# Determination of the Corrosion and Plugging Risks in Fire Sprinkler Systems

# Perforation, Acidification and Plugging (PAP) risk protocols FSP 0808

**Prepared by**:
Roy Cullimore PhD Registered Microbiologist

***DROYCON BIOCONCEPTS INC***
315 Dewdney Avenue, Regina, Saskatchewan, Canada S4N 0E7
(306) 585 1762 Fax: (306) 585 3000
www.dbi.ca

August 2, 2008

# Determination of the Corrosion and Plugging Risks in Fire Sprinkler Systems

**(PAP risk)** **FSP 0808**

## Executive Summary

Fire sprinkler systems are designed to provide instant response to sudden combustive acts (e.g. flames, overheated equipment) by spraying water over the hazard to cool it down and put out any flames. This means the systems have to have an adequate reserve of water at pressures that can douse the flames and contain the fire with the least amount of damage. Sprinkler systems are constructed of steel pipe to take the additional stresses imparted by the high water pressures involved. Additionally the water is subject to make up from a storage tank that also ensures that there is an adequate amount of water to fight the fire successfully. Some water losses from the system are tolerated (typically 30 litres/minute) and pressure is sustained commonly at around 8bar. These environmental conditions do however provide opportunities for bacteria to grow within the pipes and in the storage tanks. This bacteria growth will first create biomass which would then impact the sprinkler system in two ways: (1) the steel walls of the pipe may corrode leading to leakages and structural failures in the piping system; and (2) the biomass may grow sufficiently to reduce the flow of water through the pipes leading in extreme cases to the pipes becoming completely plugged with biomass.

There are two basic sprinkler systems generally called “dry” or “wet”. Dry systems employ galvanised pipes while wet systems have the normal iron pipe painted black on the outside and prime coated on the inside. Dry systems begin having over-pressurised air at approximately 3 bars. When any one of the sprinklers pops to open then the air pressure is relieved and a pressure differential valve opens to fill the pipes with water. Because of the difficulty of creating slopes in the pipes some water will end up perching along the lines. While the dry systems would appear to have an initially low potential for biofouling, the movement of water through the system will end up causing water to pool at low spots and support microbiological influenced fouling and corrosion. In the wet system there is always some water pooled within the pipes and that can cause corrosion

(lower down in reductive conditions) and plugging (higher up in the oxidative conditions). Both sprinkler systems are prone to fouling but the form will be affected by the nature with which water pools in the pipes and sprinkler heads.

This protocol uses three of the BART testers to monitor for the occurrences of corrosion (either leading to site-specific perforation of the pipe; or lateral corrosion leading to increased porosity in the steel and leaking) and then plugging. The protocol involves the development of a PAP (perforation, acidulolytic and plugging) risk determination that can be applied on a routine basis (e.g. every two months) to the wet or dry sprinkler system of concern.

Additionally some attention is paid to the potential for the sprinkler systems to be leaking from perforations, porous pipes and failing seals on the sprinkler heads and creating bacteriologically rich aerosols that could cerate a potential health risk Three bacterial genera are recognized as being potential opportunistic pathogens that could be in the aerosol and be capable of affecting the health of aerosol-exposed people.

# Determination of the Corrosion and Plugging Risks in Fire Sprinkler Systems

**(PAP risk)** **FSP 0808**

## 1 Introduction

Fire sprinkler systems have the common property of holding water in line for release to fight fires in buildings. These systems commonly include steel piping to hold the resident water head with a back up water storage tank to provide an immediate supply of water in the event of a fire. Water is dispersed to the area at risk from the fire by a sprinkler system. In general the water is held under pressure in the pipes and is recycled through the system (wet) or water enters the system after a sprinkler has popped and then pools at low points within the system (dry). Losses in water circulating through the system are primarily from evaporation from storage tanks but this can be supplemented with leakages of water through the pipes caused by corrosion. Essentially the water moves from a low surface area: volume (SA: V) ratio in the storage systems that are oxidative to a high SA: V ratio in the piping that are reductive. This provides two distinct basic environments within which biofouling can occur. A further environment is created by the pumps moving water through the system. Here one major effect would be one of transient compression and turbulence as the water moves through the wet system. Dry systems are more likely to included zones where water has pooled at low points and it would be here that major biofouling could occur.

In the sprinkler systems there would commonly be present two distinct environments (oxidative low SA: R; and reductive high SA: V). In general the first fouling of wet or dry systems during operation would be the result of bacteriological activity. Here there is a high probability of bacteriologically influenced biomass would be most intense at the oxidative – reductive interfaces (redox fronts) where these occurs[12]. Generally in groundwater situations about 80% of the biomass and most of the biofouling problems concentrate at the redox front. In fire fighting systems it may be expected that similar microbiological growth concentrations would occur at these redox fronts as occurs in water wells[21]. The net effects of this biomass concentrating at the redox front can be summarised with the generation of biomass on the oxidative side (plugging), and the

generation of corrosive activities on the reductive side of the front[8]. Both of these effects can materially impact the efficiency of a fire fighting sprinkler system. Biomass growth on the oxidative side could lead to plugging of the pipes in much the same way as it occurs in water wells[23]. Plugging will reduce the ability of the pipe to transmit water at critical times of emergency demand through the generation of biofilms on the surfaces (reducing the conductive characteristics of the pipe) and through the interception of flow with a biomass that then prevents any free flow along the impacted section of the pipe.

Microbiologically influence corrosion (MIC) commonly is associated with reductive conditions at, or close to, the pipe walls. There are two recognized MIC events that are common in these reductive conditions: (1) generation of hydrogen sulphide by sulfate reducing bacteria (SRB)[11] commonly leading to a radical focussed pitting of the steel; and (2) generation of acidic conditions primarily by acid producing fermenting bacteria (APB) that lowers the pH into the range of 3.0 to 5.0 where lateral corrosion of the steel can occur. Most of the studies conducted on steel corrosion are confidential to the client and so cannot be discussed. However researches on the steels present on the *RMS Titanic* are being subjected to both pitting and lateral generalized corrosion[6]. Both through consulting and the studies of sunken steel fabricated ship wrecks it has been found that the pitting form of perforation is more commonly associated with the SRB while the lateral forms flaking types of corrosion is associated with the APB[18].

Fundamentally the variety of biofouling that can occur in fire sprinkler system can be considered to closely parallel the types of microbial activity causing plugging, clogging, corrosion and losses in water quality in water wells[9, 22, 23]. Essentially the critical connections between biofouling and the fire sprinkler system relate to interactions between steel surfaces to water. Extensive researches have been conducted on sunken steel fabricated ships from the RMS Titanic[15, 16,19,20,28] and in the Gulf of Mexico, Mediterranean Sea and North Atlantic[33]. In all of those cases steel test platforms where placed on the wrecks and the rates of corrosion and biomass generation are being

monitored. These studies led to the development of a new concept of the manner in which bacteria function at the water: steel interfaces using the *RMS Titanic* information as the prime example[7].

