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Molecular analysis of petroleum derived compounds that adsorb onto gas hydrate surfaces.

Molecular analysis of petroleum derived compounds that adsorb onto gas hydrate surfaces

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Abstract

Field observations have shown that some streams of water, gas and crude oils do not form gas hydrate plugs during petroleum production even when operating within thermodynamic conditions for hydrate formation. Also, when studied under controlled laboratory conditions, some oils are found to form hydrate dispersed systems whereas others form plugs. The oils with low tendency to form hydrate plugs are believed to contain natural hydrate plug inhibiting components (NICs) that adsorb onto the hydrate surface, making them less water wet and preventing the particles from agglomerating into large hydrate clusters. The molecular structure of the NICs is currently unknown. In this work, hydrate adsorbing components have been extracted from crude oils using freon hydrates as an extraction phase. The fractions are found to be enriched in polar material, and more polar material is associated with hydrates generated in biodegraded crude oils compared to non-biodegraded oils. Various fractionation schemes and analytical techniques have been applied in the search of molecular characterisation. The average molecular weights are found to be approximately 500 g/mole. GC-MS chromatograms show a large UCM. Thus, GC-MS has a limited potential for identification of compounds. A commercial biosurfactant has been used as a model compound in the search for similar structures in the extracts, but no structures resembling this biosurfactant could be identified. The results suggest that the type and structure of the adsorbing components are more important for hydrate morphology than the amount of material adsorbed.

Key words: gas hydrates, crude oil, adsorption, freon, inhibition, biodegraded oil

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1 Introduction

The risk of gas hydrate formation can be a "show-stopper" for the oil companies when evaluating new prospects. The cost of preventing hydrate plugs can be prohibitive, so the hydrate strategy is a critical factor for field development. Gas hydrates constitute a crystalline material of where gas molecules are trapped in a framework of water molecules (Sloan, 1998). Natural gas hydrates form at high pressure and low temperatures, i.e. typical operating conditions during production shut-ins or long tie-backs in cold sub sea environments.

Once hydrate nuclei are formed on the interfaces of the gas/oil/water system, the hydrates can either agglomerate into large hydrate clusters that can plug pipelines and valves in the platform equipment, or they can remain as unproblematical small particles flocculated in the oil as a dispersion. This variation in the hydrate morphology is possibly a function of many factors, one of them believed to be crude oil composition, as some crude oils are observed to be associated with high risks of hydrate plugging and others are not under otherwise equal conditions. Several authors have indicated that the plugging tendency is dependent on the presence or absence of natural inhibiting components (NICs), (Fadnes, 1996; Gaillard *et al.*, 1999; Bergflødt, 2001; Høiland *et al.*, 2005a). The likely mechanism would be the adsorption of special compound types onto the hydrate surface, preventing the small hydrate particles from agglomerating into large plugs. Consideration of such effects is not part of the current hydrate control strategies, which typically consider only the hydrate phase equilibrium curve of pressure and temperature relative to bulk fluid composition, making sure that the production does not enter hydrate stable conditions. Addition of large volumes of methanol or glycol are used to shift the equilibrium if necessary. Low dosage hydrate inhibitors (LDHIs) are also commercially available, but their use is often restricted by environmental regulations or the severity of the conditions.

The aim of this work is to increase the understanding of how the bulk oil composition influences the morphology of the formed hydrates, with special focus on the degree of biodegradation. Ultimately, the aim is to incorporate compositional information for the oils into models that predict the plugging tendency of a given fluid system, and thus develop differentiated hydrate control strategies. Furthermore, the identification of the molecular structures could be important for the development of new, low-concentration hydrate inhibitors.

In a previous study by Høiland *et al.* (2005a) it was demonstrated that crude oils generate freon hydrates of various wettabilities depending on the crude oil composition. The variation in wettability was attributed to variations in the type and/or amount of crude oil components that adsorb onto the hydrates, creating oil-wet, intermediate-wet or water-wet hydrate surfaces. The wetta-

bility of the hydrates is correlated to the hydrate plugging tendency because the crude oils generating oil wet hydrates will give dispersed hydrates, and not plugs. The crude oils used in the present work are the same as used by Høiland *et al.* (2005a), and the wettabilities and plugging tendencies of the hydrates formed in each oil are given in Table 1. Two of the crude oils were found to give oil-wet hydrates, whereas the rest generated intermediate wet or water wet hydrates.

Crude oil is a complex mixture composed of millions of compounds, and the search for individual molecular structures with specific properties is difficult. The NICs (natural inhibition components), if present at all, most likely occur at low concentrations in the crude oils (ppm level), which makes their characterisation and identification even more challenging. Hydrates are networks of cage-like structures, where most cages contain a small guest molecule. The surface of these structures comprises hydrogen-bonded water molecules. Organic molecular structures that are able to interact with the hydrate surface are expected to contain polar functional groups and to be surface active. Petroleum acids and bases are typical candidate compounds. As the molecular structures of the NICs are currently unknown, no standard procedure is available for the isolation and identification of these compounds.

In this work, a new method for extracting hydrate adsorbing components by the use of freon hydrates has been developed and tested. The hydrate adsorbing components are collected and molecular structures are analysed using a range of techniques. In the first step, freon hydrates are used as model systems for natural gas hydrates and contacted with crude oils to selectively adsorb the active compounds. The hydrate phases are then decomposed (melted). Solid phase extraction is used to fractionate the organic extract. To characterise the polar fractions of the extract, molecular weights are determined by Gel Permeation Chromatography (GPC). Fourier Transform Infrared (FT-IR) spectroscopy is used to identify functional groups in the fractions, and gas chromatography combined with mass spectrometry (GC-MS) is used in an attempt to determine specific molecular structures.

The fractionation scheme has been validated for a candidate NIC compound. The choice of the model compound is based on the fact that the level of biodegradation of the crude oils previously has been found to be an important indicator for the hydrate plugging tendency (Høiland *et al.*, 2005a). Our hypothesis is that biosurfactants, that are surface active products from microbial activity, could be compound types that might interact with hydrate surfaces and function as NICs (natural inhibiting components). In fact, a water soluble biosurfactant was previously found able to convert a hydrate plugging system to become a hydrate dispersion (Høiland *et al.* , 2005b). Cyclic lipopeptides are a class of biosurfactants that have been identified as products from thermotolerant bacterial strains. Such biosurfactants have been isolated

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from petroleum reservoirs (Yakimov *et al.*, 1995; Vater *et al.*, 2002). One such biosurfactant, surfactin, has been chosen as a possible NIC model structure in order to validate that the procedure developed for extraction of NICs is suitable for identification of this class of compounds.

