Chapter 4

Factors Affecting Bart Testing

4.1. Significant Factors that can affect the BART testing procedures

While the Bart testers can yield valuable information regarding the microbiological composition of the sample being tested, there are numbers of important considerations that need to be addressed.

4.2. Sampling

The success in analysis of any given water or soil sample is the dependency upon the validity of the manner in which the sample was taken and its environmental relationship to the site being investigated. Any water sample when taken is composed of suspended (planktonic), particulate (mainly in biocolloidal forms), along with sheered materials coming from attached bacterial biomass fouling surfaces, pores and fractures that are exposed to, or connected to, the site being sampled. This makes sampling a challenge since the sample could contain an uncertain mixture of “dissolved” floaters and slime forming (biocolloids) along with attached particulates that could affect precision. Here both floaters and detached biomass could significantly increase the predicted population count.

Sampling from a site where there is seemingly no disruption then it is probable, but not certain, that the majority of the bacteria detected certainly would have come from planktonic and biocolloidal sources that were not attached to surfaces. Since greater than 80% of the bacterial biomass is attached to surfaces then these would not be accounted for in the analysis of such a sample. To recover these attached bacteria then there has always to be some level of shock applied to the sampling site prior to taking the sample. Such a shock can be relatively benign (turning off the pumps for a day or dead ending the lines) or it can be aggressive leading to physically rupturing the biomass with subsequent severe short-term stress effects. It is desirable to trigger the releases of some of the attached bacteria in the biomass so that they then the water

phase from which these bacteria can then be detected using the E-tATP and Bart testers.

Porous and fractured media (e.g. soils, filter media, encrustations, sludges) present a different challenge since different bacteria will be active at different points within the environment. Major factors here would be the bound water content, charges on surfaces, size of pores and any fractures; and the availability of nutrient and toxic chemicals. These growths are therefore more challenging since any bacterial activities can be more tightly defined.

Assuming an acceptable sample has been taken then the tester provides a sensitive method for the detection of bacteria. It might be an advantage to examine for bacterial activity in the sample using the ATP technique (Section 4.13 and section 14). If little or no ATP is detected then there may be no value in applying Bart testing to such biologically "dead" samples.

Bart testers come in two formats that make them easy to be used in field or laboratory conditions. The major differences between the field and laboratory version of the tester is that the field tester has a second vial (bottle) that provides additional stability and protection to the tester when it is being transported and used in the field. Laboratory versions are more economical involving only the inner vial (bottle) and are designed to be tested in the laboratory setting using Bart racks to ensure they stay upright (section 15 gives details of the use of the visual Bart reader system, VBR, for this purpose. Field versions involve the second outer vial (bottle) to provide additional protection to the inner vial.

There are occasions when there is a need to take a water sample that would then be used to fill the inner vials (testers) while out in the field With the field testers there is the potential to use the outer vial (bottle) as the means of collecting the water sample for use in the testers. The inside contents of the field tester are sterile and so therefore, when removed, the outer vial remains effectively sterile and can be used to collect the water sample. To do this uses the following procedures using the field testers:

(1) Unscrew and remove the outer cap, remove the capped inner tester and place in the aluminum foil pouch from which the field tester was taken, and lay the outer cap down on a clean surface without turning it over;

(2) Screw the outer cap back onto the outer vial and it is now ready to be used for collecting the water sample;

(3) When collecting the water sample then remove the outer cap again and place back down on a clean surface;

(4) Add the water sample to the outer vial but do not fill beyond the fill line beneath the threads, this line denotes that 65ml of water has been added; and

(5) Put outer cap back on to the outer vial and screw down. Up to 65ml of water sample can be taken using one outer vial. This would be enough to charge four inner testers. It should be remembered that the water sample only remains valid if it has not been contaminated during collection. Therefore do not charge the outer vial in an environment that is dust laden and always handle the outer vial from the outside to avoid contaminating the inside of the sampling bottle. If sterile latex gloves are available then it is advantageous to handle the outer vial wearing thee gloves to further reduce the risk of contamination.

There are no chemicals added to the outer vial and so any chemicals present in the water (for example, chlorine) would not be neutralised. However all inner testers do include sodium thiosulfate in the chemical pellet so that any chlorine impacts on the bacteria in the sample are limited to that period of time before the samples are dispensed into the inner testers. It should be noted that all testers have to pass through a rigorous ISO 9001: 2000 certification process that includes sterility checks, the use of clean rooms to minimise contamination and full quality management procedures to ensure that the products meet all claims. All sampling procedures need to be followed in both the taking and the subsequent storage of the water sample prior to starting the testers. See Chapter 4.6 for more details on the storage of water samples if there is some delay before starting the tests. Read the “Certificate of Analysis” which accompanies every box of testers for the protocol to set up the test, for more details.

4.3. Oxidation-Reduction Potential (ORP) implications from BART tester reactions

The ORP generated in the Bart tester is a combination of the values found in the sample and the influence that this now has on the charges associated with the different reactions (Table 4.3.1.). Note that the ORP ranges (in millivolts, mv) are given (right hand column) for the established reaction (center column) generated from the BART tester (left hand column). These are presented for each reaction is the common range observed and it has to be remembered that the reaction being observed in the active biomass will involve gradients. Hence the ORP values are shown as ranges. ORP is an expression of the electrical charges within the sample expressed in millivolts (mV). Oxidative conditions have a positive mV while reductive conditions are negative. Essentially oxygen is present in +mV and hydrogen in –mV as an expression of the electrolysis of water within that environment. Remember there can be significant ORP gradients within that biomass that may affect the bacterial activities. Note that in Table 4.3.1. ORP ranges are given in millivolts, mV) are given in the eight columns to the right while the type of tester is in the first column to the left and the reaction code in the second column from the left. Each tester is shown within a thick black border. Ranges within which particular reaction codes commonly occur are shown black indicating that these ORP values (see top row for designations) will support these reaction codes in the tester being designated. Oxidative conditions will generate + mV values while reductive conditions will have –mV values. Therefore oxidative (aerobic in blue) conditions are to the left and reductive (anaerobic in red) conditions are to the right. Remember that there can also be significant ORP gradients within a biomass and so the ORP measurement taken from the outside environment may be not be valid for bacteria growing within the biomass. ORP interfaces that are close to 0mV are shown in yellow.

