APB-BART Protocol DBASOPO6

Introduction

Acid producing bacteria (APB) are formed by a variety of heterotrophic bacteria that share the common ability to produce organic acidic products when growing under reductive conditions utilizing organics. These APB cause the pH to drop significantly from neutral to acidic conditions ranging from terminal pH levels from 3.5 to 5.5. These mildly acidic conditions are sufficiently corrosive to be significant to the integrity of any metallic structures being impacted. Because of these acid-producing activities in the absence of oxygen, it has been found that the APB are very likely to be significant partners in corrosion with the sulfate reducing bacteria (SRB) particularly in the oil and gas industry. As a result the management and control of corrosion frequently involves assessing the aggressivity of both the APB as well as the well-recognized SRB.

Corrosion and APB

As a result of industrial practices over the last century it has always been considered that microbially influenced corrosion (MIC) were dominated by the sulfate reducing bacteria (SRB) because of their ability to trigger electrolytic corrosion of metals. This event occurred primarily under highly reductive conditions in the presence of adequate sulfates and organics. In general the SRB generated hydrogen sulfide (H_2S) as a metabolic product and it was this that triggered the corrosive processes. While this corrosion was primarily electrolytic it was observed that some MIC was acidulytic and was caused by bacteria able to generate acid products generally under high organic and reductive conditions. Today it is now recognized that the APB are significant contributors to corrosive processes though the compromise to the metals through a gradual dissolution of the metal under the very acidic conditions that are created. In general the APB are found to be active under reductive conditions within biofilms, slimes, encrustations, nodules and tubercles. Their activity can sometimes be noted as a lateral erosion of the metal surface that can be most clearly seen the metal surface is examined using reflective light. Much of the APB is usually located at the metal – biomass interface. If present, the surface of the metal will appear to have an irregular pattern of shallow depressions. This would mean that the most effective examination of a sample for the presence of APB would be achieved by sampling the slime / concretion / encrustation / nodule / tubercle immediately at the interface between the growth and the metal surface. This is different to the electrolytic corrosion caused by SRB which tends to cause deeper pitting

of the metals and deeper cavities. In simple terms the APB generally cause broad impacts over much of the metal surface while the SRB cause focused forms of pitting and cavitation within the metal. APB always generates organic acids that do not commonly drop the pH below 2.0.

Conditions that would suggest APB as being a possible major factor includes the creation of very reductive conditions, presence of some form of growth over the surface (biocoating), and sufficient organic materials that could break down to fatty acids. This commonly highly porous biocoating over the surface of the corroding metal or biofouled concrete would allow the APB to become active deeper down and close to the surface of the metal. This creates a challenge in the detection of APB since they are frequently only present within these coatings and would be virtually absent from any waters or other fluids passing over the biocoating It is recommended that APB-BART tests should be undertaken on samples of the biocoating preferably at the metal to growth interface where the APB will be the most active.

Composting and APB

A second environment where APB can be very active is in compost where an organic rich highly porous medium is saturated with water. Under these circumstances, which commonly will become very reductive, the APB will become extremely active degrading the organic materials with the releases of fatty acids and the creation of acidic conditions. Here the APB is commonly distributed throughout the compost growing mostly in biofilms (slimes). Sampling for the presence of APB should concentrate on the compost that is saturated with water and not the regions that are semi-saturated where a broad band of microbial degradative activities will be occurring. Flowing oxidative waters are less likely to contain APB than static reductive waters but most of the activity is most likely to be within the compost or slime growths associated with the water draining from the compost (when this occurs).

