuttings material, at conditions partly realistic for *in situ* drill cuttings accumulations. Both aerobic and anaerobic biodegradation was investigated separately from other processes known to affect biodegradation rates in these small scale experiments. The biodegradation rates obtained will be used as input to a numerical long-term model.

Series of small-scale degradation experiments were prepared to provide expected maximum degradation rates of total hydrocarbons (THC) from the Ekofisk 2/4A and Beryl drill cuttings material. In addition, available drill cuttings material from other piles was included to expand the range of input data to also embrace other pile characteristics (basically based on oil type and concentration). North Sea reference sediment was also included in the test matrix.

Conditions of test systems:

Degradation experiments were carried out under both aerobic and anaerobic conditions. The set up was designed to focus exclusively at biodegradation (as far as possible), not taking into account parameters like erosion, surface active layers or loss of THC etc. The degradation series were prepared as closed systems. Time-series data was obtained over a period of 100 and 229 days for anaerobic and aerobic series respectively.

The experiments were carried out at 10°C, somewhat higher than expected site conditions (likely range, 3-6°C). It is a general trend that microbial degradation processes become more rapid with increasing temperature, within certain limits. The higher temperature used in this experiments was selected for several reasons:

- possibly higher degradation rates (advantageous since experiments were rather short-term);
- the increase in temperature was not so large, with the rather small deviations, that not the same fraction of the microflora were likely to be active;
- and so that the experiments can be directly comparable with other task 3 experiments that have certain limitations in that the ambient seawater temperature in the lab is about 10°C.

The cuttings material, representing the top 30-40 cm of the piles, was homogenised (mixed) and the same batch was used for both degradation series. Homogenising implied that; the content of the test bottles was similar and thus comparable; the mix did not represent a specific layer, but represented the top section of the pile; existing surface active layers (SAL) was removed.

Results and discussion:

Degradation of THC was measured as changes in THC levels in the sediment fraction. Microbial related processes were further examined by including parameters such as oxygen, sulfide and pH in the water-phase, and microbial activity in the sediment phase.

The microbial related process parameters reproduced quite similar in the different treatments. The small variations seen basically corresponded to the observed changes in THC. In the anaerobic series, sulfide levels and bacterial activity showed clear correlation to decreasing THC levels for the Frøy cuttings material. In general, these parameters supported the observations of the THC levels, but only in a few cases were they clearly motivating conclusions on the biodegradation.

It should be noted that when preparing the anaerobic test series, no chemically reducing agents were added to completely remove traces of oxygen. Therefore, it might be that the THC degradation reported in these test bottles may be a combination of degradation at microaerophilic and true anoxic conditions. This is difficult to confirm from the oxygen measurements since the method used is not the best suited at very low oxygen levels.

The variability of THC measurements was high, most likely due to heterogeneity of the cuttings material on a micro-scale. Statistically, the variation in the data is in the order of 25-30%, implying that THC reductions far above this is needed to conclude with certainty that THC is degraded. However, other analyses within this sub-task and other tasks have been used to judge the appropriateness of presenting actual degradation rates. Although the inaccuracy of the data must be regarded as high, half-lives of THC and relative changes in concentration from start to end of the experiments have been calculated when possible. These calculations and measured concentrations are presented in the tables below:

Aerobic degradation series – reduction in THC and degradation half-lives:

Cuttings	C_0 (mg/kg)	C_1 (mg/kg)*	C_1/C_0	K_1 d^{-1} **	$t_{1/2}$ (days)
Beryl A 20 %	553	338	0,61	**0.006**	**120**
Beryl A 100 %	2428	2662	1,10	**ND**	**ND**
Ekofisk 2/4 A 20 %	10600	9110	0,88	**0.0005**	**1300**
Ekofisk 2/4 A 100 %	73990	61793	0,84	**0.0009**	**750**
Frøy 20 %	322	121	0,46	**0.003**	**200**
Frøy 100 %	4971	4574	0,92	**0.0005**	**1250**
Ekofisk 2/4 C 20%	647	614	0,95	**ND**	**ND**
Ekofisk 2/4 C	4763	4178	0,88	**ND**	**ND**

* C_1 is the THC concentration measured at the end of the experiment, after 229 days of incubation
** K_1 d^{-1} values are derived from 1st order kinetics. The $t_{1/2}$ has been derived from the same

Anaerobic degradation series– reduction in THC and degradation half-lives:

Cuttings	C_0 (mg/kg)	C_1 (mg/kg)	C_1/C_0	K_1 d^{-1} **	$t_{1/2}$ (days)
Beryl A 20 %	749	343	0,46	**0.01**	**70**
Beryl A 100 %	2981	3972	1,33		
Ekofisk 2/4 A 20 %	10110	10476	1,04		
Ekofisk 2/4 A 100 %	64924	64262	0,99		
Frøy 20 %	1681	997	0,59	**0.01**	**60**
Frøy 100 %	6110	5054	0,83	**0.0007**	**950**
Ekofisk 2/4 C 20%	511	570	1,12		
Ekofisk 2/4 C	4825	4108	0,85		

* C_1 is the THC concentration measured at the end of the experiment, after 100 days of incubation
** K_1 d^{-1} values are derived from 1st order kinetics. The $t_{1/2}$ has been derived from the same

In sum, the tables above indicate that:

- Reduction in THC was seen with the Frøy cuttings both aerobically (mostly for the 20% cuttings series) and anaerobically (both 20 and 100%). The other measurements (sulfide, pH, oxygen consumption) supported a presumption that this decrease was of biological origin. Most strongly was this the case in the anaerobic series.
- A decrease in THC was also seen with Ekofisk 2/4A cuttings in the aerobic series, but the decrease in THC was less than the assumed accuracy limits of the THC data. This decrease is also assumed to be due to biodegradation. No measurable decrease in THC was seen with the anaerobic series. However, there were indications of microbial processes occurring, as seen by microbial activity and sulfide measurements particularly.
- Some evidence of Beryl biodegradation was also seen, mainly in the 20% cuttings series. Lack of degradation of the 100% Beryl series may be indicating toxic THC levels reducing or preventing microbial degradation.

The degradation half-lives reported is based on 1st order kinetics calculated from rather few input numbers. The accuracy is therefore low (only the Frøy 100% of the aerobic series showed a confidence interval above 95%). However, the figures presented may be interpreted as indicative of potential maximum degradation rates at the experimental conditions applied.

Conclusion:

The calculated degradation rates can be used as input to the mathematical model, representing maximum rates under optimal conditions.

The constraint in the data, seen isolated from the other experiments of task 3 is:

- The degradation rates represent a process going on in a mixed sample. At least anaerobically, other factors than the presence of a microbial community capable of degrading THC will be limiting.

- With the mixed sample, components of the cuttings may have been redistributed in a manner that increases the likelihood of biodegradation to take place. Also, oil contained within the larger particles may have become more bioavailable due to the mechanical actions.
- At the aerobic test conditions, the sediment and water phase was constantly mixed. The whole sample can then be seen as representing a surface-active layer of a “real” cuttings pile.

Acronyms

RF	RF- Rogaland Research
SINTEF	SINTEF Applied chemistry and SINTEF
AEAT	AEA Technology Environment
ERTSL	ERT (Scotland) Ltd
CP	Cuttings pile
DNV	Det Norske Veritas
UKKOA	UK Offshore Operators Association
OBM	Oil based mud (i.a. well drilled using a OBM)
PBM	Pseudo oil based mud
WBM	Water based mud
SSE	Small scale experiment (microcosm)
THC	Total Hydrocarbon

1 Introduction

The UKOOA Task 3 work comprises several sub-tasks performed by the project team; RF, SINTEF, ERTSL and AEAT. This report deals with the sub-tasks focused on degradation of hydrocarbons associated with drill cuttings material, performed by RF.

The rationale behind the experimental focus has been to provide time-series data on hydrocarbon degradation at conditions partly realistic for *in situ* drill cuttings accumulations. Several types of 'real' drill cuttings material with a range of contaminant types and concentrations has been examined, at both aerobic and anaerobic experimental conditions.

Details of the Task 3 project outline is presented in the main report (RF-2001/220).

2 Material and methods

2.1 Experimental approach

Two experimental series were prepared, investigating aerobic and anaerobic degradation processes respectively. The same material and basic set up was used with both series, the main difference being the aeration and continuous mixing of the aerobic series, while the anaerobic series was left undisturbed during incubation.

2.1.1 Test system

Sealed glass bottles (325ml) were used for both degradation series. A set of identical bottles was prepared for each cuttings type/mixture tested. At each sampling point, one bottle from each set was terminated. A selection of analysis was then carried out on these terminated bottles. These analyses were the same with both test series. Single tests were prepared, except some selected samples were prepared in triplicate to account for statistical variations. Ideally, triplicate measurements should have been prepared and analysed for each treatment, but this would require too much resources. The statistical variation in the triplicate 100% Beryl A samples were used as a measure of the variation also in the single test series.

2.1.1.1 Aerobic degradation series

To the aerobic test series, the bottles were added about 60g of the cuttings sample, and 100ml seawater. Additional N- and P-sources were added to concentrations of 8.5mg/l KH_2PO_4, 21.75mg/l K_2HPO_4, 33.4mg/l Na_2HPO_4 x $2H_2O$, and 1.7mg/l NH_4Cl (OECD, 1992).

The bottles were primarily incubated at 10ºC. Additional series were also incubated at 4 and 20ºC respectively. The bottles were incubated in the dark. They were continuously

shaken on a mixing table (about 100-150rpm), and they were incubated lying flat, to maximise the interface between the cuttings, water and air phases. The bottles were aerated approx. once a week by injecting filtered air through the septum. Pressure build up was avoided by inserting an additional syringe through the septum. Each bottle was aerated for about 3-5 minutes every time. Five sampling points were applied; 1, 14, 28, 56 and 229 days (Table 1). Four sampling points were initially planned, but an extra set of test bottles were prepared so that it would be possible to extend the test period if considered feasible.

Table 1. Experimental set up, sampling schedule and analysis parameters, aerobic degradation experiment. 20% refer to a mixture of 20% cuttings and 80% reference sediment (w/w). Analysis included at all sampling days: THC, pH, oxygen, sulfide (water), microbial activity.

No	Content	Sampling days
1	**reference sediment**	1, 14, 28, 56, 229
2	**Beryl A 20%**	1, 14, 28, 56, 229
3	**Beryl A**	1, 14, 28, 56, 229
4	**Beryl A**	1, 14, 28, 56, 229
5	**Beryl A**	1, 14, 28, 56, 229
6	**Ekofisk 2/4 A 20%**	1, 14, 28, 56, 229
7	**Ekofisk 2/4 A**	1, 14, 28, 56, 229
8	**Frøy 20%**	1, 14, 28, 56, 229
9	**Frøy**	1, 14, 28, 56, 229
10	**Lille-Frigg 20%**	1, 14, 28, 56, 229
11	**Lille-Frigg**	1, 14, 28, 56, 229
12	**Ekofisk 2/4 C 20%**	1, 14, 28, 56, 229
13	**Ekofisk 2/4 C**	1, 14, 28, 56, 229
15	**Beryl A, 20ºC**	1, 14, 28, 56, 229
17	**Beryl A, +N+P, 20ºC**	1, 14, 28, 56, 229
19	**Beryl A, 5ºC**	1, 14, 28, 56, 229
0	**Beryl A, +HgCl2**	1, 14, 28, 56, 229

2.1.1.2 Anaerobic degradation series

The bottles for the anaerobic test series were added 80g cuttings sample and 200ml seawater. As with the aerobic series, additional N- and P-sources were added to concentration of 8.5mg/l KH_2PO_4, 21.75mg/l K_2HPO_4, 33.4mg/l Na_2HPO_4 x $2H_2O$, and 1.7mg/l NH_4Cl (OECD, 1992).

The same incubation conditions were applied as with the aerobic test series, except the bottles were incubated standing, without mixing. Four sampling points were applied; 1, 28, 56 and 100 days (table 2). No additional set of test bottles was prepared for the anaerobic series.

Table 2. Experimental set up, sampling schedule and analysis parameters, anaerobic degradation exp. 20% refer to 20% cuttings and 80% reference sediment. Analysis included at all sampling days: THC, pH, oxygen, sulfide (water), microbial activity.

