Investigation of Microbial Aspects of Groundwater Quality and Volatile By-products Related to Coal Seam Gas Development

DRAFT Microbial Analysis Research Project Programme Basis

For

Coal Seam Gas Compliance Unit, State of Queensland
Department of Natural Resources and Mines

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Ground Water Science
Science and Planning for Earth's Most Critical Resource

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MICROBIAL ANALYSIS RESEARCH PROJECT PROGRAMME BASIS

This is the first work product of the Research Project entitled Investigation of Microbial Aspects of Groundwater Quality and Volatile By-products Related to Coal Seam Gas Development. This work is prepared by Smith-Comeskey Ground Water Science LLC (Ground Water Science) at the request of the Coal Seam Gas Compliance Unit, State of Queensland Department of Natural Resources and Mines (DNRM) under a Service Agreement with the DNRM.

1.0 Introduction

Any scientific monitoring programme, particularly one to be conducted in a high-profile environment subject to energetic scrutiny, should have a durable technical basis. In the present case, both the microbiological causes and effects of microbiological activities in groundwater and water bores remain relatively unfamiliar to the technical community and the public that will review and use the results. This basis document is provided to define and explain the scientific requirements of the planned programme:

- Characterizing the microbial ecology of sulphide generation and other expected geomicrobiological processes influencing groundwater quality constituents and physical-chemical conditions in the subsurface, and formation and bore-component changes.
- Defining the scientific basis for a programme of testing for and interpretation of microbiological data that is both practical and valid for short-term problem diagnosis and evaluating causes underlying potential longer term trends in water quality.

This document provides an overview of conditions influenced by microbial activity and occurrence, how these apply in the framework of interest (coal seam gas development in geological settings that also contain groundwater aquifers), and how these processes and effects can be monitored. The work is supported by a reference list for further research. Subsequent work product will define the planned testing programme.

2.0 Review of Mixed Coal-Sedimentary Basin Hydrogeology as it Affects Water Quality in Aquifers

An important feature of studying geomicrobiological processes is the understanding that they operate within the physical (including structural and hydrologic) and lithologic frameworks of the systems (such as stratigraphic basins) of interest (e.g., Pashin, 1998; Freij-Ayoub, 2013). For example, for a coal unit developed for CSG to have a potential effect on a particular aquifer, they have to be in hydrologic contact, there has to be a flow path, and also sufficient hydrologic head (and time) for water from the CSG coal system to reach sampling points at a point of interest (such as in an aquifer).

CSG development is optimized where there are conventional and hydrodynamic traps (typically separating coals from aquifers). Areas of active recharge with downward flow potential and/or convergent flow where there is no mechanism for entrapment do not favor CSG (Scott, 2002). CSG practices, of course, alter hydrologic systems so that potential effects depend on the geometry of present and past CSG formation and development.

Systems experiencing higher hydrostatic pressure are less likely to generate usable CSG, but are more likely to influence associated systems, assuming hydrologic barriers are not in the way. Other studies indicate that patterns of partial dewatering and rewetting can encourage biogenic CSG formation (Jones et al., 2013). Patterns and engineering of CSG wells will also influence potential effects (Palmer, 2010). The composition of coal influences the type of methane produced and how it is stored and transmitted to discharge points. According to Moore (2012), CSG forms as either biogenically- or thermogenically-derived gas. The former occurs in 'under mature' (< 0.5% vitrinite reflectance) coals and is the result of bacterial conversion of coal into CO_2 or acetate, which is then transformed by microorganisms (see discussion below) into CH_4 under the appropriate conditions. Thermogenic gas is formed as part of the coalification process and is described by Moore (2012) as being purely a chemical devolatilization of coal components that releases CH_4. A thermogenically sourced methane regime is more typical of older and more deeply buried coals.

However, as described by Midgley et al. (2010) and Jones et al. (2013), reality appears to be more complicated than that summarization. Although microbial activity might be expected on surfaces of nearly pure reduced organic carbon, coal (including lignite) consists of recalcitrant biopolymers, and is actually a poor substrate for microbial growth (Jones et al., 2010; Ehrlich and Newman, 2009). For biological communities to access this massive, but recalcitrant, resource, a range of conditions and biogeochemical activities and microflora are required (Midgley et al., 2010). Biological extraction of methane and other organic compounds is of industrial interest (e.g., Engesser et al., 1994; Jones et al., 2010), and as such, a subject of study. Summarizing:

- Lower rank coals (higher H content) are more susceptible to biodegradation than higher rank coals.
- More impurities in coal make coals more accessible for biological decay, as chemoautotrophic bacterial communities "mine" the recalcitrant coal for these resources.
- Fermenting bacteria are involved in coal breakdown and certain classifications of fungi are adept at breaking recalcitrant coal polymer linkages, and make byproducts that are accessible to chemoheterotrophic bacterial communities.

Consequently, where coal is accessible for biological weathering, complex, interactive microbial communities exist (see following discussion on microbial consortia). Under any circumstances, such a diverse biogenic system is likely to harbor a wide range of geomicrobial activity.

Coal and sedimentary surfaces experiencing periods of wetting and drying may have complex microbial ecologies due to the variety of carbon resources available (Jones et al., 2013). Even more complicated would be systems being used to store CO_2 in CO_2 sequestration efforts, or where CO_2 is used to stimulate CSG production from wells (Hamawand et al., 2013).

According to Australian sources, for its large CSG basins, large volumes of produced saline groundwater and managing the large tonnage of saline byproducts are the primary concerns, summarized by Hamawand et al. (2013). In addition to salinity (NaCl) and/or sodicity ($NaHCO_3$), which are destructive to

soil and irrigated crops, salinity, sodicity, metals and other constituents in produced water can be harmful to aquatic life.

However, groundwater under anaerobic conditions and in long contact with coal and associated sandstone, shale, and siltstone can carry with it dissolved organic compounds such as polyaromatic hydrocarbons, and nuisance inorganics, including ammonia nitrogen, sulphides, and elevated levels of dissolved iron (Fe^{2+} or FeII). Minor metallic constituents include arsenic (As) and radioactive compounds eroded from surrounding highlands and retained in deposits that become coal. Depending on the saturation and redox potentials, such constituents may be carried in solution or deposited as FeII sulphides such as pyrite or other minerals. Shales in contact with coal typically also have high carbon content, and tend to serve as carbon filters retaining metals and radionuclides.

Methane and other gases such as carbon dioxide, produced by either microbial or thermogenic activity, of course, do not necessarily have to follow fluid gradients. They can rise to infiltrate and affect freshwater aquifers, assuming connections exist, and gases are not detained by reactions along the pathway. The effects on a freshwater aquifer would result from the presence of the product gas as substrate or geochemical agent in the aquifer, independent of the microbial ecology or aquatic chemistry of the source rock.

Hydraulic fracturing (HF) gases and fluids may also potentially affect groundwater (Freij-Ayoub, 2013), especially as coal seams can be relatively shallow, compared to oil and gas reservoirs where HF is also used for stimulation. Likewise, drilling fluid components can influence bore water quality locally, for example by introducing biodegradable polymers, nutrients (especially phosphate) or electron acceptors (e.g., sulphites). Other anthropogenic influences might include soluble byproducts of efforts to stimulate biogenic methane production or solution mine useful organics from coal.