2 Materials and methods

2.1 Materials

Hydrate adsorbing components were extracted from a set of 15 crude oils, spanning from heavy biodegraded oils enriched in asphaltenes to light non-biodegraded oils and condensates. Most of the oils originate from the Norwegian continental shelf and were supplied by Norsk Hydro ASA. The oils are marked with a letter, B - biodegraded oil or S - sweet, non-biodegraded oil, followed by a number indicating production field and a letter denoting different wells or different batches within one field. The level of biodegradation (Peters and Moldowan (1993) scale) for the different oils was previously reported by Barth *et al.* (2004), and is given in Table 1. In addition, the crude oils in the data set have been thoroughly characterised with respect to compositional properties such as total acid and base numbers, density and asphaltene contents (Barth *et al.*, 2004), as well as with respect to the wettability of the freon hydrates that are generated by each crude oil (Høiland *et al.*, 2005a).

The biosurfactant surfactin was purchased from Sigma (purity approx. 98%). The structure of surfactin is shown in Figure 1. Surfactin contains a seven-membered ring of amino acid units, made up of four different amino acids, linked with a hydroxy fatty acid. In order to validate the hydrate extract and fractionation processes, crude oils were spiked with surfactin at different stages in the procedure.
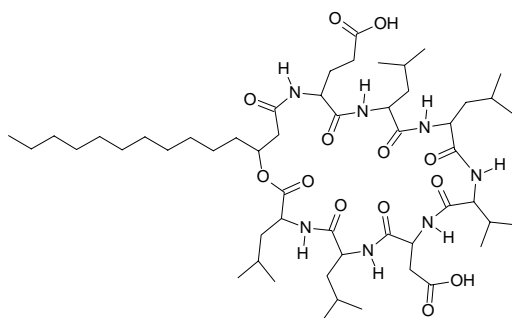

Fig. 1. The structure of a surfactin molecule, containing a seven-membered ring of amino acid units, made up of four different amino acids (leucine, glutamic acid, valine and aspartic acid), linked with a hydroxy fatty acid.

Freon (trichloro fluoro methane, R-11), supplied by Aldrich (purity ≥99%), was used as the hydrate forming compound. This compound forms structure II hydrates below 8.5°C at 1 bar (Wittstruck *et al.*, 1961), and is thus suitable for laboratory experiments without pressurised equipment. Freon R-11 has a boiling point of 23.8°C and has low solubility in water (0.124 g/100 ml). Petroleum associated natural gas also forms structure II hydrates, and so Freon is used as a model for the natural gas hydrates in this work.

The brine consists of 3.5 wt% NaCl (p.a. quality) in doubled-distilled deionized water.

Polystyrene standards used for GPC analysis were purchased from Polymer Laboratories, and had a purity of 99.5%.

All other solvents and chemicals were of p.a. quality.

2.2   Extraction of hydrate adsorbing components

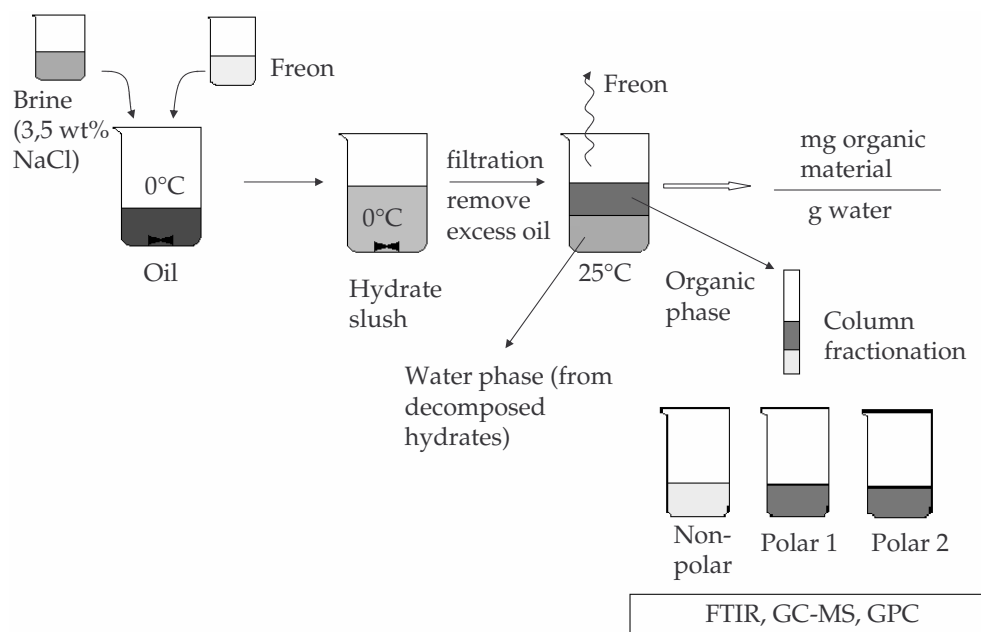A flow sheet of the experimental procedure is shown in Figure 2.

Fig. 2. Flow sheet of the experimental procedure.

A sample of 2.5 ml crude oil, 20 ml brine (3.5 wt% NaCl) and 5 ml freon are mixed in a 100 ml closed container with constant stirring at 0°C for 2-3 hours. A tiny amount of crushed ice is then added to initiate hydrate formation. The hydrate slurry is stirred for one hour and filtered under vacuum, using 50 ml of cold toluene to remove excess oil. The hydrate phase is collected, and left to melt slightly for a few minutes. Cold brine (20 ml) is added to the hydrate

phase, stirred at 0°C for 1 hour during recrystallisation of the hydrates, and again filtered with toluene. Another portion of brine (20 ml) is added to the hydrate phase and stirred for 1 hour. The last filtration is performed without toluene. The collected hydrate phase is left to decompose at room temperature. Diethyl ether (5 ml) is added, and the solution is separated into an aqueous and an organic phase, the latter containing the hydrate adsorbing components. The amount of aqueous phase is regarded as a direct measure of the amount of hydrates. Freon evaporates easily at room temperature, and it is assumed that neither freon nor toluene affects the amount of organic phase. Anhydrous Na_2SO_4 is added to the organic phase in order to remove water. The organic phase is filtered and quantified gravimetically on a Cahn electrobalance (range 0.0001-2 mg). In the quantification procedure 5 or 10 μl of the solution is placed on the weighing pan and the solvent is evaporated for 20 minutes. The amount of non-volatile organic material as a fraction of the aqueous phase is then calculated.

The ratio of organic material to amount of water (mg/g) is regarded as a direct measure of the amount of organic material adsorbed per unit of hydrates in each experiment.

2.3   Fractionation procedure

Prior to analysis the organic phases are fractionated on micro silica columns. The fractionation removes the non-polar components and separates the rest of the hydrate extract into two polar fractions, the first polar fraction containing less polar compounds (typically carboxylic acids) than the second polar fraction (typically polyfunctional compounds). Two types of columns are used; Type A: packed in the laboratory in a long pasteur pipette using 0.063 - 0.200 nm sized SiO_2 grains (pre-washed with dichloromethane (DCM) and activated by drying at 140°C for at least 16 hours), or Type B: commercially available Silica 1cc columns from Waters Sep-Pak. Different solvent systems are used for the two columns. The packed column is eluted with approximately 26 ml hexane:DCM 90:10 (v/v), 12 ml DCM:methanol 93:7 (v/v) and 12 ml methanol:DCM 70:30 (v/v) (Method 1). The Silica 1cc column is eluted with approximately 6 ml hexane:DCM 90:10 (v/v), 26 ml DCM and 12 ml DCM:methanol 93:7 (v/v) (Method 2). All the fractions are evaporated under a gentle N_2-flow and redissolved in small amounts of the respective eluents. The amount of organic material in the fractions is determined gravimetically using a Cahn electrobalance.