No	Content	Sampling days
1	**reference sediment**	1, 28, 56, 100
2	**Beryl A 20%**	1, 28, 56, 100
3	**Beryl A**	1, 28, 56, 100
4	**Beryl A**	1, 28, 56, 100
5	**Beryl A**	1, 28, 56, 100
6	**Ekofisk 2/4 A 20%**	1, 28, 56, 100
7	**Ekofisk 2/4 A**	1, 28, 56, 100
8	**Frøy 20%**	1, 28, 56, 100
9	**Frøy**	1, 28, 56, 100
10	**Lille-Frigg 20%**	1, 28, 56, 100
11	**Lille-Frigg**	1, 28, 56, 100
12	**Ekofisk 2/4 C 20%**	1, 28, 56, 100
13	**Ekofisk 2/4 C**	1, 28, 56, 100
15	**Beryl A, 20ºC**	1, 28, 56, 100
17	**Beryl A, +N+P, 20ºC**	1, 28, 56, 100
19	**Beryl A, 5ºC**	1, 28, 56, 100

2.1.2 Cuttings material

Drill cuttings material from five different cuttings piles were compared. These included:

- Beryl A, an oil based cuttings pile sampled as part of the UKOOA Task 1 survey in September 2000 (Westerlund et al., 2001),
- Ekofisk 2/4 A, a water based/ part pseudo-oil based pile also sampled as part of the UKOOA task 1 survey,
- Ekofisk 2/4 C, water based/ part pseudo-oil based (mainly ester) pile sampled during 1998 Ekofisk survey for PPCoN (Cripps et al., 1999),
- Frøy, water based/ part pseudo-oil based (alphaolefines) pile sampled for Elf (TotalFinaElf) in 1999 (Westerlund and Cripps, 1999), and
- Lille-Frigg, purely water based pile also sampled for Elf in 1999.

The material used had been stored refrigerated and dark since it was collected. The Ekofisk 2/4C, Lille-Frigg and Frøy samples were all part of core-samples not previously sectioned. The material had been sealed, and was still moist when used for these experiments. The Ekofisk 2/4 A and Beryl samples were sub-collected from two box-corer samples each (see Westerlund et al. 2001 for sample identification). The reference sediment sample used was collected during the 2000 survey, at a set reference location in the North Sea (Westerlund et al., 2001).

The cuttings material used was homogenised/mixed as well as practically feasible. This implied taking sub-cores from the large box-core samples (Ekofisk 2/4A and Beryl) penetrating the about 30 cm thick sample, from two boxes each, and mixing by rotating it in a sealed box in a cement blender for 30 minutes. The other cuttings samples were of a smaller volume, and these were mixed using a muddler, mixing the sample thoroughly for about 20-30 minutes. The same mixed batch was used for both degradation studies.

To the aerobic and anaerobic 100% cuttings series, 60 or 80g were weighed directly from the mixed batches into the bottles. The bottles added 20% cuttings were prepared by weighing the mixed cuttings (20% w/w) and the reference sediment (80% w/w) separately into the bottle. The bottles were then mixed by rigorously shaking for 1-2 minutes once the seawater was added.

2.1.3 Sampling and analysis

For both experimental series, test bottles were terminated at the indicated sampling points (table 1 and 2). Some of the parameters were analysed directly or within hours after sampling, while others (mainly THC) were frozen and stored for later analysis.

The test bottles of the aerobic biodegradation series were allowed to stand still overnight prior to sampling to allow suspended material to settle. Sulfide and oxygen were measured in the water phase before other parameters were analysed.

After sampling/analysing the water fraction, the residual water phase was decanted or filtrated away from the cuttings/sediment phase. The cuttings/sediment phase was then used for bacterial activity measurements (2.1.3.3), and sub-samples for analysis of i.a. the organic fraction were taken and stored frozen until analysed (THC/ other GC-FID identifiable components of organic fractions after extraction, 2.1.3.4).

2.1.3.1 Sulfide measurements

Sulfide was measured on sub-samples of the water phase that were taken through the septum of the bottles. Sulfide was measured according to a method described by Munson (1977):

1. To a 50ml volumetric flask, 10ml of a 2% zinc acetate solution and 0,5ml of the sample are added. Distilled water is added to approximately 40ml.
2. Four ml of a dimethyl-p-phenylenediamine sulfate solution (0,2% in 20% H_2SO_4) is added, and the bottle is swirled gently.
3. Next, 250µl of a $FeNH_4(SO_4)_2$ solution (10% in 2% H_2SO_4) is added, and the bottle is shaken and allowed to stand for approx. 10 minutes.
4. The volumetric flask is then filled up to the 50ml mark, and the optical density against a blank at 670nm (A670) is measured in a spectrophotometer. The blank is prepared accordingly, replacing the 0,5ml sample with distilled water.

The sulfide concentration is calculated from a previously prepared calibration curve.

2.1.3.2 Oxygen measurements

Oxygen in the water phase was measured either directly in the bottles (anaerobic series) or by transferring part of the water phase into a proper vial (aerobic series).

Oxygen was measured using a YSI Model 59 Dissolved oxygen meter. Oxygen was measured at ambient air conditions.

2.1.3.3 pH measurements

pH was measured in the same lot as the oxygen, after the oxygen measurements were completed. The pH was measured using an Orion 20 pH-meter.

2.1.3.4 Bacterial activity measurements

Bacterial activity was measured by a method modified after Schnürer and Rosswall (1982). The method is based on measuring bacterial degradation of carbon by enzymatic hydrolysis of FDA (3', 6'-diacetylfluorescein). FDA is hydrolysed by several 'general' enzymes (i.a. lipases, proteases and esterases), yielding the fluorescent product fluorescein. The product is quantified spectrophotometrically. The method is modified for use with seawater and sediment samples contaminated by hydrocarbons.

Approx. 1g (exact weight) of the sediment/cuttings material is weighted into appropriate sealable tubes (volume approx. 15mL), and 10mL of a 60mM TRIS base (pH 8.1, filtered through a 0.22µm filter) is added. The enzymatic reactions were started by addition of 250µl of FDA (Sigma Chemical CO) dissolved in acetone (2mg/ml).

The samples were incubated on a shaker/magnetic stirrer for approx. 90 minutes at 10ºC (or ambient room temperature for the test bottles incubated at that temperature). After incubating for about 115 minutes, the samples were filtered through a paper filter (Munktell no. 3). The filtrate was further filtrated through a 0.22µm filter directly into the cuvette, using a syringe. After exactly 120 minutes, the absorbance at 490nm was measured.

2.1.3.5 Organic fraction processing and analysing

Degradation of hydrocarbon and other organic compounds of the cuttings material was assessed analysing for THC in the samples. Samples for extraction and analysis by GC-FID were stored frozen until analysed.

(1) Sample processing

Thawed cuttings/sediment samples were thoroughly mixed in the glass beaker used for storage. Sample for THC measurements were transferred to appropriate extraction thimbles and weighed. The water content of the samples varied significantly. It was therefore crucial to measure this accurately to be able to compare samples. The water content were measured on the same mixed lot by weighing approx. 5 grams into a separate beaker, and then dry this at 110°C overnight, or until stable weight was obtained.

The THC sample was added internal standards, a mixture of deuterated nC20 (0.020504g/ml) and nC30 (0.010308g/ml). The amount of the internal standard mixture added was varied during the course of the analysis to meet changing conditions of the samples and to improve the output based on current experiences. The volume range used was 25µl – 100µl (or 0.05126-0,20504 mg/g sediment (d-C20), or 0.02577-0.10308 mg/g sediment nC30). The THC sample was then extracted using methanol and dichlormethane.

Equipment used:

-Soxterm 2000

-Visiprep Solid Phase Extraction Vacuum Manifold (Supelco)

-Solid Phase Extraction Tubes, Normal Phase packing LC-Si, 3ml (Supelco)

Extraction characteristics:

Extraction temperature:	150 °C
Boiling time:	30 minutes
Solvent reduction A:	5 * 15 ml
Extraction time:	60 minutes
Solvent reduction B:	8 minutes
Solvent reduction C:	5 minutes

After cooling the samples were transferred to a 250ml beaker and dried overnight.

(2) Sample cleanup

The extracted samples were cleaned for contamination possibly interfering with the GC analyses by use of 3ml LC-SI Bond Elute columns.

The columns were placed on top of 10-15ml vials. The dried extracts were added about 3mL n-hexane:dichloremethane (1:1) and transferred to the columns. The column material was not allowed to dry out. Two additional portions of 3ml n-hexane:dichloremethane (1:1) were successively used for washing the evaporation beaker, and transferred onto the column. Then 3ml n-hexane:dichloremethane (1:1) were added to the column.

The eluate volume was reduced to about 0.5ml by use of Na. The sample material was transferred to a vial suitable for GC-analysis before being sealed and stored in a refrigerator until analysis by GC.

3 Results and discussion

3.1 General observations of drill cuttings samples

The cuttings samples from the different pile locations had different characteristics. This was evident from visual observations directly – the colour and texture varied – and also from the varying water content and apparent density of the material as observed when the material was stirred. Such differences influenced on i.a. removal of water from the sediment phase before further analysis etc.

The reference sediment behaved differently from the cuttings samples. As normal with fine sand/sediment, the particles settled much more rapidly through the water column, and it was also easier to homogeneously mix the reference sediment. Samples containing both reference sediment and cuttings material behaved more like the reference sediment, which could be expected since only 20% of the sample was cuttings.

3.2 Aerobic degradation series

3.2.1.1 General observations (aerobe)

The variability of the cuttings samples became especially apparent in the aerobic degradation series. Even though the test bottles were left standing overnight before sampling, separation of the mixture into a clear water phase and a cuttings phase was more or less absent in some of the 100% cuttings samples. This became more and more expressed during the course of the experiment. The Beryl and Ekofisk 2/4 C samples separated least.

3.2.1.2 Sulfide (aerobe)

No sulfide was measured in any of the test bottles at any sampling point (detection limit: 0.2ppm).

3.2.1.3 Oxygen (aerobe)

The bottles were not aerated before the day 14 sampling. It then became evident that the oxygen supply was low, and weekly/bi-weekly aeration was introduced. It was shown that this was sufficient to keep the oxygen level at a level allowing aerobic processes to occur. Not all bottles had large enough water phase to allow oxygen measurements. Data are presented in figure 1 and in Appendix 1.

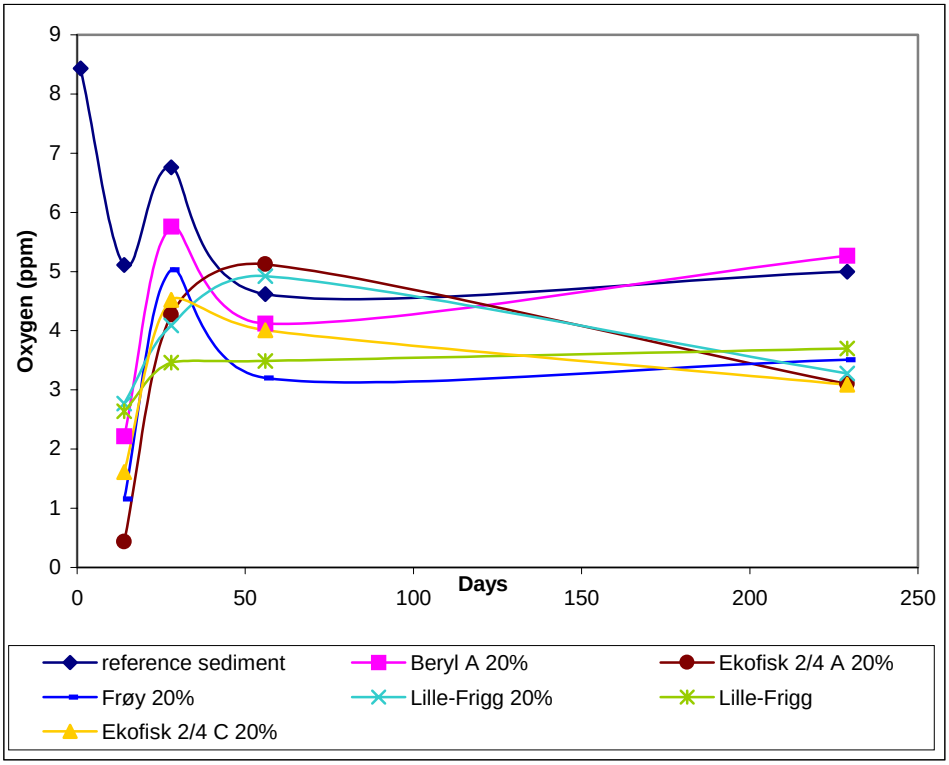


Figure 1. Oxygen levels at sampling in aerobic degradation series. The test bottles incubated at 20°C was accidentally not aerated before the 28 day sampling.

3.2.1.4 pH (aerobe)

The starting pH of the samples varied from about 7.7 to 9.0. As with the oxygen measurements, enough water for measurements could not be withdrawn from all test bottles. In most of the test bottles the pH decreased with time until the day 28 sampling. The pH changed least in the 100% cuttings of Beryl. Only limited data is available due to the difficulties in separating a water phase from the sediment. But this trend was seen for Beryl at 5 and 20°C, and with some limited data at 10°C. A similar tendency was seen with Lille-Frigg. For the other cuttings types, no water samples could be withdrawn at the 100% cuttings. Data are presented in figure 2 and Appendix 1.