3.0 Coal and Related Geomicrobiology, Microbial Biogeochemistry, and Microbial Ecology and Influences on Water Quality

With the previous discussion, the topic of geomicrobiology has already been introduced thematically, as it is this and allied disciplines that have provided insight into how microbes and earth materials interact to produce observed results. Geomicrobiology is a broad field of inquiry that can be defined as encompassing aquatic (by and large freshwater, including groundwater) microbiology, the microbiology of rock and earth materials, thermal and other extreme conditions, and the interaction among microbes, their substrates, and the cycling and transforming of materials. The authors of what is perhaps the single most important textbook on the subject, *Geomicrobiology* (Ehrlich and Newman, 2009, is the current, fifth edition), prefer that the term “geomicrobiology” be reserved for the study of the role of microorganisms in fundamental geologic processes. In this framework,

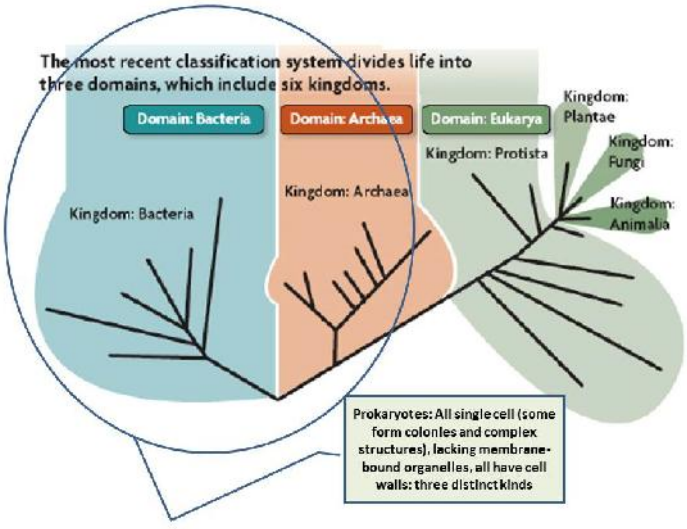
- Microbial ecology is the study of microbial ecological interrelationships more than interactions with the physical and chemical environment.
- Microbial biogeochemistry is the study of microbially influenced geochemical reactions.

- Environmental microbiology is related to both, and functionally is microbiological analysis of natural environments – more emphasis on technique and less on microbiological processes and relationships. Industrial microbiology is applied environmental microbiology primarily focused on engineered systems.

It should be apparent that these fields naturally overlap one another and related disciplines such as geochemistry, sedimentology, and hydrogeology. The methods recommended for use in this project (discussed in the following) are essentially tools of environmental (and industrial) microbiology.

This report does not presume to describe the whole of geomicrobiology, biogeochemistry, and microbial ecology. Ehrlich and Newman (2009), *Geomicrobiology*, is one comprehensive reference, which is recommended for further review to familiarize the reader with the breadth of the subject. Actually, this text itself certainly addresses a wider range of topics than the authors' limited definition of "geomicrobiology". There is a journal *Geomicrobiology* , that ranges through the overlapping fields, and a Geological Society of America division is devoted to the subject (http://www.geosociety.org/divisions/gbgm/). An internet search will reveal various research centers with an interest in geomicrobiology and related disciplines. For the purposes of the present work, in this present document, we will use more of a "lumper" definition of "geomicrobiology" that encompasses biogeochemical functions, and we will take an active interest in microbial ecology for reasons that will become apparent.

Geomicrobiology (focused on microbe-substrate-geochemical interactions) is especially relevant in CSG effects analysis since coal, and sedimentary rocks usually associated with coal, have abundant highly reactive surfaces containing carbon, nutrients and electron acceptors needed for microbial metabolism, and metallic substances typically involved in various microbial processes, including respiration and detoxification. Because coal is largely organic carbon (but with other materials abundant in the coal matrix), and also in contact with oxidized minerals and groundwater, there are likely to be distinct oxidation-reduction (redox) potential gradients that microbes utilize in their activities (see Ehrlich and Newman, 2009). Exploitation of redox gradients by microbes goes back to the beginning of life on earth (Russell et al., 2013).



3.1 Types of microbes and their functions

Modern classifications of life by microbiologists have undergone a transformation in the past 20 years, and can be very different from classifications learned or written about before approximately 1990. Figure 1 is one version.

Fig. 1: Classification of life

Underground life is primarily microbial, and consisting of the prokaryotes (single-celled organisms with cell walls and lacking organized organelles in their interiors). The biological classifications are still somewhat in debate. One way to organize prokaryotes is as Domain Prokarya, organized into the superkingdoms Bacteria and Archaea. Some refer to these two superkingdoms as domains (as in Figure 1). These appear to dominate underground life in both mass and genetic diversity. Bacteria themselves are divided into the Gram positive and negative groups, which have entirely different cell wall construction. For details of microbial phylogeny, we refer to a good general environmental microbiology textbook such as Pepper et al. (2015).

Protists and fungi (Protista and Fungi, which are eukaryotes, classified with the Domain Eukarya, see Figure 1) are present in smaller amounts, but locally important. Bacteria seem to be the most genetically diverse and flexible forms of life, and also have the widest range of respiration options. Archaea are likewise diverse, but much less well-known than Bacteria. They are different enough from bacteria to warrant their own superkingdom or domain, and they also share some biochemical characteristics with eukaryotic cells. Figure 1 illustrates the presumed relationship among the different cell types. Many archaeans are adapted for relatively extreme conditions (salinity or heat). Methanogens (forming biogenic methane) are all archaeans (Ehrlich and Newman, 2009). Bacterial and Archean classification is still a developing science (Fraser et al., 2009).

A clear understanding of the true range of microbial (especially bacterial and archean) genetic diversity was long hampered by the limited analytical tools available in microbiology. Until the very end of the 20st Century, truly effective genetic analysis tools were limited to academic research. Prior to that, microscopic observation and culturing (physiological) methods were the available tools, and remain important. Culturing remains highly useful in environmental microbiology, and still widely used for taxonomic or functional identification. These will be discussed further in the following.

Commercially available biochemical or “microbial fingerprinting” methods (analyzing components of cell walls and nucleic materials for use in assigning identity) became available in the late 1990s (e.g., White and Ringelberg, 1997; Reeves, 1997). These permitted a broad, large-scale understanding of microbial diversity in both space and time. For example, Ground Water Science utilized such analyses in several practical projects in the last 12 years for several purposes (Smith, 2015):

- Analysis of aquifer core samples to determine evidence of a past organic chemical release in the aquifer matrix by analyzing the geographical distribution of microbial diversity using biochemical markers (phospholipid fatty acid analysis, PFLA).
- Analysis (again with PFLA) of aquifer (Geoprobe) direct push core next to a recharge bore to define the size of iron-oxidizing and sulphate-reducing zones around the frequently clogging bore.
- Analysis of deep water bore and core samples (using nucleic acid methods) to determine the diversity and possible origin of biofouling in newly constructed bores.

Studies of groundwater and the subsurface in general have revealed tremendous genetic and physiological diversity and heterogeneity (e.g., Sirisena et al., 2013), which are reflected in the above-mentioned studies.

The reasons this is important to an understanding of CSG field and aquifer microbiological effects include:

(1) Genetic studies of microbial diversity in the subsurface have revealed a biological universe that is much larger in mass and diversity than was imagined before the availability of microbial genomics (large scale genetic study, e.g., Handelsman, 2004). Thus, conclusions about the limitations of microbial activity have had to be extensively revised.
(2) Microbiological activities have known effects on water bores and aquifers, as well as hydrocarbon reservoirs and wells, at many scales (e.g., Smith and Comeskey, 2009; Youssef et al., 2009).
(3) Such relationships and activities (over time and in three dimensions) can be analyzed using commercially available methods to better understand how CSG development may affect aquifers and water bores, and how geomicrobiological activities affect CSG development.

