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The Use of an Anaerobic Closed Bottle Test to Determine the Relative Biodegradability of Synthetic Base Fluids.

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The current demand for energy in the US underscores the need to utilize oil reserves from the Gulf of Mexico. An important aspect of deep-sea drilling is the environmental impact of rock cuttings contaminated with synthetic base fluids (SBF) that are discharged into the Gulf. SBF can be composed of vegetable esters, low viscosity esters, or unsaturated hydrocarbons including linear-alpha olefins or linear-internal olefins. They are a component of drilling fluid and are needed to cool and lubricate the drill bit and to help bring rock cuttings to the surface. At the surface, the cuttings, contaminated with residual SBF, are separated from the drilling fluids and discharged into the marine environment. In response to concern about the environmental impact of the disposal of SBF laden cuttings off-shore, the EPA has enacted rules requiring that all SBF must be proven biodegradable in marine sediment before they can be licensed for use in the Gulf (Federal Registry / Vol. 66, No.111, / Friday, June 8, 2001/Rules and Regulations/30807).

The American Petroleum Institute (API) and the EPA have chosen the Closed Bottle Test (Modified ISO 11734) to evaluate SBF biodegradability. The test reflects the biodegradability of SBF under anaerobic conditions that are prevalent at sites where large amounts of cuttings contaminated with SBF have been disposed of overboard. Although marine sediments may be aerobic initially, the deposition of large amounts of degradable organic carbon quickly caused a depletion of available oxygen necessitating that any further degradation occurs under anaerobic conditions. The Closed Bottle Test is similar to other anaerobic biodegradability tests (for review see Roberts, 2001) and is based on the correlation between anaerobic gas production and the biodegradation of SBF in marine sediment.

The Closed Bottle Test is designed to provide a reliable, reproducible, and relatively uncomplicated procedure for determining the relative biodegradability of SBF. Relative biodegradability is determined by comparing anaerobic biodegradation of SBF to surrogate compounds and to a C16C18 internal olefin. The test requires the use of three surrogate compounds which act as controls to validate the response of marine sediment to SBF. Ethyl oleate is a surrogate for ester-based SBF, and represents a positive control. The olefin-based surrogate is 1-hexadecene, and represents the intermediate control, while squalane, a complex branched paraffin, represents the negative control.

The Closed Bottle Test method provides a procedure whereby SBF or surrogate fluids are spiked into marine sediments (2000 mg carbon/kg sediment dry weight) collected along the Gulf of Mexico shoreline. The sediments chosen should have a water content less than 60% (w/w) or a wet to dry ratio of 2.5, a pore water salinity between 20-30 ppt, and should have a minimum organic matter content of 3% (w/w). Spiked and unamended (control) sediments are mixed into a slurry using synthetic sea water (Forty Fathoms) and then transferred into 125 mL serum bottles. Each serum bottle will contain 30 g of sediment (dry weight) with sufficient sea water for a final volume of 75 mL. The air in the headspace of the bottles is removed by sparging with nitrogen gas, and the bottles are sealed airtight using butyl rubber septa and aluminum crimp seals. The bottles are incubated at $29 \pm 1^{o}C$ in the dark.

At bimonthly intervals, total anaerobic gas (carbon dioxide and methane) production in each bottle is determined using a pressure transducer. Headspace pressure (PSI) is converted to mL of gas volume using a standard curve. Methane gas production can also be monitored using gas chromatography. Prior to gas sampling the bottles should be brought to room temperature, and the barometric pressure and room temperature should be recorded. Gas production by the sediment is expressed in terms of the volume (mL) of gas at standard temperature ($0^{o}C = 273^{o}K$) and pressure (1 atm = 30 inches of Hg). Anaerobic gas production in SBF- or surrogate-spiked sediments is corrected for gas production in the control (unamended) sediment.

Standardizing the amount of sediment in each serum bottle (30 g dry sediment) and the amount of SBF or surrogate compound (2000 mg carbon/kg dry sediment) results in the addition of 60 mg of carbon substrate to each bottle. Based on the theory that anaerobic microorganisms will convert 1 mole of carbon substrate into 1 mole of total anaerobic gas production, the total moles of anaerobic gas production can be calculated, and then converted into a volume (112 mL) of gas. Anaerobic gas production from each bottle can then be expressed as the percentage of predicted total anaerobic gas production. The amount of predicted methane gas production can be calculated separately from carbon dioxide production using Buswell’s equation (Symons and Buswell, 1933).

In order for a particular SBF to pass the Closed Bottle Test, the biodegradation of the SBF, as indicated by the total anaerobic gas generated once gas production has reached a plateau, or at the end of 270 days, which ever is first, must be greater than or equal to the volume of gas produced by the C16C18 type internal olefin.

