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The influence of structural components of alkyl esters on their anaerobic biodegradation in marine sediment

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Abstract

Ester-based organic compounds are one type of synthetic base fluid added to drilling mud used during offshore oil-drilling operations in the Gulf of Mexico. Concern over the environmental impact of synthetic base fluid (SBF) contaminated rock cuttings discharged into the Gulf of Mexico has prompted the promulgation of EPA regulations requiring that all SBF be tested for biodegradability in marine sediment prior to their use in the Gulf. In order to allow the design or selection of suitably biodegradable esters, the anaerobic biodegradability of a variety of ester compounds was tested using a marine sediment inoculum to reveal the effect of: (a) increasing the chain length of the acid moiety, (b) increasing the chain length of the alcohol moiety; (c) alternating the relative size of the alcohol and acid moieties, (d) branching in the alcohol moiety, and (e) the presence of an unsaturated bond in the acidic moiety. The chemical structure of esters was found to affect the completeness and rate of anaerobic biodegradation, and would affect their ability to be certified for use as an SBF in the Gulf of Mexico. Recommendations for ester usage include using esters that have a total carbon number of between 12 and 18 and avoiding the use of branched alcohols (or acids by inference). The presence of an unsaturated bond in the acid (or alcohol by inference) increased biodegradability of the ester.

Introduction

The drilling of exploration and production wells beneath the sea floor requires a drilling mud medium, which is used to lubricate the drill bit, transfer rock cuttings to the surface, and control reservoir pressure. One component of the drilling mud is the synthetic base fluid (SBF), which are non-aqueous phase liquids composed of olefin- or ester-based compounds. SBF enter the aquatic environment as a coating on rock cuttings, or tailings, discharged from the drilling platforms. The US-EPA has enacted rules requiring that all SBF be certified as biodegradable by anaerobic microorganisms indigenous to marine sediment before the SBF can be licensed for use in the Gulf of Mexico. To accomplish this, an anaerobic bottle test system, known as the Closed Bottle Test (CBT) has been adopted as the standard method for determining relative biodegradability of SBF in marine sediment (Federal Register 2004). The test is similar to other anaerobic biodegradability test systems (Battersby & Wilson 1988; Colleran et al. 1992; Owen et al. 1979; Roberts 2002; Shelton & Tiedje 1984). The CBT system determines the biodegradation potential of SBF in marine sediment under anaerobic conditions relative to reference compounds (Federal Register 2004, Herman & Roberts 2005). Anaerobic biodegradation of SBF is indicated by an accumulation of anaerobic gases (CO_2 and CH_4) in SBF-spiked sediments, corrected for the gas production in

control (free of SBF) sediments. To be classed as biodegradable the average % of expected total gas production from the test SBF must be equal to the average % of expected total gas production from an industry standard reference compound (C1618 IO for internal olefins and Petrofree or Petrofree LV for ester compounds) (Federal Register 2004). The use of percent of expected values rather than absolute gas production values was chosen to allow sediment to sediment and inter lab comparisons. The biodegradation of SBF is verified by determining the removal of SBF from the sediment based on gas chromatography with a flame ionization detector (GC-FID) analysis of solvent extracted sediment samples. Any SBF intended for use in drilling mud must be certified as biodegradable on an annual basis before they can be used in drilling mud in the Gulf of Mexico.

In previous tests of many commercial olefin- or ester-based SBF, it was found that the ester based SBF showed the most differences in biodegradation potential. Some esters were degraded very rapidly, while others were degraded only very slowly. In some cases, degradation was rapid at first and then unexpectedly came to a stop. Since there are a number of different esters that can be used commercially, a systematic testing of the biodegradation potential of different esters is called for. In order to allow the selection of readily biodegradable ester compounds for use as SBF, an examination of the relationship between the chemical structure of 16 different esters and their biodegradation potential in marine sediment was performed.