Chasing microbial genetic diversity is, of course, very interesting but not necessarily the most useful pathway in achieving the CSG Compliance Programme's objectives, although it can have very real practical applications ("where did these microflora come from, and how are they introduced?" or "what happened to the methane we expected to find here?" or "how can we determine what is living here when we cannot culture it?").

Microorganisms across the range of genetic classification have adopted similar metabolic and physiological strategies, such as using oxygen or sulphate as electron acceptors in respiration, filamentous growth, metal oxidation or reduction, biofilm formation, etc. The occurrence of such strategies is often closely related to groundwater chemistry and redox potential (Sirisena et al., 2013). Others, prominently sulphate-reducing bacteria (SRB), have dual or multiple metabolic mechanisms that turn on or off depending on local conditions (Plugge et al., 2011), including switching to sulphite as the electron acceptor (Stueber et al., 1994). Likewise, there appears to be archaeal geochemical cycling in parallel to the better known bacteria, but the extent is poorly understood. Focusing on what the microorganisms "do" can often simplify the analytical strategy in problem-solving diagnosis (Cullimore, 2008; Smith, 1992; Smith and Comeskey, 2009).

3.2 The role of microbial consortia and syntrophy in geomicrobiology and microbial biogeochemistry

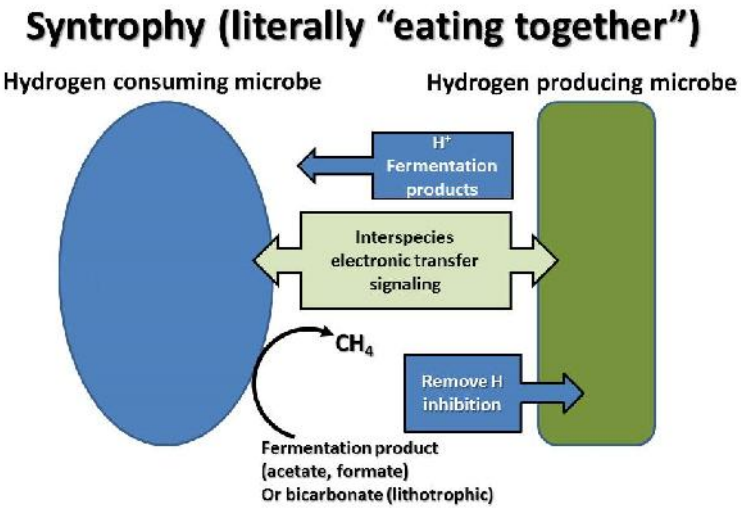
It is now well-understood (e.g., Cullimore, 2008, Ehrlich and Newman, 2009) that microbial species work together in cooperative groups of species known loosely as microbial consortia. These may occur as tight aggregates of microflora, or may be loosely associated. A good example is the above-mentioned cooperative assemblies that degrade coal and oxidize methane (Marlow, 2015). Most of the biogeochemical cycles or even corrosion structures on metal discussed in the following involve consortia

that collectively perform the necessary functions. Such cooperation is typical in more familiar animal, fungal, and plant multi-species ecosystems (lichens being a common example).

The term syntrophy (syntrophic) is used for consortia of microorganisms (including associations of archaea and bacteria) involved in tightly coupled mutualistic interactions in which the syntrophic partners exchange essential compounds. The term syntrophy was first used to describe the exchange of sulfur compounds between phototrophic, green-sulfur bacteria and chemotrophic, sulfur-reducing bacteria. Recent ecological and physiological studies show that syntrophy plays a far larger role in carbon cycling than was previously thought (McInerney et al., 2008; McInerney et al., 2011).

In biogenic methane production, hydrogen and formate, acetate and other short-chain compounds are exchanged in methanogenic environments between fermentative syntrophic metabolizers and methanogenic archaea. One partner produces hydrogen and formate that the methanogen partner consumes, keeping the intermediates at low concentrations that support the degradative reactions in this partnership (McInerney et al., 2011). Acid-producing fermenters (or some other source of H^+) also would benefit chemolithotrophic methanogens "mining" carbonates for bicarbonate to reduce to CH_4. Figure 2 is a summary of the processes.

Fig. 2: A basic syntrophy example



Another variation on syntrophic cooperation of interest in biogenic methane production is the capacity of certain assemblages of microbes to work together form methane from more than one carbon source by direct electron transfer from one microbe to another. For example, the Archaean methanogen *Methanosaeta harundinacea* forms associations with the common bacterium *Geobacter metallireducens* in which *Geobacter* metabolizes ethanol, producing acetate, utilized by *Methanosaeta* in methane production, but the bacterium also supplies electrons directly to support a direct CO_2 to CH_4 reduction pathway (Rotaru et al., 2014). Figure 3 illustrates this system.

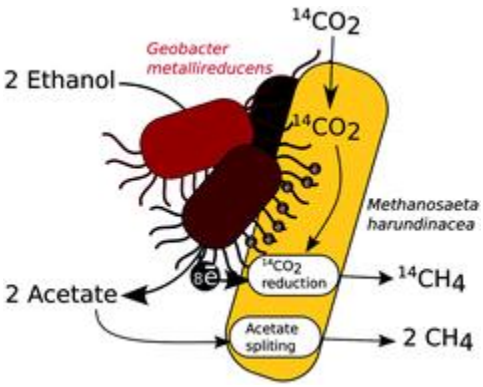


Fig. 3: Syntrophic methane production system (source: Rotaru et al., 2014)

3.3 Why aquifers (broadly defined) are favorable microbial habitats

In contrast to the widespread view in the 1970s (even among microbiologists) that aquifers (i.e., other than karst, where macroscopic life is evident) should be largely devoid of microbial life due to filtration and lack of available organic carbon, aquifers are now understood to be home to large and diverse microbial populations. This should not be surprising in that aquifers offer the following habitat advantages:

- Vast surface area – This varies all over the scale by lithology but for discussion purposes, consider 25 % of saturated rock volume. The vast majority of microbes live on surfaces in biofilms. Very large surface area is also typical of coals.
- Intergranular living space – even in relatively nonporous sedimentary rock, bacterial are known to live (actively metabolizing, usually slowly) within the rock matrix itself (known as *endolithic* microorganisms). Among these are active communities of interest to CSG development and its effects.
- While the saturated subsurface deeper than the soil zone is relatively lacking in abundant, readily available organic carbon and nutrients (especially P), many types of microorganisms are well-adapted to this *oligotrophic* (low nutrient and C availability) lifestyle. These include methanotrophs, lithoautotrophs fixing CO_2 (sometimes as bicarbonate), and various chemoheterotrophs.
- Groundwater habitats are characterized by hydrological, chemical and geological heterogeneity. These provide gradients that favor arrangements of mutually beneficial respiration and metabolic activities. Transitions between lithologic units often appear to support abundant microbial growth for these reasons, as electrons flow between nodes in contrasting environments such as relatively oxidized sandstones and reduced shales.
- However, within lithologic zones, conditions are highly stable – microorganisms in groundwater are stressed by sudden changes in conditions, but well-adapted to stasis.
- The distribution of microbial life in subsurface environments such as aquifers is controlled by ecologic and physiological factors, and not only thermodynamics (Bethke et al., 2011).

- The scales of biomass and activity are difficult to determine at this stage and probably highly variable.
- Sheer biomass is likely to be large – perhaps 40 % of the planet's total prokaryotic biomass is found in aquifers (typical density of $10^3 – 10^6$ cells/cm^3).
- The species distribution, what metabolic mechanisms are used, and biomass are highly dependent on carbon and nutrient availability. For example, species diversity and overall biomass are higher around a carbon source such as a petroleum release, and distinctly different from those in a pristine part of a freshwater aquifer. Likewise, infiltration of water of different salinity or geochemical quality will change microbial profiles. Such differences can be used to evaluate impacts from various sources.