## 2 Biofouling of Steels

From research on the biofouling of steel it became evident that the bacteriologically influenced biomass incorporated a number of distinct communities that appeared to have unique and potentially definable biochemical structures[4]. While it has been accepted since the 1980s that biofilms were commonly formed on surfaces including steels, the role of bacteria in the formation of ferric rich deposits (commonly referred as ochres) was not clearly understood until a basic definition of iron related bacteria was developed in 1978[27]. From these various studies it became clear that oxidative conditions tended to create orange-red to brown ferric rich biomass in the presence of iron while reductive conditions created grey and black iron sulfide rich slimes and encrustations. This was confirmed in the laboratory using ochre mesocosm apparati[32] and in the field[30, 31]. From these studies it was determined that bacteria were intimately involved in the formations of slimes, nodules, tubercles, encrustations, floating biocolloidal particulates as well as in the generation of gases. Various groups of bacteria were found to be precursors to the occurrence of corrosive MIC events, plugging and even clogging. This led to the development of a new bacterial classification system[10] in which the bacterial genera were displayed within a two dimensional atlas.

## 3 BART testing, history

Directions for investigations were initially focussed towards the diagnosis and rehabilitation of biofouling water wells[13, 29] and the development of suitable bacteriological testing systems that would declare not only the activity levels for specific bacterial communities but also which ones were present. This was addressed in 1986 with

the development and subsequent patenting of the biological activity reaction test (BART™) and reported as being effective in 1990[21, 24]. Initially the BART testers were used to determine the risk of biofouling in water wells and the impact of rehabilitative treatments on the production characteristics of the well[1, 2, and 3]. In 2000 the potential to use BART testers were explored in novel manners for the detection of other biofouling-related problems in water was addressed[17]. Here claims were developed around the clear advantages of the BART testers over the more conventional test methods that primarily utilised agar culture media that proved to restrict the bacteria recognized to only those that would form colonies or generate distinctive colour reactions on, or within, the cultural agar medium being applied. Comparison of BART testers was described in 2005[e] with the various other standard microbiological methods.

Concepts used in the BART tester that allowed it to be very sensitive and accurate was examined [f] and the major advantages primarily relating to the generation of an oxidation-reduction gradient during the test, the gradual diffusion of the selective culture media into the liquid phase and the utilization of a floating BART ball to stabilise the lateral environments within the sample being tested. Of all of the factors one of the most important is the composition of the selective cultural medium that diffuses up the BART during test. This is described [g] and allows the effective investigation of various bacterial communities within a common water sample. Comparisons between time lapse and reaction data for the HAB-BART system was compared to the colony forming units for three strains of American type culture collection (ATCC) strains of bacteria and good statistical correlations were reported[5].

Manufacture of the BART testers began in 1990 and since that time 1.7 million BART testers have been sold primarily into the water industry with secondary sales in the chemical, gas and oil industries. In 2001 Droycon Bioconcepts Inc became registered as an ISO 9001:2000 certified company and all stages of the manufacture were included to assure effective quality management. General information on the BART testers is

periodically updated and the last update was in 2006 with the advisory DBIMANG06 providing general information. Three BART testers have been through the Canadian Environmental Technology Verification in 2002 for the IRB-BART [m] and the SRB-BART [q]. In 2004 the HAB-BART system was also taken through and approved using the Environmental Technology Verification [k] and the system was described [j] which included the first electronic reading system for the reactions. Critical in the establishment of precision is the interpretation of the time lapse (in seconds, hours or days) to the recognition of a reaction. This gives a direct evaluation of the population primarily as predicted active cells per ml and equivalent to the traditional colony forming units. These relationships were updated in 2006 for the bulk of the BART testers and the error incorporated when only daily readings were performed [n]. Over the last five years there has been developed a BART-SOFT program now in version 6.08 [f] which allows the automatic interpretation and archiving of entered data for later evaluation using Excel spread sheets and .rtf files formats.

## 4 Standard Protocols

Standard protocols exist for all three of the BART tester products recommended for the determination of the primary PAP risk analysis. Latest protocols for the BART testers that may be of interest to the fire sprinkler system maintenance are listed as: (1) APB-BART protocol DBASOP06; (2) DN-BART protocol DBDNSOP06; (3) HAB-BART protocol DBHSOP06; (4) IRB-BART protocol DBISOP06; (5) SLYM-BART protocol DBLSOP06; and SRB-BART protocol DBSSOP06. These protocols commonly include various alternate scenarios to allow the evaluation of soils, encrustations, scales and various forms of solids.

## 5 Relationship of BART tester technologies to the monitoring of fire sprinkler systems

Fire sprinkler systems commonly employ a coated steel pipe for holding the water at a dead pressure of 3 to 8 bars. Integrity of the industrial sprinkler systems is determined and the alarm sounded when there is a loss by covert or direct demand discharge losses exceeding 30 litres / min. The setting of this alarm threshold at 30 litres/min does not take into account lower levels of covert water losses that could be associated with evaporation (from the storage tanks), initial point source bleeding of water through MIC pits in the steel pipe (primarily from the distribution system), more generalised bleeding of water through sections of the steel pipes made more porous by lateral forms of corrosion (primarily in the distribution system), and leakages through the sprinklers as a result of physical failures of the seals accelerated by microbiologically influence fouling (MIF). All four types of covert losses from the sprinkler system would not trigger the alarm until the total demand created by the covert “bleeding” exceeded the tolerance and triggered alarm set at 30litres/min. Such covert losses of water could be from a combination of the above events assuming that the sprinkler system does not have any mechanically induced failures from improper fitting to physical stresses on the lines. If the water loss is from any of the above sources then water from the sprinkler system is likely to enter into protected environment in the forms dripping water or an aerosol. Dripping water is most likely to arise from a specific site that has become perforated by some forms of pit that has either passed through, or around the steel. Where a section of the steel pipe has become more porous due MIC events then the leaking water might escape from a longer section of the pipe but at levels that do not create dripping but keeps the pipe damp. Here both cases this water in escaping from the sprinkler system now becomes involved in the formation of aerosols. These aerosols would be supporting the survival of the various micro-organisms that have essentially escaped from containment in the pipes. These microbes would include not only the organisms directly involved in the MIC and MIF events but also other microbes able to survive under the environmental conditions in the

sprinkler system (e.g. *Legionella*). To the integrity of the sprinkler system, microbiological challenges may be summarised as either MIC or MIF events. Here the MIC events generally attack the steel containment system causing pitting/perforation or porosity in the steel walls of the pipe. MIF on the other hand causes problems through the generation of biomass that can be at specific sites causes plugging or more generally located along the length of the pipe causing losses in transmissivity.

