Chapter 11

Risk Analysis using BART testers

11.1 Introduction

Risk, whether it relates to health, the environment or an engineered system; is commonly presented in numeric terms. The term risk can be presented in so many forms but here it primarily addresses the health risk and nuisance bacteria (HRNB). Health in the classical sense refers to the condition of humans and whether they are suffering from symptoms that could be related to a specific infestation which would most commonly be microbial in origin. In the broader context health can also relate to soundness (e.g. manufacturing process, financial state, and system functioning as claimed in the design). For this discussion of risk health would be limited in scope to economically significant harvestable foods and the human populations. Health risk relating to the environment is by default diverted to the activities of nuisance microbes that in some manner impact the environment. Bart testers have been developed to examine the risks from this wide variety of nuisance microbes.

Nuisance with regards to the microbes refers to those that are able create circumstances causing trouble or annoyance to the user. In general terms the two terms (annoyance and trouble) are inter- related since commonly the microbiologically generated annoyance would automatically lead to trouble. The best general all-embracing term for the problems caused by nuisance bacteria is biofouling. This could be interpreted to mean biologically instigated conditions causing offensive properties to be generated (e.g. filth, odors, and slimes) or damaging effects (e.g. corrosion, plugging, scaling and thinning). In designing the suite of Bart testers one principal aim has been differentiate the nuisance microbes that cause these various significant problems. In the evaluation of risk of risk it is primarily based on the reaction pattern signatures and additionally the predicted population as pac/mL generated by the time lapse. Risk in this case is not specific to some specific form for biofouling but rather on the severity of the risk. Chapter 13 addresses these risks particularly as they pertain to corrosion, plugging and health

Many people observe the level of risk simply by a number generated by some standard method. In the development of the various tester protocols it has been expedient not to include specific numbers (of populations) as being indicative of risk. Because these risks, in the first instance, are the results of trying to determine what risks to human health, health of the well and the efficiency of the system. Perhaps also the health of the microorganisms functioning within the biomass should also be something to concern. It is perhaps because the testers are used to detect a variety of microbial functions in different environments that these testers work well combinations with each other. Through the traditional roles of the testers it should be possible to effectively use the data comparatively between each sampling site. In these cases deterioration would normally be seen through increases in populations while effective treatment would show (hopefully) population decreases. Shifts in the dominant microorganisms would be seen in changes in the reaction patterns observed including the sequence in which they occur. Risk analysis is therefore divided for each tester into risks to human health, risks to sustainability of the producing source, and then general environmental risks relating to the bacterial activity detected.

Each tester has the risk defined separately using both the product name and the parameter code (Pcode). Risk ranges are provided based upon the population count (room temperature, $22\pm2^{o}C$) for human health, sustainability of the water source, and potential environmental consequences posed by the risks. Risk assessment is based on the potential for the particular population to be representative of normal background that might be expected to occur in the sample problematic populations. These may be sufficiently large to offer potential problems and so severe that detected populations indicate that threats to the functionality of the environment do exist. Therefore for each tester the generated time lapse (converted to predicted population) and reactions can be fitted into a range from normal background, to potentially problematic, and then to severe. In the latter event of severity then the population detected would already be displaying consequences resulting from the population activities. Risk analysis is first generated in a single table that defines critical populations (as predicted active cells per ml, pac/ml) that can be taken as equivalent to colony forming units per ml (cfu/ml) using the classical agar methodologies.

In addition to a generalised risk analysis based upon population there is an additional risk analysis based upon the reaction code(s) that were generated during the test. These reaction

codes (multiple codes are formed into a chronologically sequenced reaction pattern signature, RPS) that can be evaluated as affecting the risk analysis. This analysis is performed for each tester type and is included in the risk analysis interpretation for each tester type.