Griebler and Lueders (2009) provide a highly useful and relatively current review of these and related topics regarding microbial life in groundwater systems.

3.4 Some relevant microbially influenced geochemical cycles

How microbes interact with and affect both CSG wells and water bores is mostly about how microbes affect fluids and formations/surfaces by enhancing weathering, making minerals soluble, and depositing or co-depositing minerals in insoluble deposits. The microbial cycling of materials is the subject of biogeochemistry, but affects weathering and deposition of earth materials. Among the prominent cycles:

(1) Carbon (obviously) – CSG is largely gaseous methane derived from carbon solids. The methane may be thermogenic or biogenic, as discussed. Methane (CH_4) is also oxidized to CO_2 by methanotrophic bacteria and archaea. CO_2 then can be transformed to participate in the inorganic carbon system (carbonates and bicarbonates). In these forms, the methane-origin C can become insoluble. Within the biological sphere, the C is trapped as biomass or may again become converted to CO_2, CH_4, or complex organics. Microbes also form carbonate structures, alter them (for example by dolomitization), or soften and dissolve them.

(2) Hydrogen – H is likewise processed by microbes, with some generating H, some able to oxidize and respire H_2 and H is obviously an important component of CH_4 and other organic C compounds such as hydrocarbons. Much H is bound up in biomass.

(3) Sulfur – Sulphate reduction is often associated with coal and organic-rich shales due to sulfur minerals co-precipitated with coal or limestone or trapped in shales and sandstones. S is found in various redox states (Figure 4) with varying degrees of solubility. S species tend to be highly reactive. The oxidized forms are commonly soluble and used as electron acceptors in microbial respiration. Reduced forms (especially sulphides) readily react with metals such as iron and uranium, forming minerals and ultimately ores, and are likewise oxidized by bacteria and archaea for metabolic processes. Some bacteria hoard soluble electron-neutral S due to S utility in cellular biochemistry, and large amounts of S are incorporated into biomass.

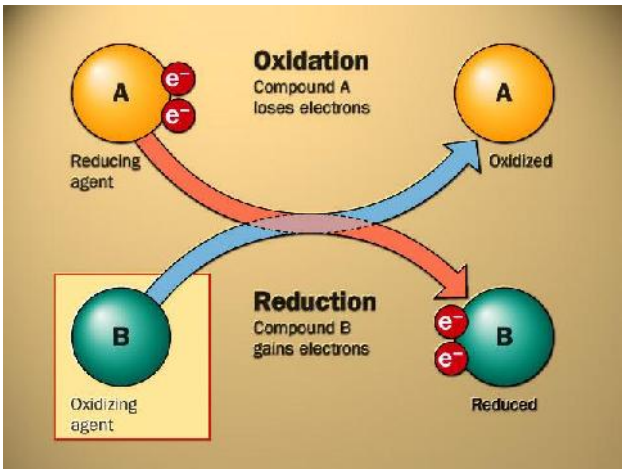
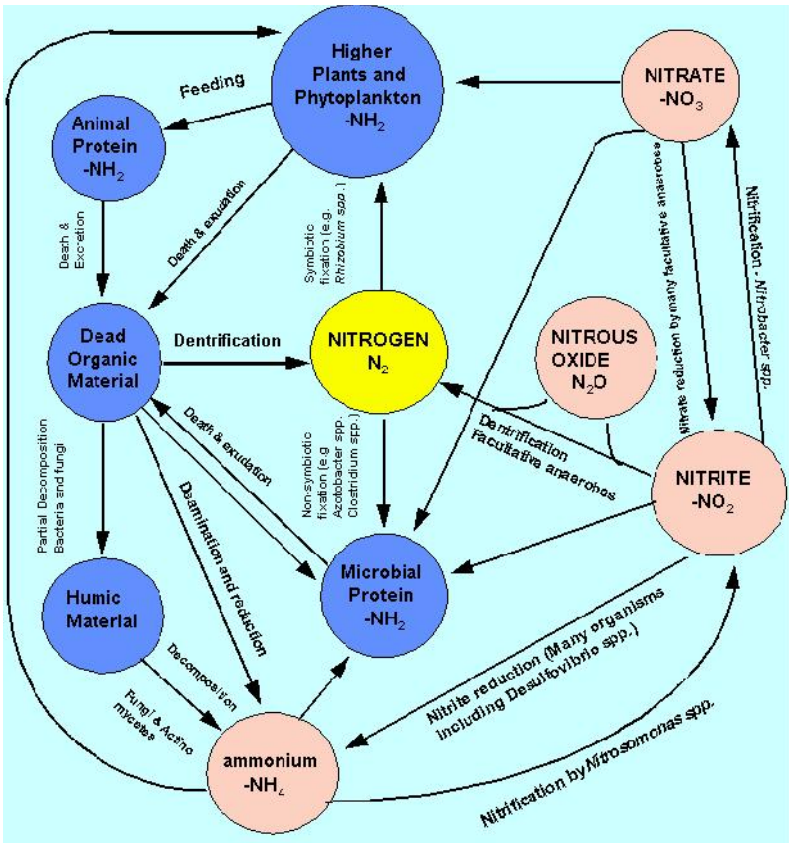


Fig. 4: Reminder – electron flow in oxidation-reduction (redox) reactions (source: www.studyblue.com)

(4) The nitrogen cycle is biologically crucial and entirely dominated by biological oxidation, reduction, and transformation. As with S oxidation and reduction, discrete collections of microbes mediate specific oxidation-reduction steps such as reduction from nitrate to nitrite. Microbes working in symbiosis with fungi and plants are essential to N_2 fixation from the atmosphere reservoir to bring N into the biological system, and for producing nitrate that plants can use for forming amino acids. In groundwater systems, N species reduced (also biologically) to ammonia can be aesthetic nuisances. Figure 5 illustrates the multi-stage N cycle, each mediated by specific bacterial oxidations and reductions.

Fig. 5: Nitrogen geochemical cycle mediated by bacteria (source: http://dwb.unl.edu/teacher/nsf/c09/c09links/bordeaux.uwaterloo.ca/biol446/chapter8.htm)