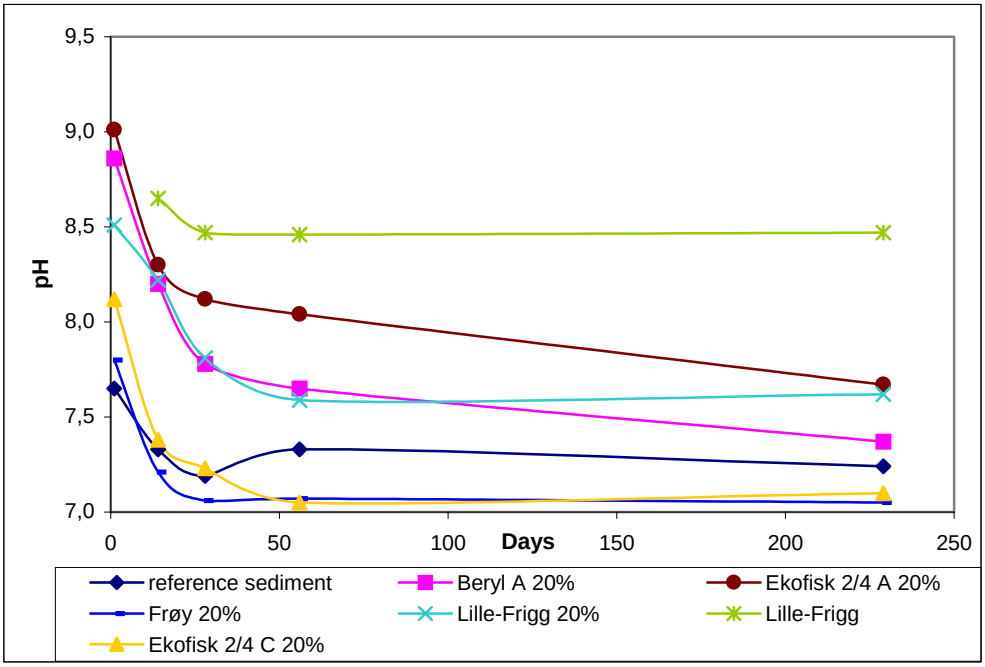
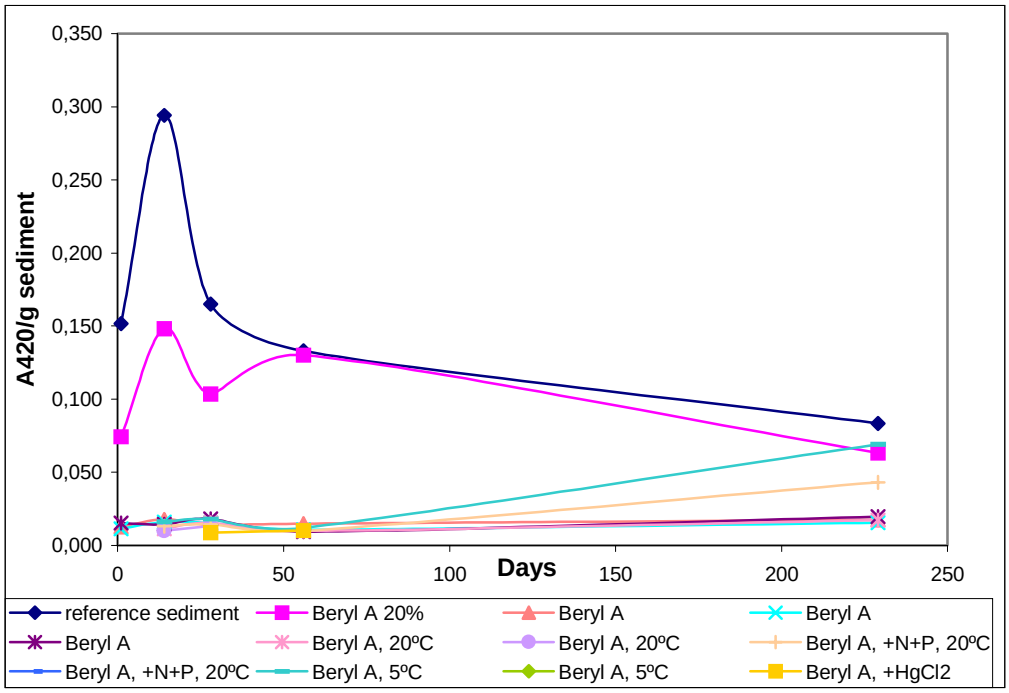


Figure 2. pH in aerobic degradation series.

3.2.1.5 Microbial activity (FDA) (aerobe)

The microbial activity assay is very general, measuring enzymatic responses found in many common metabolism pathways. The assay does not specifically relate to hydrocarbon degradation, and it is possible that the presence of active hydrocarbon metabolising microorganisms is underestimated with such an assay. Nevertheless, the assay is empirically found to give a broad picture of general microbial activity.

Sources of microorganisms in the samples are the cuttings, the reference sediment and the seawater. Differences in activity may partly reflect differences in the starting point with respect to the pool of microorganisms present. The microbial activity varied (figure 3). More details can be found in Appendix 1. Highest activity was found in the reference sediment and Frøy cuttings. The activity of the Beryl samples was low in all cases, also when only 20% cuttings were added. This may indicate that the Beryl cuttings had a negative (toxic) effect on the microflora associated with the reference sediment making up 80% of the mixed Beryl samples.



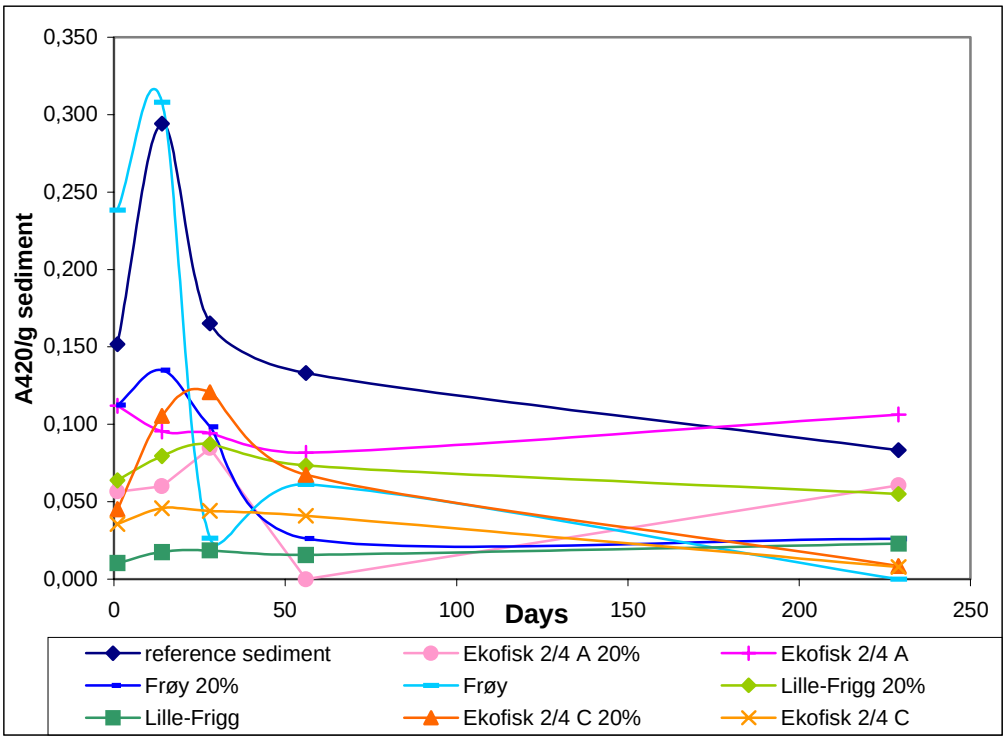


Figure 3a and b. Microbial activity (FDA) of the aerobic degradation series. Beryl treatments (a) and others (b).

3.2.1.6 Organic fraction (THC) (aerobe)

The THC measurements were intended to demonstrate noticeable degradation (as loss of total THC) over the course of the experiment. Any decreases seen would then be used to estimate a degradation rate specific for the cuttings type and conditions.

Within the time frame of the aerobic degradation experiment, noticeable degradation could not be seen in any of the tests. The change (decrease in THC from day 1 to day 229 is illustrated in table 2. The accuracy of the data is discussed in section 3.3.4.

The calculated THC data are presented in table 1 and figures 4 and 5 (details of the Beryl data, average of several analyses).

Table 1. Measured THC levels in the aerobic degradation test at the given sampling days. Values in mg/kg dry sediment.

mg/kg (DW)	1	28	56	229
reference sediment	**0**	**0**	**7**	**14**
Beryl A 20%	**553**	**216**	**267**	**338**
Beryl A*	**2428**	**2092**	**2649**	**2662**
Ekofisk 2/4 A 20%	**10382**	**9835**	**11582**	**9110**
Ekofisk 2/4 A	**73986**	**64536**	**67578**	**61793**
Frøy 20%	**261**	**402**	**289**	**121**
Frøy	**4971**	**4437**	**4353**	**4574**
Ekofisk 2/4 C 20%	**647**	**1496**	**569**	**614**
Ekofisk 2/4 C	**4763**	**5266**	**4259**	**4178**
Lille-Frigg 20%	**12**	n.a	**19**	n.a
Lille-Frigg	**5**	n.a	**45**	n.a
* Average of 2 - 5 analysis				

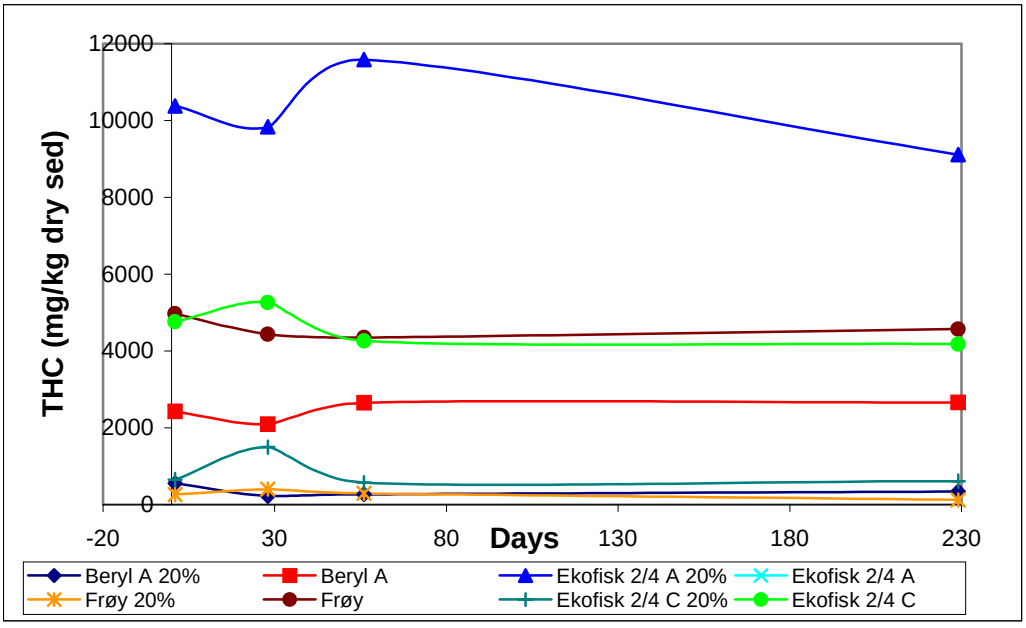


Figure 4. THC of the aerobic test (mg/kg dry sediment). Beryl A data is average of 3-5 extracted samples.

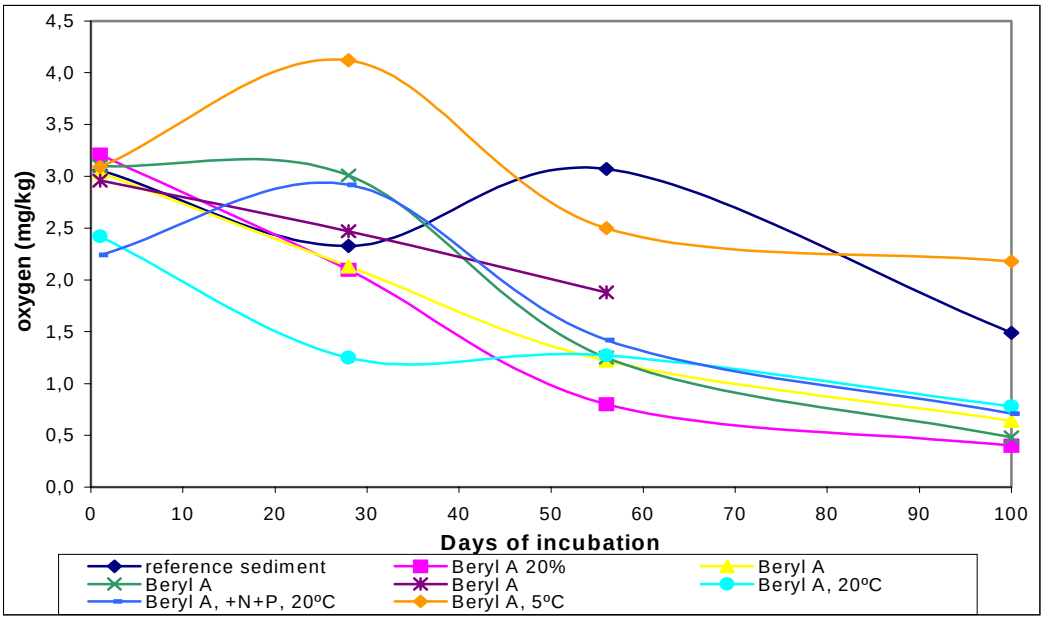


Figure 5. THC of the aerobic test, all Beryl samples (mg/kg dry sediment). Data presented are average of 1-5 separately extracted samples.