Table 4.3.1 ORP ranges (mV) for the major reaction patterns in the Testers

Tester	Code	+200	+100	+50	0	-50	-100	-150	-200
IRB	CL								
	FO								
	BR								
	BC								
	BG								
	RC								
	GC								
SRB	BT								
	BB								
SLYM	DS								
	SR								
	CP								
	CL								
	BL								
	TH								
	PB								
	GY								
HAB	UP								
	DO								
APB	DY								
DN	FO								
N	PP								
	RP								
	DR								
ALGE	GG								
	FG								
	OB								
	YB								
	GF								
	DG								

4.4. Visual BART reader (VBR) test systems

From the beginning of commercial use of the testers there has always been a frustration with getting an accurate time lapse for significant reactions when daily observations were conducted. This limits the level of accuracy that can be applied and in the last ten years technology has moved forward rapidly. The Visual BART Reader (VBR) system have evolved out of these technologies into two distinct models (VBR I and VBR II) that are addressed in Chapter 15 along with the current software packages. Many users have developed their own systems to allow the observation of the testers to be automatic and reduce the need to come in to read the testers at weekend or take them home to read. Advantages of the VBR system are much improved precision of the time lapse data (commonly to the nearest fifteen minutes rather than to the nearest day). Another major advantage of the VBR system is that the camera allows storage of jpg images for future reference and better determination of the time lapse and reaction signatures that are observed. The two VBR systems are briefly described below. Both utilise two racks which hold nine lab testers to give a total of eighteen testers in a single VBR unit. These testers are bottom illuminated using daylight LED lights that provides permanent light and achieves clear detection of the various reactions that can occur.

VBR I systems are designed to either be used at room temperature ($22\pm1^{o}C$) or in a temperature controlled incubator or room in which case the systems will operate at any incubation temperature between +2 and $+45^{o}C$. Currently the VBR I system has been customised for use with lab versions of the IRB-, SRB-, SLYM-, HAB-, DN-, and APB- testers.

VBR II systems have the two racks placed in a temperature controlled incubator that can routinely be operated from +25 to $+62^{o}C$ when the system is placed at room temperature. If the VBR II unit is placed in a cooled incubator or room then there needs to be at least a $4^{o}C$ differentiation between the cooled room temperature and the desired incubator temperature. For example a VBR II functioning at $12\pm1^{o}C$ would need to have the background temperature in the incubator / room at $< 8^{o}C$.

4.5. Biofilms, Biomass and Tester terminology

Two common terms used to describe microbial activity are biofilm and biomass although slimes and encrustations form common alternative terms. Biofilm growths are defined as being attached films of microbial growth on surfaces to which water has become bound into the growths (hence this can now be considered "slime" once the biofilms become thick enough to be obvious). If the slime forms a biomass with a high inorganic content then this become a scale or encrustation. As biofilms begin to develop they go through a number of changes:

(1) Stratification with reductive environments underpinning the oxidative;

(2) Excessive accumulations particularly of metallic cations and carbonates; and

(3) Decreasing porosity and increasing density.

One common feature of all of these stages of biofilms is the presence of general heterotrophic bacteria and so the most useful tester would be the HAB-. Detection of aerobic, oxidative bacteria can be recognized by the UP reaction and anaerobic bacteria can be recognized through the reductive (DO) reaction. The activity level (population) may be determined by how fast the reactions occur (time lapse). Young biofilms would generally give UP reactions while fragmenting aging biofilms are more likely to give DO reactions sporadically instead of UP.

Biomass is a common term applied to the total growth of mass at a defined site. Oak trees and humans for example both have a clearly defined biomass by outline. Microbial biomass is a little more difficult to assess as definable structures since they do not have easily distinguishable edges (they are often fuzzy in form). The microbial biomass associated with an oak tree is actually around the roots and forms ill-defined structures that may extend even into the woody roots, trunks and up into the leaves! In humans the biggest active microbial biomass is actually in the intestine! Around inanimate objects such as water wells then the defined biomass forms within and around that well below grade. As water is pumped into (injection), or out of (extraction), the well then this biomass remains sight unseen but it does significantly affect water flows and qualities. This "hidden" biomass functions to support the activities of those microbes functioning around that well. When this leads to changes in water quality and reduced flows then

often the cause is thought to be geo-hydrological and linked to clogging. If a biomass impact is involved causing plugging then traditionally this has been neglected as important. Bart testers provide a means to detect whether plugging is significantly occurring with water wells.

Sometimes a growing biomass can impact on surfaces to which it is attached. For example metal surfaces can begin to corrode, lose strength and finally fail. Such negative effects can also be referred to as biofouling where there is a negative impact created by that biomass. Here corrosion is one of the most important economic factors generated by microbial biomass (see also section 4.8). If you want to examine the various bacteria present within a natural biofilm then the most likely bacterial communities (other than HAB-) would be the sulfate reducing (SRB-), acid producing (APB-) and the iron related (IRB-) bacteria. DBI software QuickPop, VBR and %CBR software allows conversion the time lapse to predict active cells per ml (pac/ml) as populations SRB- testers will define reductive and oxidative activity through the generation of a BB (black base) and a BT (black top) reaction respectively. BB reactions can be expected deeper inside the biofilm while BT is more likely to occur on the outside of the biofilm. IRB- testers are the more challenging of the three commonly used testers to use simply because it is reactive to the iron oxidizing and iron reducing bacteria that may be commonly present in the biofilm in different layers. Most commonly the first reaction under reductive conditions is foam (FO) while for oxidizing conditions then the first reaction is clouding (CL). If the biofilm is forming ochre then the reaction seen first is a basal gel (BG) where a darkened green gel forms in the bottom of the tester.

4.6. Collecting and storage of water samples for Bart testing

There is always a concern as to how long may water samples be stored before beginning testing for bacterial activity. This concern stems from the fact that the sample should always be tested immediately once it was taken. This is a more of a dream than a reality. Samples that are stored for any length of time prior to testing will begin to degrade (and change) as a result of the microbiological and chemical activities in the sample. The challenge now becomes how long can you realistically delay starting the testing from taking the sample? There is a fine line between the gradual onset of these detrimental activities and the achievement of precision in the test

method. These impacts stem from changes in the environmental conditions particularly temperature, oxygen content, and turbulence all of which will cause negative or positive influences on the bacteria in the sample and thus potentially reduce precision. Temperature shifts, for example, can have a major impact since bacteria adapt to specific ranges associable with the source environment and may not adapt to those temperature changes. Generally if the temperature shifts upwards or downwards by more than a couple of degrees (Celsius) then the bacterial communities in the sample may to become unstable. In either event the bacteria would be likely to shift activity levels that could then affect precision.

Oxygen shifts during sample storage can also lead to critical conditions where the oxygen levels may become stressed (e.g. going to <1.4ppm O_2) on down to technically absent (i.e. <0.04ppm O_2). Here the stress increases on the aerobic (oxidative) bacteria and decreases on the anaerobic (fermentative, reductive) bacteria. Net results of shifting oxygen levels can therefore lead to biasing in the community activities towards those favouring either oxidative or reductive conditions depending upon the shift in oxygen concentration.