2.4 FTIR analysis

FTIR analysis is performed on a Nicolet Protege 460 FTIR spectrometer with a Diamond Attenuated Total Reflection (ATR) - Dura sampler cell (from SensIR). The samples are dissolved in the solvent from the fractionation procedure before the FTIR-analysis. A small amount of sample (one drop) is placed on the ATR diamond, and the solvent is evaporated before the analysis. The spectra are recorded from 600 to 4000 cm^{-1}, using 32 scans and a resolution of 4 cm^{-1}.

2.5 GPC analysis

GPC analysis is performed using a PL-gel 3 μm MIXED-E column. Tetrahydrofuran (THF) is used as the mobile phase and the flow rate through the column is 0.5 ml per minute. The detector is a Sedex 55 light scattering detector (S.E.D.E.R.E, France), and the detector temperature is 40°C.

The molecular weights of the samples are calculated from a standard curve based on seven model compounds, with molecular weights spanning from 122 g/mole (benzoic acid) to 599 g/mole (vanadyl-octaethyl porphyrin, synthesised by R. Ocampo at the Louis Pasteur University, Strasbourg) in addition to eleven polystyrene standards with molecular weights from 162 to 70000 g/mole.

2.6 GC-MS analysis

The samples are analysed by GC-MS on a HP5890 Series Gas Chromatograph Plus, with one Flame Ionisation Detector (FID) and one Mass Sensitive Detector (MSD), HP5971, and a WCOT fused Silica, ultra 2, 25 m column with i.d 0.2 mm and film thickness 0.33 μm. The injection temperature is 250°C, the detector temperatures 300°C (FID) and 280°C (MSD), and the temperature program is: 60°C for 1 minute, 10°C/minute to 250°C, 20°C/minute to 300°C, held for 3 minutes, and 20°C/minute to 320°C, held for 10 minutes. Helium is used as a carrier gas and 1 μl of the prepared sample is injected.

Due to high molecular weights many of the compounds in the samples are outside optimal GC-MS scope. The samples are therefore hydrolysed to decompose large molecules into smaller, identifiable compounds. In the hydrolysis procedure, the hydrate extract is evaporated to dryness with N_2-gas, 100 μl 7 N HCl is added, and the sample is kept at 80°C for 17 hours in a closed 20 ml sample vial. After cooling, 100 μl 0.1 M HCl, 100 μl 1 mM trichloroacetic

acid and 0.5 ml chloroform are added, and the mixture is kept at 50°C for 30 minutes. The water phase is separated from the organic phase. Approximately 1 ml of chloroform is added to the organic phase which then is neutralised by addition of a small amount of $CaCO_3$ (approximately 0.02 g), according to the neutralisation procedure described by Castro *et al.* (1997). The $CaCO_3$ is filtered off and the organic phase is evaporated with N_2-gas. The sample is finally redissolved in a 50 μl mixture of water:ethanol:pyridine (5:4:1 v/v/v).

After hydrolysis, the water phase and the organic phase are derivatised with chloroformate as described by Liebich *et al.* (1992). The amounts of reagents are doubled compared to this procedure. Thus, 100 μl of a buffer solution (Merck pH 9, dilluted 1:6 with water), 10 μl of ethanol, 290 μl of acetonitrile, 20 μl of ethyl chloroformate and 30 μl of pyridine are added to 10 μl of a standard solution with a concentration of 0.2 mole/L. After 1 minute 300 μl of chloroform and 400 μl of saturated $NaHCO_3$ are added. The phases are separated with a pasteur pipette and the organic phase is dried with anhydrous Na_2SO_4 before GC-MS analysis.

After the methods described above had been validated by positively identifying a standard solution of surfactin, they were tested on hydrate extracts to search for lipopeptides in the oils.

The most accurate method for identification of surfactin is GC-MS analysis. To test the detection limit of surfactin on GC-MS, samples of surfactin of 1, 10 and 100 ppm in ethyl acetate were analysed. The detection limit was between 1 and 10 ppm, meaning that samples of 10 and 100 ppm are easily detected, but not samples of 1 ppm.

3   Results

3.1   Quantification of adsorbed oil

Hydrate adsorbing components were extracted from all the crude oils in the data set. The amount of organic material in the different hydrate extracts are given in Table 1. The data includes two blank runs; Petroleum ether (100-140 °C) mixed with freon and brine (blank 1) and a mixture consisting of freon and brine only (blank 2). The values for the total amount of organic material extracted from the oils are averages of 3 to 4 parallel extractions.

Rather large variations in the extracted amounts are sometimes observed from this extraction procedure (see Table 1). While for some oils the deviation was less than 1 percent of the amounts, others were associated with deviations

Table 1
Average amounts of organic material in the hydrate extracts and polar fractions of the hydrate extract given in mg organic material per gram water (fractionated with Method 1). The level of biodegradation on the Peters and Moldowan (1993) scale is given in the fifth column (reported by Barth *et al.* (2004)). Wettability and plugging tendency of the crude oils are taken from Høiland *et al.* (2005a).

Oil	Hydr. extr. mg org.mat./ g water	Polar 1 mg/ g water	Polar 2 mg/ g water	Bio- deg. level	Wetta- bility [a]	Plugging tendency
B1a	102 ± 5	22.6	0.6	2	water	high
B2a	67 ± 28	9.2	0.7	6		low
B2b	115 ± 40	21.0	1.3	6	oil	low
B3a	82 ± 38	15.8	1.0	8	water	high
B4b	92 ± 1	10.1	0.4	8/2 [b]	int.	high
B4c	71 ± 18	7.9	0.5	2	oil	low
S1a	85.7 ± 0.1	11.1	0.4	0		
S2a	58 ± 18	3.5	0.2	0		high
S2b	86	3.6	0.2	0	water	high
S3a	95 ± 6	6.4	0.4	0	int.	high
S3b	60	6.0	0.6	0	int.	high
S4a	24 ± 4	0.8	0.1	0		high
S4b	101 ± 6	5.9	0.4	0	water	high
S5a	54 ± 18	3.4	0.3	0	water	high
S6a	20 ± 4	0.3	0.2	n.a. [c]	water	high
Blank1	2 ± 1					
Blank2	0.2 ± 0.1					

[a] Wettability denotations: oil: oil wet particles, water: water wet particles, int.: intermediate wet particles. (Høiland *et al.*, 2005a).
[b] The B4b oil is a mixture of a more biodegraded oil and a less biodegraded oil.
[c] n.a. not applicable.

of more than 40 percent. The reason for this difference can be attributed to variations in amounts of co-extracted oil creating large deviations between parallel samples, due to e.g. variations in crude oil viscosity.