## 6 MIC events likely to occur in steel sprinkler systems

MIC may cause pitting leading to perforation of the steel pipe wall or it can be associated with lateral losses in the structural integrity of the pipe with associated increases in the porosity of the steel wall. In general the pitting perforation phenomenon is considered to be primarily a result of the activity of sulfate reducing bacteria (SRB) that generate hydrogen sulfide from sulfate and it is this gas that causes an electrolytic form of corrosion. This form of corrosion will cause the steel in the wall to literally dissolve into soluble products leading to pitting and then perforation of the steel wall and loss of integrity (leaking). Hydrogen sulfide can also be generated by many proteolytic bacteria under reductive from organics including the sulfur amino acids. SRB generally dominate in waters that have a low organic content (e.g. < 5 mg/l TOC) while the proteolytic bacteria can become dominant when the water has a higher organic content (e.g. >10 mg/l TOC). Generation of hydrogen sulfide is likely to generate black iron sulfide daughter products that may appear in the biofilms coating the walls of the pipe. Pitting leading to perforation is likely to be as a result of the generation of hydrogen sulfide from one of these two groups of bacteria.

Wet and dry sprinkler systems are likely to develop different patterns of bacteriologically influenced fouling. Dry systems would imply that the internal voids in the pie are free of water and this therefore would reduce the potential for the growth of bacterial biomass in the form of ochres, slimes, nodules and other recognized growths. Plate one was taken

from a pipe in a dry sprinkler system and shows extensive coatings of ochrous-like materials attached to the walls of the pipe.

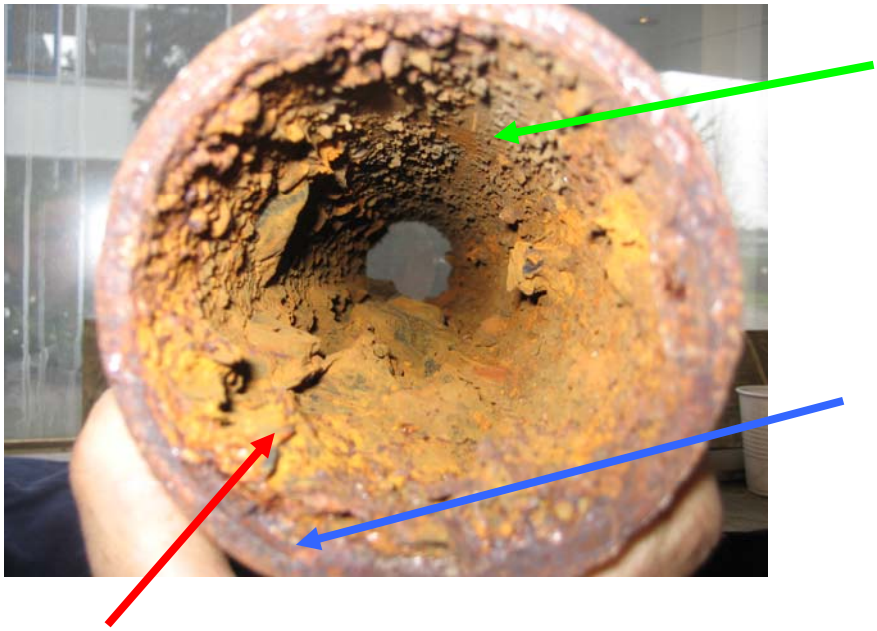

**Plate One, Cross section of a 2" pipe taken from a dry sprinkler system showing ochrous forms of growth**

Plate One shows most of the ochrous growths as thick ferric-rich biomass mainly on one side of the pipe (red arrow) while directly across from this biomass the pipe walls are still relatively free of any ochres (green arrow). Even though there are heavy ochrous growths on one side which is attached to the pipe's walls some of the pipe wall can be seen (blue arrow) indicating that the walls at that point had not been physically compromised to a major extent.

Dry systems begin life by being over-pressurised with air to approximately 3 to 8bars. However when any one of the sprinklers pops to open then this air pressure is relieved and a pressure differential valve opens to fill the pipes with water as a fir control mechanisms. Because of the difficulty of creating slopes in the pipes some water entrancing the pines will end up perching along the lines. The type of microbiological

activity that might be experienced in such a dead volume of water would more likely be associated with plugging events. Here the dominant bacteria are likely to be aerobic since there would also be perched pockets of air in the system as the movement of water through the system will end up causing water to pool at low spots and support microbiological influenced fouling and corrosion. Such pooled water would tend to be deficient in nutrients and the bacteria would turn towards the steel as the potential source of nutrients (particularly phosphorus and sulfur) together with iron which would be oxidized to various forms of ferric oxides and hydroxides. These chemicals would then be employed in the form of crystalline structures to provide protection to the developing bacterial growths that take on the form of ochres. In plate one the presence of the ochrous deposits on one side would suggest that this was the base of inside of the pipe and the ochres tended to collect there since they are relatively dense (1.2 to 1.8 is a typical range). The fact that the roof of the pipe is relatively free may be explained by two possible factors: (1) as ochre grew on the roof then its density caused it to fall off onto the floor of the pipe; and (2) the roof of the pipe was outside of the pooled water and so did not attract so many ochrous growths.

In the wet system there would always be water pooled within the pipes and that can cause corrosion (lower down in reductive conditions) and plugging (higher up in the oxidative conditions). It is possible that there would be an gas cavity above the water reducing contact between the water and the pipe walls at these places. Plate Two illustrates the types of biofouling that might occur in such a wet sprinkler system.

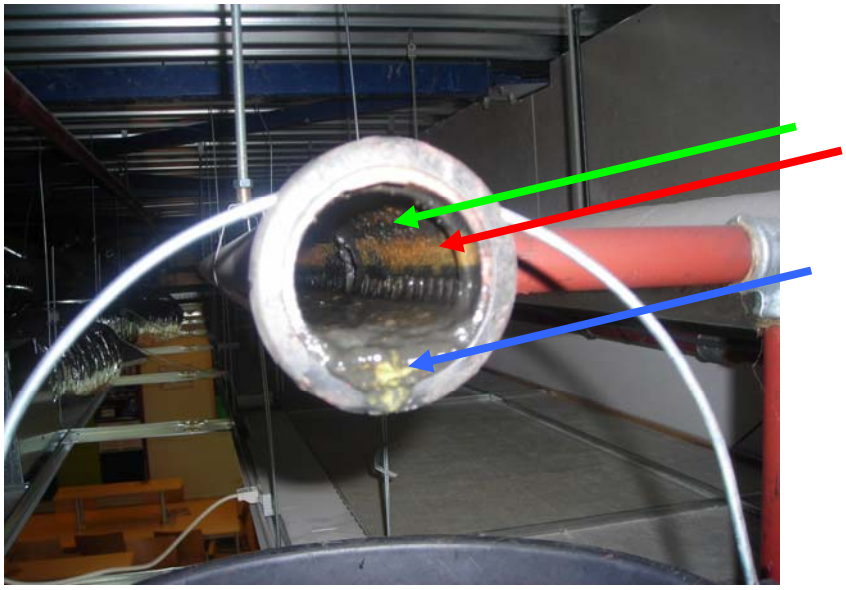

**Plate Two, Cross section of a pipe from a Wet Sprinkler System**

Plate two shows the cress section of pipe from a wet sprinkler system and there are many differences from the growths observed in the dry system. The most noticeable difference is that there are three clear zones of growth which imply that the pipe was not full of water but had a gas / air head. Uppermost on the inside wall of the pipe was a series of ferric-rich nodules that could have grown above the nominal water line but in the highly humid atmosphere, these growths (green arrow) appear to be all of a similar size and spread evenly along the upper arc of the pipe. Below these nodules is a zone that laterally extends along the walls of the pipe as a ferric-rich smear (red arrow) that relates to the changes in the height of the standing water present in the pipe. Essentially these ferric rich growths occur at the water – pipe wall – air interface that would changes slowly over time. In the bottom of the pipe (blue arrow) is a viscid clear slime that is gradually oozing out of the pipe. There are some white to yellow crystalline inclusions in the slime. This slime may be reflective of a biomass growing in the water and forming dense gels. It is possible that the water contained sufficient nutrients including organic carbon, phosphorus and nitrogen to support this biomass without the need for the bacteria to