Turbulence in the sample is another (third) major factor that can affect the precision of bacteriological testing. Very commonly some bacteria will grow attached the surfaces and tend to be less affected while remaining in the biofilm and perhaps not detectable in the sample's water. For these bacteria to be present in the sample's water then they must have been sheered away through turbulence from the biofilms into the water.

Additionally bacteria within floating slime formations (as "dissolved" biocolloids) may also break up due to turbulence rendering a greater number culturable units (e.g. as colony forming units or pac/mL). These factors together mean that there is a probability that samples being tested may contain more evidence of bacterial activity (turbulence) or stress (temperature shifts or changing oxygen levels) than was present in the original sample at the moment of collection. The longer the sample is stored before analysis then the greater the potential becomes for this variability to occur. The common practise of placing a water sample over ice prior to testing can exaggerate these stresses and will temporarily slow down activities at the same time. "Over ice"

for transport of waters samples brings the sample's temperature down to within the range from 0 to 8 ^{o}C and is thought to "preserve" the sample for a longer period of time. However such temperature shifts downwards are likely to inflict severe stress on the bacteria leading to lengthy increases in the time lapses when Bart testing is performed.

When out in the field collecting water samples for bacteriological testing there is the challenge of not being able to start the testing until the samples are back in the laboratory for testing. The challenge now arises as to how you keep the water samples until you get back to the place where you can do the testing. Keeping the water sample for longer than one day creates serious problems. Here the bacteria in the water sample will change in their activity levels and even the dominant communities could have shifted over that time. There can be no doubt that changes will occur in bacterial activity but to level the "playing" field all of the samples should go through a common protocol when the storage time is reasonably consistent. It is well known that most bacteria start to enter into a dormant state when temperatures are reduced below 7 ^{o}C. This can be done by placing the water samples into a refrigerated environment (4±3 ^{o}C) using a small portable refrigerator or putting the samples over ice. Make sure that the sample bottles are not packed in too tightly. If they do touch due to tight packing or stacking then there could be greater variations in storage temperatures. This would be because some sample storage bottles would not cool down as effectively as others thus causing additional variability in activity levels. Depending upon the original temperature of the water, there would be different degrees of impact on the bacterial activity in the sample. However at storage temperatures of 4±3 ^{o}C, most bacteria become much less active. This means that the samples stored for longer (generally up to three weeks) before testing will cause **variability since at least some of these bacteria will require extended periods of** adaptation before becoming active again. Whether the sample has been kept for one day or as long as three weeks then all would all have been reduced to a level of inactivity (static state). Comparisons cannot be made between samples stored for only one day with those stored for as long as three weeks and then the comparison would have limited value.

To examine a series of samples taken over time or, from different locations, it is essential to

follow a common method for handling for all of the samples These may be coming from a single site as a series of sequenced samples, or from different locations within the same sampled region. There are a number of acceptable options:

1. **Hold the water sample for no longer than four hours** in a manner that would bring the sample up to **room temperature (22±2^{o}C)**. This is very important since any supersaturated oxygen present in colder samples would vent off and not affect the reactions in the testers. If there remains supersaturated oxygen in the sample then this would be most likely to vent at the start of the test causing gas bubbles to form primarily on the inside wall of the tester. To make sure all sample bottles are at room temperature it is important not to stack the bottles since would cause irregular equilibration to room temperature. This could affect the precision of the testing since not all samples would have reached room temperature and gassing of supersaturated oxygen could then still occur and/or the temperature of the sample would be cooler at the start of the testing which would affect time lapses being observed.

2. **If the water sample has to be held for longer than four hours** but **less than 24 hours** before the onset of testing then the samples should be placed over ice. By lowering the temperature down into the range of 4±3^{o}C then the bacteria in the samples will become less active or totally inactive. If the samples have been kept over ice then it can be expected that oxygen would now supersaturate the samples and it becomes important to allow the samples **to acclimatize to room temperature for four hours**. This time frame also aids in allowing the bacteria in the samples to adapt back to room temperature.

3. In the event of water samples having to be held over ice **for longer than 24hours and up to three days** then it can be expected that the recorded time lapses will lose some precision. To compensate for this, the value of the data collected has less value than testing samples in less than 24hours from sampling. However such data remains valuable if either: (a) all samples were collected from the sampling sites following the same storage routine: or (b) the same routine was applied for all of the testing even if this took place over extended periods of time (e.g. monthly or biannual sampling).

The ideal option (1) is to perform testing within four hours of taking the sample and allowing the samples to equilibrate to room temperature (22±2^{o}C) first. Delays in testing due to a need to store for longer than four hours (options 2 and 3) can reduce the comparative value of the data being generated during testing. With these two options comparisons become more limited to other testing even when employing the same option for storage.

In setting up the testers using these samples it is very important to have allowed the water samples to have returned to room temperature (preferred option 1). To do this put the sample bottles out on a bench without stacking them or pushing them together. There needs to be a good flow of air around each bottle to ensure that all of the water samples have come up to room temperature (22±2 ^{o}C) before beginning the testing. Of course all of the water samples would be impacted by a cooling and then warming cycle which would affect the levels of bacterial activity but hopefully in a relatively common manner. It may be expected that the time lapses would normally have lengthened due to the additional time that the bacteria have now taken to adapt. While reactions may not be affected by the prolonged storage it could be expected that the time lapse (and hence the prediction of the pac/mL) would have lengthened with smaller predicted populations. However these data can be used comparatively for the various samples subjected to the same storage regimen. Generally any storage time of greater than 24hours even over ice limits the value of the data to semi-qualitative and quantitative values. If water samples are involved in one to four days of transport to the laboratory for testing then bacterial activity and populations can be measured but may only be used in a comparative sense with data from other sample sets subjected to the same conditions.

Bottom line is that you can store water samples in a refrigerator for as long as three weeks and you will be able to determine which testers could be used for the major bacterial communities. Remember that the bacteria would be affected and some might actually thrive at these low (storage) temperatures while others would take some time before flourishing. That is the reason for putting a three week upper limit on refrigerated. Also if the water sample is from a very cold source (e.g. (8±4 ^{o}C) then these bacteria might adapt quickly to refrigeration temperatures and

then dominate even when the testing is being performed at room temperature.

Under ideal conditions then triplicated testing (i.e. three replicates from the same sample) would be needed to improve precision. In the DBI laboratories duplicated or triplicated testing is common using the VBR I or II systems using time lapse photography with images taken every fifteen minutes . When this is done with triplicated testing it is common for the time lapse, reaction signature and predicted population to fall within a maximum variance of 5% which is similar to that achieved for many chemical test methods.