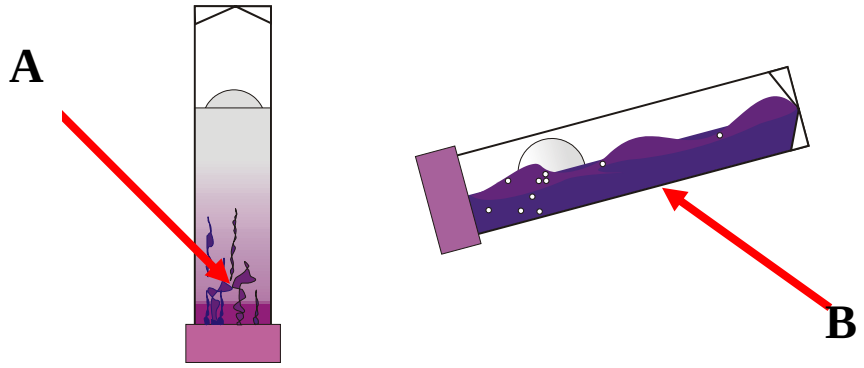
DBALSOPO6

Standard Operating Procedures for the APB-BART

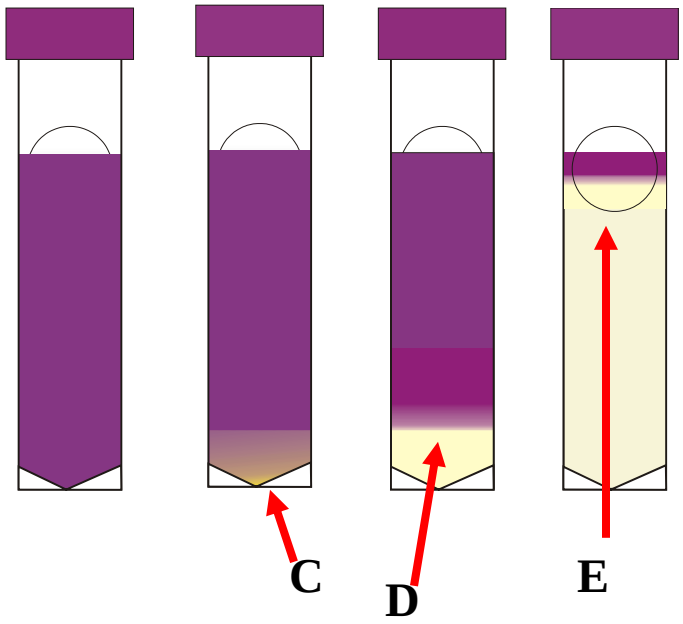
Standard operating instructions are applicable to all APB-BART tests with the minor exceptions laid down in DBALSO6 (when testing sludges and soils), DBALWO6 (when testing waters and leachates, and DBALOO6 (when testing oils and hydrocarbons). In using the APB-BART tester, the sequence of tests involves a number of steps including the addition of the sample (along with water if the sample is “dry”) and then inverting the tester in order to dissolve the pH indicator that is crystallized into the cap. This standard operating procedure is designed for use with samples of water or leachate and has to be modified for the examination of sludges, soils, oils and hydrocarbons.

For the testing of a water or leachate sample, the protocol calls for:

1. Remove the cap from the APB-BART lab tester and place the cap down on a clean dry surface without turning the cap over.
2. Add sufficient sample to reach the fill line indicating that 15mL of sample has been added.
3. Screw the cap back firmly on the tester.
4. Turn the tester upside down for 30 seconds. This allows the pH indicator that is dried on the inside of the cap time to dissolve and begin to diffuse up into the liquid (see A).
5. Shake the tester by a rotatory manner twice to ensure that the pH indicator is completely mixed with the sample under test (see B).



To conduct the test the tester is placed upright at room temperature and observed on a daily basis. If APB is present in the sample and become active then there will be a drop in the pH in the lower part of the tester (the more reductive region) and the color of the culturing fluid will shift from purple to a dirty yellow. Determination of a positive for APB is recognized when a yellow front begins to rise up from the floor of the tester (C). This should not be confused with the time early in the test when the crystallized medium is dissolving and there may be a small zone of dark yellow forming in the base of the tester (D). This color change to yellow indicates that the pH has dropped to less than 3.5 to 3.9 and it will progress over time until the bilk of the liquid in the tester has turned yellow (E). A time lag can be declared when there is clear signs that the yellow (acid) is moving up the tester (C).



Time lag to a positive recognized by the yellow fringe rising (D) should be taken as being that point when the yellow fringe moves upwards 15mm above the base of the APB-BART tester. This time lag can be used to predict the size of the population of APB in the sample using the table below if the tests were undertaken at room temperature.

Time Lag (days)	APB Population (p.a.c./mL)
1	251T
2	89T
3	15T
4	2T
5	422
6	126
7	53
8	27
9	12
10	1

Note: p.a.c. refers to predicted active cells and the letter “T” following a number indicates that this number is in thousands. Fuller interpretation can be accessed on QuickPop available at the web site www.dbi.ca.

DBALSO6

Testing for the presence of APB in sludges and soils.

This protocol follows the same format as DBHLSO6 although it is recommended that 0.5g of sample be used if it is a soil or a sludge and 1.0g of sample used in the case of

hardened deposits such as an encrustation. In common with that format two APB-BART testers would have to be used. One would have the ball rolled out following the protocol and the sample placed in the floor over the crystallized deposit (this one would be used for the test itself) while the other would employ 15mL of sterile distilled water (at a pH of 6.8 to 7.4). This water would be added to the second tester, capped, and then subjected to the standard protocol DBASOPO6 after which the water (now a purple color because of the pH indicator) would be transferred to the first tester (holding the sample). Once the water has been added then the ball can be dropped back into the tester and the cap screwed down to start the test.