With the accuracy of the analysis (see section 3.4) the Beryl A 20% and Frøy 20% samples appears to be considerably degraded (table 2). However, the THC levels in these bottles are quite low, and so the accuracy of measurements may be even less. Also the variation from one sampling point to the next may point in both directions. There are indications of decreasing THC levels also with the other cuttings materials, although the significance of this is debatable. However, as discussed in the joint report (RF 2001/220), results from other experiments substantiate an assumption that 100% Ekofisk 2/4A may be degradable, although slow, while the 100% Beryl cuttings seems to be less degradable. Based on these observations, THC half-lives ($t_{1/2}$) has been calculated for Beryl, Ekofisk 2/4A and Frøy as presented in table 3 and figure 6. The half-life figures are derived from 1st order kinetics as seen in figure 6, and are based on only 4 input data points. None of the curve-fitting data produced a 95% confidence interval (as compared to a straight line representing no degradation).

Table 2. Relative changes in THC from day 1 to day 229, aerobe degradation experiment.

mg/kg (DW)	1	229	relative change
Beryl A 20%	**553**	**338**	0,61
Beryl A	**2428**	**2662**	1,10
Ekofisk 2/4 A 20%	**10382**	**9110**	0,88
Ekofisk 2/4 A	**73986**	**61793**	0,84
Frøy 20%	**261**	**121**	0,46
Frøy	**4971**	**4574**	0,92
Ekofisk 2/4 C 20%	**647**	**614**	0,95
Ekofisk 2/4 C	**4763**	**4178**	0,88

Table 3. Calculated half-lives of THC as indicated by the aerobic experimental results.

Cuttings	C_0 (mg/kg)	K_1 d^{-1}	$t_{1/2}$ (days)
Beryl A 20 %	553	0.006	120
Be A 100%, 20°C,+N,P	2294	0.002	430
Ekofisk 2/4 A 20 %	10600	0.0005	1300
Ekofisk 2/4 A 100 %	73990	0.0009	750
Frøy 20 %	322	0.003	200
Frøy 100 %	4971	0.0005	1250

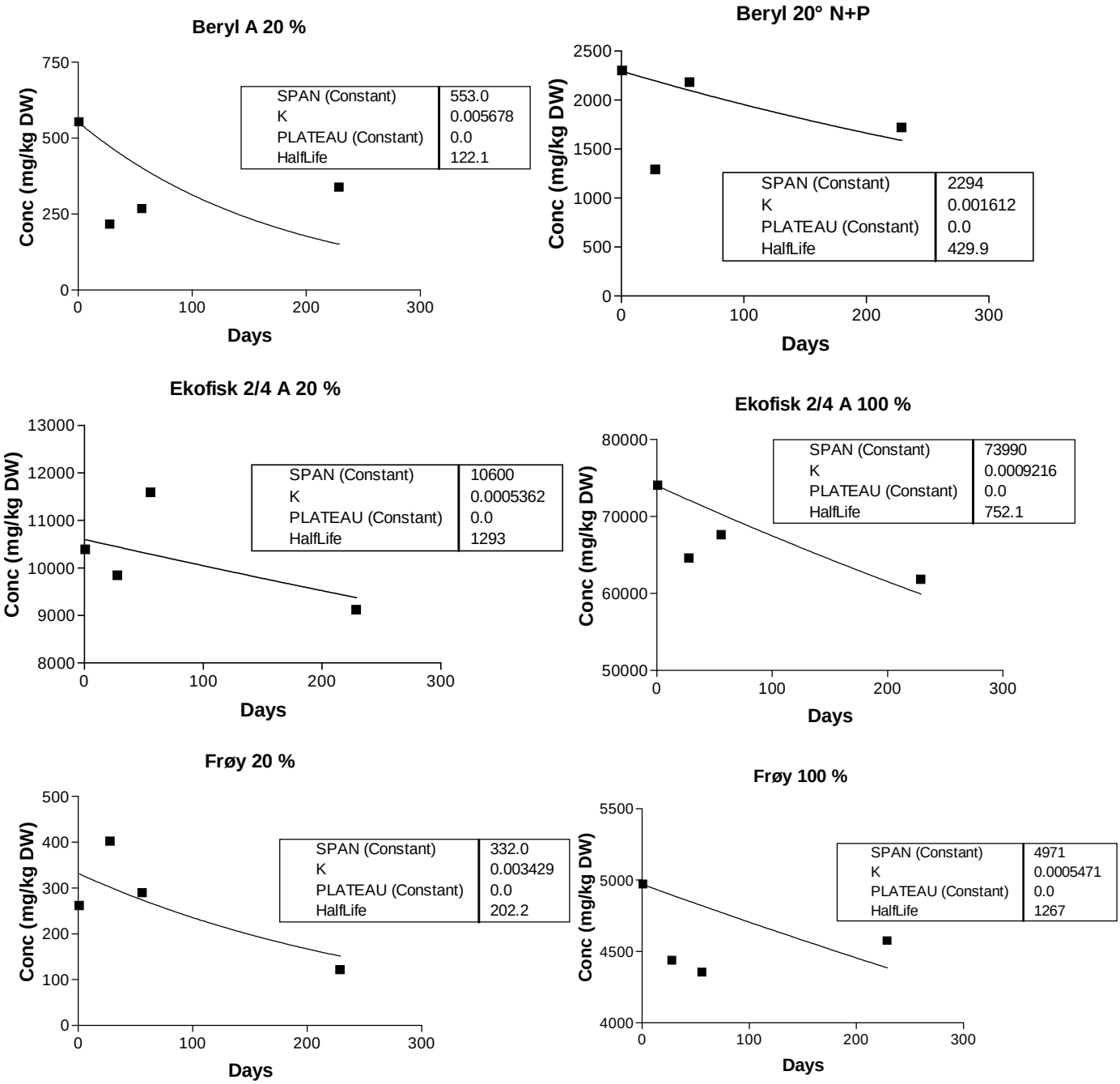


Figure 6 (a-f). 1st order kinetics of THC degradation of cuttings samples from aerobic degradation experiment.

3.2.2 Summary and discussion aerobic degradation series

Indication of microbial activity is seen from the rapidly falling oxygen levels before aeration was started, stable or slightly increasing bacterial activity and falling pH. From the THC measurements, only the Frøy 20% and Beryl A 20% series appear to show significant degradation after almost 8 months of incubation when considering the relatively low accuracy in the THC measurements as shown in section 3.4. Due to the low THC concentrations found in these two series, it may be that the actual accuracy is even less. For these two series, this can neither be supported nor disproved by the other parameters investigated.

When combining the THC results with the other parameters, it is possible that some limited degradation may have taken place also with the other cuttings types, particularly the Ekofisk 2/4A material (even though the reduction in THC is less that the suggested accuracy limit of the THC measurements). Such assumptions are supported also by other studies within task 3, as discussed in the summary report (RF 2001/220).

3.3 Anaerobic degradation series

3.3.1.1 General observations (anaerobe)

To thoroughly mix and distribute the cuttings and/or reference sediment in the test bottles, they were vigorously shaken (by hand) for about one minute before incubation. With this procedure, it was seen that there was a tendency for finer particles to accumulate in the surface layer. The same observations were seen with all cuttings types, but were most expressed with the reference sediment.

Changes to the surface layer during the course of the experiment were apparent. Some bottles formed a black/red/brown layer on top of the sediment surface. This was more expressed with time.

3.3.1.2 Sulfide (anaerobe)

Sulfide was detected in some of the test bottles, but only very low levels were seen before the day 56 sampling (Figure 7). The most marked changes were seen with the Frøy cuttings, which showed a significant boost in sulfide level after the 28 day sampling. Increasing sulfide levels were also apparent with the Ekofisk 2/4 A cuttings, although at a much smaller scale. No sulfide levels above the detection limit were seen for any of the Beryl samples (all incubation temperatures) or Lille-Frigg and Ekofisk 2/4C samples.

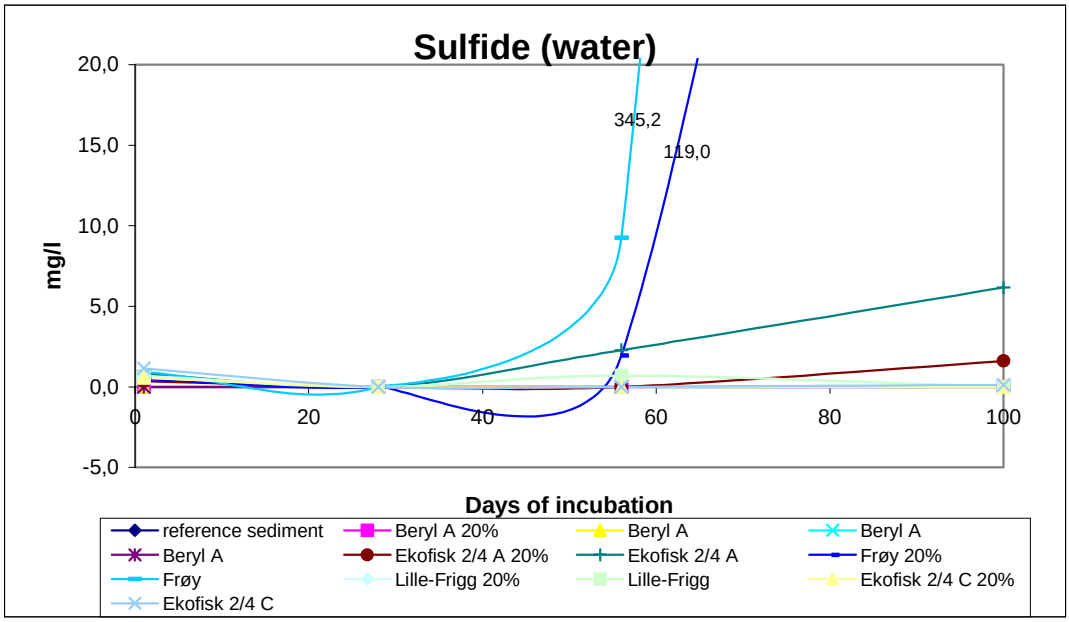
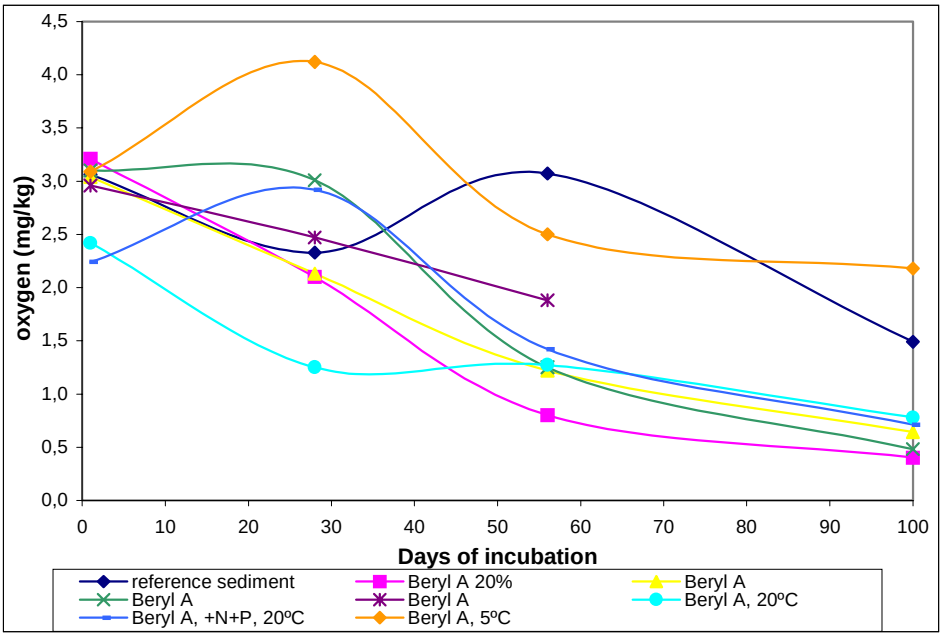


Figure 7. Sulfide levels of anaerobic degradation series (apparent negative values are only a result of the curve fitting function in the software, not real observations).