11.2 Product name: IRB- BART Pcode: iron biotester

In the iron biotester (IRB-) the objective is to detect the broadest possible range of bacteria that can interact with iron causing ferric accumulation (oxidative) or ferrous dispersion (reductive). In this test there are broad ranges of bacteria functioning under various ORP conditions. Risk to human health relates particularly to the enteric and pseudomonad bacteria which often form integral parts of the biomass. For the sustainability of an engineered system (e.g. water well, cooling tower, heat exchanger) then the risk relates to plugging of the system with biomass or corrosion. Environmental risks can be associated with many factors ranging from degeneration of the surface environment (e.g. reducing water quality, obnoxious slimes and seeps) to impacting the natural flora, fauna and microflora. One of the major challenges of the iron tester (IRB-) is that there are eight recognized as phase two, three and four reactions (see Table 3.1.3) each of which can generate both a population prediction (as the first observed reaction) and can these also form a part of the reaction pattern signature. In assessing the IRB- risk then there are two aspects that should be considered: (1) population size; and (2) reaction patterns reported. In the rest of the chapter this approach will be employed for each risk assessment. Risk analysis is shown in Table 11.1 and gives the general risk pattern associated with all observed recognized reactions and then specifically the risks associated with particular reactions.

Table 11.1, Risk Analysis for the Investigations of IRB population (pac/mL)

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
All (a)	50 - 499	500 - 9,999	100,000+
BC BR (b)	10 - 199	200 - 19,999	20,000+
GC RC (c)	1 - 99	100 - 9,999	10,000+
BL (d)	1 - 9	10 - 999	1,000+
BG (e)	10 - 99	100 - 4,999	5,000+

Notes: (a) All reactions principally refer to the CL and FO reactions that commonly trigger the start of the iron biotester (phase two reactions); (b) brown clouded and brown ring reactions relate more specifically to the activities of oxidative (aerobic) iron related bacteria that would increase the risk of iron related plugging; (c) both green and red clouded reactions relate to potential health risk bacteria such as enteric and pseudomonad groups and these may increase the risk to health; (d) black liquid is a terminal reaction that may be triggered by either enteric and organic reducing bacteria and (e) would signal either severe health risk or extremely reductive environments which could impact the water quality severely and affect production often with the presence of ochres.

11.3 Product name: SRB- BART Pcode: sulfide biotester

Traditionally hydrogen sulfide production has been linked to the reduction of sulfates. Relatively little attention has been paid to the other principal source of hydrogen sulfide that are the sulfur amino acids that will, in a reductive environment, not only release ammonium as a daughter product but also release hydrogen sulfide. Thus there are two sources for the generation of hydrogen sulfide which relate to the source of sulfur-containing chemicals that are then reduced by the bacteria. These two reactions have a different risk analysis and this is shown in Table 11.2. Risk relate primarily to the generation of hydrogen sulfide that can impact water quality, production as well as corrosion.

Here the BT reaction would normally indicate the risk involves all of the generating biomass which is often oxidative (aerobic) but contains parts that are reductive (anaerobic). These activities can include blackening in waters, rotten egg odors, fouling of the natural conduits or pipes carrying the water, and corrosion. This is commonly electrolytic and generally involves very distinctive black biomasses growing in the affected environment that may be either reductive or oxidative. It is common for the BT reaction to involve complex bacterial consorms in which sulfide producers are but one small activity within the reductive regions of that biomass.

By contrast the BB reaction relates to covert sulfate reducing bacteria that commonly are more active deeper down in the reductive zones (commonly with ORP values from -10 to -150mv).

Risk analysis for the BB therefore relates more directly to corrosion risks (generated by the covert biomass dominated by sulfate reducers) rather than the more general biofouling and water quality issues. It is for this reason that there is primary separation of the risk into the BT and BB groups. However when observations of the biotesters are daily then it could be that both the BB and BT reactions may occur in which case this is called a BA (black all) reaction. When this occurs, risk interpretation defaults to the BB type of reaction. Greater precision is obtained using the VBR systems and two jet black reactions around the ball (BT) or in the basal cone at the bottom of the tester (BB) are easily recognised.

Table 11.2 Risk Analysis for the Investigations of the SRB populations (pac/mL)

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
BT (a)	50 - 499	500 - 99,999	100,000+
BB (b)	1 - 9	10 - 4,999	5,000+
BA(c)	20 - 99	100 - 9,999	10,000+

Note: (a) risks associated with the black top, BT reaction and normally this would be associated with the degradation of protein-rich organics; (b) risks relate the reduction of inorganic sulfates with the subsequent releases of hydrogen sulfide; and (c) if a black all, BA, reaction is observed without the preceding BT or BB reaction then the risk analysis defaults to a combination of BT and BB reactions. Note that a BA reaction was preceded by either a BT or BB but there were not sufficient observations to allow that differentiation.