Populations can be calculated using the table above but a correction factor would have to be applied since the sample was either 0.5 or 1.0g. For the soil or sludge the correction factor to be applied to the population generated from the time lag would be x30 while for the hardened deposit then the correction factor would be x150 and the final population would be p.a.c. APB/g.

DBALWO6

Testing for the presence of APB in waters, saline waters and leachates.

This protocol is essentially the standard protocol DBASOPO6 in which a 15mL sample of water or leachate is added directly to the APB-BART prior to the inversion and mixing to dissolve the pH indicator. The test starts immediately the mixing has been accomplished and would be considered positive for the detection of APB when condition D is observed (using the figure above). There is no need to apply a correction factor to the predicted population. It should be noted that if the water sample is colored or very cloudy then it may be necessary to dilute the sample tenfold with sterile distilled water in order that any reaction can be clearly recognized. Predicted populations would then have to be multiplied by a factor of x10 to correct for the dilution of the sample. Note also that if the sample is saline then the sterile distilled water should be amended with seawater salt to the same salinity as the original sample to assure a minimal impact on the APB in the sample.

DBALOO6

Testing for the presence of APB in oils and hydrocarbons.

This protocol follows the DBASOPO5 standards except that 15mL of sterile distilled water is used to mix the pH indicator in the tester. The oil or hydrocarbon sample is added over the floating ball once this has been done. For oils the normal volume to be added would be 0.1mL while for hydrocarbons it would be 0.5mL. Because of the lower density of these samples than water and also the fact that these samples are immiscible with water would mean that the sample would float at the interface between the liquid and the headspace around the floating ball. Once the cap has been screwed back down

after the admission of the sample then the test would have started and the time lag to a positive would be obtained when condition D occurs in the tester. Correction factors for converting the 0.1ml of oil or 0.5ml of hydrocarbon are x150 and x30 respectively with the population being predicted as p.a.c. APB/ml hydrocarbon.

Note that the APB-BART tester is constructed with polystyrene components that would make this test method unsuitable for gasoline and volatile hydrocarbons. For diesel fuels the integrity of the tester is limited to a seven day test before the vial becomes significantly damaged. All testers used for these tests should be disposed of quickly using acceptable laboratory practices.

Disposal methods

APB-BART testers, when charged with a sample and incubated, are likely to contain active bacterial populations whether the tester has gone positive for iron related bacteria or not. Such testers may be used for confirmatory tests in a certified microbiology laboratory but most would then be disposed off since they are single use disposable test methods. Disposal may vary with the location of the completed testers. In the laboratory setting, the testers should be placed in a biohazard bag which would then be sealed prior to steam or gas sterilization. Once sterilized then the testers do not present a health risk issue and should be disposed of with the regular laboratory solid wastes.

In the event that the testers have been used at too great a distance from a suitable certified microbiology laboratory or do not any arrangement to get the samples to such a laboratory then the testers do have the potential to contain active bacterial cultures. To eliminate the hygiene risks from these bacteria to the general society through disposal as domestic garbage, the testers need to be disinfected or pasteurized prior to final disposal. Recommended methods for this are listed below:

Disinfection of Used Testers. Take an 10.5” x 11.25”(27 x 28.5 cms) plastic freezer bag that has a zip lock that can securely open and close the bag. Open the bag and place six 8.5” x 11” (22.5 x 29 cms) sheets of household paper towel which have been folded along the longer side of the sheet to make a “v” shape fold. These sheets are placed in fold side down and opened so that there are six sheets on each side of the bag. Up to 9 field testers or 15 laboratory testers can be placed in the center of the bag lying on their side (make sure the outer caps have been screwed down tightly onto the outer vials). Once the testers have been added then 125ml of household bleach is poured into the bag. This bleach is soaked up by the paper towel. The bag is now sealed and can be disposed of with domestic garbage. Note that the normal function in trash collection includes compressing the garbage which would cause the plastic vials to fracture and leak. There is sufficient active disinfectant in the bleach to assure the disinfection of the contents so that the risks are no greater than for the rest of the domestic garbage.

Pasteurization of Used Testers. Heat can be employed to kill the bacteria that have grown in the tester. The recommended method involves the use of dedicated 800 to 1,000 watt microwave that would only be used for this purpose. To perform this treatment the initial steps are the same as given above for disinfection using a plastic freezer bag and placing the finished testers inside the bag but here there are two differences: (1) the testers are set up right; and (2) the caps are not screwed down tightly. The microwave should be activated for 50 seconds for up to 9 field testers or 65 seconds for up to 15 laboratory testers. This amount of heat would be sufficient to pasteurize the contents and cause sufficient distortion in the plastic vials to allow the contents to leak out and be absorbed by the paper towel. After the heat treatment then the sealed bags can be disposed with domestic garbage.