Materials and methods

Anaerobic closed bottle test

The test was performed as described previously (Candler et al. 2000, Herman and Roberts 2005). Marine sediment was collected from Galveston Bay, 60 miles south of Houston, TX from a water access point near the intersection of Interstate 45 and Regional Highway 146. The sediment was sieved through a 2 mm mesh sieve, followed by a 0.5 mm mesh sieve, and stored at 4 °C before use. Each ester was added to the sediment to a final concentration of 2000 mg carbon/kg dry sediment. Synthetic seawater (Crystal Sea/Forty Fathoms Marine mix, Marine Enterprises International, Baltimore, MD) was added to make a slurry, and 75 ml of this slurry, containing the equivalent of 30 g/dry sediment, was added to 125 ml serum bottles. A drop of resazurin solution (0.5 g/l) was added to indicate the redox conditions inside the bottles. The headspace of each bottle was then flushed with a stream of nitrogen gas for 1–2 min to remove oxygen from the headspace, and the bottles were sealed with 20 mm diameter rubber stoppers (Bellco Glass, Vineland NJ) and crimp cap seals (Fisher Scientific). The headspace within the bottles was vented to establish an internal pressure equivalent to atmospheric pressure. The serum bottles were incubated at 29 ± 1 °C and anaerobic gas production was monitored for 112 days.

Anaerobic gas production measurements

Every 2 weeks the bottles were removed from the incubator and allowed to cool to room temperature. Total gas production was measured as gas pressure inside the bottles using a pressure transducer (Biotech International, Sugarland TX). The pressure was converted to a volume using a calibration curve based on a series of sealed 125 ml serum bottles containing 75 ml of water. The pressure was created by forcing a volume of air (1, 5, 10, 20, and 40 ml) into the headspace of the bottles. The calculated volumes were corrected to STP using the atmospheric pressure and temperature on the day of sampling. The predicted total anaerobic gas production was calculated based on the amount of carbon substrate added to the sediment. Each bottle received test fluid to a final concentration of 2000 mg carbon/kg dry weight. Given that each bottle contained the equivalent of 30 g dry sediment, then each bottle contained 60 mg (0.005 moles) carbon as test fluid, which is predicted to make 0.005 moles of gas (CO_2 or CH_4). At standard temperature and pressure, 1 mole of gas occupies 22.4 l, then 0.005 moles of gas will occupy 112 ml of total gas (CO_2 and CH_4 combined). The equation of Symons and Buswell (1933) was used to calculate the proportion of carbon dioxide to methane produced during anaerobic metabolism of each substrate based on the hydrogen and oxygen content of the substrate. The predictions of total gas and methane for the commercial products were performed using an

elemental analysis provided by the supplier. The results are presented in terms of percent of total expected gas to allow a determination of the completeness of the degradation process, to normalize for the differences in behaviors of sediments and to allow for inter lab comparisons.

Methane gas production was measured using GC-FID (HP 6890 Series GC system) using a 30 m HP-5 (0.32 mm ID, 0.25 micron film) column. The flow rate of helium was 1 ml/min. The oven and injector (using a 1/50 split) temperatures were set at 50 °C, and the detector temperature was 275 °C. A methane calibration curve was prepared by adding known volumes of pure methane to sealed bottles, and then injecting a 0.1 ml onto the GC column. A headspace sample volume of 0.1 ml was analyzed for each sample bottle.

Sediment extraction and GC-FID analysis

The concentration of the ester compounds in the sediment initially and after the incubation period was determined by GC-FID analysis of sediment extracts. An aliquot (20 g wet weight) of sediment from each bottle was transferred into a 400 ml Pyrex beaker and spiked with 1 ml internal standard solution (500 mg/l of hexamethylbenzene and heneicosane in dichloromethane). The internal standard solution was mixed into the sediment sample using a glass rod. The water in the sediment was then removed by adding anhydrous sodium sulfate and mixing with a glass rod until the sediment sulfate mixture gained an evenly distributed sand-like texture. This prevents the formation of emulsions during the extraction procedure. The dried sediment was extracted with approximately 100 ml of dichloromethane, and sonicated in a water bath (Fisher Scientific) for 20 min, three times. The solvent for each extraction was decanted through a sodium sulfate filter and combined in a 500 ml round bottom flask. Rotary evaporation was used to reduce the solvent volume to 1.8 ml that was transferred into an auto-sampler vial. GC-FID analysis (HP 6890 Series GC system) was performed with a 30 m HP-5 (0.32 mm ID, 0.25 micron film) column. The flow rate of helium was 1 ml/min, and the injector and detector temperatures were set at 275 °C. A ramped temperature regime was used, which had an initial temperature of 100 °C for 1 min, then was increased by 2 degrees per minute to a final temperature of 225 °C, which was held for 2 min. Total run time was 66 min. The concentration of ester present in the samples was determined by comparing the response ratio to internal standard calibrated using a 5-point curve.