For similar reasons, it is difficult to differentiate between biodegraded and non-biodegraded oils on basis of the amount of hydrate extract. The average

yields for the biodegraded oils is 88 ± 18 mg/g, and 65 ± 30 mg/g for the non-biodegraded oils, giving approximately 30% higher yields for biodegraded versus non-biodegraded oils. A simple analysis of the variance showed however that the difference probably is not significant.

The hydrate extracts were fractionated by Method 1 (see Section 2.3) into one non-polar fraction (not shown), and two polar fractions and quantified. The results are given in Figure 3. The polar 1 fraction contains significantly more material than the polar 2 fraction. For both fractions a considerably higher amount of polar material is extracted from the biodegraded oils compared to the non-biodegraded oils. Here, the influence of co-extracted oil is minimised since the non-polar components have been removed. Hence, less uncertainty is associated with these values. The fractionation procedure showed good reproducibility, with deviations less than 10% for parallel fractionations. Average yields for biodegraded oils are 14 ± 6 mg/g for the polar 1 fraction and 0.7 ± 0.4 mg/g for the polar 2 fraction. The corresponding average yields for non-biodegraded oils are 5 ± 3 mg/g (polar 1) and 0.3 ± 0.1 mg/g (polar 2), giving approximately 69% and 59% higher yields, respectively, for biodegraded oils compared to non-biodegraded oils.
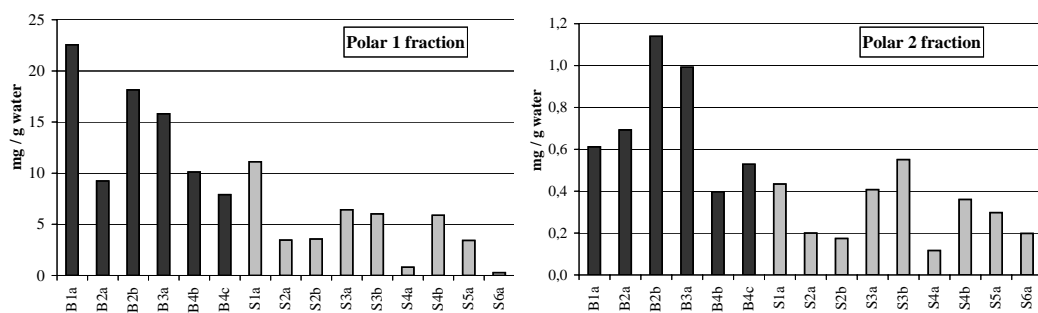

Fig. 3. The amount of organic material in the hydrate extracts after column fractionation of the hydrate extracts into one non-polar (hydrocarbons, not presented) and two polar fractions by the use of a silica column (Method 1). Left: Polar 1 fraction, Right: Polar 2 fraction. Black bars (B-oils): Biodegraded oils, and grey bars (S-oils): Non-biodegraded oils.

The fractionation procedure was also applied to selected crude oil samples in order to compare the original content of polar material in oil with the corresponding fractions of the hydrate extracts. The comparison showed that the hydrate extracts were significantly enriched in polar compounds, which supports the intention of the procedure, namely a selective extraction of compounds with affinity for hydrate surfaces.

Fractions from all the hydrate extracts were analysed by FTIR. Spectra of the first polar fraction of a biodegraded oil and a non-biodegraded oil are shown in Figure 4. The first polar fraction of the hydrate extracts all had similar FTIR spectra. The spectra shown in Figure 4 contain two bands at 2853 to 2922 cm^{-1} and bands at 1456, 1376 and 745 cm^{-1} indicating C-H bonds, and a band at 1604 cm^{-1} indicating C=C bonds. In addition they have a band at approximately 1705 cm^{-1} which probably is C=O stretching from carboxylic acids. Alternatively, saturated ketones also absorb at this wavenumber, but are considered less probable constituents in polar fractions. The spectra also have weak broad bands at approximately 3400 cm^{-1}, assigned to O-H bonds possibly due to alcoholic OH, phenolic OH, and/or carboxylic OH. Control analysis of the non-polar fraction showed no bands at these wavelengths, and the spectra were dominated by C-H band and C-C band absorptions.
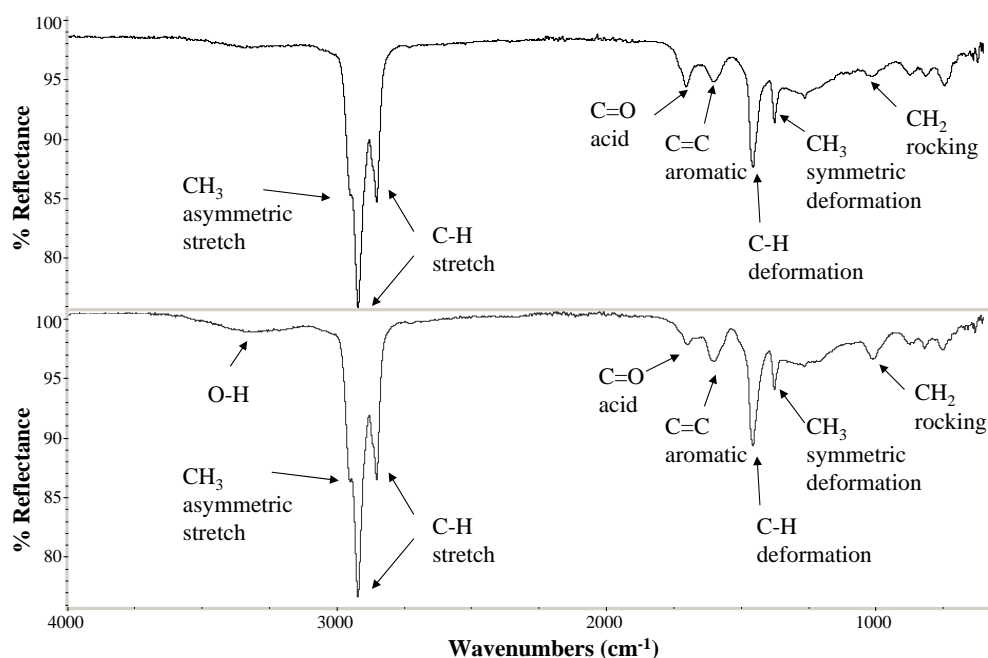

Fig. 4. FTIR spectra of the polar 1 fraction of the hydrate extracts of one biodegraded oil, B4b (upper spectrum) and one non-biodegraded oil, S2a (lower spectrum). The oils are fractionated by Method 1.

Some differences in the relative intensities of the bands are observed when comparing hydrate extracts from different crude oils. The B2 oils (B2a and B2b) and the B4 oils (B4b and B4c) deviate from the rest of the data set by having a slightly higher intensity of the C=O band at 1705 cm^{-1}, which can be seen in Figure 4.

