Protocol DBSLOO5

Using the SRB-BART (laboratory) tester to test for SRB in oil

For the purposes of this test procedure oil may be defined as any fluid hydrocarbon that has a density low enough to float on water. More reactive fractions such as those present in gasoline may compromise the polystyrene walls of the tester but fractions of diesel fuel, motor oils and some of the lighter grades of crude may be tested using this technique for the presence of SRB.

This procedure uses the same procedure as DBSLWO5 but modified by the replacement of the water sample with 15mL of sterile phosphate buffer. The sequence for this test is:

1. Unscrew cap of the laboratory SRB-BART tester and place on a clean dry surface without turning over.
2. Aseptically pipette 15 mL of sterile phosphate buffer into the tester at which time the ball should float up to the fill line.
3. Dispense 0.1 mL of the oil under test aseptically over the interface between the ball and the wall of the tester. Here the oil should form a ring at the air-liquid interface.
4. Screw the cap back down firmly onto the tester and incubate with daily observations for reactions for 7 days.

There are four reactions types that need to be recorded as to the day at which they were first observed. These include the BT and BB reactions that are indicative of the presence of SRB. Additionally there is a ring of hazed plastic that forms above the air-liquid interface and gradually extends upwards if hydrocarbon degraders are present and degrading the hydrocarbons with the release of volatile daughter products that rise and react with the polystyrene to cause the haze to form. Between day 2 and day 7 there may also be a wool-like growth that forms across the base of the tester in the culturing fluids. This would mean that fungi are also active in the oil sample. These four reactions can be interpreted as follows:

BT, SRB are present as a part of the biofouling bacteria in the oil

BB, SRB are present but are dominant under reductive (anaerobic) conditions

Haze, indicates that is a significant level of aerobic bacterial breakdown of the hydrocarbons occurring in the sample.

Fungi, the presence of the wool-like growths generally in the base of the tester would indicate that aerobic degradation of the hydrocarbons was occurring with the fungi being involved