3.3.1.3 Oxygen (anaerobe)

No special measure was taken to remove residual oxygen from the test bottles at set up, except the seawater and headspace of the bottles was flushed with nitrogen during filling. Oxygen levels measured are presented in figure 8a and b and Appendix 2. In most bottles, the oxygen level decreased towards 1mg/l or less to the day 56 sampling. Only the Lille-Frigg reference and Beryl 5°C series still had levels above 1.5mg/l at the day 100 sampling.



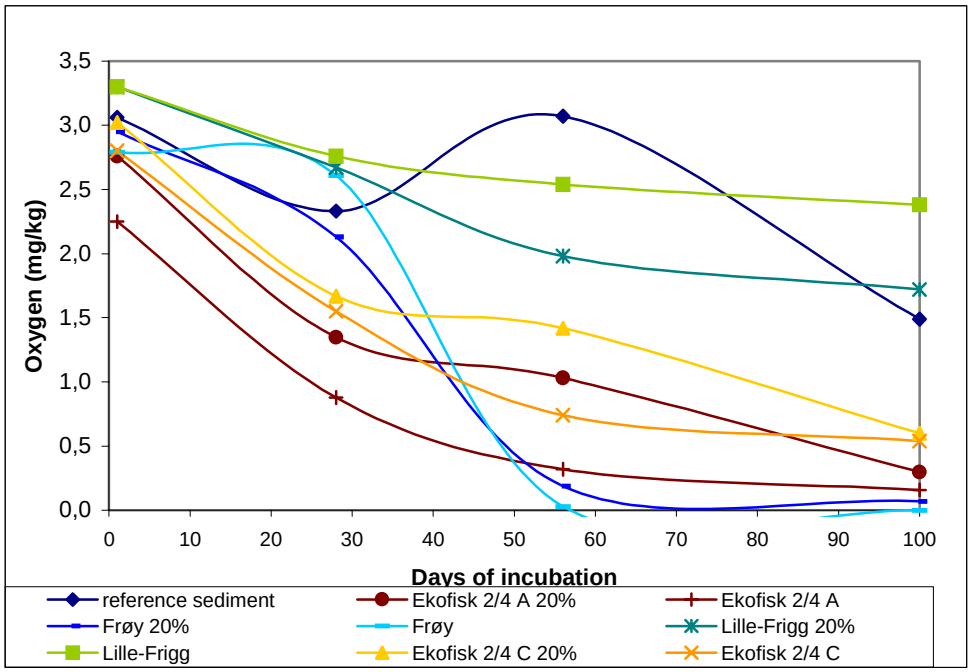
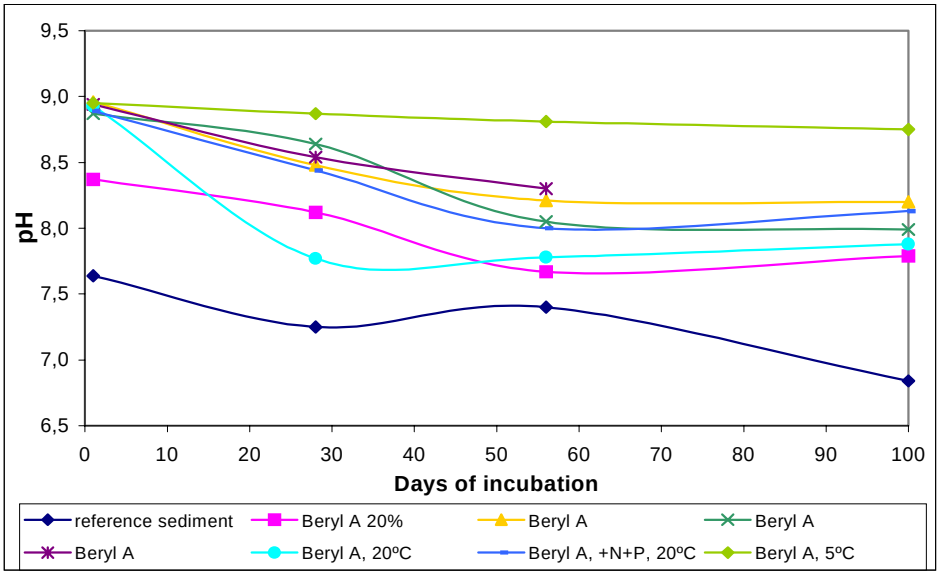


Figure 8a and b. Oxygen levels in water phase of anaerobic degradation series samples.

Some oxygen was measured in the water phase of most of the bottles, also towards the end of the experiment. Oxygen was measured by inserting the electrode into the bottle, at ambient air conditions. The measured oxygen levels may therefore partly reflect introduction of oxygen during measurement. Alternatively, the low oxygen levels seen may indicate that some oxygen has been available for microbial processes during the experimental period. If that is the case, the measured THC reductions may partly be due to aerobic or microaerophilic processes. Increasing sulfide levels in the Frøy and partly Ekofisk 2/4A bottles indicate however that anaerobic processes have taken place. It is likely that traces of oxygen present very soon have been limited to the water phase above the sediment fraction and the upper few mm of the sediment fraction. The reduction in THC levels is similar to that observed in the aerobic test series. In the aerobic series, basically all sediment and the water phase were in contact due to the constant mixing. There is no clear evidence that THC removal in the anaerobic series is restricted to aerobic processes and limited to the surface layer only. If that were the case, lower total THC removal would be expected, reflecting the reduced size of the oxic sediment layer.

3.3.1.4 pH (anaerobe)

The starting pH of the samples varied from about 7.6 (reference sediment) to 9.2. In most of the test bottles the pH decreased with time until the day 28 sampling (Figure 9a and b). The decrease was less towards day 56 and 100 for most samples. There was a tendency of slightly increasing pH again towards the end, especially in the series where sulfide was produced (Frøy and Ekofisk 2/4 A). Data are also presented in Appendix 2.



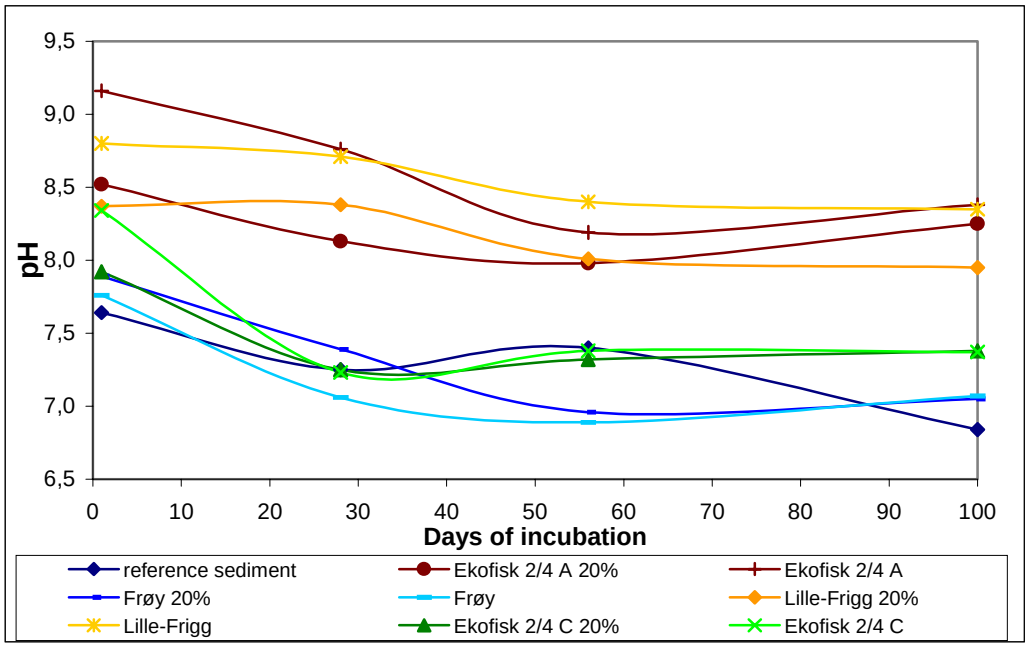
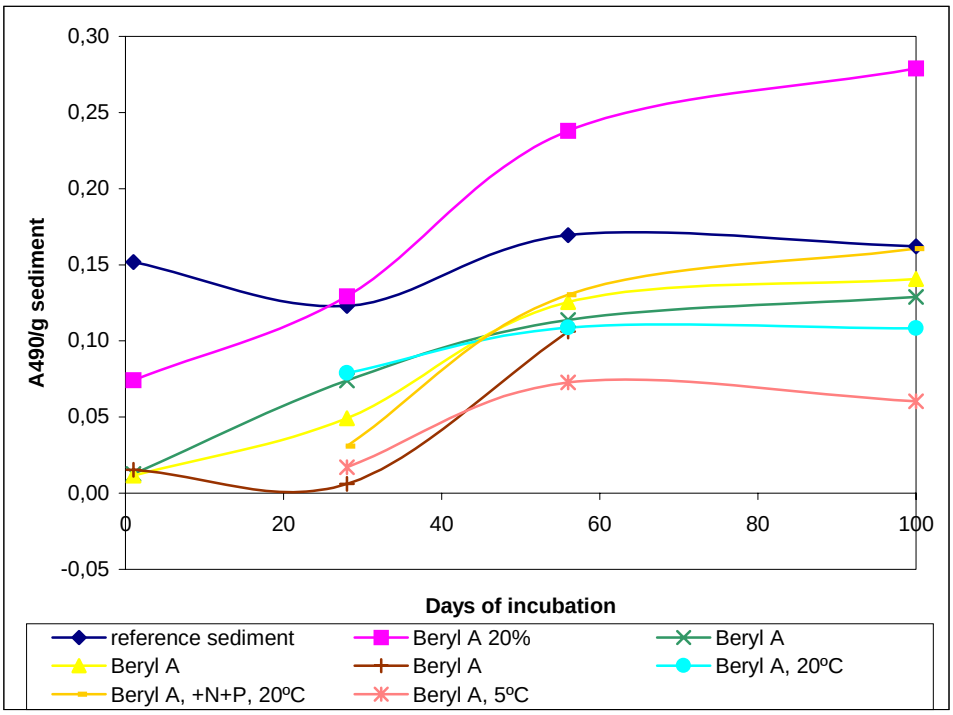


Figure 9a and b. pH of anaerobic degradation series.

3.3.1.5 Microbial activity (FDA) (anaerobe)

The microbial activity assay is very general, measuring enzymatic responses found in many common metabolism pathways. The assay does not specifically relate to hydrocarbon degradation or aerobic/anaerobic processes specifically. It is possible that the presence of active hydrocarbon metabolising microorganisms is underestimated with such an assay. Nevertheless, the assay is empirically found to give a broad picture of general microbial activity.

The microbial activity varied (figure 10a and b). More details can be found in Appendix 2. Highest activity was found in the Frøy cuttings and in Ekofisk 2/4 A. Unlike the aerobic series, the activity in the reference sediment was very low. The activity of the Beryl samples was low in all cases, but slightly higher in the 20% cuttings samples.



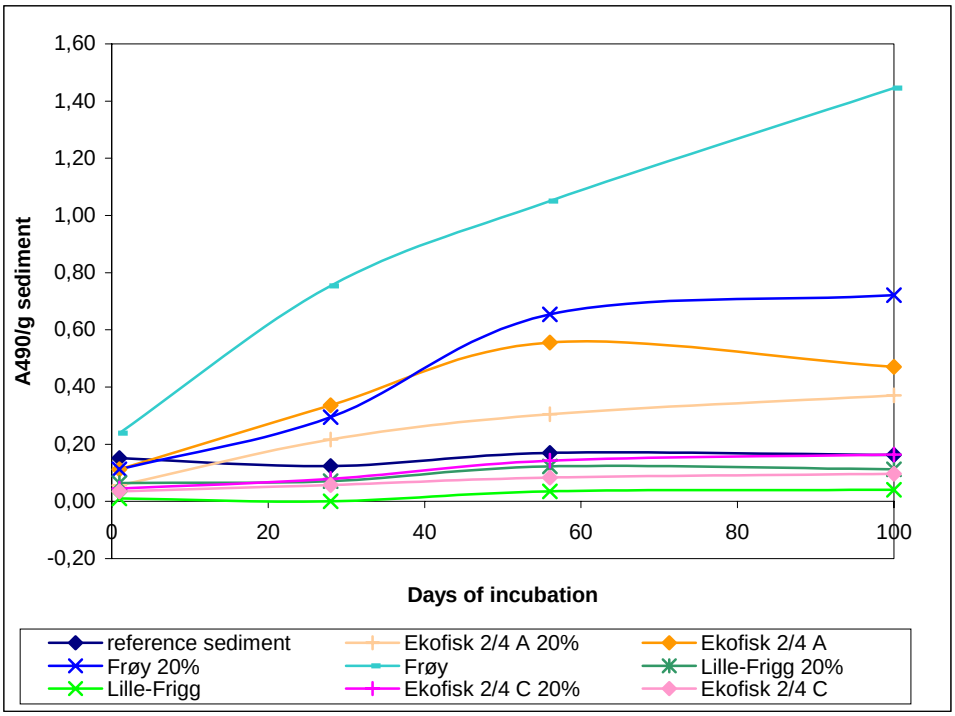


Figure 10a and b. Microbial activity (FDA) of the anaerobic degradation series. NB! Note the different scales.