extract nutrients from the steel pipe and lay down ferric rich ochres. The walls of the pipe appear to have a black coating that may be attributable to black iron sulfides in the biofilm.

Both wet and dry sprinkler systems provide unique closed environments in which different bacteria will thrive depending upon those conditions. All of these systems are not sterile and the water used provides an environment within which various nuisance bacteria can propagate causing plugging (such as the ochres in Plate One) or corrosion.

To detect these corrosion causing bacteria from perforated steel pipe or pits then the SRB-BART tester can be used. Here the true SRB (generating $H_2S$ from sulfates) is most likely to trigger a BB reaction (black base) in which the sulfide products are mostly accumulated on the floor of the tester as a jet black deposit. If the MIC leading to perforation involves both the SRB and proteolytic bacteria in the production of $H_2S$ then the reaction pattern in the tester is more likely to be a BT reaction (black top) where the jet black sulfides form around the BART ball floating in the tester. While these reactions (BB, BT) are the first reactions observed in the SRB-BART tester a secondary reaction can occur as the $H_2S$ continues to be produced and the whole of the contents of the tester turn black.

Pitting can also lead to (confidential consulting project) to perforations of steel in gasoline storage tanks was found by experimental testing to range from 5.3±3.8mm/yr to an outlier generating 10.6mm/yr in a strong electrical field. Similar rates of perforation could be expected in sprinkler pipes under extreme test conditions.

Lateral forms of MIC leading to a more generalised loss in the integrity of the steel walls leading to greater porosity in the steel is generally caused by the acid producing bacteria (APB). Here conditions have to be low in sulfur organics and sulfate (otherwise the SRB are likely to dominate) and have sufficient organics to form a biomass over the surface of

the steel. Once the biomass has formed then the reductive conditions follow within that biomass which allows fermentation to begin. Here the daughter products are commonly fatty acids that then cause the pH at the steel's surface to drop from 8.4±4 (in a typical functional biomass) to a pH range from 3.6 to 5.5. At these acidic pH values there can be a slow solublization of metals including iron that then allows the bacterial biomass to penetrate through the fissures deeper into the steel wall. This then allows the steel to begin to become porous as water is able to move through these corroded channels in the steel. For a number of steel fabricated ship wrecks, steel test platforms have been placed by DBI for later recovery. For the *RMS Titanic*, four test platforms have been recovered and the rates of lateral embrittlement of the steel coupons have been assessed. Here the rate of losses of iron from the steel is at 0.03±0.01gFe/sq.cm/yr. These are extreme environmental conditions with high salt and pressures with low temperatures and limited available nutrients for growth.

Under the conditions of an active biomass attached to the surface it has been found that commonly the APB dominate and here the APB-BART tester has been effectively employed. Detection of APB activity is through the generation of acid (fatty acid) daughter products. These are detected by using a pH indicator that shifts from blue-purple to a dirty yellow color when acidic conditions are generated in the tester. Generally (like the SRB) the APB commonly will occur deeper down within the biomass and may not be detected unless the biomass is disrupted to move these bacteria into the water. It is more probable that the APB and SRB will be detected in any drips, water films or aerosols generated from perforated or low porosity pipes.

**7 MIF events likely to occur in steel sprinkler systems**

The efficiency of a sprinkler system can also be affected by any biomass forming within the pipes. There are two principal effects: (1) biomass coating the inside wall of the pipe causing reduced transmissivity due to a smaller bore hole in the pipe (coating see also

Plate One); and (2) biomass forming across the pipe and blocking the free flow of water along the pipe (plugging). The type of biomass can be very variable depending upon the chemistry of the water under dead pressure in the pipes. These can range from scaling (high in carbonate); encrustations (high in ferric iron); nodules and carbuncles (capping that are high in ferric with the contents high in organics); to slimes (high in bound water and organics see Plate Two, blue arrow). There are two bacterial groups commonly associated with MIF events: iron related bacteria (IRB) that tend to dominate where ferric iron is accumulating (e.g. nodules, encrustations); and heterotrophic aerobic bacteria (HAB) that dominate particularly when the biomass is slime like.

For MIF events the chemistry of the water is particularly important. Higher organics in the water (whether natural or added) are likely to stimulate the growth of HAB. High pH values are likely to increase the potential for scaling while iron in the water from the natural source are likely to stimulate ferric deposits within the biomass. These ferric deposits would then also be generated from the iron being released from the steel pipe once MIC events begin to occur. Two BART testers are commonly used to detect these events: HAB-BART tester for pipes in which slimes and plugging are thought to be important factors; and IRB-BART tester for ferric rich problems. These two tester types will be discussed separately.

HAB-BART tester is a simple test in which bacterial activity is detected by a blue color being bleached and replaced with a dirty clouded clear or yellow solution. This blue color may disappear from the bottom up indicating that the bacteria are active in an oxidative environment and more likely to be associated with a plugging or slime event and possibly a scaling. If the blue color disappears from the top (just below the BART ball) down then this indicates a reductive environment and a greater probability for corrosion to be occurring.

IRB-BART tester is more complex in the manner that it generates reactions. It does commonly as first reactions generate either clouding (oxidative) or gassing (reductive) and it can also detect the risk of scaling. Here the base of the tester will turn white with carbonates commonly generated during the first twelve hours of the test (WB, white base). At this time the WB is not recognised as a bacteriologically influenced reaction but it is used in the water well industry as a marker for the types of chemical treatment that would be applied. Secondary reactions of significance to the biofouling of sprinkler systems are: (1) generation of brown cloudy, BC, and/or brown ring, BR that indicates oxidative conditions with encrustations and ferric rich scaling probable; (2) occurrence of brown gels, BG in the lower third of the tester that indicates potentially reductive biomass that would support APB-type activities; and (3) black all, BA which means that the tester contents have turned black and this means a greater potential for proteolytic bacteria to be actively generating $H_2S$ and generating pitting and perforation of the steel. For the routine PAP detection it is believed that the IRB-BART provides a very important back-up service to conform the presence of ferric rich ochres. Reaction of most significance here is the BR which is clearly generated during the latter stages of the testing. Both the BG and BC reactions can also be used to confirm the presence of ochres with the BA reaction indicating that a broad range of bacteria are active in the sample.