Statistical analysis

A statistical comparison of the final total gas production was performed using SigmaStat software. The cumulative total gas amount (background subtracted and computed as a percentage of expected) produced by each of the three replicates on Day 112 were compared for all of the replicates using a one way ANOVA. The Holm-Sidak all pair-wise multiple comparison procedure was used with an overall significance level at 0.05.

Chemical list

Hexamethylbenzene, heneicosane, and all of the esters were purchased from Sigma–Aldrich (Milwaukee, WI) with the exception of octadecyl acetate that was purchased from ChemService (West Chester, PA). The three commercial esters were supplied by their manufacturers and are labeled as ester 1, 2 and 3.

Results

The application of the anaerobic biodegradability test using three commercial esters revealed a significant difference in the performance of the different products (Figure 1), which is not generally observed for the olefin based SBF (Herman and Roberts 2005). This observation, along with the observation that some commercial esters did not perform the same in every test, prompted the investigation of how different ester properties could affect their biodegradation.

The anaerobic biodegradation of fifteen different esters was examined. GC-FID analysis of sediment extracts revealed a >95% removal of all esters tested after 110 days of incubation, well within the 9 month duration of Closed Bottle Testing. However, differences in the extent and rate at which esters were biodegraded were evident, as indicated by differences in the extent and rate of anaerobic gas production. Example data are presented in Figure 2. Up to 80% of the

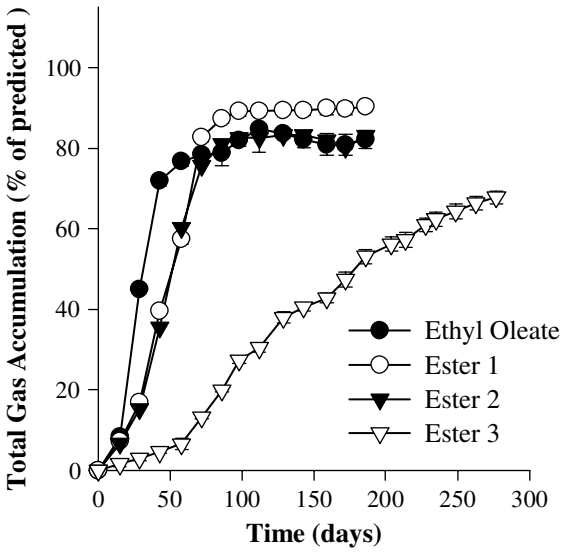

Figure 1. Anaerobic gas production from marine sediments spiked with commercial esters and ethyl oleate as a reference ester. Anaerobic gas production is expressed as the percent of expected total gas production determined from the known amount of carbon added to the culture. Gas production in spiked sediments is corrected for the gas production in control sediments. Each point represents the mean (±standard deviation) of quadruplicate bottles.