3.3.1.6 Organic fraction (THC) (Anaerobe)

The THC measurements were intended to demonstrate noticeable degradation over the course of the experiment. Any decreases seen would then be used to estimate a degradation rate specific for the cuttings type and conditions.

Within the time frame of the anaerobic degradation experiment, noticeable degradation could not be seen in any of the tests, with the possible exception of the Beryl 20% and Frøy tests. The change (decrease in THC from day 1 to day 100) is illustrated in table 5. The accuracy of the data is discussed in section 3.4.

The calculated THC data are presented in table 4 and figure 11. Details of the Beryl data are presented in figure 12. Some data points are average of several analyses, others are based on 1 point only. The average curve plotted is the average of the other data points presented in the figure.

Table 4. Measured THC levels in the anaerobic degradation test. Values in mg/kg dry sediment.

mg/kg (DW)	1	28	56	100
reference sediment	**12**	n.a	n.a	n.a
Beryl A 20%	**749**	**594**	**349**	**343**
Beryl A	**2981**	**3434**	**2389**	**3972**
Ekofisk 2/4 A 20%	**10110**	**10429**	**11268**	**10476**
Ekofisk 2/4 A	**64924**	**67358**	**67084**	**64262**
Frøy 20%	**1681**	n.a	**442**	**997**
Frøy	**6110**	**6154**	**6201**	**5054**
Ekofisk 2/4 C 20%	**511**	n.a	n.a	**570**
Ekofisk 2/4 C	**4825**	n.a	n.a	**4108**
Lille-Frigg 20%	n.a	n.a	n.a	n.a
Lille-Frigg	n.a	**110**	n.a	n.a

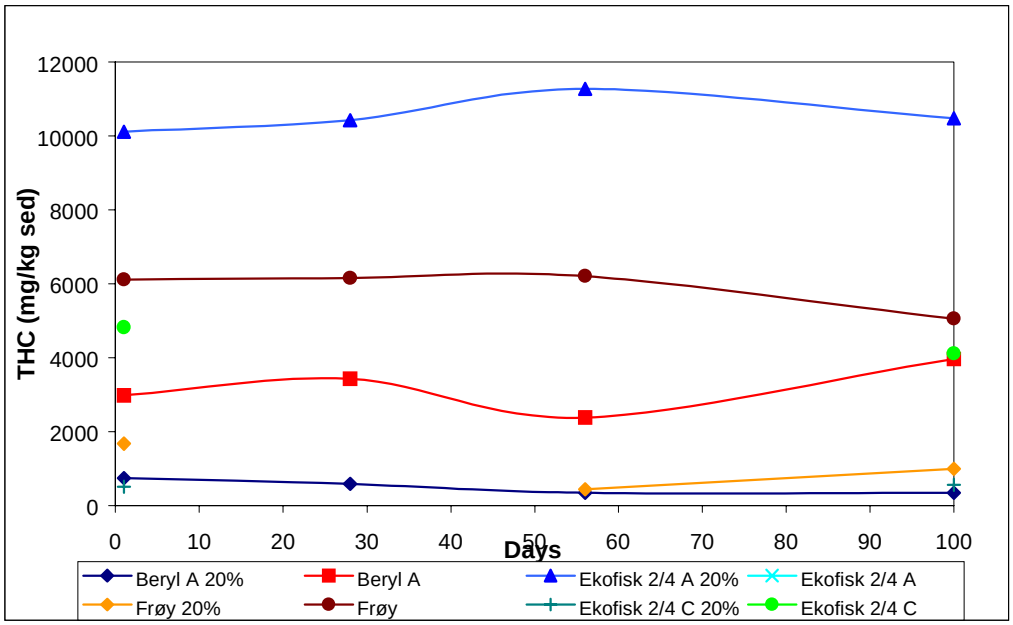


Figure 11. THC of the anaerobic test (mg/kg dry sediment).

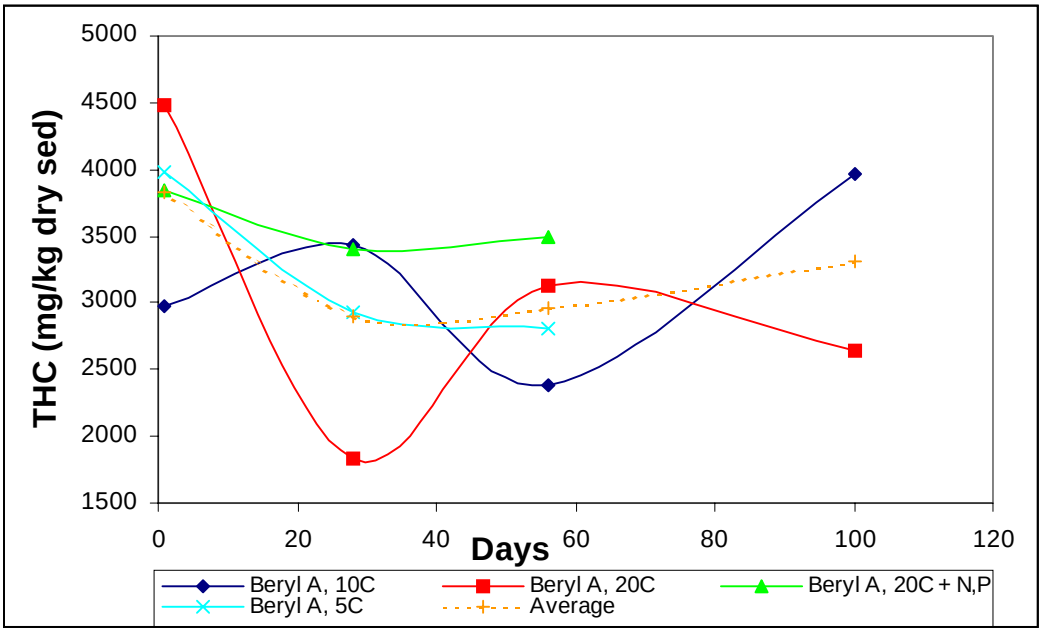


Figure 12. THC of the anaerobic test, all Beryl samples (mg/kg dry sediment).

With the accuracy of the analysis (see section 3.4) the Beryl A 20% and Frøy 20% samples appears to be significantly degraded (table 5), as is also observed in the aerobic experimental series. However, the THC levels in these bottles are quite low, and so the accuracy of measurements may be even less. Also the variation between sampling points in each series may vary much, and hence point in opposite directions. However, observations also from other task 3 sub-tasks as well as other analytical parameters with these series (sulfide, microbial activity etc.) may substantiate an assumption that biodegradation is taking place. There are few indications of decreasing THC levels with the other cuttings materials in this anaerobic series, except Frøy 100%, which is also decreasing. Some evidence of decreasing THC concentration in Beryl 100% incubated at 20°C was seen (data shown in Smedberg, 2001).

Based on these observations, THC half-lives ($t_{1/2}$) has been calculated for Beryl, and Frøy as presented in table 6 and Figure 13. The half-life figures are derived from 1st order kinetics as seen in figure 13, and are based on only 4 input data points. None of the curve-fitting data except Frøy 100% (P=0.0411) produced a 95% confidence interval (as compared to a straight line representing no degradation).

Table 5. Relative changes in THC from day 1 to day 100, anaerobe degradation experiment.

mg/kg (DW)	1	100	relative change
Beryl A 20%	**749**	**343**	0,46
Beryl A	**2981**	**3972**	1,33
Ekofisk 2/4 A 20%	**10110**	**10476**	1,04
Ekofisk 2/4 A	**64924**	**64262**	0,99
Frøy 20%	**1681**	**997**	0,59
Frøy	**6110**	**5054**	0,83
Ekofisk 2/4 C 20%	**511**	**570**	1,12
Ekofisk 2/4 C	**4825**	**4108**	0,85

Table 6. Calculated half-lives of THC as indicated by the anaerobic experimental results.

Cuttings	C_0 (mg/kg)	K_1 d^{-1}	$t_{1/2}$ (days)
Beryl A 20 %	749	0.01	70
Frøy 20 %	1681	0.01	60
Frøy 100%	6110	0.0007	950
Beryl 20°C	4477	0.008	80

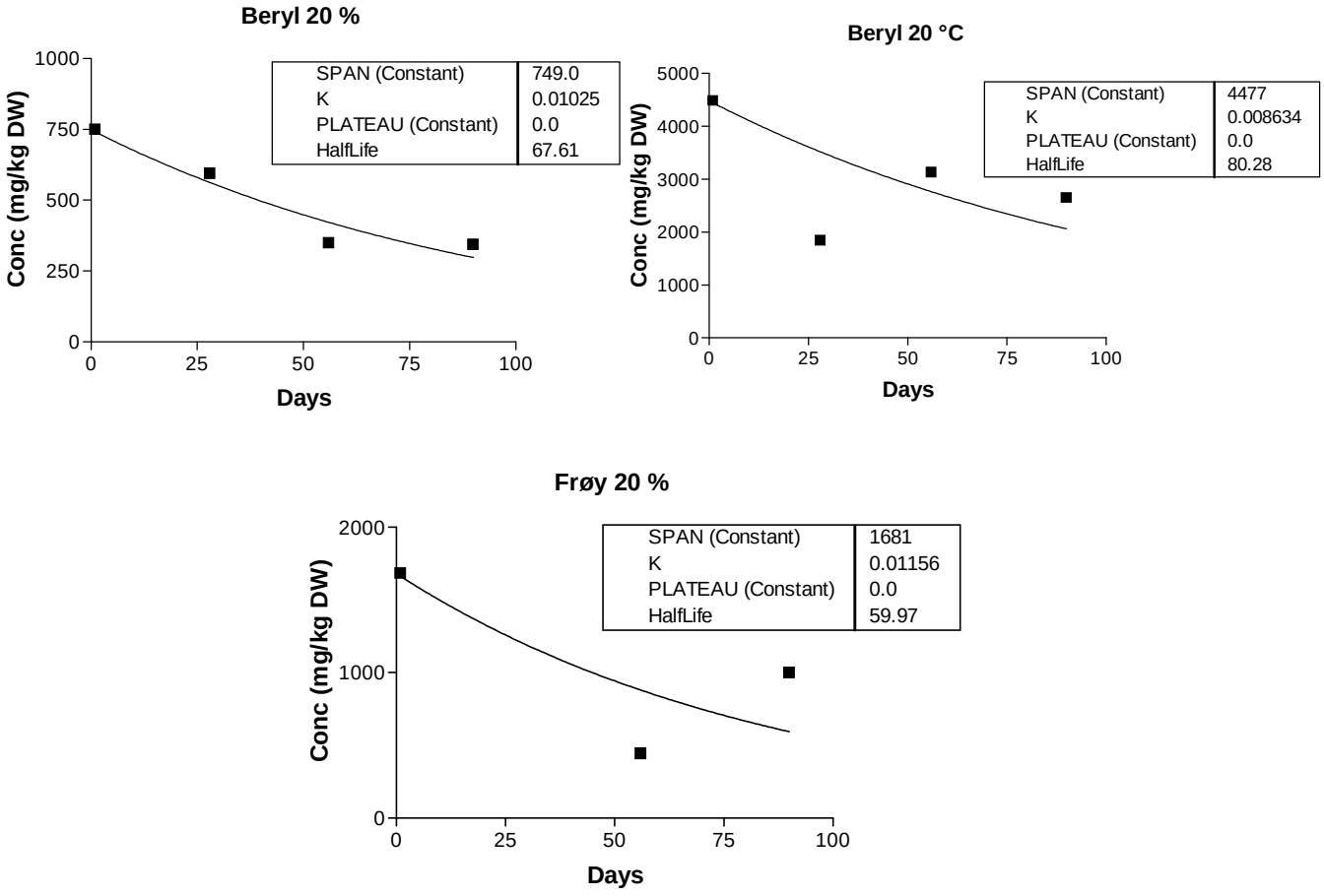


Figure 13 (a-c). 1st order kinetics of THC degradation of cuttings samples from the anaerobic degradation experiment, Beryl 20% at 10°C, Beryl 100% at 20°C and Frøy 20% cuttings are shown.