The method for evaluating the data to determine whether a fluid has passed the biodegradation test uses the equations:

$$BRR = X_{IO}/(X_T+K)$$

BRR = Biodegradation Rate Ratio
X_{IO} = Mean per cent theoretical gas production of the IO1618 standard
X_T = Mean per cent theoretical gas production of the test material
K = Kinetic constant for the historic 95% confidence limits of compounds that biodegrade comparable to IOs. This value is currently under development but has been tentatively set at 4.

If the BRR $\leq$ 1; then the test fluid passes the biodegradation test.

The amount of SBF or surrogate compounds spiked into the sediment is determined at the beginning and end of the incubation period. The procedure involves drying an aliquot (approximately 20 g) of spiked sediment with sodium sulfate, extracting the sediment with dichloromethane, and analysis using GC-FID. Internal standards, typically hexamethylbenezene and pentacosane (C25), are used to quantify the amount of test fluid.

Figure 1 shows typical results from an anaerobic bottle test currently in progress. Ethyl oleate, the positive control and surrogate ester-based SBF, showed rapid biodegradation, as indicated by a plateau in anaerobic gas production after 60 days of incubation. Two of the ester-based commercial SBF products tested, ester1 and ester2, showed biodegradation rates similar to ethyl oleate, while a third ester product, ester3, had a slower rate of biodegradation. Headspace gas analysis for ethyl oleate, ester1, and ester2 was stopped after 185 days, and sediment extract analysis revealed the complete removal of these substrates from the sediment.

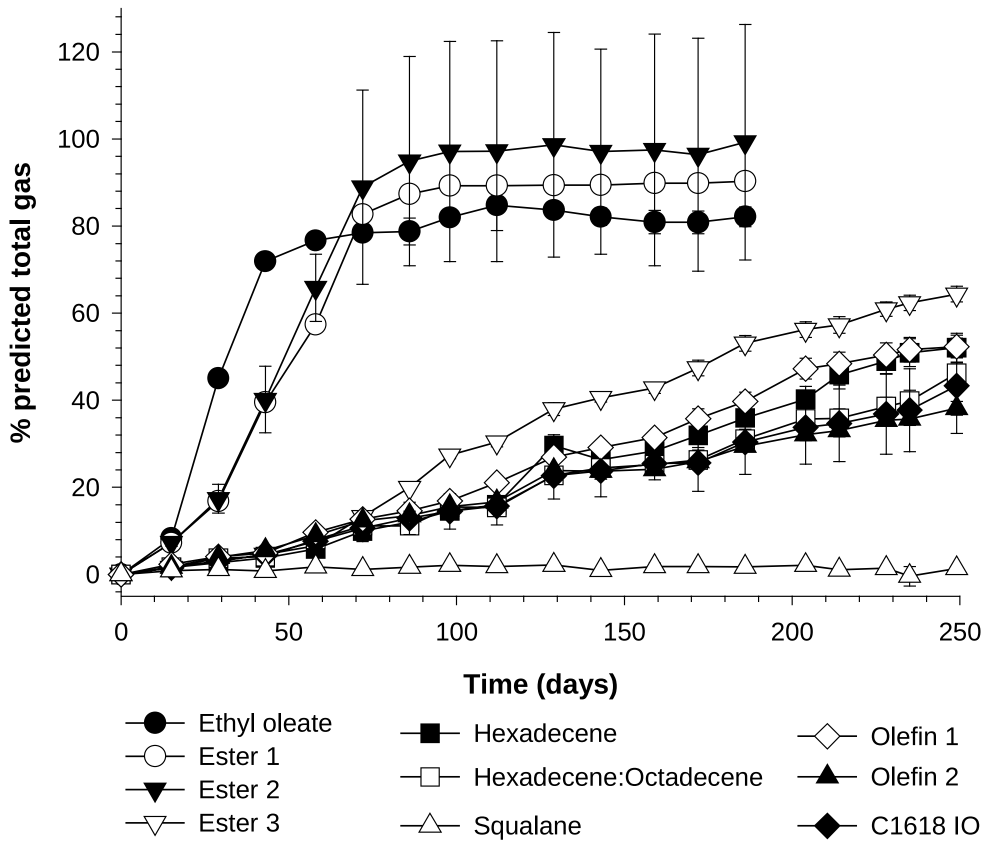

Figure 1. Total anaerobic gas production, expressed as percent of predicted gas production, for test compounds added at 2000 mg carbon/kg dry weight. Each point is the mean (± standard deviation) of 4 replicate bottles. Gas production was corrected for the level of gas production in the controls (unamended bottles).

There was no biodegradation of squalane, the negative control surrogate, as indicated by the absence of anaerobic gas production. The olefin-based SBF products, olefin1, olefin2, and C1618 IO, showed anaerobic biodegradation at rates similar to the intermediate surrogate hexadecene, and a mixture of hexadecene and octadecene (50:50). Gas production measurements will be continued for 270 days, and then the sediment will be extracted to determine the final test fluid concentration. Other tests performed by our lab have shown a 67% to 96% reduction in the concentration of olefins in the sediment.