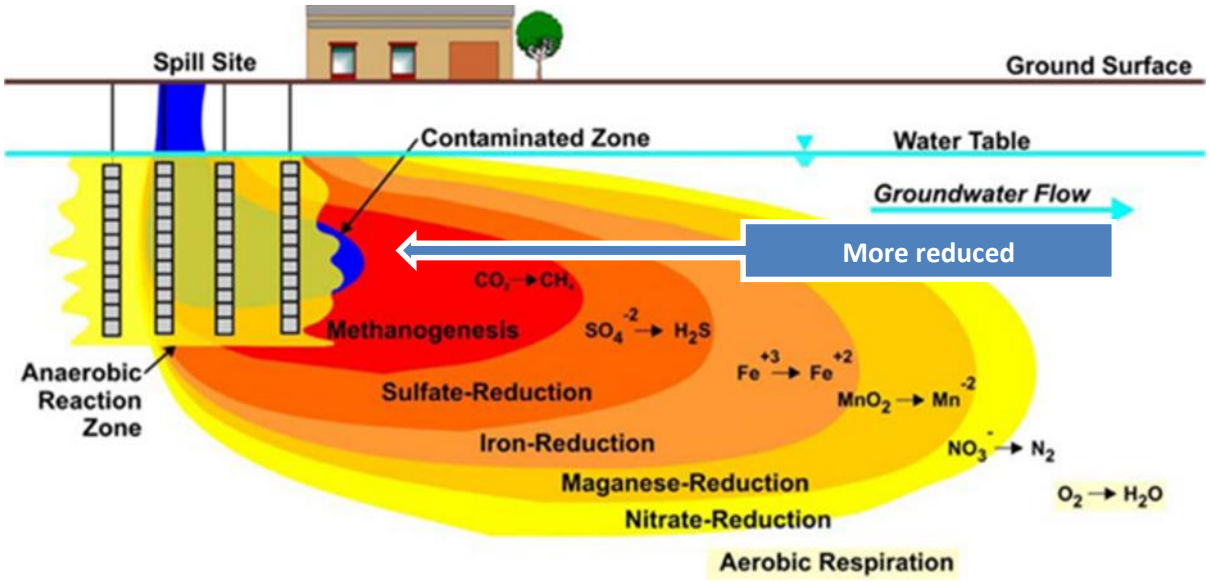


(5) Iron, manganese, and arsenic are examples of common metallic species that have differing solubility depending on redox state. All are poorly soluble in oxidized forms and soluble in reduced forms. All are highly reactive with other species. While arsenic (As) is not known to have a biochemical cycle, both Fe and Mn are oxidized and reduced metabolically by microbes, and As co-mobilizes or precipitates with them. FeIII to FeII reduction is one of the "big four" (oxygen, sulfur, nitrogen, and iron) bacterial respiration processes. Fe, Mn, and As oxidation and reduction serve a variety of metabolic and detoxification purposes.

(6) Microbial processes likewise affect other redox-sensitive metals such as uranium. Unlike Fe, Mn, and As, U has the characteristic of being more soluble in the oxidized form and less soluble in the reduced form. Sulphate reduction, inducing a reduced environment, favors formation of "roll front" U ores. These can be economically important sources of U ore if large enough and accessible for mining or nuisance sources of radionuclides impacting drinking water.

(7) A variety of other metals are likewise manipulated and used by microbes for various purposes. Figure 6 illustrates the sequence of electron acceptor choices in microbial respiration using the example of a hydrocarbon spill plume (from most reduced to most oxidized, L to R).

Fig. 6: Summary of microbial respiration electron acceptor options (source: U.S. EPA)



(8) And of course, various cycles affect one another. For example, sulphate reduction is described as reducing the amount of soluble As in groundwater (Kirk et al., 2004). Although reduced As (As III) is soluble, it has a high affinity for Fe_x sulphides. Typically, methanogenesis occurs where sulphate is depleted, so SRB can make conditions favorable to methane formation. Also anaerobic methanotrophy is performed by syntrophic consortia consisting of anaerobic methanotrophic archaea (ANME) and SRB, which consume an estimated 80 % of methane generated beneath the ocean floor (Marlow, 2015; Marlow et al., 2014). Figure 7 is an illustration of interactive geochemical cycling (in this case, coupling of methanotrophy and S and N reduction).

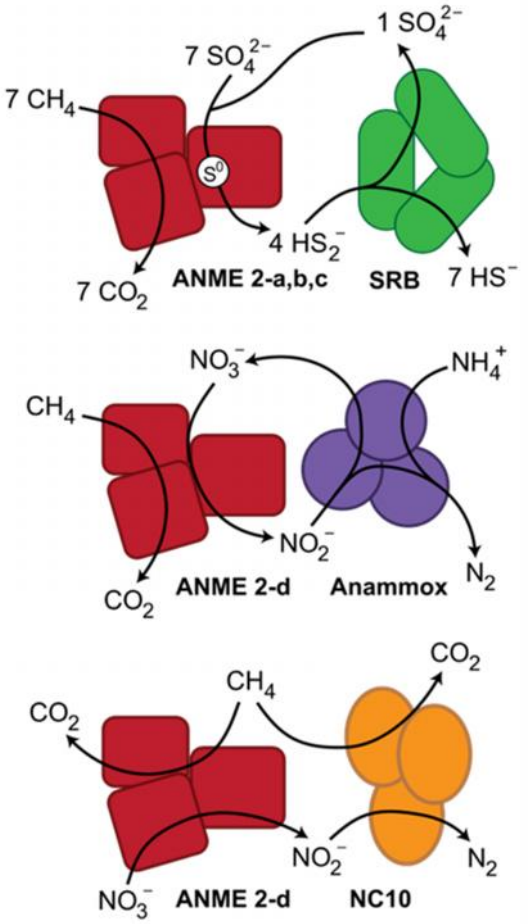
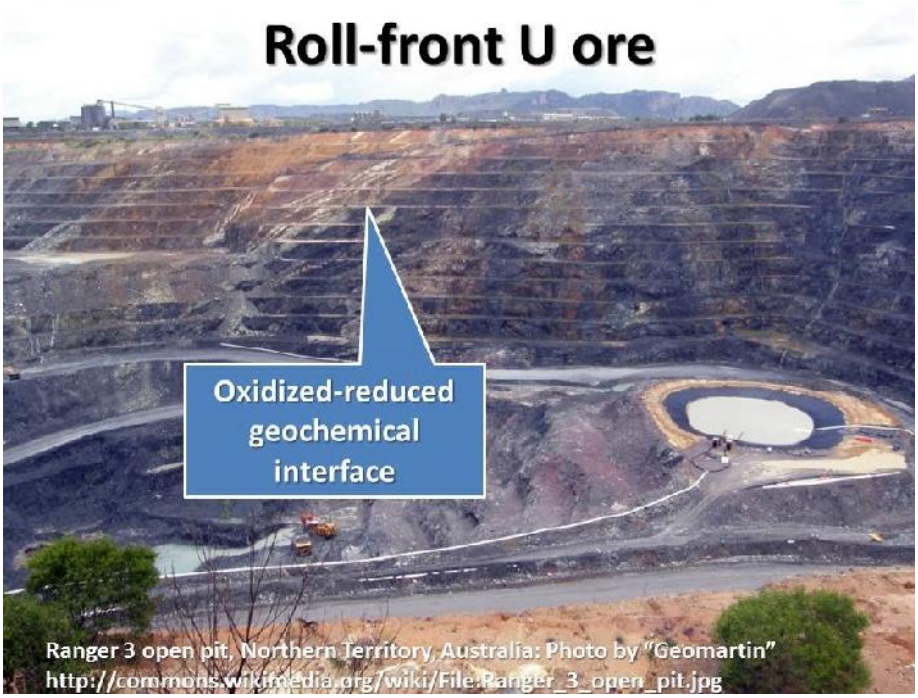


Fig. 7: Syntrophic coupling of methanotrophy and geochemical reduction (source: CtSKinnerton in Wiki Commons (no further information available) http://en.wikipedia.org/wiki/Anaerobic_oxidation_of_methane)

Ehrlich and Newman (2009) is a comprehensive reference for understanding the range and complexity of microbially affected cycles. Figure 8 is a composite of photographs illustrating lithologic expressions of redox interfaces, and Figure 9 is an example from an Australian uranium pit.