Another BART tester that has value is the SLYM-BART tester which is the most sensitive of the tests to be vast array of bacteria able to use organics. Here the tester signals a positive from the generation of cloudiness and it usually is the first tester to react with a positive detection.

## 8 Recommended Detection Protocols for the Detection of MIC and MIF events

Several key factors affect the recommended protocol. These will be dealt with separately below.

**8.1 Temperature**, it is commonly recommended that all BART tests be performed at room temperature (21±2$^{o}$C) as the incubation temperature of convenience rather than an optimal temperature. Optimal incubation temperature common for the bacteria associated with biofouling sprinkler systems is 28±1$^{o}$C. Ambient temperatures in sprinkler system set in the headspaces of rooms is probably within 5C$^{o}$ of that optimal temperature which means that faster culture-results would be obtained at the higher temperature (28±1$^{o}$C) rather than at the lower (21±2$^{o}$C) which is often more convenient. Where a sprinkler system is being operated at an artificially low or high temperature because of the environment being protected then incubation should be within 5C$^{o}$ of the average temperature experienced for the water in the sprinkler system. For example if the water in the sprinkler system was functioning at 3±2$^{o}$C then the optimal BART incubation temperatures would need to be within the range of -2 to +10$^{o}$C in order to assure cultural detection of the biofouling bacteria using the BART testers. In this particular example possibly the ideal temperature for testing would be in a refrigerator at 4±3$^{o}$C. Such a relationship is eligible for water sprinkler systems operating with water in the range of 0 to 60$^{o}$C. At lower temperatures than 0$^{o}$C then the testers may still function provided that the water has been treated to prevent freezing. The constraint here would be that the antifreeze is not toxic to the nuisance bacteria causing the corrosive biofouling events and it is quite possible that the bacteria will have adapted to the antifreeze and hence be less affected by its presence. Generally the lower limit for bacterial activity is considered to be -18$^{o}$C. At temperatures higher than 60$^{o}$C the polystyrenes used in the manufacture of the BART testers loose structural integrity and weaken.

**8.2 Time lapses** will vary when testing at temperatures other than room temperature (21±2$^{o}$C). Droycon Bioconcepts Inc have developed relationships between time lapse for the major incubation temperatures of 12±2$^{o}$C (for cooler environments); 28±2$^{o}$C (for warmer environments); and 37±2$^{o}$C (for tropical and blood heat environments) as well as the standard 21±2$^{o}$C (room temperature).

Generally the length of time that a BART tester should be monitored before it may be considered negative for significant bacterial activity varies with the incubation temperature being used. For cooler environments (12±2$^{o}$C optimal or down to 4±3$^{o}$C close to freezing) it is recommended that positive detection could still occur up to 20 days after the start of incubation. For warmer environments the temperature (28±2$^{o}$C) will usually yield any positive detection in 8 days after the start of incubation. For tropical and blood heat environments operating at 37±2$^{o}$C then any positive detection should occur within 5 days after the start of incubation. For the standard 21±2$^{o}$C at room temperature it is normal for detection to be complete by 10 days for most BART testers but the SRB-BART which should be incubated for a further five days (to 15 days) in order to detect low levels of the more covert SRB.

Where the operating temperature for the sprinkler system is over 60 $^{o}$C but is not under significant pressure (i.e. water is still liquid) then a different form of BART tester would have to be employed. These testers incorporate polypropylenes and glass to replace the polystyrenes.

**8.3 Sample storage prior to BART testing,** sample storage times prior to BART testing should be kept as short as possible since the bacteria within the sample are commonly in a state of shock. It is suggested that these samples follow the common protocol developed as Appendices H to K in the second edition of the Practical Manual of Groundwater Microbiology[12]. This would mean that the sample would be kept discrete, protected from evaporation, and cooled down to 4±3$^{o}$C if storage is to exceed for hours. For shorter

times then the sample may be kept as close as possible to the original ambient temperature of the sample.

## 9 Validation of Sample

This is actually a serious challenge since frequently the water in the sprinkler system may be relatively convenient to sample but it may contain a non-representative number of the bacteria being targeted and therefore have more limited significance. This would be because most of the bacterial biomass within the sprinkler systems would be parts of attached communities that may not be present in the waters pooled through the pipe. Endoscopic examination will physically show whether there is defined forms of biomass attached to the pipe walls (commonly in the form of biofilms, nodules, tubercles, encrustations, ochrous scaling); or the biomass may be floating in the water (as particulates commonly referred to as “snots” or “shit”) or creating a biomass dam in the water that then plugs up (restricts or prevents flow) the lines. Floating particulates within the water are probably the easiest to recover during sampling with the other forms of biomass becoming progressively more difficult based upon fragility and structural integrity, form of the surfaces, density of the bacterial populations within the biomass. In cases where it is desired to determine the nature of any pitting leading to corrosive perforation of the pipe, then it is important to take samples that would include the biomass that is supporting the corrosion process. This may be achieved by changing the environment in the pipe to be inspected. Commonly changing the environment in a radical manner (e.g. turning off the recirculation of the water for an extended period of time; applying a low level of non-toxic detergent or penetrant, taking the temperature of the pipe to be inspected up, or down, by at least $10^{o}$C). In all of these examples it can be expected that the bacteria active within the biomass will become traumatised and it is a common experienced that bacteria under these types of stresses will move out of the biomass into the water and then recovered with the sample.

## 10 BART Evaluation of Corrosion Risk in Sprinkler Systems

The BART tester offers a simple set of tools for monitoring and diagnosing the risk of corrosion events in sprinklers systems. For monitoring corrosion risk there are three types of BART testers that have a role to play and are very easy to recognize the reactions. In summary these are: (1) SRB-BART (for the detection of pitting and perforation in steel); (2) APB-BART (for the detection of more diffuse forms of lateral corrosion that can lead to the pipe becoming weaker and more porous); and (3) HAB-BART (for the detection of biomass size and activities linking to plugging).

Each of these three recommended BART testers generate in the case of a positive, both a time lapse (commonly in days) and a very clear single reaction signature (commonly as two letter acronyms). Time lapse gives an indication of the level of activity that was detected in a sample. In this case the shorter the time lag then the more active the bacteria are recovered in the sample. For the reaction signature (limited to one or two reaction types in the recommended BART testers, this can be used to determine the nature of the corrosion risk directly (in the case of the SRB- and APB- BART) and of plugging risk due to biomass generation (in the case of the HAB-BART). In most cases it would be expected that incubation would be at room temperature ($21\pm2^{o}$C) and the risk assessment below is established with that in mind. Note that IRB-BART is not included in the suite of recommended test since it is more associated with the maturing biomass under oxidative conditions where high ferric accumulation has occurred.

Because of the nature of the three recommended BART testers, the diagnosis of risk is taken on the basis of the time lapse and reaction signatures observed for each BART tester separately.