4.7. Testing at sea

Ships have two major internal bacteriologically influenced problems with water that can affect ongoing operations. These problems relate to: (1) the potable water supplies for any crews and passengers, and (2) the bilge waters that collect between the two hulls plates and are sometimes used to improve stability to moving bilge water between the compartments. Bart testers can be used to determine the extent and risks that can be associated with the risks that can be generated from too high a level of bacterial activity. To address these two problems, it is recommended that the following three testers be employed to test the activity of:

- General heterotrophically active bacteria using the HAB- tester and remember to pre-dissolve the methylene blue in sterile distilled water where the salinity is greater than 4%;
- Sulfate reducing bacteria using the SRB- tester; and acid producing bacteria using the APB- tester.

While the HAB- tester will detect unacceptably high levels of bacteria in potable waters, the SRB- and APB- testers can when used together monitor bilge waters for potential corrosion-related risks. To conduct each of these tests then 15ml of the sampled water needs to be added to each tester following recommended procedures. It is recommended that the more economical laboratory testers be used in the slotted VBR I system which would allow eighteen testers to be monitored in one system. These racks need to be held down to prevent ship movements from affecting the testers. Individual testers can be positioned within the slots by placing a 4” ¼” wide

rubber band along the slot holding the nine testers. When the rubber band is tightened then the two stretched sides of the band prevent the testers from moving. Reading the BART testers is very simple using the VBR I system:

- HAB- tester starts with the sample solution becoming blue at the start of the test. If the blue disappears from the bottom up (UP reaction) then aerobic bacteria dominate and there is a lot of bacterial activity in the sampled water. If the blue disappears from the top down then there is strong likelihood that the bacterial activity could be supporting corrosive reductive (anaerobic) events. Here the population activity is directly linked to the time lapse before a reaction is seen using the VBR I or II systems (see Chapter 15. The longer the time lapse then it may be linked to the smaller the active population of general HAB bacteria. Time lapses are usually measured in days for potable water supplies the blue color should stay for at least four days (preferably six). If the blue color bleaches in less than two days for a potable water supply then disinfection of the water should be a considered option. Where bilge water is being tested with the HAB-tester it can be expected if larger bacterial populations are active. The occurrence of a down reaction in the tester could be taken as a warning sign that corrosive processes are under way within the sampled parts of the bilge. This may be associated with more odors. Generally for bilge waters a time lapse of less than two days may be considered significant particularly with a down (DO) reaction. Refer to the data from the other two Bart tester types for clarification of the corrosion risk in the bilges.
- SRB- testers determine the corrosion risk from pitting and eventual perforation of the steel. There are two reactions that can occur: (1) blackening around the ball called a BT reaction; and (2) blackening in the conical base of the tester called a BB reaction. BT links to widespread pitting (erosive corrosion) and BB links more to perforation (penetrative corrosion) of the steels. Both of these are warning signs that corrosion is affecting the safety of the ship.
- APB- testers have only one reaction which is a dirty yellow (DY) color that may reverse (DYB) that relates to the formation of organic acids that can aid in the erosive corrosion of the steel. Time lapses are significant with less than 5 days for an SRB- and 3 days for the APB- tester indicating a significant corrosion risk may exist in the bilges. pH of the bilge water would confirm this where the pH is acidic (i.e. <5.5 pH units)

4.8. Microbiologically influenced corrosion (MIC) and the Bart testers

Corrosion is more commonly recognized by its effects than its cause. Effects include leaking tanks and pipes, sudden pressure drops in an industrial complex, increases in the treatment costs, reduced efficiencies in the system, and increased secondary environmental impacts. These are commonly summarised as being either pitting, perforative or erosive (thinning) forms of corrosion. All of these events can become acute problems requiring immediate expenditures to achieve (at least temporary control). The chronic causes of corrosion (e.g. thinning and dishing) are often forgotten while the acute symptoms (e.g. perforation) are more easily recognized but correction may be at a high cost. Acute causes of corrosion most commonly are reflected in sudden onsets of perforations leading to leakages and plant system failures. Recognition of these risks is often achieved by building a greater corrosion allowance in the materials but this does not address the cause but merely controls (slows down) the final failure.

Corrosion is defined commonly as the effect of the wearing away of surfaces (commonly a metal alloy or concretion) as a result of biological or chemical activities. Causes of corrosion are fundamentally two fold. First the microbes associated with corrosion (i.e. MIC) would need to be present and active. Secondly the environment would need to be conducive to the development of the various events that can lead to corrosion. Detection / diagnosis of corrosion can involve three stages that are not necessarily always performed ideally in the same order:

(1) Determine the presence of active MIC communities or chemical precursors that could lead to corrosion;

(2) Diagnose the corrosion risk potential based upon the observed levels of MIC and any chemical precursor activity; and

(3) Evaluate the nature of the corrosion through its form and function and what preventative or rehabilitative strategies need to be employed.

If corrosion has already occurred then it is necessary to determine the effect and then undertake the establishment of cause. In the determination of the cause of corrosion through a recognized

MIC activity, the first step should be to determine whether there is any detectable microbial activity. One simple first step methodology is biochemical and fast involving the assessment of the enhanced total ATP (adenosine triphosphate, E-tATP) in samples from the site (see also 4.13 and section 14). If there is biological activity then there would also be ATP activity (as the prime energy driver) associated with the growing MIC biomass. Broad spectrum testing should be undertaken using the enhanced total ATP protocol that offers better precision. Once ATP activity is confirmed then one of two MIC bacteria can be identified. There are two cultural methods that can determine the activity of the sulfate reducing bacteria (SRB) and the acid producing bacteria (APB) using the tester system. These Bart methods allow corrosion risk to be assessed on the basis of the activity (recorded as time lapses) and observed reactions. If E-tATP levels are high (i.e. over 500pg/mL) and the SRB- and/or APB- data shows very active bacterial communities (positive reactions with short time lapses of less than three days) then the causative agents can now be confirmed.

Diagnosis of the corrosion risk in the sampled environment is based firstly on the E-tATP which is measured in picograms with significant MIC presences being at greater than 500pg/g or pg/ml with marginal levels ranging from 50 to 499pg/mL E-tATP. For the SRB- and APB- testers data can be considered to have created a critical risk when the time lapse is less than three days. In the SRB-Bart test then a BB reaction would indicate that it would be more difficult to manage because of the more covert (pitting perforation) nature of these growths. BT reaction is generally more manageable since here the SRB are sited deeply within the biomass and these can be treated more effectively by disruption of that biomass. APB- Bart data has one reaction (DY) and this type of MIC is more associated with lateral slow growing biofilms (associated with organic acids generation) that eventually lead to more generalized failures such as erosive corrosion and increased porosity in the steel.