Sediment properties have been shown to influence the rate of SBF biodegradation in Closed Bottle Testing. Fig. 2 compares the anaerobic gas production from hexadecene for 10 sediments collected from different locations along the Gulf of Mexico. Sediment will differ in both the rate of hexadecene biodegradation and in the amount of anaerobic gas produced. For example, Freeport sediment showed the fastest rate of anaerobic hexadecene biodegradation, while Sportsman's Road sediments showed the slowest rates. However, Sportsman's Road 2 sediment showed a greater amount of gas production than Sportsman's Road 1 and 1b sediments. The Closed Bottle Test accounts for the apparent variability between sediments by always including the testing of surrogate SBF compounds and the C16C18 type internal olefin in order to verify the relative rates of biodegradability of commercial SBF products.

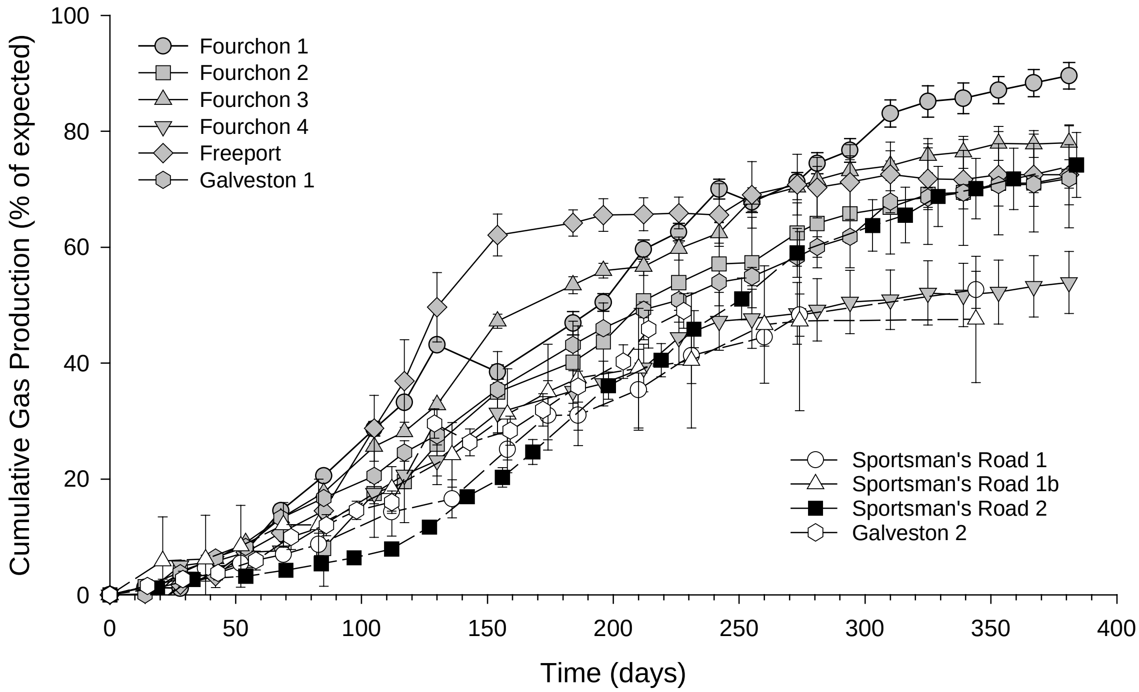

Figure 2. Total gas production, expressed as a percentage of predicted gas production, from 1-hexadecene spiked to a final concentration of 2000 mg carbon/kg dry sediment. Each point is the mean (± standard deviation) of 4 replicate bottles. Gas production was corrected for the level of gas production in the controls (unamended bottles).

One drawback to the Closed Bottle Test is the length of time required (9 months or longer) to complete the anaerobic biodegradation of olefin-based SBF. We have begun a process of identifying sediment parameters that are predictive of relatively rapid SBF biodegradation. There was no evident correlation between sediment organic matter content and the rate of SBF anaerobic biodegradation. For example, Freeport sediment, containing 3.3% volatile solids, showed a faster rate of hexadecene biodegradation than Fourchon#2 sediment, which contained 9.9% volatile solids. However, the results did indicate that sediments should contain at least 3% volatile solids in order to maintain relatively fast anaerobic biodegradation rates. Other factors, possibly microbiological parameters or prior exposure history of the site, may be important in predicting the anaerobic biodegradation potential of a sediment.

In conclusion, the Closed Bottle Test provides a reliable, reproducible, and relatively uncomplicated means to determine the relative biodegradability of SBF in anaerobic marine sediments. It should be recognized that the test does not replicate environmental conditions that exist in deep-sea sediments that receive SBF-contaminated cuttings discharged during oil drilling in the Gulf of Mexico.

References

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