Fig. 8: Visible redox reactions at lithologic interfaces (clockwise from upper L: Carboniferous SS and shale (Ohio USA), SS with Fe-rich flow (Ohio USA), biogeochemical "varnish" on Precipice SS, Carnarvon Gorge, Mossy Grotto, Carnarvon Gorge

Fig. 9: Visible oxidized-reduced interface, uranium mine, Northern Territory

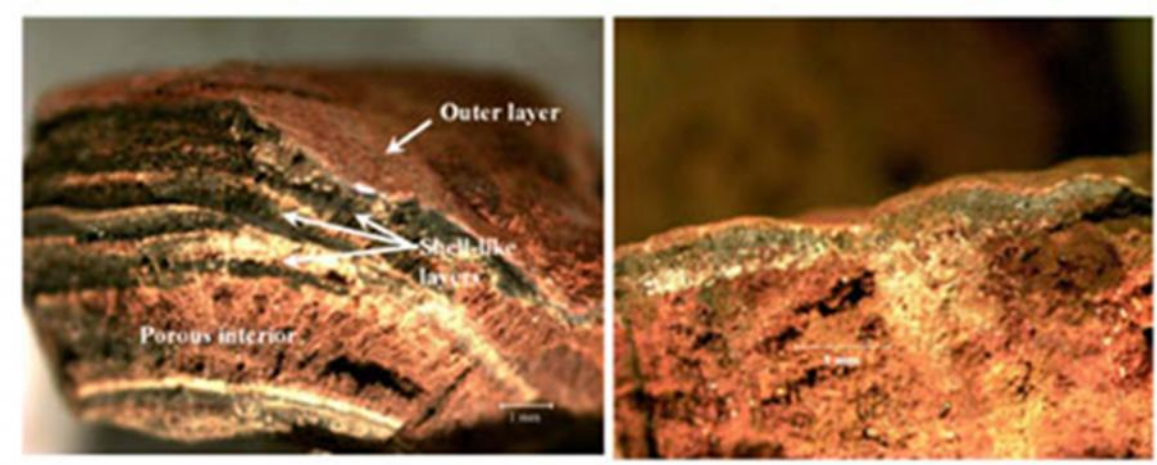


4.0 Constituents and Conditions Generated as a Result of Microbial Activity Associated with CSG

Methanogenesis, the process of interest in developing CSG fields, as described previously, is often (although maybe not always) a biological process in coal. Of course, biogenic methanogenesis is not exclusive to coal or even hydrocarbon source or reservoir formations. Indeed, it is common in wetlands, digestion, anaerobic refuse tips, and wastewater treatment. As discussed, where sulphate is depleted and sulphate reduction is not favored, methanogens can have ecological advantage, favoring methane formation. In either case, low redox-potential environments are favored. Depending on local mineralogy, microbial populations, etc., Fe and other metal reduction can be favored.

Besides H_2S production by SRB that makes water noxious, low redox potential environments are widely known to be associated with corrosion of steel alloys. Both mild or carbon steel and most stainless steel alloys depend on oxidized surfaces to repress corrosion. Where redox potentials favor Fe or sulphate reduction or methane formation, oxidized and metallic Fe and other alloyed metals are stripped from metal surfaces. Corrosion ensues, and product water contaminated by over-abundant metals and sulphides. On carbon steel surfaces, corrosion tubercles may form. These are formed at anodes on metal surfaces such as pipe by consortia of SRB and neutral-philic iron-oxidizing bacteria, which serve one-another's needs for FeII and a protective anaerobic environment, respectively. These processes are discussed in Smith and Comeskey (2009) in the context of effects on bores developed in aquifers and downstream components. Figure 10 shows cross-sections of corrosion tubercles on mild steel pipe, showing layers of iron-oxidizing and sulphate-reducing bacteria.

Fig. 10: Cross-sections of iron pipe (mild steel) corrosion tubercles (U.S. EPA)



Likewise, where produced methane is oxidized to CO_2, typically by methanotrophic microorganisms, groundwater can become gassy or supersaturated with respect to bicarbonate. Groundwaters typically maintain dissolved solids and gases in solution, even when supersaturated, and CO_2 is over 50 times as soluble in water as other common air gases, so it does not degas easily.

A small fraction of aqueous carbon dioxide, CO_2 (aq), reacts with water forming carbonic acid, H_2CO_3 (aq). Carbonic acid may loose protons to form bicarbonate, HCO^{3-}, and carbonate, CO_3^{2-} or dissolve $CaCO_3$ (also generating HCO_3^-). Depending on local lithology and microbial ecology, the reactions can become complex. Where protons are liberated to the water, pH may decrease, or H^+ may be taken up by microbes and either used in electron transfer or stored as biomass. Where H^+ are scavenged, pH increases.

In most cases, a delicate equilibrium is achieved in native ground water, but pumping a well can upset this equilibrium. CO_2 degassing would be most likely if the pumping water level is significantly deeper than the static water level (Figure 11). These changes can result in dissolution from solids, a problem with "hard" water, and subsequent mineral precipitation and incrustation. In some cases, physico-chemical transformations can alter aquifer hydraulic properties**,** especially when pumping begins (lowering pressure) or changes.

Fig. 11: CO_2-enriched groundwater collected during a pumping test in Ohio USA (not flammible)

Redox and pH shifts toward oxidation and alkalinity, respectively, may result in the deposition of iron and manganese oxides and carbonates. Carbonate clogging of extended hydraulic structures is known from antiquity. A notable example was the circumstances of carbonate clogging in the Roman aqueduct systems. Much the same phenomena occur in modern dam geotechnical drains (Smith and Hosler, 2001 and 2006). Iron and manganese oxide clogging is well known in the groundwater field, affecting the performance of both pumping and monitoring bores and downstream water system components.

Microorganisms accelerate the formation of such deposits. In particular, dissolved MnII resists chemical oxidation in groundwater of a pH suitable for potable use and requires a microbial mediator for Mn oxides to form (see the following discussion). Where Mn oxidation and deposition are common, aquifer microbial ecology is flourishing.

In groundwater systems, as discussed, microbes occur predominently in biofilms on the abundant surfaces in aquifers and bores and on equipment in bores. Where S- , Fe- , and Mn-oxidation (and some other phenomena, including carbonate formation) occur, biofilms attract and accumulate such oxides and carbonates, as well as particulate matter such as clay, silt, and sand. Where these collect and build up to the point of influence on systems, biofouling occurs. Depositional biofouling and biocorrosion typically occur together on corrodible metal surfaces. Further discussion of these processes and their effects may be found in Cullimore (2008) and Smith and Comeskey (2009).

5.0 Potential Drilling and Stimulation Fluid Contributions

A potential factor in poor CSG well and water supply bore water quality are constituents of drilling fluids and development and hydraulic fracturing fluids, both for the water supply bores and CSG wells. Among complaints both in CSG fields and oil and gas fields elsewhere are hydrogen sulphide, other odors, tastes, and discoloration of bore water. In general, use of biodegradable drilling and development fluids is discouraged in water bore production, especially as high-quality Na bentonite and suitable nondegradable polymer additives such as acrylamides are widely available for both water- and air-based fluid systems (ADITC, 1997; NGWA, 1998). Leading groundwater industry sources also discourage the use of phosphorous-containing products, especially phosphates, which are scarce in groundwater, so efficiently scavenged by groundwater microflora to promote growth (Smith and Comeskey, 2009). However, biodegradable natural polymeric gum products (e.g., guar gum) and cellulose-based viscosity enhancers, as well as P-polymer additives, remain in the inventory (Sterrett, 2007).

Oilfield-type fluids employ a wider range of additives, but also can employ aggressive biocides not usable in potable water aquifers in Australia and most countries that protect groundwater quality. Also, various Na or K sulfites, bisulfites, and sulfates are used as fluid modifiers. This situation: the possibility of oil-based fluids with emulsifiers and cellulose or gum polymers, electron donors for common bacteria in groundwater (sulfates and sulfites), and essential nutrients (P), can result in environments where microbial activity can produce growth and undesirable products such as hydrogen sulphide. Recall that, as discussed previously, various microorganisms are versatile in metabolic pathways.

Also as discussed previously, if the drilling environment involved is a CSG well, a pathway and hydrostatic head have to be sufficient in order to affect aquifers, or the effects are localized. If the

pressure is insufficient and the pathway unlikely, the cause is more likely to be at or near the affected water bores.