**11 SRB-BART testers**

This tester may function for as long as 15 days with two possible reactions (BB, BT) defined above. Table SRB.1 lists the relative corrosion risk using the time lapse (days) and reaction signature. Risk is generated from this table.

**Table SRB-1, Corrosion Risk Analysis based upon time lapse and reaction type**

| | Day 1 | Day 2 | Day 3 | Day 4 | Days 5 -8 | Days 9-10 | Days 11-15 |
|---|---|---|---|---|---|---|---|
| **BB** | 9 | 8 | 8 | 7 | 5 | 3 | 1 |
| **BT** | 9 | 9 | 9 | 6 | 4 | 2 | 1 |

Notes: Day number refers to the number of days preceding the observation of a positive reaction; reactions are shown as being either of the BB (black base) or BT (black top) forms; risk numbers are shown for each cell in the table but the risk relates to a different forms of corrosion; BB risks are defined to relate to a rapid perforation of the steel by $H_2S$ generated by the SRB principally from sulfates; BT may also be related to the rapid perforation of the steel but with the $H_2S$ being generated proteolytic bacteria; Corrosion risk analysis is shown in each cell as a single digit from 1 to 9 depending upon the (ascending) severity of the corrosion; only one digit may be used for the calculation of the corrosion risk with priority being given to the reaction that is first observed; In the event of both reactions (BB and BT) being observed then the risk assessment is based upon the first reaction observed. In the event that both reactions are observed on the same day then priority is given to the BB reaction for calculating the corrosion risk.

Calculation of the corrosion risk for pitting-induced perforation (PIP) of the steel pipe would consist of a single digit based upon the notes for table SRB-1. In the event of a BB reaction it may be expected that there would be a relatively low biomass involvement and the pitting would lead rapidly to perforation. Where BT reaction was first observed then

the type of pitting would be likely more extensive in association with the greater biomass involved in this type of event. Perforation would remain the most probable outcome but the biomass may act to restrict water leaking through the hole in the steel.

## 12 APB-BART testers

This tester may function for as long as 10 days with one possible reaction (DY, dirty yellow) defined above. Table APB-1 lists the relative corrosion risk using the time lapse (days) to the reaction signature (DY). Risk is generated from this table.

**Table APB-1, Corrosion Risk Analysis based upon time lapse and reaction type**

| | Day 1 | Day 2 | Day 3 | Day 4 | Days 5 -8 | Days 9-10 |
|---|---|---|---|---|---|---|
| **DY** | 9 | 9 | 8 | 6 | 3 | 1 |

Note: Day number refers to the number of days preceding the observation of a positive reaction; reactions are shown based on the development of acidic conditions through fermentation with fatty acids as the daughter products; risk numbers are shown for each cell in the table and relates to a different forms of acidulolytic corrosion. This type of corrosion would be more lateral and generalized and would more likely lead to a micro-fracturing of the steel pipe which would cause increases in porosity of the pipe wall.

Acidulolytic corrosion (AC) would have a more generalised effect on the steel but would not necessarily cause perforations before there was a general structural collapse in the pipe. This would be represented as a separate risk to the perforation risk.

## 13 HAB-BART testers

This tester may function for as long as 8 days with one of two possible reactions. Table HAB-1 lists the relative plugging risk (PR) using the time lapse (days) to the reaction signatures (UP, aerobic; DO, anaerobic). Risk is generated from this table for the risk of biomass generation that could lead to either biofilms forming on the walls and reducing flow rates or plugs that can prevent flow completely. With this biomass generation there is also a much greater potential for the expending biomass to cause radical changes to the water quality and also cause unacceptable dispersion of bacteria from the pipes through leaking sites particularly in the form of aerosols. Two bacterial genera that can be active in the pipe biomass and then pose health risks to people exposed to these aerosols are *Pseudomonas* and *Legionella*.

**Table HAB-1, Plugging Risk Analysis based upon time lapse and reaction type**

| | Day 1 | Day 2 | Day 3 | Day 4 | Days 5 | Days 6 | Days 7-8 |
|---|---|---|---|---|---|---|---|
| **UP** | 9 | 9 | 8 | 5 | 3 | 1 | 1 |
| **DO** | 9 | 7 | 5 | 5 | 4 | 4 | 1 |

Note: Day number refers to the number of days preceding the observation of a positive reaction; reactions are shown based on the development of reduction in the tester from the bottom up (UP) in the case of aerobic bacteria dominating the sample or from the top down (DO) in the event of anaerobic bacteria dominating; UP reactions are commonly associated with oxidative conditions that commonly means the free presence of oxygen in the water; DO reactions are associated with reductive conditions; only one reaction can occur in the HAB-BART tester; plugging risk (PR). In common with the PIP and AC corrosion assessments the PR generates scales of risk from 1 to 9 with the most severe risk having the higher numbers.

## 14 Sprinkler Corrosion and Plugging Risk Potential Determination.

Where the three BART tests are used for the determination of biofouling risks to the sprinkler systems then three numbers are generated. Pitting induced perforation (PIP) is generated from Table SRB-1 and gives and indication of the risk of pipe wall perforation from the activities of the sulfate reducing bacteria. Acidulolytic corrosion (AC) forms the second assessment using Table APB-1 and generates the risk of the pipe wall weakening and failing through losses in structural strength and gains in porosity. Plugging risk (PR) relates to the generation of biomass within the pipe in the water and is determined using Table HAB-1 which now gives a potential risk assessment for the pipe losing flow through the generation of biofilms and daughter products on the side walls of the tube.

To be effective in monitoring the biofouling risks to the sprinkler system then a risk analysis needs to be performed using the three BART testers (SRB-, APB-, and HAB-BART). Completion of the test will generate three numbers from 1 to 9 if a risk is seen and 0 where the tests are negative. In the determination of the biofouling risk then the three numbers are calculated from the tables and then presented as a perforation (PIP), acidulolytic (AC) and plugging (PR) risk. This would be summarised as the PAP (perforation, acidulolytic and plugging) risk and would be shown as three numbers separated by dashes (e.g. 5-9-0 would mean that the PAP risk was moderate for perforation, high for acidulolytic events, and no evidence for plugging). The example shown above would mean that there was a high probability that sprinkler system included pipe sections that were beginning to leak due to increased porosity in the steel. Table PAP -1 gives some examples of PAP generated risks.

## 15 Water Storage Tank Risks

One of the major factors affecting the potential for PAP risk events in a sprinkler line is the nutrient feedstock that may enter with the make-up water to the system. The greater the amount of make-up that is applied then the greater the probability that some form of biofouling will occur since biomass growth within the system (including the tank) is a reflection of the nutrients entering the system. Critical components in this make up water include (brackets display the typical range from minimal critical concentration to optimal for biomass generation given in mg/l): total organic carbon (1.0 to 20), total nitrogen (0.5 to 5); total phosphorus (0.1 to 2.0). Iron is another component that should be avoided in make up water with the critical concentrations that can cause ferric accumulation in the biomass under oxidative conditions being (minimal to optimal, mg/l) as total iron would be 0.1 to 5.