Spectra of the second polar fraction of one biodegraded oil and one non-biodegraded oil are shown in Figure 5. Like the spectra of the polar 1 frac-

tions, the spectra of the polar 2 fractions contain bands at 3400 cm^{-1} and just below 3000 cm^{-1}. The relative intensities of these bands were different for polar 2 fractions from different oils. The B2a and B2b oils had larger C-H bands relative to O-H bands than the other oils, indicating that these oils contain compounds with more alkyl character. Bands identified at approximately 1735 and 1250 cm^{-1} can correspond to C=O and C-O stretching in an ester compound. These two bands are much more evident in the spectra from the B2 and B4 oils than the others. Note that FTIR analysis of the second polar fractions were difficult due to low concentrations and contamination.
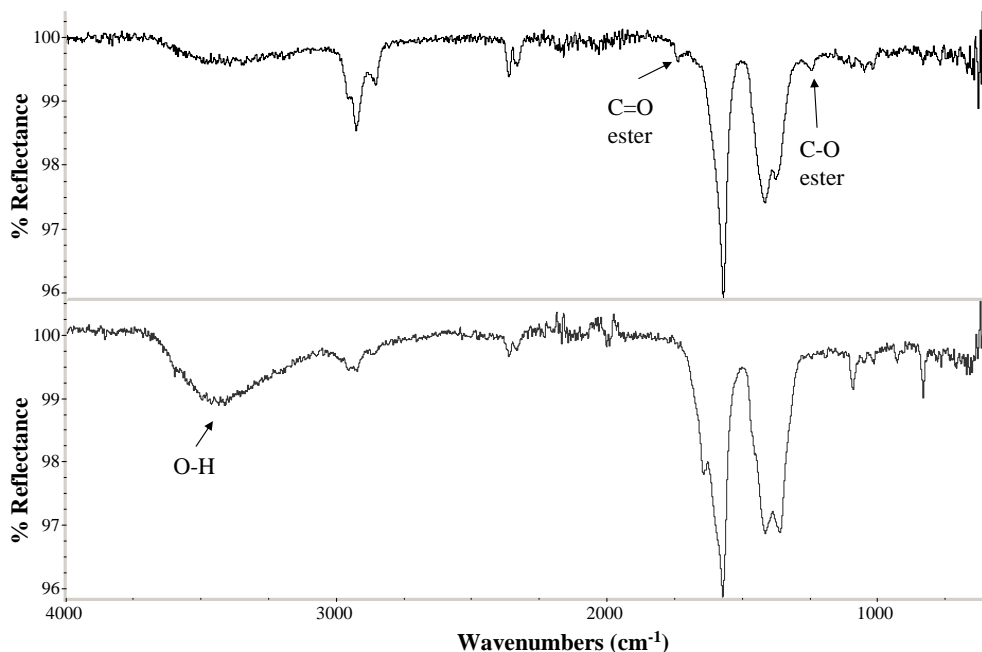

Fig. 5. FTIR spectra of the polar 2 fraction of the hydrate extracts of one biodegraded oil, B2a (upper spectrum) and one non biodegraded oil, S2a (lower spectrum). The oils are fractionated using Method 1.

3.3 Molecular weight of compounds in the hydrate extracts by GPC

The range of molecular weights in the hydrate extracts was determined by GPC. The molecular weights calculated from the maximum intensity of the GPC-chromatogram peaks are given in Figure 6. The values of most of the hydrate extracts were within a range from 400 to 600 g/mole. The exception is the hydrate extract from S5a which deviates from the rest of the data set with a Mw of 900 g/mole. The other extracts are quite similar in molecular weights, although the extracts from two biodegraded oils (B1a and B2b) are observed to be 100-200 g/mole higher in molecular weights than the rest. Some degree of uncertainty is connected to these data due to variations within parallels. The data are therefore given as the nearest 100 g/mole. The reason for the deviation of S5a is not known.
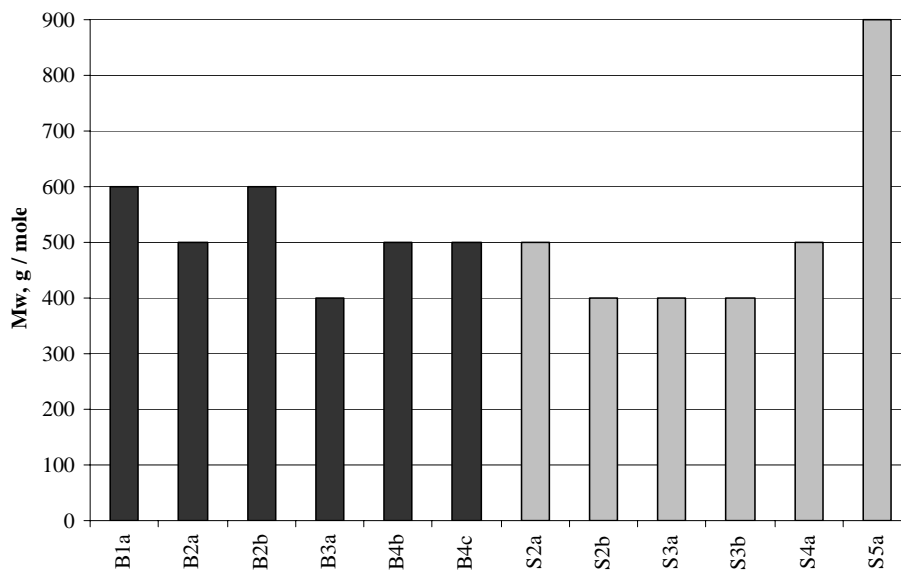
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Fig. 6. Average molecular weights of the different hydrate extracts determined by GPC. Black bars (B-oils): Biodegraded oils, Grey bars (S-oils): Non-biodegraded oils.

The molecular weight range for some of the sub-fractions of the hydrate extracts were also determined. No significant differences were observed when comparing the different oils. All the fractions had molecular weights within the range from 500 to 1000 g/mole. The non-polar fraction had a lower molecular weight range than the two polar fractions.

3.4 Structural analysis of the hydrate extracts, GC-MS results

Since the high molecular weight and complex nature of the target compounds make them unsuited for GC-MS analysis (molecular weights $\geq$ 400 g/mole), the polar hydrate extract fractions were hydrolysed before derivatisation and GC-MS analysis. Hydrolysis decomposes compounds containing ester bonds into smaller structures. For example, hydrolysis of surfactin (see Figure 1) liberates the amino acids and the hydroxy fatty acid. Non-hydrolysed samples were also analysed in order to compare with hydrolysed samples, and to identify compounds generated by the hydrolysis procedure.

Some selected polar fractions from fractionation Method 1 were hydrolysed, and both the water phases and the organic phases were derivatised using chloroformate. The concentrations of recovered compounds were low, but the results showed positive identification for alkanoic fatty acids (C9-C18) and methyl fatty acid esters. This corresponds well with the findings from FTIR analysis.