6.0 Microbiological Analysis

Microbiological analyses of causes of nuisance and sanitary water quality issues were among the first applications for the available techniques, stretching back to the invention of the microscope. Study of deposits in water system pipes and natural springs and bog ores extend to the early 19th Century. Methods for studying iron- and sulfur-related bacteria were developed beginning in the late 19th Century. We are using sanitary and heterotrophic bacteriological techniques that are virtually unchanged since the 19th Century.

6.1 Purpose drives selection

In a programme such as the planned Microbial Analysis Programme being developed for the CSG Compliance Unit (CSGCU), the tools and techniques specified should match the mission of the programme, and we suggest that they should not (1) duplicate other monitoring programmes (e.g., sanitary monitoring routinely conducted by public health authorities) or (2) be unnecessarily experimental. The compliance oversight programme is subject to public and scientific scrutiny and its work subject to rigorous technical review by interested parties. The programme also has a certain amount of resources, and how these are distributed must be prioritized. Where possible, methods selected should meet multiple objectives (e.g., the stated need for both short-term and ongoing testing). Methods selected should be accepted and reliable for the purposes assigned. Finally, methods and systems selected should be practically useful as integral parts of the wider monitoring programme. The Sampling and Analysis Plan developed for CSGCU (Smith-Comeskey Ground Water Science, 2015) reflects these priorities.

When we choose the wording “unnecessarily experimental,” we anticipate that monitoring relatively deep bores in a complex biogeochemical setting such as described previously may require some creativity within responsible scientific experience. Microbial diversity is likely to be high, so the spectrum of methods chosen should not be unduly restricted. While methods that are likely to be employed are described in peer-reviewed technical literature and standard documents, many are relatively new and not in widespread use. For example, if we are going to test for methanogens or many methanotrophs (sources of volatiles), we may be developing a microbial ecology laboratory in support. Finally, such a monitoring programme can be an opportunity for advancing technique, where appropriate.

6.2 Methods candidates and applications

Based on past experience both in Queensland and other hydrogeochemical environments that resemble the Queensland CSG fields, several water quality issues and effects on bores and bore equipment that have a microbial biogeochemistry component can be anticipated:

(1) Occurrence of biogenic methane and CO_2 in bore water, with associated gassiness and changes in bicarbonate and carbonate saturation.

(2) Reduction in redox potential and associated stimulation of sulphate and iron reduction.
(3) Where the redox potential of water is reduced, soluble FeII or MnII are available in an oxidized water column in a bore, where oxidation of the Fe or Mn can occur.
(4) Nitrate reduction also provides a mechanism for FeII oxidation.
(5) Where sulphide is available in groundwater supplying an oxidized water column in a bore, sulphide oxidation occurs.
(6) Biofouling associated with subjects 1 to 5.
(7) Corrosion associated with 2 and 3 and within established biofouling deposits.
(8) Tastes, odors, increase in metals (including As and U) and discoloration associated with 1 to 7.

Thus, methods that could provide information on microbial activity on a range of redox potentials from methanogenesis to MnII → MnIV oxidation (from below -330 to > +700 mv) might be needed, at least selectively. However, the microbes behind the generation of CH_4 and CO_2 might be assumed, unless confirmation of presence, and information on species composition, consortia, or population numbers are needed. The necessity to include Mn oxidation or other metallic ion oxidation may depend on the geochemical occurrence or relative absence of Mn and other compounds of interest in groundwater.

Standard Methods for the Examination of Water and Wastewater (currently APHA, AWWA, WEF, 2012) has published sampling and analytical methods for iron- and sulphur-related bacteria (oxidizing and reducing) for decades in Section 9240. While the subject of the section is iron- and sulphur-related bacteria, methods and media for Mn-related bacteria are also included. While not the only source of microbiological analytical methods, *Standard Methods* is widely used as a reference in water laboratories, and its methods sometimes have quasi-legal status.

Sampling and analytical methods in Section 9240 were viewed as inadequate and antiquated for years (Smith and Tuovinen, 1985; Smith, 1992; Smith, 1996; Tuhela et al, 1993). By the 19th Edition of *Standard Methods*, the disconnect between methods published in Section 9240 and physiological and sampling methods in widespread use in practical analysis of water bore problems and environmental microbiology had become quite large (Smith, 1992; Gariboglio and Smith, 1993; Cullimore, 2008). From the 20th to the 22nd editions, the gap gradually narrowed. The current edition (22nd) Section 9240 includes surface collection methods and a modern suite of physiological methods. It lacks reference to biochemical methods of analysis, and thus large fractions of available microflora and their functions may be overlooked in analyses.

6.3 Microbiological methods for biofouling and biocorrosion testing

The following methods can answer the following questions:

- Are biofouling and/or biocorrosion present?
- What types of biofouling and biocorroding organisms and activities are present?
- Is the bore or associated systems more or less biofouled than before? What is the pace of corrosion? The answer to these latter questions requires monitoring over time.

For bore maintenance and problem diagnosis purposes, methods that provide rapid, general insight into biofouling and biocorrosive conditions (what is happening?) are generally preferred over methods that characterize genetic make-up or metabolic capabilities (who is doing it and how?).

6.4 Types of biological analyses employed in diagnostic and maintenance monitoring of bores

(1) Examination by light microscopy: This has traditionally been the method of choice for confirming and identifying "iron bacteria" (APHA, AWWA, and WEF, 2012, Section 9240). However, in many instances, biofouling as a cause of well problems may be difficult to diagnose via microscopy alone, even with very good tools and skills (Smith, 1996) and identification by microscopy alone is prone to error.

(2) Cultural enrichment and physiological indication: Culturing can provide a means of detecting non-filamentous, metabolically active biofouling microflora, and also to profile the ecological physiology niches occupied by microorganisms. Among the available methods in the cultural approach for routine maintenance monitoring purposes is the BART™ Method developed by Droycon Bioconcepts Inc., Regina, Saskatchewan, Canada (Cullimore, 2008; Smith, 1992; Smith, 1996; Smith and Comeskey, 2009). This method was found by Smith (1992) in field trials to provide useful qualitative information in well biofouling events and is increasingly accepted as a standard biofouling monitoring method. Figure 12 illustrates a selection of BART reactions: black cap SRB-BART, red IRB-BART, and purple are APB-BART.

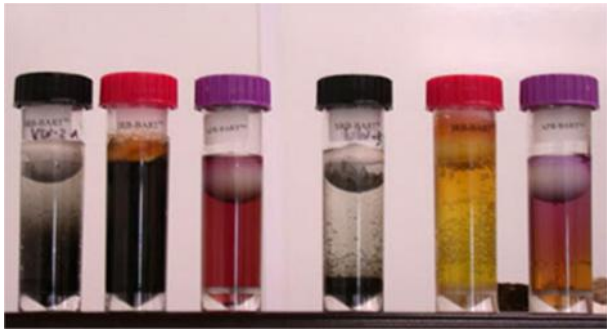


Fig. 12: A selection of reacted BART tubes (Hudson River Valley aquifer, New York, USA, angled municipal bores)

A similar system, employing bottled liquid cultures, is marketed by Laboratorio MAG in Argentina (Gariboglio and Smith, 1993; Smith and Comeskey, 2009). BRS-MAG (SRBs) tests and the sample injection method are illustrated in Figure 13.