11.4. Product name: SLYM- BART Pcode: slime biotester

Slime forming bacteria is the name given to those bacteria that are active within environments where they bind water into extracellular polymeric substances (EPS). This bulking of the biomass using bound water can often exceed 95% or more of the total biomass weight. Generally, slime forming bacteria grow under oxidative conditions as floating particulates (bio-colloids) or attached biofilms maturing to slimes and encrustations. These types of biomass are often growing at the oxidative-reductive interface. Heat exchangers, cooling towers and filters

are also frequently sites where these bacteria grow and are usually recognized by the abundance of slimes attached to surfaces, as floating bio-colloidal particles, or as slime threads forming slime webs throughout the water. Primary risks relate to the engineered efficiency of the impacted system resulting in reduced efficiencies or water flows through the impacted site. Secondarily the impacted water may take on a clouded appearance and may even show slimy web threads or floating slime particles that make the product water less acceptable. Primarily the slime biotester is a monitoring system for the health of an engineered system to assure that efficiency is not impaired by the slime forming bacterial biomass. Table 11.4.1 gives the risk analysis on the basis of all reactions and then supplementary risks if a dense slime (DS) or black liquid (BL) reaction are observed. These two reactions change the risk analysis. For the DS reaction there is a probability that there is a biomass impacting the water in a manner that is affecting flows (due to plugging) and quality (primarily linked to clouding the water). Because of the limited location of the DS biomass activities, the effects can be more serious than the numbers would suggest. For the BL reaction which is generally a terminating reaction for the testing then the biomass would have become impacted by very reductive conditions that can severely impact water quality and may also pose a health risk.

Table 11.3, Risk Analysis for the Investigations of the SLYM population (pac/mL)

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
All (a)	50 - 999	1,000 – 99,999	100,000+
DS (b)	1 - 99	100 - 9,999	10,000+
BL (c)	20 - 99	100 - 999	1,000+

Note: (a) refers to the risk analysis for all recognized reactions except DS and BL; (b) dense slime growths are indicative of a tight biomass being formed commonly at the oxidative-reductive interface and this can cause restrictions in water flows and to quality; and (c) relates to terminal black liquid, BL, reaction that do occur when conditions are reductive with a relatively high organic burden.

11.5 Product name: HAB- BART Pcode: bacterial biotester

These bacteria are essentially the "organic busters" and play major roles in the degradation of organics (such as at bioremediation of hazardous waste sites). This HAB tester specifically determines the activities of the heterotrophic bacteria using methylene blue as the redox indicator for oxidative (blue) and reductive (clear) conditions. Here the tester always starts in an oxidative state as blue and then moves to a clear state when the bacteria become active and utilises the oxygen by respiratory processes to create a reductive state. There are two major reactions which separate the bacteria into dominantly oxidative (aerobic) as the UP reaction; and reductive (anaerobic) conditions as the DO reaction. UP reactions begin in the base of the tester and the blue shifts to clear from the base upwards. Risk analysis for this reaction would primarily relate to aerobically active bacteria. DO reactions commonly begin just below the floating ball when transient clearing may first occur and then shift back to the original blue color. As the anaerobic activity continues then the reduction of the methylene blue becomes more permanent and a clear zone now forms below the ball and moves downs. There are two risk analyses in Table 11.5.1 recognizing the different nature of the two reactions.

Table 11.4 Risk Analysis for the Investigations of the HAB populations (pac/mL)

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
UP (a)	10 - 999	1,000 - 99,999	100,000+
DO (b)	1 - 99	100 - 9,999	10,000+

Note: (a) UP reactions are a clear signal that the HAB- are dominated by aerobic (oxidative) activities and these bacteria tend to grow prolifically within the biofilms and biocolloids meaning that high populations can be found in degradable organic-rich waters; and (b) dominated by reductive (anaerobic) activities that would mean that the populations may be smaller but capable of causing problems from the daughter products such as fatty acids (causing the pH to shift to acid) and gases (particularly carbon dioxide, hydrogen, and sometimes methane) which then can become perched with a foam rich biomass and cause changes in the hydraulic flows through the impacted region.