GC-MS analysis were also performed on some selected extracts fractionated

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by Method 2. These polar fractions were not hydrolysed, but directly derivatised using chloroformate before GC-MS analysis. The results show that the biodegraded oils have larger UCM (Unresolved Complex Mixture) in the chromatograms than the non-biodegraded oils. This is illustrated by the two chromatograms given in Figure 7.
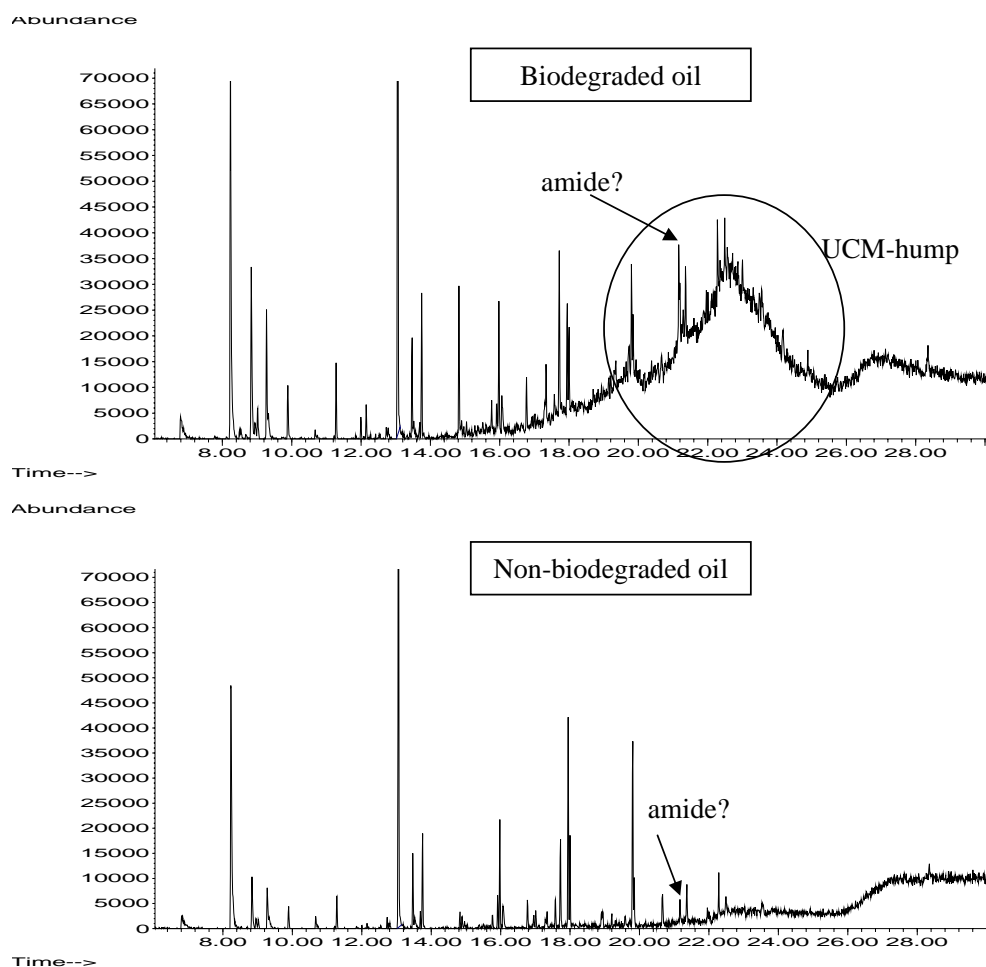

Fig. 7. Total ion chromatograms of the second polar fraction of hydrate extracts from; upper chromatogram: a biodegraded oil, B2b, and lower chromatogram: a non-biodegraded oil, S2b. The hydrate extracts were fractionated by Method 2 and the polar fraction were derivatised by chloroformates, but they are not hydrolysed.

In the first polar fraction some diaryl ketones were found (closest match: 1-1,1-biphenyl-4-yl-ethenon and (4-acetylphenyl)-phenylmethan). These compounds were only found in the B2b oil, the most biodegraded oil of the samples tested by this procedure. In the second polar fraction a compound that resembles a unsaturated amide (closest match: octadecenoic amide, possibly derived from a hydroxy substituted structure) was identified in hydrate extracts from all the oils investigated (see Figure 7).

GC-MS chromatograms from analysis of the second polar fraction of the hydrate extract from B4c with and without hydrolysis are shown in Figure 8.

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The chromatograms show that the hydrolysis gives a trace with well separated identifiable peaks, and no UCM. The chromatogram of the directly analysed sample contains significant UCM, and the composition is more complex. This indicates that the hydrolysis releases carboxylic acids from larger structures, and makes them available for identification.
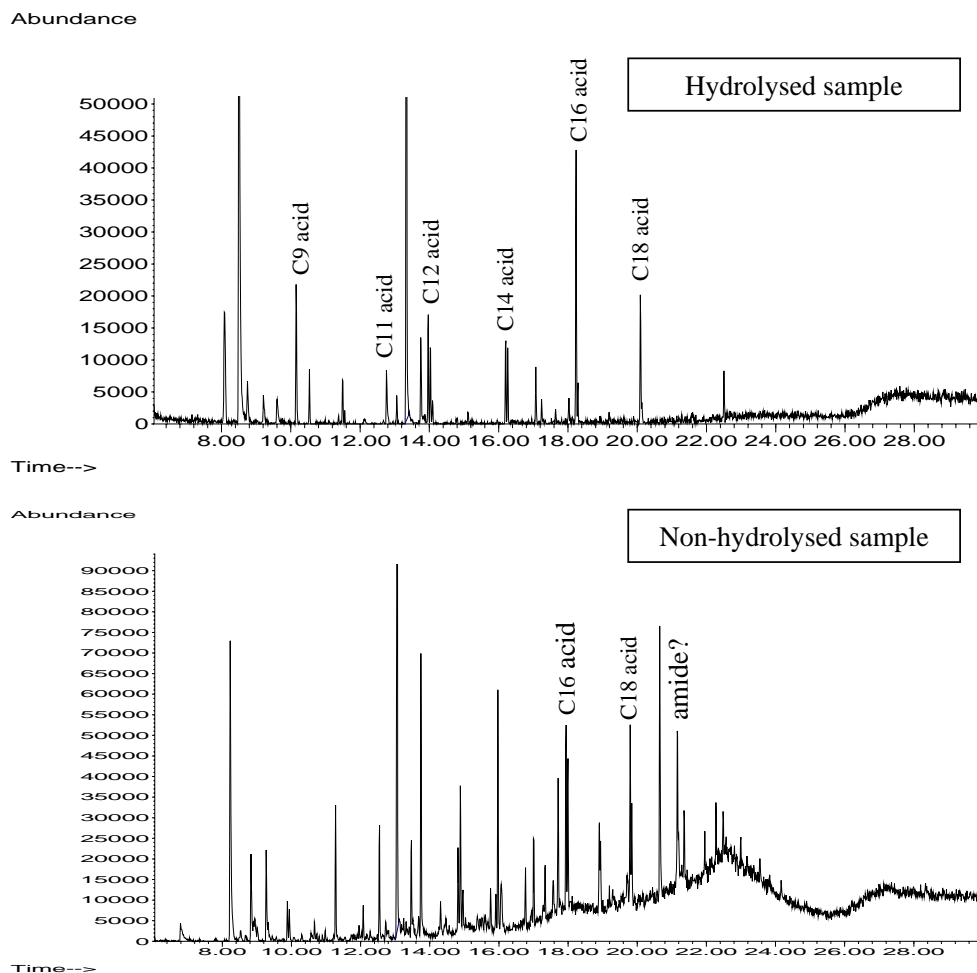

Fig. 8. Total ion chromatograms of the second polar fraction of hydrate extracts from; upper chromatogram: organic phase of a hydrolysed sample, B4c (fractionation Method 1), and lower chromatogram: non-hydrolysed sample, B4c (fractionation Method 2).