**Table PAP – 1, Examples of PAP risk interpretation**

| PAP | Interpretation |
|---|---|
| **0-0-0** | No detectable PAP risk |
| **9-9-9** | Severe PAP risk with sprinkler pipe failing a very high probability |
| **9-3-0** | Perforation of the pipes imminent due to SRB activity |
| **1-9-4** | Acidulolytic corrosion a significant threat with pipes becoming more porous |
| **1-3-9** | Plugging of the pipes occurring with reduced water flows |
| **3-5-4** | General moderate risk of corrosion through perforation or lateral pipe failures |

Where conditions inside the storage tank are oxidative then it can be expected that oxidative (aerobic) conditions will exist that could then lead to biomass generation within the tank. This may be seen by the water going cloudy (due to bacteria growing within the suspended colloidal particles) and then the wall of the tank developing slimes (biofilm)

coatings. Such growths may then cause high bacteriological loadings with the make-water and stimulate the PAP risk. It should be considered that the storage tank therefore forms a reservoir for the bacteria that will then enter the sprinkler system and increase the PAP risk. BART testing using the standard three BARTs used for the detection of PAP in the sprinkler system is recommended with the same generation of a PAP risk factor. It might be appropriate to include the IRB-BART if the make up water has a total iron content of greater than 0.5mg/l Fe since the IRB would now become an active part of the bacteriological community within the tank.

## 16 Aerosol Generations from Sprinkler Systems

As pipes being to leak from PIP events as the perforation penetrates the steel wall but "scabs" over with biomass, or in an AC event where the water moves through the porous steel, then some of the water moves out into the surrounding environment. Water can also be released into the environment from the sprinklers is the seals are beginning to fail due to aging and/or the growth of biomass within the seals. In all three cases (PIP, AC and through leaking sprinklers) will generate both water films and droplets that, through air movement, can enter the local atmosphere as aerosols (suspended water droplet). These droplets are likely to have a high bacteriological content made up of bacteria directly associated with the leakages but supplemented by any other bacteria that were present in the water but now moving out with the diverted water flows associated with the leakages.

Aerosols arising from leaking sprinkler systems are likely to contain not only the bacteria directly associated with the leaking event (e.g. PIP, AC or failed seals) but also from the bacteria that may be surviving or active within the sprinklers system and the storage tanks. Health risks can arise if those bacteria in the aerosol from any source have the potential to be pathogenic (see Figure 10.33 to 10.35[10]). Of the normal bacterial flora recovered from such systems the two genera most like to present a direct risk would be species of *Pseudomonas, Legionella* and potentially *Klebsiella.* Some species within

these genera are capable of establishing pulmonary infections through the hosts breathing in the bacteriologically laden aerosols from the sprinkler systems.

Pulmonary infections in staff operating within an environment known to be serviced by sprinkler systems that are beginning to fail (i.e. are creating a demand for up to 30litres/min of makes up water) may also be such health risks. Of these bacteria both of the genera *Pseudomonas* and *Klebsiella* may be a part of the normal bacterial communities associated with the growth of biofouling biomass using to plugging events. On some occasions particularly when the water is in the 35 to 55$^{o}$C range then species of *Legionella* can become major components in the biofouling and these species are capable of causing Legionnaires disease in humans.

# Determination of the Corrosion and Plugging Risks in Fire Sprinkler Systems

**(PAP risk)** **FSP 0808**

## A References


1) Alford, G. and Cullimore, D. R. (1999) The Applications of Heat and Chemicals in the Control of Biofouling Events in Wells, Monogram in the Sustainable Water Well Series (D. R. Cullimore, series editor) published by Lewis Publisher / CRC Press, Boca Raton. Florida 205 pages

2) Alford, G., Mansuy, N. and Cullimore, D.R. (1989) The Utilization of the Blended Chemical Heat Treatment (BCHT$^{TM}$) Process to Restore Production Capacities to Biofouled Water Wells. Proc. of the Third Outdoor Action Conference on Aquifer Restoration, Ground Water Monitoring and Geophysical Methods. Association of Ground Water Scientists and Engineers. Orlando, Florida. May, p. 229 – 237.

3) Cullimore D.R, Johnston, L.A and G. Alford (2007) Evaluation of the effectiveness of selected rehabilitation processes applied to wells impacted by microbially influenced biofouling, proceedings of the American Water Works Conference, Toronto, Canada

4) Cullimore D.R. (1990) Microbes in Civil Engineering Environments: Biofilms and Biofouling In Microbiology in Civil Engineering (P. Howsam, ed.) E & F.N.Spon, London ISBN 0 419 16730 1, p. 3 - 12.

5) Cullimore, D. Roy (2008) Preliminary Comparison of the Applicability of the HAB-BART system and the Agar Spreadplate Methods for the Quantification of Bacterial Populations in Selected Dilutions of Pure Cultures of Three Bacterial species. Journal of Environmental Micropaleontology, Microbiology, and Meiobenthology Volume 3 31 to 43

6) Cullimore, D. Roy and Johnston, Lori (2007) "Rusticles on the *RMS Titanic"* JMBA published in Global Marine Environment May 2007, pages 10 to 12.

7) Cullimore, D.R, Pellegrino, C and Lori Johnston (2001) RMS Titanic and the Emergence of New Concepts on Consortial Nature of Microbial Events; Rev Environ Contam Toxicol 173:117 – 141

8) Cullimore, D.R. (1990) An Evaluation of the Risk of Microbial Clogging and Corrosion in Boreholes In Water Wells Monitoring, Maintenance, Rehabilitation (P. Howsam, ed.) E & F.N.Spon, London ISBN 0 419 16750 1, p. 25 - 34.

9) Cullimore, D.R. (1993) Practical Manual of Groundwater Microbiology. Lewis Publishing, Chelsea, Michigan ISBN 0-87371-295-1, pp. 403. (it has been designated as a best seller by the publishers)

10) Cullimore, D.R. (2000) Practical Atlas for Bacterial Identification published by Lewis Publishers / CRC Press, Boca Raton, Florida. (it has been designated as a best seller by the publishers)

11) Cullimore, D.R. (2005) Sulphur Reducing Bacteria chapter 4 in Anonymous (1995) Problem Organisms in Water: Identification and Treatment (organized by Organisms in Water Committee), AWWA Manual M7, published by American Water Works Association, Denver, Colorado. 153 pages

12) Cullimore, D.R. (2008) Practical Manual of Groundwater Microbiology. Lewis Publishing, Chelsea, Michigan, pp. 590.

13) Cullimore, D.R. and Legault, T. (1997) Microbiological Investigations of Water Wells in the Municipal District of Kneehill, Alberta. Rural Water Development Program, PFRA, Canada Agriculture, p. 27.