Nature of the MIC at site may be examined by looking for pits and perforations, in encrustations, nodules, tubercles, ochres, and various forms of biomass. It is also important to determine whether there are any significant electrical motive forces (e.g. buried power cables) that might be attracting the activities of MIC.

4.9. Chlorine disinfection and potential impact on testing

Chlorine disinfection treatments in water commonly use different strengths of bleach as standard treatments for water wells, storage tanks, and distribution systems. Chlorine is commonly used to treat systems suffering from forms of production loss, perceived hygiene risks and/or quality control problems. These symptoms of failure could at least be partly caused by the forms that the biomass takes within the water environment. Natural growths and activities of bacteria can cause plugging, encrustation, slimes, corrosion, discoloured water, smells and can even affect the amount of water being pumped as well as the basic hygiene issues. Within water, the microbes are commonly dominated by various communities of bacteria and chlorine has been found to affect most of these bacterial growths and activities with reductions in determinable symptoms.

Of the chlorine products it is sodium hypochlorite as a nominally 5.5% solution that is most readily available (as domestic bleach). It should be remembered that bleach solutions do degrade over time simply upon storage. This product is however a very economical way to apply shock chlorination to control the various risks. Symptoms that commonly cause problems for the water users include losing flows (production), offensive odors (such as rotten eggs), dirty or discoloured water, and frequently equipment failures due to corrosion, scaling issues or plugging. Testers can be used to identify the majority of the bacterial communities that are the principal cause of these failures. To achieve this all Bart testers in regular production contain chemical inhibitors / neutralizers most commonly sodium thiosulfate. This chemical in practise prevents the chlorine from interfering with the activities and reactions generated by bacteria during Bart testing. If testers do show activities and reactions indicating bacteria are present before treating with chlorine then successful treatment could be confirmed by repeating the tests and finding either much longer time lapses (smaller active populations) and/or shifts in the reaction patterns (different communities). Testers showing reactions can determine the types of active bacteria and these reactions can be used to crudely determine whether how much chlorine treatment has impacted on active bacterial activities. Remember to follow all of the recommended safety procedures (example, safety goggles be worn and that the hands be protected by wearing latex or rubber gloves) when handling bleach solutions. These procedures may also be good safeguards (e.g. gloves and goggles) when setting up testers on chlorinated

samples.

If the chlorine is effective at reducing bacterial activity then changes may be seen through lengthening time lapses and changes in the form of reactions seen in the tester. Common effects of chlorine are that the reaction colors may change, growths break apart more readily, and some level of clarity returns to the water in the tester. It should be noted that chlorine (at concentrations of up to 5,000ppm) would be normally neutralized in the tester. Furthermore remember that the positive testers may contain active microorganisms and disposal should follow the standard recommended procedures as described on the Certificate of Analysis that can be found in all boxes of testers.

One of the potential concerns in all of the regular Bart testers is that sodium thiosulfate has been added to negate the potential for residual chlorine impacts once the sample to be tested is places in the tester. In the standard testers the potential impact of any residual chlorine is negated in the moments after the sample (that has chlorine residual) is charged into the tester. Some clients using the testers on waters with chlorine residuals are concerned because the Bart may show activity and reactions that would otherwise not be present if the residual chlorine was still present (it has been neutralised by the sodium thiosulfate). This is particularly critical when the impacts of some selected chlorine treatment are being evaluated. Early Bart testers were unintentionally vulnerable to residual chlorine and it was found, at that time, there was a lack of precision which was resolved by the addition of sodium thiosulfate. For customers needing to examine the longer termed impacts of residual chlorine the use of the standard sodium thiosulfate in the testers to buffer and eliminate residual chlorine may not be achievable. Four Bart testers (HAB-, IRB-, SRB- and APB-) are available as chlorine vulnerable (CV) testers to meet the needs of customers to examine the longer termed effects. The CV series are available only by special order and are only available as specialty products. While the VBR I and II systems may be used to monitor the reactions, activities and interpret the populations. It needs to be recognized that the data so generated will be different to that from the standard Bart testers. These differences would be reflections of the impact of the residual chlorine slowing the time lapse, shifting the reaction signatures, and generally showing smaller active bacterial populations.

4.10 Relationships between time lapse and predicted bacterial populations, colony forming units (cfu) and predicted active cell (pac) comparison.

Bart testers work on the concept that specific bacteria within a given sample would be able to generate activities or reactions in which the time lapse which generates the active population of those bacteria being examined. Here less active populations would involve longer the time lapses before activity and reactions are generated. The formulae involved are integrated into the VBR, CBR and Quick-Pop software. Furthermore it would be only such active bacterial populations within the sample that would generate the time lapse that would be detected to the first recognised reaction for that Bart tester type. This time lapse is converted to predicted active cells per mL (pac/mL) using the standard equations. To achieve these relationships, correlations were made with data from conventional agar spread plate technologies employing serial dilution to obtain comparable populations as colony forming units using agar plate techniques. Measurements using colony forming units per ml (cfu/mL) has been around for more than a century and this data has been based on the convenience of being able to count the numbers of distinct bacterial growths (called colonies). The more colonies that are counted then the greater the population estimate of detectable culturable bacteria in that sample. This has become a standard for reporting in bacteriology with colony forming units per ml (cfu/mL) being accepted as the standard term. In predicting the active population of bacteria in the sample (pac/mL), replicate testing needs to be undertaken so that the known population size can be confirmed statistically from the time lapses. In DBI laboratory practice triplication has been commonly employed both for quality management and support for project. When re[placation is performed using the VBR I or II systems then variance can be down to between 2 to 5% which is very good for a microbiological monitoring system and far superior to using the agar spreadplate / dilution protocols when applied to natural or industrial samples. In testing the maximum replication used during QM is twenty seven replicates. These analyses generate statistical relationships between the time lapse and the size of the active population of bacteria in those samples.

Using the agar spread plate methods has a number of drawbacks which include:

(1) The common need to dilute the sample so that the sample being tested contains commonly between 30 and 300 culturable cells which limits sensitivity;

(2) The agar surface provides an unfriendly environment for many bacteria to grow on and form colonies since these “unculturable” bacteria are being excluded (not counted) simply because they did not form colonies;

(3) Agar plates generate restrictive environments in which the water is bound up under highly oxidative interface making the water more difficult to “mine”; and

(4) Spread plates do not offer a variety of environmental sites within which colonies can form.

These factors limit the sensitivity of the agar culture media due to the inability of many bacteria to form colonies and be counted.