3.5 Method validation for a biosurfactant

Surfactin was used as a representative biosurfactant standard. Surfactin eluted in the second polar fraction in both fractionation methods and was identified by FTIR and GC-MS. The FTIR spectrum and the GC-MS chromatograms of surfactin are shown in Figure 9 and 10.

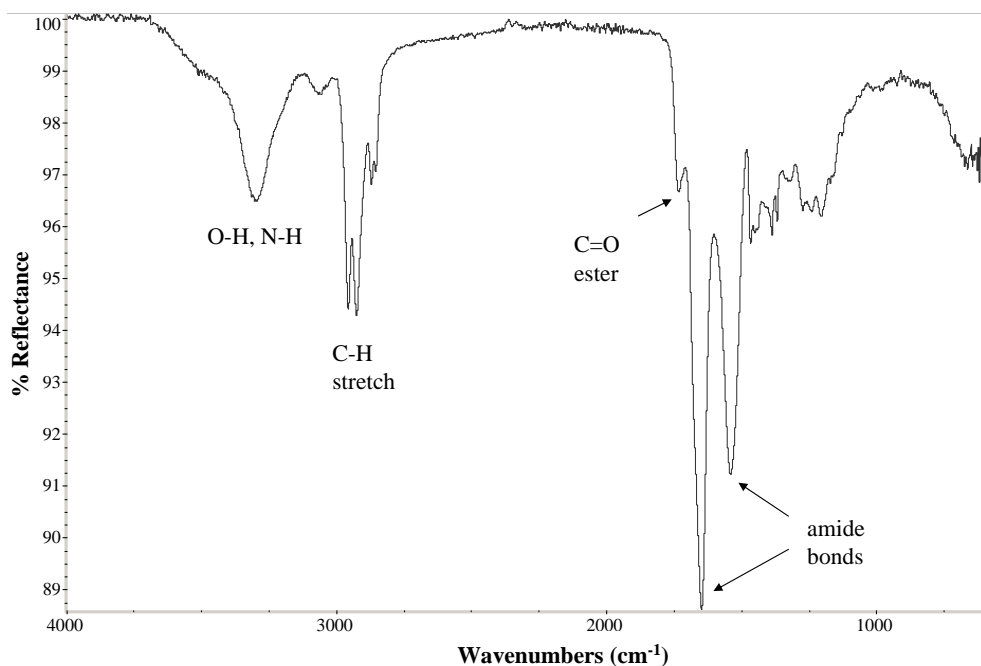The FTIR spectrum of surfactin was compared to spectra found in the liter-

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Fig. 9. FTIR spectrum of the second polar fraction after fractionation of surfactin by the use of Method 2.

ature (Arima *et al.*, 1968; Ferré *et al.*, 1997). The spectrum shows absorption bands from O-H and N-H stretching at 3060 and 3300 cm^{-1} with a shoulder at 3500 cm^{-1}, and bands just below 3000 cm^{-1} from C-H stretching. The band at 1732 cm^{-1} corresponds to C=O in ester bonds, and the bands at 1646 and 1540 cm^{-1} are amide bands.

The GC-MS analysis were performed after hydrolysis and derivatisation of the surfactin sample. In the water phase three of the four amino acids present in surfactin were easily identified, and hydroxy fatty acids were found in the organic phase.

In order to validate the procedure, one hydrate extract (from S3a) was spiked with surfactin in a concentration of 8350 ppm. The sample was fractionated using Method 1 and analysed by FTIR and GC-MS. Surfactin could be identified in the second polar fraction both in the FTIR and the GC-MS analysis. This means that if present in sufficient amounts in the hydrate extract, the lipopeptide structures should be detected by the applied procedures.

In addition, the crude oil S2b, was spiked with surfactin in a concentration of 1350 ppm prior to hydrate extraction. The hydrate extract was analysed in the same way as the other non-spiked hydrate extracts (fractionated by Method 1). Surfactin was clearly identified by GC-MS analysis, which shows that surfactin adsorbs onto the hydrate surface, and that a concentration of surfactin of 1350 ppm in an oil is sufficient to be detected by the applied methods. Using FTIR, the concentration were at the detection limit for the
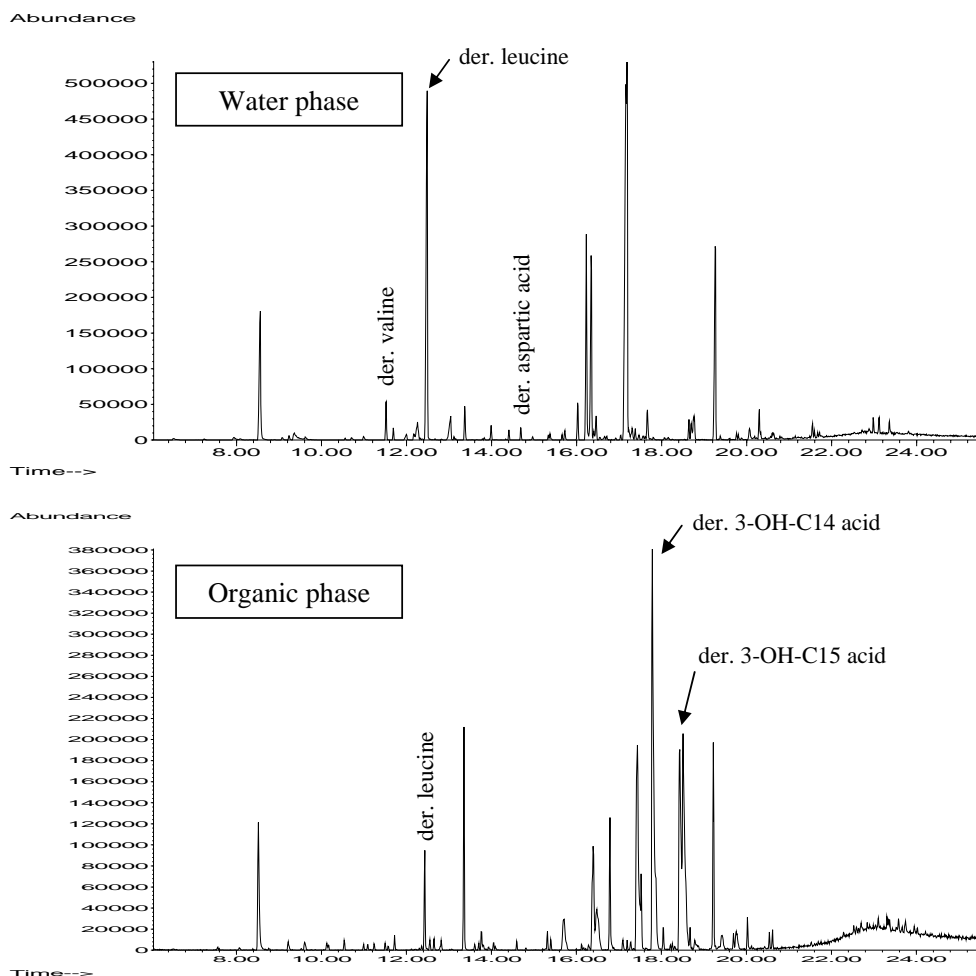
16