11. 6 Product name: APB- BART Pcode: acidogenic biotester

Under organic-rich reductive conditions it is likely that fermentative bacteria will dominate the growing biomass generating daughter products that will almost always include fatty acids as well as gases. Under these circumstances the normal outcome is the pH indicator turning dirty yellow (DY). There can sometimes be a temporary drop in pH into the acid range which the biomass buffers the acidity back to neutral (DYB**)**. Where buffering occurs then there would be a broader spectrum of heterotrophic bacteria present in the biomass. If the fermentative function generates stable acidic pH values then this is likely to have a traumatizing effect of the biomass which would then become less active. In the acidogenic APB tester the objective is simply to determine fermentative activities under reductive conditions that generate organic acids. Here the pH indicator turns yellow but the reaction is clouded by the generally intense bacterial activity. Hence the yellow color is commonly made dirty by these bacterial activities. The only time that a bright yellow reaction will be observed is when the fermentative activity focuses around, or just below, the ball. Here the DY reaction would be declared once the bright yellow reaction appears stable around or below the ball. In such cases there is a descending DY reaction until the whole tester has a uniform yellow color. Buffering is a condition where the DY reaction now reverts to the purple colors as the acids are bacteriologically neutralised. This buffering commonly begins in the base of the tester or around the floating ball where there is an abundance of headspace (diffusing) oxygen. This tester is very relevant to sites where there is some evidence of potential for erosive forms of corrosion since APB- will cause an acidulolytic form of corrosion which can be as significant as the hydrogen sulfide induced electrolytic corrosion influenced by the presence of sulfide producers (e.g. SRB-).

Table 11.5 Risk Analysis for the Investigations of the APB population (pac/mL)

Reaction	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
DY (a)	10 - 999	1,000 - 99,999	100,000+
DYB (b)	1 - 99	100 - 9,999	10,000+

Notes: (a) is the generation of a "dirty yellow" reaction which may begin around the ball or further down in the culturing sample and indicates the bacteria are anaerobically functioning within a reductive environment as an active biomass; (b) the symbol "DYB " indicates that there is a buffering of the pH once fermentation has been finished with the pH returning to the more neutral values. Here the biomass essentially acts to correct (buffer) the pH which then returns towards neutral. In the latter (buffering) event a relatively small but adaptable bacterial consorm can be involved.

11.7 Product name: N- BART Pcode: nitrate biotester

Nitrifying bacteria are unique within the bacterial kingdom because of their ability to oxidize ammonium (from the reductive anaerobic degradation of proteins) into nitrate. Nitrate, when present in potable waters, can present a threat to the very young and the elderly and so strict regulated guidelines are enforced to limit nitrates (and hence the activities of nitrifying bacteria). Nitrifying bacteria are relatively slow growing and often require long adaptation times before they become active (e.g. three days). In the oxidation of ammonium through to nitrate these bacteria create demands for oxygen. This has been a problem in the biochemical oxygen demand (BOD) test since its inception. This is because this test takes five days of incubation and so there is the potential for any ammonia to become oxidized to nitrate with a very significant demand for oxygen. It is considered that such demands distort the functioning and precision of the BOD test and inhibitors have been applied to control this nitrifier influenced excessive oxygen demand. The nitrate tester is, in its simplest form, a presence-absence test for nitrifiers. It is possible to semi-quantify the nitrifier activity by undertaking the test on 10^{-1}, 10^{-2}, 10^{-3} dilutions of the sample. After five days incubation the test for the presence of nitrite is used to measure the

activity of the nitrifiers (Table 3.7.2.1.). Nitrite is used as the prime detection of nitrification since it was found that the nitrate generated by the nitrifiers (group 2) was commonly rapidly reduced to nitrite (as a result of denitrification). Essentially the nitrate biotester focuses on the emergence of nitrite during the test as a result of the activities of the nitrosofiers (group 1). In the application of a dilution series it is possible to now semi-quantitatively predict nitrosofiers populations (e.g. in sanitary wastewater treatment plants) without the loss of precision generated by the denitrifiers reducing nitrate through to nitrogen gas.