Bart testers have the advantage in offering a wide variety of lateral dynamic environments within which the bacteria can become active. These environments are generated primarily along the oxidation-reduction potential gradient along with the selective nutrient culture medium diffusion fronts. The tester is basically filled directly from the sample (15mL) from the sample that has not been diluted. This means that there is a minimum of trauma for the bacteria (particularly this can be caused by dilution). This means that the test begins immediately the sample is added to the tester and positive detection relates to the time lapse before the first recognized activities or reactions are observed. These activities or reactions relate to the types of bacterial communities detected. Once the time lapse has been generated then it is possible to statistically convert that data to predicted active cells per ml (pac/mL). In generating pac/mL the statistics have been established using pure bacterial cultures, natural samples and commonly employing the agar spread plate / dilution techniques in which the data is generated in cfu/mL. There is therefore a direct link between the cfu/ml in the statistical formulation of the pac/mL using the Bart tester technologies. For this reason it may be taken that pac/ml can be considered equivalent to cfu/ml on the understanding that is some ways the Bart tester format offers improved sensitivity and better precision. Limits of detection using the testers are 67 active cells per litre which is equivalent to one active cell in every 15mL of the sample.

4.11. Health risks to users of the water and the managed environment.

Health risks can mean one of two things. Firstly there are the direct health risks to the people directly and indirectly using the water supply. Secondly there are the health risks to the environment within which the water system is situated (e.g. water well, storage tank, treatment process). Public health addresses the former risks of concern with associated extensive monitoring programs and so will not be discussed further here. The health risks to the water system itself are another concern. Traditionally the health of water has been addressed in engineering terms relating to production rates and acceptable quality maintenance issues. Newer concepts relating to such systems should also have to consider risks from biomass infestations associated with both (upstream) water source, and the on-site management of the water which could affect the acceptability of that water for the consumer. There will almost inevitably be some level of microbiological activity associated with the source and management of that water that could affect water quality and production. This microbial activity can relate to the product water quality through bioaccumulation of chemicals from the water (natural bio-filtration effect); releases of microbes and their daughter products into the water (sheering effect); and the direct impact of the growing biomass in flowing water (plugging effect).

These three factors can all contribute to the deterioration in water quality and production and thereby affect the bottom line economics for the processing of the water for consumption. Additionally the growing biomass is likely to including reductive zones where there could develop the corrosion of support equipment that is likely to occur. This forms yet another challenge to the "health" of the water system. What is happening here is primarily that bacteria are concentrating through forming a biomass generally at the oxidation-reduction fronts where oxygen is coming from the oxidative sides and chemicals and nutrients from the reduced side. This focussing of the biomass initially starts as a natural biological filter improving the quality of the water bio-accumulating chemicals such as iron, and/ or degrading recalcitrant organics. As this biomass grows not only is there commonly a reduction in the flow / specific capacity but also the chemistry of the produced water changes. This secondary decline in water quality is more a result of the biomass beginning to fail to function as a "natural" filter but releasing back into the water flow some of the chemicals that had been accumulated. At the same time some of the bacteria active within the biomass will also be impacted by these destabilizations and which

would include periodic releases of bacteria into the produced water. Water quality and production therefore can show periodic spikes in undesirable bacterial numbers and chemical content. This can lead to step-wise increases as the biomass grows before it collapses. Chemical testing of the impacted product water commonly may show irregular increases in the metal content (particularly iron) along with similar increases in the particulates and total organic carbon. Testing impacted product water generally first displays just “shadows” of this more erratic behaviour that increases over time with the accelerating bacterial activity. This is recognized by shortening time lapses and often changing reaction patterns. Shortening time lapses means the bacteria are becoming more active and this may likely to be affecting the “health” of the water supply.

4.12. Zones of Interrogation (ZIP), Microbe hunting using Testers

We live (thank goodness!) on the oxidative surface side of a water-rich planet that primarily has a reductive crust underneath and an oxidative atmosphere above. This means that when groundwater is extracted from a reductive state (in the crust) it moves towards oxidative states closer to, and on, the surface. Biological activity occurs in oxidative state for plants and animals but many microbes can "enjoy" growing under reductive conditions. Here there has been found to be some preference for growing at the interface between oxidative and reductive conditions and this is one preferred location for biomass activities. In examining environments supporting different bacterial communities (e.g. ground water extraction wells) it has often been found that these various communities do actually grow preferentially at different sites along the ORP gradient that exists between strongly oxidative and reductive conditions. In general bacterial communities will cluster along this gradient in the following order (from oxidative to reductive): N-, IRB-, HAB-, SLYM-, SRB-, and DN-. These bacterial communities listed above are ones that can be routinely monitored for using Bart testers.

In groundwater investigations it has been found that these six communities can all be detected by their location and activity in sequences using timed pumped water samples from extraction wells. Here it is critical to first disrupt the biomass to maximize the sheering of bacteria during this disruptive phase. For a producing well this may be as simple as turning off the well to break the production cycle. This action then causes the oxidative-reductive interface to shift in relation to the borehole. Once such disruption through manipulating the interface has occurred then pumping the well continuously will release the sheering biomass to come out in the pumped water in the same sequence. For example the early pumped bacteria would have come from close to the well (e.g. IRB-) while later pumped samples would be from further away (e.g. HAB-, and SLYM-) from the borehole followed by the more deeply entrenched anaerobes (e.g. SRB- and DN-).

By conducting testing on the sequence of pumped samples it is possible to determine where the various bacteria communities are in relation to the borehole. BART-SOFT allows the data to be entered as ZIP interpretations. This use of ZIP allows the relative positions of the bacterial communities to be determined. Data entry includes the sampling time into the continuous

pumped water cycle, time lapses, and reaction patterns that then allows the generation of the zones of interrogation. Here the borehole is presented as a series of concentric rings and the activity of each bacterial community is shown by color (red – very active; yellow – moderately active; green – present at background levels; white – not detected).

4.13. ATP testing for microbial activity

Adenosine triphosphate (ATP) is the principal molecule used for the storage of high energy within phosphate bonds in all living organisms. ATP is therefore universal in all living cells and performs the primary high energy storage functions (similar in function to an electrical storage battery). When cells are metabolically more active, the ATP concentration tend to rise with the increased storage of energy in this form. Concentration of ATP is measured in picograms per gram (pg/g) and the methodology is described in Chapter 14 as the E-tATP protocol. Dormant cells have virtually no ATP activity while active cells generate concentrations of ATP in relationship to their activity level. Thus testing for ATP provides a rapid indicator of bacteriological activity within environmental samples can be achieved in at least a semi-quantitative manner. Methodologies for the detection of ATP have focused on the ability of the enzyme, luciferase (also referred to luminase) which breaks down ATP quickly with the generation of light directly in relation to the amount of stored high energy phosphate bond. The greater the amounts of light generated then the greater the amount of phosphate bonds that were broken down. The source for luminase enzyme was initially the firefly (*Photinus pyralis*) or the bioluminescent bacteria (*Photobacterium*). This test can be conducted very quickly which has made the ATP test a "gold standard" for the detection of quantifiable biological activity.