Fig. 10. Total ion chromatograms of; upper chromatogram: the water phase after hydrolysis of surfactin and lower chromatogram: the organic phase after hydrolysis of surfactin. Both phases are derivatised with chloroformate.

method, but the expected bands were present though with low intensities.

The results indicate that units incorporated in biosurfactant structures of the lipopeptide type are recovered in the applied procedure.

3.6 Relation to bulk oil composition

The crude oils constituting the data set presented in this work have previously been characterised with respect to a number of crude oil compositional properties, such as total acid number (TAN), total base number (TBN), asphaltene content and density (Barth *et al.*, 2004). The data set is not large enough to support a detailed statistical analysis, but even so a set of linear correlation coefficients between the various parameters can be helpful. The amounts of hydrate extract as a whole, and after fractionation into the polar 1 and polar

Table 2

Correlation coefficients (R) by comparing crude oil properties to the amounts of hydrate extract as a whole and after fractionation into two polar fractions.

	Hyd.filt. amount	Polar 1 amount	Polar 2 amount
Asphaltene content	0.31	0.68	0.61
Total base number (TBN)	0.38	0.72	0.59
Total acid number (TAN)	0.40	0.76	0.76
Biodegradation level	0.34	0.65	0.85
Density	0.53	0.84	0.73
Hydrate filtr. (total amount)	1	0.73	0.57
Polar 1 (amount)		1	0.80
Polar 2 (amount)			1

2 fractions, have been correlated to the compositional properties as well as the degree of biodegradation (see Table 1). The correlation coefficients are shown in Table 2.

The results show that the amount of hydrate extract and polar fractions have poor correlation with most of the crude oil compositional properties, which supports that the hydrate extract is a separate fraction compared to normal fractionation schemes (asphaltenes, petroleum acids, etc.). This supports the premise that the hydrate extract consists of compounds with special affinity to the hydrate surfaces. Density has the strongest correlation to the amount recovered in the polar fraction 1 with R = 0.84, so this fraction may represent a certain part of the heavier oil components, and the bidegradation level has the highest correlation with the polar 2 fractions, which thus may be linked to biodegradation products.

4   Discussion

The results from this work indicate a relation between the level of biodegradation for a crude oil and the amount of polar material that adsorbs onto hydrates, i.e. heavily biodegraded equals large amount adsorbed polar material, see Figure 3.

Applying these results to the wettability classification in Table 1, it is found that the wettability of the hydrate surfaces does not correlate to the amount of material extracted onto the hydrates. Biodegraded crude oils that generate

water wet hydrates, e.g. B1a and B3a, give similar yields of adsorbed compounds as the crude oils generating oil wet hydrates (B2b and B4c). Hence, it is likely that the type and structure of the adsorbing material is more important for hydrate wettability, and thus the hydrate plugging tendency, than the amounts of material adsorbed.

This observation corresponds well with the conclusions in another previous work by Høiland *et al.* (2001), where petroleum acid types and structures were found more important to wettability alteration of silica surfaces than the acid concentrations in the oils.

For the two oils that form dispersed, oil wet freon hydrates, B2b and B4c, FTIR analysis of the hydrate extracts shows that they deviate from the rest of the data set with respect to their internal relation between functional groups. This supports the idea that the type of compounds present may be more important than the amount. However, more complex relationships between the solvent properties and polar compound content for each crude oil can also be envisioned.

Biodegradation seems to be a necessary condition for the formation of oil wet hydrates. In the whole set of oils investigated, there are no non-biodegraded oils that are able to produce oil-wet hydrates. However, other factors must also be important, as many of the biodegraded oils still produce water-wet hydrate surfaces. As of now, the search for specific lipopeptide biosurfactants in the dispersing oils that produce oil-wet hydrates has not been successful. The amino acids that would identify lipopeptide structures, such as surfactin, could not be detected (in the samples investigated), and neither could hydroxy fatty acids in the organic phase be identified. The results indicate that molecular structures of lipopeptide type are either a) not present in the hydrate extracts, b) present in concentrations below the detection limit of the GC-MS (i.e. less than 10 ppm, see experimental section 2.6), or c) not liberated by the applied procedure. However, oil degrading bacteria produce a number of different biosurfactants depending on type of bacteria and growth conditions, so such compounds cannot yet be eliminated as the determining factor for hydrate particle wettability in oil/water/gas systems.

The GPC results show that the major part of the components in the hydrate extracts are of intermediate molecular weight (see Figure 6). Thus, neither high-molecular compounds such as asphaltenes nor simple, low-molecular petroleum acids and bases are prominent in the adsorbed matter.

The molecular weight range of the extracted compounds in part explain the analytical problems. With molecular weights in the range of 500-1000 g/mole, combined with polar functional groups, the volatility of the compounds is too low for analysis and identification by GC-MS, which in general is the

most powerful identification method for components of mixtures of organic molecules. More advanced separation methods, like liquid chromatography combined with mass spectrometry (LC-MS) may show a better capacity for characterising such mixtures.

The results from the GC-MS show that fatty acids are found in the hydrate extracts (see section 3.4). The distribution of the fatty acids corresponds well with a biological source, with hexadecanoic and octadecanoic acids dominating. However, these acids are very common and are found in many samples, and they cannot be used as a definite indication of biomolecules. Some unusual ketones and amids have also been found in the GC-MS chromatograms, and these may be products from biodegradation. At the moment these compounds cannot be directly related to biosurfactants or other surface active compounds.

5 Summary

The method for extraction of compounds with affinity for a hydrate surface has been shown to selectively extract specific polar compounds from crude oils. The yield and composition of the extracted polar compounds is a function of the oil composition, but it is different from the bulk polar fraction of the oil.

The results indicate that petroleum acid type and structure are more important for hydrate wettability, and hence the hydrate plugging tendency, than the amounts of material adsorbed onto the hydrate surface. The previous indicated link between biodegradation and hydrate plugging tendency may thus arise from the importance of biodegradation for the acid profile in the crude oils as discussed by Barth *et al.* (2004).

Molecular description of the extract is extremely challenging, and the analyses reported here do not result in molecular identifications. However, the method provides a tool for further study and characterisation of NICs.

Acknowledgements

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