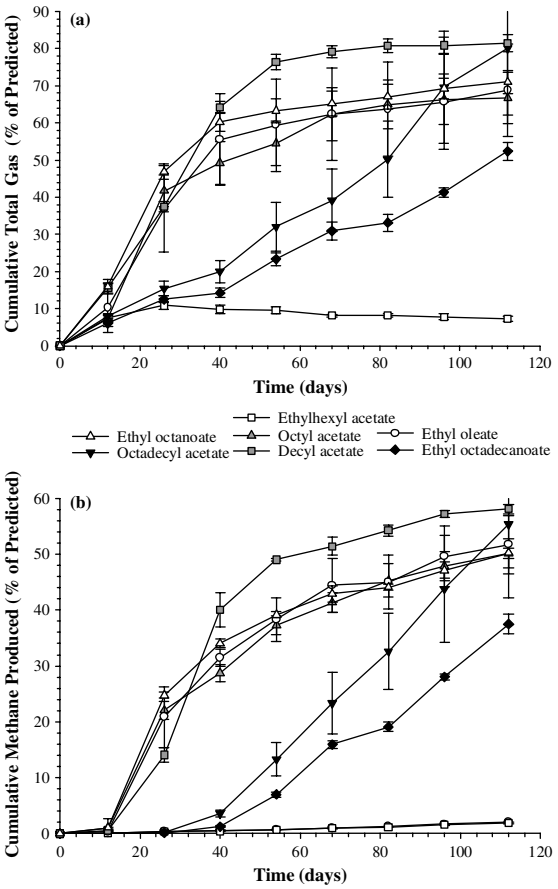

Figure 2. Cumulative (a) total anaerobic gas and (b) methane production from representative alkyl esters tested. Anaerobic gas production is expressed as the percent of expected total gas production determined from the known amount of carbon added to the culture. Gas production in spiked sediments is corrected for the gas production in control sediments. Each point represents the mean (±standard deviation) of quadruplicate bottles.

expected total gas and up to 60% of the expected methane were produced from most of the esters tested. Methane production was typically detected after 10–40 days lag phase, depending on the ester tested. This is to be expected since sulfate would act as the initial electron acceptor and would be exhausted rapidly because of the carbon overload of the system, leaving methanogenesis as the major electron sink.

The results of the incubations were analyzed to determine the rate and extent (% of expected gas on day 112) of gas production. Using just the maximum rate of gas production to compare relative biodegradability would be misleading due to the fact that some esters showed an initial rapid increase in gas production and then reached an early plateau compared to other esters. For example, comparison between ethylhexyl acetate and ethyl octadecanoate (Figure 2) revealed that the extent of gas production by 112 days of incubation was a more accurate parameter to compare relative biodegradability.

Five different structural features of ester compounds were identified as possibly having an affect on anaerobic biodegradation. The first feature examined was increasing the chain length of the acidic moiety using six different ethyl (C2) esters with an 8, 10, 12, 14, 16 and 18 carbon long acidic moiety (group 1, Table 1). Increasing the chain length of the alcohol moiety was examined using 1, 2, 4, 6, or 8 carbon alcohol esters with octanoic acid (group 2, Table 1). Alternating the relative size of the alcohol and acidic moieties was examined by comparing ethyl decanoate vs. decyl acetate (group 3, Table 1), and ethyl octadecanoate vs. octadecyl acetate (group 4, Table 1). The presence or absence of branching in the alcohol moiety was examined by comparing C10 esters containing a C8 alcohol moiety, which was

Table 1. Maximum gas production from ester compounds

Comparison group[a]	Ester compound	# of Carbons	Average gas Produced % of expected (SD)	Statistical grouping[b]
1, 2	Ethyl octanoate	2 + 8 = 10	71 (3.2)	D
1, 3	Ethyl decanoate	2 + 10 = 12	72 (7.4)	D
1	Ethyl dodecanoate	2 + 12 = 14	82 (2.4)	E
1	Ethyl tetradecanoate	2 + 14 = 16	86 (3)	E
1	Ethyl hexadecanoate	2 + 16 = 18	83 (4.4)	E
1,4, 6	Ethyl octadecanoate	2 + 18 = 20	53 (2.4)	B
2	Methyl octanoate	1 + 8 = 9	64 (2.4)	C
2	Butyl octanoate	4 + 8 = 12	82 (2.6)	E
2	Hexyl octanoate	6 + 8 = 14	87 (2.5)	E
2	Octyl octanoate	8 + 8 = 16	82 (2.9)	E
3	Decyl acetate	10 + 2 = 12	82 (2.2)	E
4	Octadecyl acetate	18 + 2 = 20	71 (3)	D
5	2-Ethylhexyl acetate	8 + 2 = 10	7 (0.7)	A
5	Octyl acetate	8 + 2 = 10	67 (6.8)	C, D
6	Ethyl oleate	2 + 18 = 20	75 (0.4)	D

[a]Structural features compared are described in the Section Results.
[b]Significantly different amounts of gas were produced from compounds assigned different letters.

branched (2-ethylhexyl acetate) or had a straight chain (octyl acetate) (group 5, Table 1). The presence or absence of an unsaturated bond in the acidic moiety was examined using ethyl octadecanoate (C18 saturated acidic moiety) compared to ethyl oleate (C18 unsaturated acidic moiety) (group 6, Table 1).