3.3.2 Summary and discussion anaerobic degradation series

For the anaerobic degradation series, there seem to be consistency between low oxygen, increasing sulfide, decreasing pH and high microbial activity. The sulfide production is high in the Frøy samples, which is also the samples that show most reduction in THC over the 100 days incubation period. Based on these facts, it seems evident that some limited biodegradation has occurred at anaerobic conditions in the Frøy series.

The Ekofisk 2/4 A series (both 100 and 20%) show indications of microbial activity by all parameters except THC. For the Beryl A 20% it is opposite, The THC changed considerably, but it can not be directly supported by the other parameters. Also, the THC levels of the Beryl 20% sample is low, and hence may be even less accurate than the other THC measurements.

The fact that oxygen was not totally removed from the sediment and water fraction at start up raises doubt whether the anaerobic degradation indicated was truly an anaerobic process. The way oxygen was measured in the test bottles, the accuracy in measuring very low levels of oxygen is limited. However, since the measured, low O_2 levels did change from the start of the experiment, it is plausible to assume that some oxygenic/microaerophilic activity has occurred. There is however clearly evidence of true anaerobic processes taking place in some of the test bottles, as shown by the increasing sulfide levels, and partly also the microbial activity that, with the method used, was more evident with the anaerobic set up.

Assumptions of anaerobic biodegradation taking place is partly substantiated by other task 3 sub-tasks as discussed in the summary report (RF –2001/220).

3.4 Statistical variance and accuracy of measurements

The original experimental set up was prepared to allow for statistical accuracy to be tested. This was achieved by preparing triplicate tests of the Beryl A (100%).

3.4.1 Oxygen, pH and bacterial activity

For the parameters pH, oxygen and bacterial activity, statistical accuracy were good, with deviation in the range 0-2 % for pH and oxygen. The standard deviation was higher (12-20%) with the FDA (bacterial activity), but this merely reflects the low activity seen. The variability in the triplicate readings for each sample (same test bottle) was in the same range as between the triplicate Beryl A tests sampled each day. The relative variability in the triplicate readings from each sample (all tests) decreased when the bacterial activity increased, e.g. the Beryl A 20% test showed a standard deviation of the triplicate readings of about 8-10%.

3.4.2 THC and estimation of degradation rates

The accuracy of the THC measurements is more of a concern. Relatively large variations are in some instances seen with duplicate and triplicate samples as well as

multiple analysis of the same samples. These discrepancies complicate the interpretation of the data, and the estimation of degradation rates.

There may be several explanations for the relatively large variation seen. Basically, two reasons for the high standard deviation was envisioned, either the sample heterogeneity was high despite attempts to mix the sample, or the processing and analysis methods were too inaccurate.

In order to test the accuracy of the sample processing and analytical methods used, a set of Beryl A samples was re-analysed with different protocols etc (3.4.2.1). Multiple analysis with the same protocols has also been carried out to verify the statistical accuracy. This has then addressed whether heterogeneity of the samples has been a major complication in assessing the THC data (3.4.2.2).

It should be noted that there might be differences between the types of cuttings material. Except for duplicate analysis in some instances, the major THC validation work is carried out with the Beryl cuttings. By looking at the THC data, it seems reasonable that the accuracy is as good or better with the other cuttings types.

3.4.2.1 Assessment of sample processing and analytical approach

Since considerable variation were seen in the data produced, and since some of these variations were difficult to explain merely by reasoning, the sample processing and analytical approach were assessed.

Except for the Beryl A 100% samples, only one data set was available for each variable with respect to cuttings type and concentration. The expected results, based on the set up, was that:

- either degradation occurred, which would result in decreasing THC levels with time, or
- no or very little degradation took place, giving none or small changes in measured THC levels.

Observations made showed that the THC levels could vary considerably with time, and also tended to increase compared with the starting concentration in some instances. To test whether this observation was a consequence of insufficient methods for processing, different processing methods (e.g. the extraction step) were tested (table 6). The analytical method (GC-FID application and data processing) were also checked by re-analysing and re-processing of samples.

It can be seen from table 6 that the variability in the data material was in the same range with all four processing/extraction methods, except when the sample was dried and mixed in a mortar before sub-sampled into three aliquots. This then confirms that the sample processing and extraction methods used are satisfactory. It also indicates (as discussed below) that the in-homogeneity of the starting sample was significant. The Beryl sample analysed is from the same mixed batch that was used for preparation of the degradation test bottles (e.g. the day 0 sample used for both the aerobic and anaerobic test series).

The accuracy level based on the analytical testing indicates a 15-20% deviation in measurements.

Table 6. THC measurements of triplicate samples after different extraction/processing approaches.

Processing (extraction)	THC (mg/kg DW)
As before	**2454**
ST DEV	487
ST DEV (%)	20
separately mixed	**2785**
ST DEV	412
ST DEV (%)	15
separately dried and mixed	**1914**
ST DEV	379
ST DEV (%)	20
Dried and mixed before separated	**2770**
ST DEV	226
ST DEV (%)	8
Average all samples	**2481**
ST DEV all samples	407
ST DEV (%) all samples	16

3.4.2.2 Variability and standard deviation of data

From the data sets of the 100% Beryl A samples analysed, it is evident that no significant degradation, as measured by THC, has occurred. The total data set available is quite extensive, including the aerobic and anaerobic degradation experiments (with triplicate samples), the processing/extraction step testing (see previous section), as well as the work included in the master thesis of Inger Smedberg (Appendix 2).

The standard deviation estimations of the data are presented in Table 7 below. The data are used in more combinations to illustrate different aspects.

Standard deviations between 10 and 28 % were seen in the individual data sets (i.a. data from repeated extraction/analysis of the same test bottle or homogenised material batch). The standard deviation for duplicate and triplicate samples (i.a. triplicate test bottles), as well as samples from the same test bottle being re-analysed was generally high. The individual standard deviations for one experiment at a specific sampling day was in some cases as high as the standard deviation of all Beryl A data regardless of experiment type and sampling point.

The implication of these data is that in general, to conclude that degradation has occurred in any of the test series of the aerobic or anaerobic degradation test, the decrease in THC from the starting point should exceed 25-30%. On this basis, degradation *rates* are very difficult to estimate also when the decrease in THC exceeds this 25-30% limit.

Table 7. Selection of average THC levels with standard deviation measures of Beryl A samples from all degradation experiments and sample processing/extraction test.

Source data	# samples	Aver. THC (mg/kg DW)	% ST DEV
Aerobe test*, day 1	5	2308	23
Aerobe test*, day 28	3	1885	27
Aerobe test*, day 56	5	2649	16
Aerob test, day 1, all T**, only Smedberg	4	1986	10
Aerob test*, day 1, 28 and 56 combined	13	2383	23
Aerob test, day 1, 28 and 56, incl. Smedberg	25	2219	23
Anaerob test, day 1, all T** , incl. Smedberg	5	3651	18
Anaerob test, day 28, all T** , incl. Smedberg	5	3004	23
Anaerob test, day 56, all T** , incl. Smedberg	5	2839	17
Anaerob test, day 1, various T, only Smedberg	4	3819	16
All day 0 and 1 samples, aerobe + anaerobe***	26	2597	28
All day 0, 1, 28 and 56 samples, aerobe + anaerobe***	53	2552	27

* not including data from Smedberg 2001 (Appendix 3)
** including tests at 10°C, 20°C, 5°C and 20°C with additional nutrients
*** including aerobe and anaerobe tests from all experiments, inclusive data from Smedberg and test of sample processing/extraction

3.4.3 Summary and discussion THC data interpretation

It is evident that much of the variation seen in analysis results of the same sample is due to the sample itself, e.g. that the sample is quite heterogeneous on a micro-scale despite the attempts to mix and homogenise the sample initially. Previous analysis of THC content of cuttings material (e.g. Westerlund et al., 2001 and more) has not indicated this, and as such, heterogeneous samples were not expected to complicate the interpretation of the THC data to the extent observed. It is likely that some of the complexity of extraction, analysis and interpretation of the THC data are caused by the nature of the cuttings material, and its heterogeneity. Even when properly mixed, the particle structure of the material is likely to cause large variations in the extractable THC-fraction. Hydrocarbons are probably not evenly distributed in the cuttings matrix. The hydrocarbons may preferentially be trapped within larger particles, or more heavily associated with certain particle fractions than others. Such differences on a micro-scale are difficult to account for in the experimental set up, sampling and sample processing. Changes in hydrocarbon distribution within a sample may also occur with time as the samples in question are exposed to shear stress etc. (like in the aerobic degradation set up).

Several attempts to clarify whether the large variations and low accuracy seen are due to routine errors, sub-optimal procedures etc. have been made. None has however been found. We therefore presume that the variability lays within the material itself more than with the procedures used.

3.5 Additional work supplementing UKOOA task 3

3.5.1 Master thesis, University College Stavanger

As part of the task 3 work, a thesis with the University College of Stavanger has been carried out. This thesis is attached in full in Appendix 3. The thesis, by Inger Smedberg, is entitled 'Fluorescence as a screening method for THC in sediment samples'. A short synopsis of the work is presented here.

The aim of the thesis work was twofold. One aim was to test whether there is correlation between the total hydrocarbon concentration (as THC) and the aromatic content of a sample as measured by fluorescence. If so, the intention was to see whether fluorescence measurements could be used as an inexpensive alternative to screen sediment samples for THC, or as an addition to know more about the specific fraction of the THC pool. The second goal was to measure and compare both parameters (THC by GC-FID and fluorescence) on a data set on Beryl cuttings material, as part of the degradation test series.

The THC degradation data is presented in the previous sections along with the rest of the degradation series THC data.

The analytical work on fluorescence revealed that several factors were affecting the fluorescence response of a sample. Some of these factors, such as inner filter effects, resonance energy transfer and self-absorption were investigated further. It was found that the relationship between concentration and fluorescence response was not necessarily linear, and that the complex mixture of components within a sample (e.g. the cuttings samples) can affect the fluorescence result. With the current sample set, and with the low degradation seen, it was not possible to see any correlation between the THC and fluorescence approaches.

3.5.2 Master of science, Marine Resource Development and Protection, Heriot-Watt University

In connection with the master thesis work (MSc in Marine Resource Development and Protection, Centre for environmental Reseource Management) of Elaine Tooth at Heriot-Watt University, drill cuttings samples were requested. Her work focused at investigating toxicity of cuttings material applying Microtox ®.

The Beryl A, Frøy and Lille-Frigg samples, representing OBM, PBM and WBM piles respectively were analysed. In addition, some samples from the aerobic and anaerobic degradation series were also included; 20% Beryl A, 20% Frøy and 20% Lille-Frigg from the 56 days aerobic series, and 20% Beryl A and 20% Frøy from the 100 days anaerobic series. The EC50 values obtained are presented in table 8. A spiked reference sample was also prepared and analysed, as reported in the table.

Table 8. Microtox ® EC50 values for individual cuttings extracts

Cuttings source	Cuttings type	THC, mg/kg	EC50 100%	EC50 20% aerobic	EC50 20% anaerobic
Beryl A	OBM	5000*	3,700	27,200	18,800
Frøy	PBM	3000*	4,900	49,500	8,100
Lille-Frigg	WBM	10*	28,600	49,500	N/A
Reference		46,500**	46,500	N/A	N/A

* approximate concentration reported by RF

** THC obtained using Brent Crude Oil as standard (IR Spectroscopy analysis)

As expected, highest toxicity was reported with the OBM Beryl cuttings. The Lille-Frigg WBM sample showed low toxicity, also as expected. It is difficult to conclude whether the toxicity measured in the extracts of the degradation experiment series reflects degradation of the THC-fraction or not. There does however seem to be less toxicity (e.g. higher EC50 values) with the aerobic derived samples than the anaerobic samples. The results reported here is an extract of the thesis work only, and currently no attempts have been made to discuss the meaning of the reported toxicity values.

3.5.3 Work of Dr. Lisa Gieg, University of Oklahoma

During the Task 1 offshore survey, samples were collected to be used for investigations of metabolites for anaerobic degradation, to be studied by Dr. Lisa M. Gieg at the laboratory of Dr. Joseph M. Suflita, Department of Botany and Microbiology and Institute for Energy and the Environment, University of Oklahoma, USA.

Interstitial water from the two samples (large box-cores of Beryl A, and Ekofisk 2/4 A, respectively) were extracted and analysed by GC-MS to look for putative metabolites of anaerobic hydrocarbon decay.

Several anaerobic degradation pathways of different hydrocarbon compounds are known from the literature. Based on earlier investigations at University of Oklahoma and others, metabolites of such degradation pathways were investigated. Possible anaerobic degradation of n-C17 was found for the oil-based cuttings (Beryl A) material. In addition several other metabolites (acids, diacids etc.) were identified, but it is not obvious whether these are aerobic or anaerobic derived. Putative metabolites for anaerobic phenanthrene, naphthalene and decalines were also sought for, but were not detected.