Initial research on the potential use of bioluminescent as microbiological activity detection method was proposed in 1968 for use in waters and then in foods by 1970. Since that time ATP methodologies have tended to replace the traditional spread plate techniques where the bacteriological activity levels in the sample are of prime interest. Since that time luminase testing for bacterial activity has undergone significant improvements in precision towards being now fully quantitative (second generation testing). ATP assays measure the amount of ATP in the sample commonly as relative light units (RLU, also sometimes called relative luminase units) which can now be more directly related to the viable active bacterial population. ATP has now also been adopted for the presence/absence detection of bacteria on surfaces and also for the detection of the number of active cells. This approach does not necessarily differentiate the source of the ATP activity beyond prokaryotic (bacterial) and eukaryotic (higher organisms) cells but lacks precision in the evaluation of specific groups of microorganisms. Thus the ATP test can be used to confirm in a semi-quantitative manner that there is activity within a sample.

RLU is measured by the amount of light emitted during the interaction between luciferase and ATP in the presence of oxygen. This is summarized in the reaction: Luciferine + ATP + O_2 in the presence of luminase and magnesium generate Luciferine (oxidized) + AMP + CO_2 + Light. These reactions require magnesium (Mg^{++}) and luciferase to generate light from the conversion of ATP to AMP with the release of energy. This ATP detection has been accomplished using the second generation QGA – Quench Gone test kit that is available from LuminUltra Technologies (440 King St., King Tower, Suite 630, Fredericton, New Brunswick, Canada E3B 5H8, www.luminultra.com). This method uses a luminometer to measure the amount of light produced during the test, along with Luminase (luciferase) solution that should be kept cold in a refrigerator, UltraLute for dilution of the sample, Ultralyse 7 and tubes A, B and C for each test. Calibration of the Luminase is important before starting each set of test since the luciferase will weaken over time. To do this calibration two drops of (100µl) of Luminase are added to 2 drops (100µl) of UltraCheck 1 in a small (12x55mm) assay tube. This is now mixed gently and then immediately inserted into the powered up luminometer and the enter button pressed. After ten seconds the screen will display a number which the calibration RLUCL value. If the value obtained is less than 5,000RLU then the Luminase is spent and a new calibration would need to be done with a fresh bottle of Luminase. If the number is greater than 5,000 then this should be recorded and used for calculating the ATP. These protocols are described in detail in Chapter 14.

For liquid samples (or suspensions of solid samples with a 5 or 10% dilution (recommended) the technique is:

(1) For EACH sample, add 1ml of Ultralyse 7 into a 17x100mm extraction tube (TUBE A), 9ml of UltraLute into a 17x100mm dilution tube (TUBE B), and 2 drops (100µl) of Luminase into a 12x55mm assay tube (TUBE C);

(2) Thoroughly mix the sample, and then add 1ml to TUBE A with the Ultralyse. Cap the tube and mix thoroughly;

(3) Allow sample to sit for at least 5 minutes to allow solids to settle;

(4) Carefully remove 1ml of the supernatant and dispense into TUBE B. Extra caution is required at this point to ensure that any sediment at the bottom of the tube not be

disturbed as this will add to an anomalous reading. Cap the dilution tube and mix thoroughly;

(5) Transfer 100µl of diluted sample to TUBE C and immediately place assay tube into the luminometer, press Enter, and record RLUI displayed after 10 seconds;

(6) Convert RLUI to Total ATPI (pg/ml) using the following formula:

$$\text{Total ATP (pg/ml)} = (\text{RLUI} / \text{RLUCI}) \times 20{,}000$$

When calculating the total ATP as pg/mL (if liquid sample) or pg/g (if a solid sample) then the total ATP would have to be corrected for any dilution factors used in the preparation of the sample. Normal range of ATP found in bacteriologically active samples would range from a low of 250 to more than 1,000,000pg/ml or pg/g. Generally total ATP values of less than 2,000 pg would be considered relatively inactive, <200pg virtually inactive, 5,000 to 20,000pg active, and greater than 20,000pg very active. In the event of virtually none (<200pg) and relatively inactive (200 to 2,000pg) of total ATP by the methodology described above then there may a need to enhance the potential for metabolic activity by stimulation through enrichment. This enrichment technique is designed to determine if there is a potential for greater ATP activity if the consormial sample was stimulated. The company, Luminultra Technologies, has announced a new generation of tests including a luminometer (PhotonMaster) which renders the analysis more convenient since it is directly coupled via a USB port to a computer carrying the analytical software.

4.14. Temperature influence on Testing

Temperatures from which samples are taken can have significant impact on the precision in the microbiological cultural test data based on the specific predetermined incubation temperatures used. One factor that has to be considered in microbiological testing is the incubation temperature to grow (culture) the microbes. There are some differences based upon where the microbes are active. Microorganisms living in, or around water wells tend to grow at relatively very stable temperatures. This is unlike conditions in soils and surface waters that are subjected to some level of day-night (diurnal) temperature fluctuations. In the last one and a half centuries (from 1874), microbiology has been dominated by the search for microbial pathogens of warm blooded animals (particularly humans!) that function at temperatures ranging from 35 to 45 ^{o}C. One unfortunate outcome of this was the idea that all microorganisms would grow best at these temperatures (35 to 45 ^{o}C). By the 1930s it was realised that many microorganisms had lower optimal (most favourable) growth temperatures and by the 1970s, 28 to 30 ^{o}C was considered to be the most suitable. There are now four temperature ranges that can be considered for the detection of microorganisms by cultural testing (including Bart testers). These are: 12±2 ^{o}C; 22±2 ^{o}C; 28±1 ^{o}C; and 36±1 ^{o}C. Each of these temperatures is used for different communities of microorganisms that can function in different environments. This does not eliminate the extreme importance of other temperatures often used in industrial processes involving water.