When the acidic chain length was less than 14 carbons, the total anaerobic gas accumulation increased with increasing chain length (Table 1), although the rate of gas production did not change significantly (Figure 2, not all results shown). Increasing the chain length of the acidic moiety of these ethyl esters to greater than 14 carbons, namely hexadecanoate and octadecanoate, resulted in a slower production of anaerobic gases, even though the gas production from the ethyl hexadecanoate did eventually reach levels comparable to the other esters with shorter acids by 112 days. It should be recognized that ethyl esters containing a C8–C14 acidic moiety are liquids at room temperature, while ethyl esters containing a C16–C18 acidic moiety, namely ethyl hexadecanoate and ethyl octadecanoate, are solids at room temperature and had to be heated to a liquid state before spiking into the sediment. Therefore, the slower production of anaerobic gas from ethyl hexadecanoate and ethyl octadecanoate was likely due to mass transfer limitations. Commercial SBF are liquids at room temperature, and therefore would not contain solid compounds, although it is possible that the higher molecular weight esters could be present in a mixture with other liquid-phase esters.

The effect of the length of the alcohol moiety was not as dramatic compared to the effect of the length of the acidic moiety. When octanoate was used as the acidic moiety, less complete gas production was observed when the alcohol was a methyl or ethyl group. The sediment produced the most gas from hexyl octanoate. Statistically similar gas production was observed for the butyl and octyl esters.

When the total length of the ester was constrained to C12 or C20 compounds and the relative sizes of the acid and alcohol moieties were changed within that constraint, it was found that the longer alcohol group resulted in faster and more complete gas production. The decyl acetate and octadecyl acetate (C10 and C18 alcohol groups, respectively) were degraded more completely than their ethyl decanoate and ethyl octadecanoate counterparts were. Again, the ethyl octadecanoate was a solid at room temperature, so

mass transfer limitations may have played a role in its slow degradation.

The change in branching of the alcohol moiety caused the most significant impact on gas production from esters. There was very little gas production from the branched 2-ethylhexyl acetate during the 115 days of the test, while the unbranched octyl acetate resulted in rapid gas production.

The presence of an unsaturated bond in the acid portion of the ester also had a significant impact on gas production during the anaerobic test. Gas production from the unsaturated ethyl oleate was more rapid and more complete than from the saturated form ethyl octadecanoate. Again, the ethyl octadecanoate was a solid at room temperature, so mass transfer limitations may have played a role in its slow degradation.

The greatest amount of gas produced in the 112 day test was approximately 87% of what was expected and occurred in the bottles spiked with hexyl octanoate. This was not significantly different from the amounts of gas produced from six other esters. It is interesting to note that the esters in the group that saw the highest gas production all contained from 12 to 18 total carbons. Only ethyl decanoate (C12 ester) did not make it into this group. The esters that did not result in the highest level of gas production contained either $\leq$10 or $\geq$18 carbons total.

Discussion and conclusions

Closed bottle testing revealed that some commercial ester-based SBF are more amenable to anaerobic biodegradation than others are. The actual composition of poorly degraded esters, such as Ester 3 in Figure 1, is proprietary knowledge and therefore the structural features that affected biodegradation are unknown. The establishment of a structure/activity relationship for the anaerobic biodegradation of ester compounds would be a useful tool in the designing of ester-based SBF for use in difficult drilling activities. All of the ester compounds tested were biodegraded under anaerobic conditions in marine sediment, including esters that are solids at room temperature. These results indicate that a wide variety of ester compounds would be acceptable components of SBF. One exception was the branched 2-ethylhexyl acetate, which appeared to be highly recalcitrant to anaerobic biodegradation. In general, branched hydrocarbons are known to be more persistent in the environment compared to straight chain hydrocarbons (Britton 1984; Salanitro 2001).