For the partly PBM/WBM cuttings (Ekofisk 2/4A), no metabolites indicating anaerobic alkane addition metabolites were identified. Other metabolites were detected, but it is not clear how any of these may be forming. In general, more metabolites were identified in the oil-based sample.

From laboratory incubations with sediment from the sample, sulfate-reducing microorganisms were active within three months when adding lactate. Similar enrichments with C17 did not, during 10 months, result in any sulfate consumption indicative of active sulfate-reducing bacteria.

4 Conclusions

The accuracy of THC measurements was low, most likely due to high heterogeneity of the cuttings material on a micro-scale. Statistically, the variation in the data is in the order of 25-30%, implying that reductions in THC less than or close to this may be the result of sample variability as much as THC degradation or removal. However, based on other observations within this sub-task as well as other sub-tasks within task 3, degradation rates and half-lives have been calculated when possible.

Some indication of biodegradation (measured as reduction in THC) was seen with the Frøy cuttings both aerobically (mostly for the 20% cuttings series) and anaerobically (both 20 and 100%). Biological degradation of the Frøy material was supported by the other test parameters (sulfide, pH, oxygen consumption) as well, particularly for the anaerobic series. Half-lives of the Frøy series were calculated to about 200 and 1250 days respectively for Frøy 20% and 100% at aerobic conditions respectively. The half-life of Frøy 20% at anaerobic conditions was calculated to only about 60 days.

A similar trend was seen with Ekofisk 2/4A cuttings in the aerobic series, but the decrease in THC was in this case less than the assumed accuracy limits of the THC data. Half-lives of the Ekofisk 2/4A series were calculated to about 1300 and 750 days respectively for Ekofisk 2/4A 20% and 100% at aerobic conditions.

Some evidence of Beryl biodegradation was also seen, mainly in the 20% cuttings series. Lack of degradation of the 100% Beryl series may be indicating toxic THC levels reducing or preventing microbial degradation. The half-life of Beryl 20% at aerobic conditions was calculated to be about 120 days, at anaerobic conditions this was only about 70 days.

The degradation half-lives reported is based on 1st order kinetics based on rather few input numbers. The accuracy must therefore be considered to be rather low. However, the numbers presented may be taken as indicative of possible maximum degradation rates at the experimental conditions applied.

5 References

Cripps, **S.J., Westerlund S., Jacobsen T.G., Hovda, J., Kjeilen, G., Olsen, S.D., Eriksen, V., Aabel, J.P.** 1999. Ekofisk 1 Drill Cuttings piles characterisation and management plan. Report RF-1999/041.

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Appendix

Appendix 1	Aerobic degradation trial data
Appendix 2	Anaerobic degradation trial data
Appendix 3	Master thesis of Inger Smedberg

Trial 1 - aerobic

Oxygen

No	Content	day 1 ppm	Day 14 ppm	Day 28 ppm	Day 56 ppm	Day 229 ppm
1	**reference sediment**	8,43	5,11	6,76	4,62	5,00
2	**Beryl A 20%**		2,22	5,76	4,12	5,27
3	**Beryl A**			4,10		
4	**Beryl A**	6,21		4,37		
5	**Beryl A**			3,95		
6	**Ekofisk 2/4 A 20%**		0,43	4,27	5,12	3,10
7	**Ekofisk 2/4 A**		1,82			
8	**Frøy 20%**		1,16	5,03	3,20	3,51
9	**Frøy**					
10	**Lille-Frigg 20%**		2,77	4,09	4,92	3,28
11	**Lille-Frigg**		2,64	3,46	3,49	3,70
12	**Ekofisk 2/4 C 20%**		1,61	4,52	4,01	3,09
13	**Ekofisk 2/4 C**					
15	**Beryl A, 20ºC**		1,90	0,63		
16	**Beryl A, 20ºC**					
17	**Beryl A, +N+P, 20ºC**		1,07			
18	**Beryl A, +N+P, 20ºC**					
19	**Beryl A, 5ºC**		1,83	0,88	3,43	
20	**Beryl A, 5ºC**					
0	**Beryl A, +HgCl2**			2,7	2,30	

pH

No	Content	day 1 pH	Day 14 pH	Day 28 pH	Day 56 pH	Day 229 pH
1	**reference sediment**	7,65	7,33	7,19	7,33	7,24
2	**Beryl A 20%**	8,86	8,20	7,78	7,65	7,37
3	**Beryl A**	9,07		8,34		
4	**Beryl A**	8,92		8,47		
5	**Beryl A**	8,94		8,53		
6	**Ekofisk 2/4 A 20%**	9,01	8,30	8,12	8,04	7,67
7	**Ekofisk 2/4 A**		8,75			
8	**Frøy 20%**	7,80	7,21	7,06	7,07	7,05
9	**Frøy**					
10	**Lille-Frigg 20%**	8,51	8,22	7,81	7,59	7,62
11	**Lille-Frigg**		8,65	8,47	8,46	8,47
12	**Ekofisk 2/4 C 20%**	8,12	7,38	7,23	7,05	7,10
13	**Ekofisk 2/4 C**					
15	**Beryl A, 20ºC**		8,60			
16	**Beryl A, 20ºC**					
17	**Beryl A, +N+P, 20ºC**	8,77	8,60	8,47		
18	**Beryl A, +N+P, 20ºC**					
19	**Beryl A, 5ºC**		8,70	8,78	8,58	
20	**Beryl A, 5ºC**					
0	**Beryl A, +HgCl2**				8,33	

Microbial activity (FDA)

No	Content	day 1 A490/g	Day 14 A490/g	Day 28 A490/g	Day 56 A490/g	Day 229 A490/g
1	**reference sediment**	0,152	0,294	0,165	0,133	0,083
2	**Beryl A 20%**	0,074	0,148	0,104	0,130	0,063
3	**Beryl A**	0,013	0,018	0,014	0,015	0,017
4	**Beryl A**	0,011	0,016	0,015	0,011	0,016
5	**Beryl A**	0,015	0,015	0,018	0,009	0,020
6	**Ekofisk 2/4 A 20%**	0,056	0,060	0,085	0,000	0,061
7	**Ekofisk 2/4 A**	0,112	0,096	0,094	0,082	0,106
8	**Frøy 20%**	0,112	0,135	0,098	0,026	0,026
9	**Frøy**	0,238	0,308	0,027	0,061	0,000
10	**Lille-Frigg 20%**	0,064	0,080	0,087	0,073	0,055
11	**Lille-Frigg**	0,010	0,018	0,019	0,016	0,023
12	**Ekofisk 2/4 C 20%**	0,045	0,106	0,121	0,067	0,009
13	**Ekofisk 2/4 C**	0,036	0,046	0,044	0,041	0,008
15	**Beryl A, 20ºC**		0,011	0,016	0,010	0,017
16	**Beryl A, 20ºC**		0,010	0,013		
17	**Beryl A, +N+P, 20ºC**		0,013	0,015	0,010	0,043
18	**Beryl A, +N+P, 20ºC**					
19	**Beryl A, 5ºC**		0,016	0,018	0,012	0,069
20	**Beryl A, 5ºC**					
0	**Beryl A, +HgCl2**			0,009	0,010	

Trial 2 - anaerobic

Sulfide

No	Content	day 1 ppm	Day 28 ppm	Day 56 ppm	100 ppm
1	**reference sediment**	<0,2	<0,2	<0,2	<0,2
2	**Beryl A 20%**	<0,2	<0,2	<0,2	<0,2
3	**Beryl A**	<0,2	<0,2	<0,2	<0,2
4	**Beryl A**	<0,2	<0,2	<0,2	<0,2
5	**Beryl A**	<0,2	<0,2	<0,2	
6	**Ekofisk 2/4 A 20%**	0,4	<0,2	<0,2	1,6
7	**Ekofisk 2/4 A**	0,8	<0,2	2,3	6,2
8	**Frøy 20%**	0,5	<0,2	1,9	119,0
9	**Frøy**	0,9	<0,2	9,3	345,2
10	**Lille-Frigg 20%**	0,7	<0,2	0,7	<0,2
11	**Lille-Frigg**	0,6	<0,2	0,7	<0,2
12	**Ekofisk 2/4 C 20%**	0,6	<0,2	<0,2	<0,2
13	**Ekofisk 2/4 C**	1,1	<0,2	<0,2	0,1
15	**Beryl A, 20ºC**		<0,2	<0,2	<0,2
16	**Beryl A, 20ºC**				
17	**Beryl A, +N+P, 20ºC**		<0,2	<0,2	<0,2
18	**Beryl A, +N+P, 20ºC**				
19	**Beryl A, 5ºC**		<0,2	<0,2	<0,2
20	**Beryl A, 5ºC**				

Oxygen

No	Content	day 1 ppm	Day 28 ppm	Day 56 ppm	100 ppm
1	**reference sediment**	3,06	2,33	3,07	1,49
2	**Beryl A 20%**	3,21	2,10	0,80	0,40
3	**Beryl A**	3,04	2,13	1,22	0,64
4	**Beryl A**	3,10	3,01	1,25	0,48
5	**Beryl A**	2,96	2,47	1,88	
6	**Ekofisk 2/4 A 20%**	2,76	1,35	1,03	0,30
7	**Ekofisk 2/4 A**	2,25	0,88	0,32	0,16
8	**Frøy 20%**	2,95	2,13	0,19	0,07
9	**Frøy**	2,79	2,61	0,03	0,00
10	**Lille-Frigg 20%**	3,30	2,67	1,98	1,72
11	**Lille-Frigg**	3,30	2,76	2,54	2,38
12	**Ekofisk 2/4 C 20%**	3,02	1,67	1,42	0,60
13	**Ekofisk 2/4 C**	2,80	1,55	0,74	0,54
15	**Beryl A, 20ºC**	2,42	1,25	1,27	0,78
16	**Beryl A, 20ºC**				
17	**Beryl A, +N+P, 20ºC**	2,24	2,92	1,42	0,71
18	**Beryl A, +N+P, 20ºC**				
19	**Beryl A, 5ºC**	3,09	4,12	2,50	2,18
20	**Beryl A, 5ºC**				

pH

No	Content	day 1 pH	Day 28 pH	Day 56 pH	Day 100 pH
1	**reference sediment**	7,64	7,25	7,40	6,84
2	**Beryl A 20%**	8,37	8,12	7,67	7,79
3	**Beryl A**	8,96	8,48	8,21	8,20
4	**Beryl A**	8,87	8,64	8,05	7,99
5	**Beryl A**	8,94	8,54	8,30	
6	**Ekofisk 2/4 A 20%**	8,52	8,13	7,98	8,25
7	**Ekofisk 2/4 A**	9,16	8,76	8,19	8,38
8	**Frøy 20%**	7,89	7,39	6,96	7,05
9	**Frøy**	7,76	7,06	6,89	7,07
10	**Lille-Frigg 20%**	8,37	8,38	8,01	7,95
11	**Lille-Frigg**	8,80	8,71	8,40	8,35
12	**Ekofisk 2/4 C 20%**	7,92	7,25	7,32	7,38
13	**Ekofisk 2/4 C**	8,34	7,23	7,38	7,37
15	**Beryl A, 20ºC**	8,93	7,77	7,78	7,88
16	**Beryl A, 20ºC**				
17	**Beryl A, +N+P, 20ºC**	8,89	8,44	8,00	8,13
18	**Beryl A, +N+P, 20ºC**				
19	**Beryl A, 5ºC**	8,95	8,87	8,81	8,75
20	**Beryl A, 5ºC**				

Microbial activity (FDA)

No	Content	day 1 A490/g	Day 28 A490/g	Day 56 A490/g	Day 100 A490/g
1	**reference sediment**	0,152	0,123	0,169	0,162
2	**Beryl A 20%**	0,074	0,129	0,238	0,279
3	**Beryl A**	0,013	0,074	0,114	0,129
4	**Beryl A**	0,011	0,049	0,126	0,141
5	**Beryl A**	0,015	0,006	0,106	
6	**Ekofisk 2/4 A 20%**	0,056	0,217	0,305	0,371
7	**Ekofisk 2/4 A**	0,112	0,336	0,555	0,471
8	**Frøy 20%**	0,112	0,295	0,654	0,721
9	**Frøy**	0,238	0,754	1,050	1,445
10	**Lille-Frigg 20%**	0,064	0,071	0,123	0,112
11	**Lille-Frigg**	0,010	0,000	0,035	0,041
12	**Ekofisk 2/4 C 20%**	0,045	0,079	0,143	0,164
13	**Ekofisk 2/4 C**	0,036	0,058	0,084	0,097
15	**Beryl A, 20ºC**		0,079	0,109	0,108
16	**Beryl A, 20ºC**				
17	**Beryl A, +N+P, 20ºC**		0,031	0,130	0,161
18	**Beryl A, +N+P, 20ºC**				
19	**Beryl A, 5ºC**		0,017	0,073	0,060
20	**Beryl A, 5ºC**				

Appendix 3