Natural incubation temperatures to culture cold-loving microorganisms can be most effective at 12±2 ^{o}C with severe trauma setting in on often when the temperature gets up to greater than 16 ^{o}C. Many of the microorganisms growing under these cold loving conditions can also grow at higher temperatures and these can also be cultured at 22±2 ^{o}C (room temperature). Microorganisms cultured at room temperature would not be growing at their maximum rate (optimal) but there are broader spectra of microorganisms able to grow including many of the cold loving and some of the warm loving microbes. This temperature range (22±2 ^{o}C) has the convenience of being normal room temperatures in most countries and so easy to set up. That is one of the main reasons why the original testing was recommended to be done at room temperature. Care should however be taken to ensure that temperature does not fall below 20 ^{o}C since the range from 16 to

19 °C can produce erratic cultural activities and a lower precision in the data generated. For microorganisms growing in temperate environments where temperatures range from 16 to 34 °C, the optimal incubation temperature has been found to be 28±1 °C. Using incubation temperatures are higher than 29°C or lower than 27°C then there tends to be losses in precision. While that temperature may produce the fastest growth that does mean that temperature gives the better precision. Speed and precision do not necessarily go flagella in flagella (hand in hand in human terms!). For warm ground waters in tropical climates and mildly geothermal extraction wells, the microorganisms would operate over ranges similar to the warm blooded animals and the most suitable temperature for culturing these microbes would be 36±1 °C.

Population counts (achieved by agar spread plates as colony forming units, cfu/ml), or predicted active cells (achieved by testing systems generating time lapses convertible to predicted active cells, pac/ml) are both responsive to the applied incubation temperature in different ways. For the agar spread plate, lower incubation temperatures do significantly affect the length of the incubation time to generate colonial growths. Generally the times before counting colonies are: 12±2 °C, 21 days; 22±2 °C, 10 days; 28±1 °C, 7 days; and 36±1 °C, 5 days. For colonies to be countable, they first of all have formed a large enough visible size to be countable diameter (0.2mm to >2mm). Colony counts will generally underestimate the number of microorganisms because of two factors:

- Only a small fraction of the microorganisms are able to grow under the conditions that are generated in the agar; and
- Competition between rival colonies as they form will cause some colonies to be destroyed before becoming countable.

4.15. Impact of salts on bacterial activity

Chemical salts, often dominated by sodium chloride, are a common component in many environments from the oceans to salt flats and to deep groundwaters. These salts are a dominating factor restricting the types of microbes that can become active. Generally it is sodium chloride that is the most dominant chemical in dissolved natural salts. It is therefore common practise to define the concentration of salts by their equivalence to sodium chloride. In the oceans the salts are commonly referred to as "sea water salts" and gauging the effects of salt is done using natural mixtures of seawater salts. Microbes tend to be more resistant to salt concentrations than plants and animals that will generally function over very restricted ranges. Ranges affecting microbial activity, in general are:

- Sensitive to total salt concentration of more than 200ppm. These microbes are extremely sensitive to salts and are active only when the water is virtually free of salts of any kind (e.g. rainwater, ice melts).

- Normal ranges are within the 200 to 80,000ppm (0.02% to 8%). Of the microorganisms it is the bacteria that appear to have the greatest tolerances within the "normal" range. Within this range there are optimal concentrations of salt that have a minimum effect on bacterial activity and outside of that range then the bacteria may become effectively inactive. However little is known of the ability of bacterial consorms (communities) to construct a biomass that effectively controls the admission of salts and, in so doing, controls the potential impact of salts on the biomass.

- Salt tolerant ranges are from 80,000 to 140,000ppm (8 to 14%). Once the environment enters a salt regime of between 8 and 14% then this becomes a major impediment to the activities of all microorganisms except those that are salt tolerant. These salt tolerant communities tend now to dominate within zones of the salt gradients.

- Salt Resistant microbes range above 140,000ppm (>14%) to saturation. These salt resistant microbes dominate the environments when salt is between 14% and saturation. Their metabolism is likely to be salt dependent which renders laboratory culture much more challenging

Most surface and marine environments are within the “normal” range for bacteria with most tolerance for salt being over the 1,000 to 40,000ppm (0.1% to 4%) range with many bacteria being unable to function in the 4 to 8% gradients. Thus in the literature different salt tolerances are seen for different species but the protective function achieved by a community could significantly affect these impacts. One unusual feature of biocolloids in water that has been observed is that they (floating slime clouds) function as desalinators. Here the biofilms on the outer edge of the slime cloud performs desalination functions so that the inside of the biomass has much lower salt concentrations. Thus you could have a high saline content solution containing these slime clouds (biocolloids) would have a lowered ability of the solution to extract dissolvable mineral ores from geological deposits.

Recent examination of client provided saturated brine samples have revealed that some bacteria are capable of remaining active under these extreme conditions. From laboratory studies it appears that the bacteria are able to create a low salt “bubble” environment within the brine to continue to function. It is hypothesised that the edge of the bubble is formed by a biofilm wall that acts to desalinate the brine and allow a lowered salt concentration within the bubble.

4.16. Testing cloudy and turbid samples

One challenge in testing water samples rises when the water sample is highly turbid (cloudy). For example when collecting casing samples from close to iron fittings or pipes it could well be that the water is heavily laden with iron debris (red water that in all probability is rich in IRB). Such samples cannot be used at full strength (15ml per tester) since the turbidity would directly impair the recognition of specific reactions (e.g. HAB- would be difficult to determine the start of the UP or DO reactions, SLYM- could not clearly differentiate the start of particularly CL reactions, IRB- would make it impossible to detect CL, BC and BG reactions, and SRB- would be marred by difficulties in the recognition particularly of the BB mainly because of sedimented debris). Filtering the sample through a 0.45, 2.0, 8 or even 12 micron porous filter would improve clarity but it would also lead to removal of many of the clusters of bacteria. This would mean that while improving sample clarity there could be severe reductions in comes at the expense of accuracy in determining the numbers of active bacteria in the sample. Here the act of filtering reduces the number of bacteria and in particular the biocolloids (slime clouds Filtering would therefore not be a very successful technique for improving clarity of very turbid waters for testing since there would be erratic removal of bacteria by the filter.

The recommended technique for cloudy or turbid water is to dilute the sample in (preferably sterile) distilled water (do not use deionised water since this water is commonly bacteriologically challenged when the resin columns begin to biofoul) with 1.5mL of original sample and adding 13.5ml of sterile distilled water (tenfold dilution). This disperses the turbidity most of the time to allow the evaluation of HAB-, SLYM-, IRB-, and SRB- testers. If the tenfold dilution does not provide sufficient clarity then go a hundredfold dilution (0.15mL sample in 14.85mL water). Correcting the population to observe the dilution then multiply the population by x10 for the 1.5ml diluted sample and by x100 if 0.15mL diluted sample was added to give a tenfold dilution of the sample. Approximate populations are given on the “Certificate of Analysis” which accompanies every box of testers manufactured. Cloudy waters may be considered to include those waters that are not discoloured but have a reflective greyness which makes printed letters.