The first step in ester biodegradation is the hydrolysis of the ester by extra-cellular esterase enzymes, resulting in the formation of an alcohol and an organic acid compound. The degradation of the resultant alcohol and acid should proceed relatively rapidly even under anaerobic conditions since metabolism of both moieties is not expected to require molecular oxygen. Both components appear to be readily biodegraded in almost all of the esters tested. In the case of 2-ethylhexyl acetate, the products of hydrolysis would be 2-ethylhexanol and acetic acid. Acetic acid would represent 20% of the carbon, and the complete mineralization of acetic acid should result in 20% of the expected total gas production. The fact that less than 10% of the expected gas production was recorded from 2-ethylhexyl acetate biodegradation may indicate that the accumulation of 2-ethylhexanol could have inhibited microbial activity in the sediment. This is an important finding since the 2-ethylhexanol is used in the majority of commercial esters (Cognis 2005). It also explains why the testing of some commercial esters produces some odd results. If the sediment used in the test had high levels of esterase, the ester would be hydrolyzed quickly releasing the alcohol faster than it can be metabolized, thus causing it to accumulate to toxic levels, shutting down further metabolism of the SBF. A peak corresponding to 2-ethylhexanol was observed in the GC-FID analysis of the time final samples of the sediment spiked with the ethylhexyl acetate.

In general, these results highlight the usefulness of the CBT to evaluate the relative anaerobic biodegradation of a variety of compounds and SBF products. It should be emphasized that the CBT is a tool for determining the relative biodegradability of SBF. The CBT does not reflect conditions in the sea floor where cuttings are discharged, where the sediment would be under hydrostatic pressure from the column of water above the sea floor and temperatures would be less than 4 °C. Examination of SBF biodegradation using deep sea sediments under conditions that simulate the sea floor environment is currently in progress in our lab.

The overall recommendation from this study would be that esters used for SBF and intended for discharge to the sea floor on cuttings should have between 12 and 18 carbons in total length and not contain branched chain alcohols (or branched acids by inference).

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References

Battersby NS & Wilson V (1988) Evaluation of a serum bottle technique for assessing the anaerobic biodegradability of organic chemicals under methanogenic conditions. Chemosphere 17: 2441–2460

Britton LN (1984) Microbial degradation of aliphatic hydrocarbons. In: Gibson DT (Ed) Microbial Degradation of Organic Compounds, (pp 89–129). Marcel Dekker, NY

Candler JE, Lee B, Rabke SP, Getliff JM, Stauffer R & Hein J (2000) Modification of a standard anaerobic biodegradation test to discriminate performance of various non-aqueous base fluids. SPE International 2000. SPE 61203

Colleran E, Concannon F, Golden T, Geoghegoan F, Crumlish B, Killilea E, Hemry M & Coates JD (1992) Use of methanogenic activity tests to characterize anaerobic sludges, screen for anaerobic biodegradability, and determine toxicity thresholds against individual trophic groups and species. Water Sci. Technol. 25: 31–40

Cognis (2005) Oilfield chemicals–original ester EQ quality. http://www.cognis.com/oilfield/pdfs/brochure_oilfieldchemicals.pdf

Federal Register (2004) Final NPDES permit for new and existing sources and new dischargers in the offshore subcategory of the oil and gas extraction category for the western portion of the OCS of the Gulf of Mexico. pp. 60150–60151

Herman D & Roberts DJ (2005) A marine anaerobic biodegradation test applied to the biodegradation of synthetic drilling mud base fluids. Soil Sediment Contam. 14: 433–447

Owen WF, Stuckley DC, Healy JBJ, Young LY & McCarty PL (1979) Bioassay for monitoring methane potential and anaerobic toxicity. Water Res. 13: 485–492

Roberts DJ (2002) Methods for assessing anaerobic biodegradation potential. In: Hurst CJ, Crawford RL, Knudsen GR, McInerney MJ & Stetzenbach (Eds) Manual of Environmental Microbiology, (pp 1008–1017). 2nd edition, ASM Press, Washington, D.C

Salanitro JP (2001) Bioremediation of petroleum hydrocarbons in soil. Advances in Agronomy 52: 53–105

Shelton DR & Tiedje JM (1984) General methods for determining anaerobic biodegradation potential. Appl. Environ. Microbiol. 47: 850–857

Symons GE & Buswell AM (1933) The methane fermentation of carbohydrates. J. Am. Chem Soc. 55: 2028–2036