14) Cullimore, D.R. and Lori Johnston (2008) "Microbiology of Concretions, Sediments and Mechanisms Influencing the Preservation of Submerged Archaeological Submerged Archaeological Artefacts" Int J Histor. Archeol. In press

15) Cullimore, D.R. and Lori Johnston, (1999). The Fate of the Iron: More Lessons from the Titanic Tragedy. Maritime Reporter and Engineering News, August Edition, 20 & 66pp.

16) Cullimore, D.R. and Lori Johnston, (2000). Biodeterioration of the *RMS Titanic*. Canadian Chemical News/ L'Actualite Chimique Canadienne (ACCN), Ottawa, Ontario, 52 (10) 14 - 15

17) Cullimore, D.R. and Lori Johnston, (2000). Microbial Issues in Water Using an Innovative Detection System. Water Conditioning & Purification Magazine. November Issue, 71-78pp.

18) Cullimore, D.R. and Lori Johnston, (1999). The Fate of the Iron: More Lessons from the Titanic Tragedy. Maritime Reporter and Engineering News, August Edition, 20 & 66pp.

19) Cullimore, D.R. and Lori Johnston. (2000). The Impact of Bioconcretious Structures (Rusticles) on the *RMS Titanic*: Implications to Maritime Steel Structures. Proceedings of the 2000 Annual Meeting of the Society of Naval Architects and Marine Engineers, Vancouver, Canada, 9-1 to 9-16pp.

20) Cullimore, D.R. and Lori Johnston. (2000). The Impact of Bioconcretious Structures (Rusticles) on the *RMS Titanic*: Implications to Maritime Steel Structures. Proceedings of the 2000 Annual Meeting of the Society of Naval Architects and Marine Engineers, Vancouver, Canada, 9-1 to 9-16pp.

21) Cullimore, D.R., Abiola, A.T., Mnushkin, M. and Reihl, J. (1990) Evaluation of the Potential Biofouling of Water Wells, Newcastle, New Brunswick Regina Water Research Institute Report # 64c, p. 45.

22) Cullimore, D.R., Lebeden, J. and Legault, T. (1997) Investigation of Bacterially Driven Biofouling of Water Wells in Central Alberta, Canada. In Proceedings of the AGWSE National Education Program "Biological Aspects of Ground Water" Las Vegas, September. pp. 39 - 40.

23) Cullimore, D.R., Legault, T, Keevil, B., and Alford, G. (1997) The Laboratory Synthesis of Iron Related Bacterial Clogging in Well Screen and Gravel Pack In Proceedings of the AGWSE National Education Program "Biological Aspects of Ground Water" Las Vegas, September, p. 33 -34.

24) Cullimore, D.R., Mansuy, N. and Alford, G. (1990) Studies on the utilization of a Floating Intercedent Device (FID) in the Biological Activity Test (BATS) to Identify the Presence and Activity of Various Bacterial Groups in Groundwater. Proc. First Int. Symp. on Microbiology of the Deep Subsurface, USDOE, Orlando, Fl., p. 55.

25) Cullimore, D.R., Pellegrino, C., and Lori Johnston,(2001). *RMS Titanic* and the Emergence of New Concepts on Consortial Nature of Microbial Events. Rev. Environ Contam. Toxicol 173:117-142.

26) Cullimore, Roy (1999) Microbiology of Well Biofouling, published by Lewis Publishers / CRC Press, Boca Raton, Florida. 435 pages

27) Cullimore, D. R., and McCann, A. E. (1978) The Identification, Cultivation and Control of Iron Bacteria in Ground Water, Publ. In Aquatic Microbiology (ed., Skinner, F.A. and J. M. Shewan), Academic Press, New York, p. 219 - 261.

28) Garzke, W.H., Brown, D.K., Matthias, P.K., Cullimore, R., Wood, D. Livingstone, D, Leighley, H.P., Foecke, T., and A. Sandiford (1997) <u>*Titanic*</u>, The Anatomy of a Disaster, A Report from the Marine Forensic Panel (SD-7) Proceedings of the 1997 Annual Meeting of the Society of Naval Architects and Marine Engineers, Ottawa, Canada.

29) Gilman G. Violette Roy Cullimore Patrick Wall (2006) Successful rehabilitation of sand and gravel wells, Wilmot Park Well field, Fredericton Aquifer, Fredericton, New Brunswick. Proceedings of the CANADIAN Water Works Association (Atlantic Region), Halifax

30) Mansuy, N., Nuzman, C. and Cullimore, D.R. (1990) Well Problem Identification and its Importance in Well Rehabilitation In Water Wells Monitoring, Maintenance, Rehabilitation (P. Howsam, ed.) E & F.N.Spon, London ISBN 0 419 16750 1, pp. 87 - 99

31) Mansuy, Neil (1999) Water Well Rehabilitation, a Practical Guide to Understanding Well Problems and Solutions (Sustainable Well Series editor, D. Roy Cullimore) Lewis Publishers & CRC Press, Baton Rouge, FL 174pp

32) Rowe, K., Hrapovic, L., Kosaric, N., Cullimore, D.R. and Quigley, R.M. (1994) A Laboratory Investigation into the Degradation of Dichloromethane, Report conducted for the Interim waste Authority Ltd., Ontario by Geotechnical Research Center, University of Western Ontario, pp. 145.

33) Church, R., Warren, D., Cullimore, R., Johnston, L., Schroeder, W., Patterson, W., Shirley, T., Kilgour, M., Morris, N., and J. Moore (2008) A Study of Living History Deep WWII Shipwrecks in the Gulf of Mexico, PAST Foundation Publications, Columbus, OH. Pp239

**B Technical Support Documents**

a) About the BART testers, general information DBIMANG06 December 20, 2006

b) APB-BART Protocol DBASOPO6 April 2, 2006

c) BART commentary from various users, June 7, 2006

d) BART-SOFT version 6.08 released June 2008

e) Comparison of BART™ Testers with Other Microbiological Tests August 20, 2005

f) Concept behind BART testers October 17, 2005

g) Confirmation of the Selective Media Composition for BART testers March 18, 2008

h) DN-BART Protocol DBDNSOP06 April 2, 2006

i) HAB-BART Protocol DBHSOP06 May 4, 2006

j) HAB-BART System™ FOR THE DETECTION OF HETEROTROPHIC AEROBIC

k) BACTERIA (HAB) Using the HAB-BART Tester™ and HAB-BART Reader™ Environmental Technology Verification document August 4, 2004 pages 23

l) IRB-BART Protocol DBISOP051 May 3 2005

m) IRB-BART™ TESTER FOR THE DETECTION AND EVALUATION OF IRON BACTERIA IN WATER Environmental Technology Verification document July 27, 2002 pages 45

n) Relationship of time lapse to bacterial populations created by daily observation of selected BART testers October 17, 2006

o) SLYM-BART Protocol DBLSOP05 March 9, 2005

p) SRB-BART Protocol DBSSOP06 May 5, 2006

q) SRB-BART™ TESTER FOR THE DETECTION AND EVALUATION OF SULFATE REDUCING BACTERIA IN WATER Environmental Technology Verification document July 27, 2002 pages 53