11.8 Product name: DN- BART Pcode: nitrite biotester

This tester is called the nitrite tester since the initiating chemical for the detection of denitrification is nitrite and the product of activity is nitrogen gas that collects temporarily as a foam ring around the ball. Detection of this denitrification function is totally dependent upon the recognition of a foam ring when it occurs during incubation. Populations are predicted based upon the time lapse generated. Essentially there are circumstances when these denitrifiers do remove nitrate (and therefore make the water "safer") but denitrification is a reductive event that occurs associated with the biomass. Secondary consequences of this activity can be the generation of foam initiated plugging that, at least temporarily, interferes with hydraulic flows. Additionally there are the nitrogen-based daughter products of these reductive biomass activities that can be problematic for the functioning of ecological systems.

Since the aerobic biomass will input a primary demand on intrinsic oxygen in the eco-system there can be a substitution of either nitrate or nitrite for oxygen under reductive conditions. This would mean that denitrifiers will become more active, or even dominant, within a biomass transitioning from oxidative to reductive conditions. One major event where the denitrifiers do dominate is in the downstream movement of sanitary wastewaters. The sequence that causes this to happen would be:

(1) Anaerobic degradation of the wastewaters with proteolytic releases of ammonium;

(2) Movement of wastewater into an oxidative environment where the aerobic biomass that now dominates over the total and fecal coliform bacteria;

(3) Nitrification of the ammonium moving into the aerobic biomass generating

nitrites and nitrates; and

(4) Denitrification of these nitrates and nitrites once the wastewater now re-enters reductive zones.

Here the denitrifying bacteria will become a greater part of the population and be indicative of the movement of products from the breakdown of the wastewater. Table 11.8.1 illustrates the risk of this occurring.

Table 11.6 Risk Analysis for the Investigation of the DN- (denitrifiers).

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
FO (a)	0 - 99	100 - 9,999	10,000+

Note: (a) is a transitory phenomenon in which the foam ring (FO) is commonly only observed for one to two days before dissipating.

11.9. Product name: FLOR- BART Pcode: glow biotester

There are two significant reactions that are recognized as relating to specific (health) risks. These are both ultra violet light fluorescing pigments that are observable for a period of time around and below the floating ball in the incubating tester. Both relate to species of *Pseudomonas* with the pale blue (PB) associated with the *Ps aeruginosa* group while the other is greenish yellow (GY) and is produced by the *Ps fluorescens* group. Here the PB reaction usually occurs in the top 20mm of the culturing fluids for just two to three days and is indicative of a potentially serious health risk. For the GY reaction there is a lower health risk and this group tends to be often associated with intense bio-degradative activities associable with the oxidative breakdown of specific organics such as the total petroleum hydrocarbons or natural gases. GY reactions tend to spread into the upper 40mm beneath the ball and have greater staying power commonly lasting four to fourteen days.

Table 11.7 Risk Analysis for the Investigations of the FLOR

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
PB (a)	0 - 99	100 - 999	1,000+
GY (b)	0 - 499	500 - 4,999	5,000+

Note: (a) involves the U.V. glowing with a pale blue color for the top 20mm around the equator and below the floating ball with the glow persisting only two to three days and confirmation of the presence of *Ps aeruginosa* strains can be achieved using 1ml of the culturing fluid taken immediately beneath the ball using a sterile Pasteur pipette; and (b) involves a more persistent (commonly 5 to 10 days with inception on day 2 or 3) green-yellow U.V. glow that can extend 40mm down the tester.

11.10. Product name: ALGE- BART Pcode: microalgae biotester

Micro-algae can be a major biomass in surface waters and are commonly dominant in phytoplankton growing as surface blooms. They are often easily recognised by turning the infested waters to shades of green. The risk can relate to the algal biomass interfering with the natural and engineered treatment processes. These blooms do also extend into soils where summers blooms can occur (although these are not easily observed nor essentially recognized as such). Water wells can be contaminated but mainly by recharges from nearby surface waters and algae moving down through localized recharges of water moving through fractures and porous formation structure outside the effectively grouted zones. Some of the micro-algae (cyanobacteria) can present the DG reaction and this may be indicative of a potential health risk primarily from the generation of toxins.

Table 11.10.1 Risk Analysis for the Investigations of the ALGE

	Population (pac/mL) to risk		
	Background	**Problematic**	**Severe**
All (a)	0 - 99	100 - 49,999	50,000+

Note: (a) refers to all six reaction types and the populations predictions are generated using Table 3.8.2.1. This risk relates only to surface water quality and shallow groundwaters being recharged from surface waters where there may have algae infesting the wells with the recharge waters.