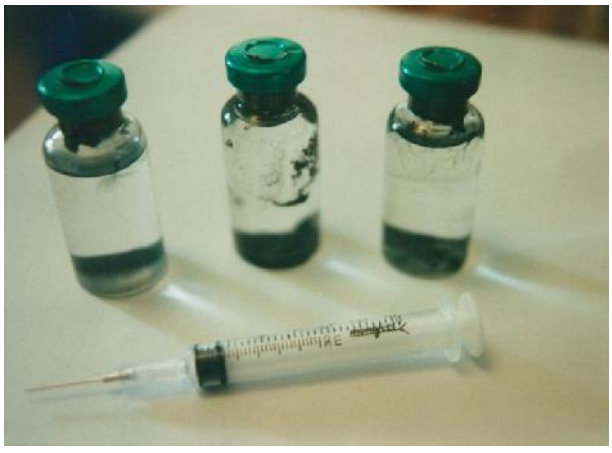


Fig. 13: BRS-MAG tests (positive for SRB, bore water samples from Ohio, USA)

Both BART and MAG iron-related and sulphate-reducing methods are now included in the 22nd Edition of Standard Methods Section 9240. Using Section 9240, a facility's laboratory can also prepare its own culture media for the same purposes, as well as for for Mn- or S-oxidizing biofouling. Some indication methods can be very simple, including using a steel washer in a bottle (sometimes amended with available spirits such as whiskey).

In addition to tests favoring dissimiliatory sulphate reduction by native SRBs, sulphide may also be produced (reduced from sulphite) by fermenting bacteria such as *Salmonella* and *Clostridia* (essentially putrifaction). There are also packaged tests (e.g., Easicult S, Orion Diagnostica) that can be used to distinguish this source of H_2S from sulphate-utilizing SRB sources.

The advantage of the commercial test products over laboratory-formulated tubes and plates include:

- Consistent formulation and shelf life
- Lack of need for the CSG Compliance Unit to invest in microbiological laboratory preparation capacity and facilities
- Lack of need for incubation and similar laboratory facilities (an exception would be testing for non-SRB sulphide producers such as *Clostridia* using Easicult S, requiring incubation)
- Portability for field use, including inoculation in the field
- Liquid incubation is more realistic than the plate environment for aquatic bacteria
- Commercial support for use and interpretation.

The BART and MAG method tubes come with a variety of media mixtures. The IRB-BART, for example (Section 9240) is designed to recover anaerobic (sulfur- and nitrate-reducing) and microaerophilic heterotrophic Fe-precipitating microorganisms. The SRB-BART (Section 9240) effectively recovers sulphate-reducing bacteria. Smith (1992 and 1996), Smith and Comeskey (2009) and Cullimore (2008) provide guidance in BART method use. Gariboglio and Smith (1993) and bulletins at www.laboratoriomag.com.ar/ address MAG use (*en español*). Both the BART and MAG media are based on published media formulations.

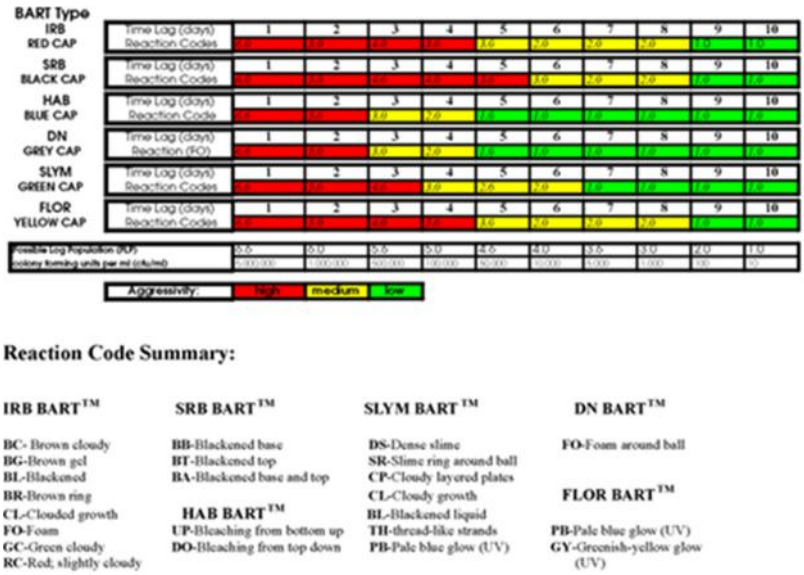
The “iron” BART and MAG tests have poor recovery for Mn biofouling, and really need to be supplemented by a Mn-related selective enrichment medium if such analysis is deemed necessary. Likewise, there is no S-oxidizing BART or MAG enrichment medium. Some users, including the author of this report (Smith, 1992; Smith-Comeskey Ground Water Science, 2012) prefer to combine methods for iron, sulfur, and manganese biofouling analysis.

There are also BART media in addition to the IRB and SRB models. These are not included in *Standard Methods* but have been reviewed by Canada Standards for quality assurance. For example, the DN-BART selectively enriches for denitrifying bacteria. Denitrification is an important pathway for FeII oxidation and the process is common in aquifers. As described in Smith and Comeskey (2009) and Smith-Comeskey Ground Water Science (2012), selecting BART methods covering a range of redox potential conditions provides a field-usable means of assaying the microbial ecology of a system of interest, such as a water bore.

Interpretation of BART and MAG: These tube-based tests do not permit the direct visual count that can be made using heterotrophic plate count methods. Laboratorio MAG does not emphasize the quantitative property for its tests, but BART are evaluated for both 1) the type of reaction and 2) the time until the reaction occurs. The type of reaction can be interpreted to provide some idea of the bacteria present. The time until the reaction has been empirically compared to the colony forming units

(CFU) per mL count developed to evaluate plate count results. So a certain "day to development" of a reaction is analogous to a CFU/mL count. Alternatively, and more favored by BART, is reference to an "aggressivity" scale. Figure 14 is an illustration. These can be compared over time in long-term monitoring (Cullimore, 2008; Smith and Comeskey, 2009).

Fig. 14: BART aggressivity scales for several types (Droycon Bioconcepts Inc.)



The horizontal axis (top) is days to a reaction. Shorter time corresponds to higher aggressivity.

At present, there is little information on how well cultural methods such as BART recover Archaea involved in S and Fe transformations.

6.5 Culturing for methanogens and methanotrophs

There are culturing methods for methanogens (e.g., Midgley et al., 2010 and Wolfe, 2011), using necessary compounds such as formate, H_2 and CO_2 as substrate, with Wolfe (2011) having a section entitled "Methanogens on a Budget." However, it is clear in reviewing the procedures that conducting such culturing is much more challenging than culturing iron-related bacteria or SRBs (using BART or MAG methods) due to the need to establish and maintain strictly anoxic conditions with a redox potential below – 330 mV. Methanogens tend to prefer circum-neutral pH but water salinity can be a selecting characteristic. Many methanogens are also dependent on syntrophic partners for essential substrates such as formate (McInerney, 2011).

Likewise, there are culturing methods for a variety of methanotrophic bacteria and archeans, including anaerobic types recently discovered (Girguis et al., 2003), and likely to be of interest to the project. Anaerobic methanotrophic archaea occur in syntrophic consortia with sulphate-reducing partners, and maintaining conditions suitable for fully functioning consortia can be challenging. Defining systems that consume CH_4 and generate CO_2, and demonstrated to be biogenic, could be part of the experimental component of the project.

6.6 Biochemical methods

This is a broad term encompassing several analytical procedures used to characterize microbial communities in samples without culturing or microscopy of any kind. Samples (which may be solid or

liquid or some combination) are collected and processed to extract chemical markers of interest. Biochemical or "microbial fingerprinting" methods in general fall into two categories widely used in the study of groundwater: 1) Signature lipid biomarker analysis (typically phospholipid fatty acid analysis, PFLA) and 2) phylogenetic methods that analyze for nucleic acids (some class of RNA or DNA) and then typically analyze for the resulting patterns. White and Ringelberg (1997) and Reeves (1997) give overviews from early in the time of widespread use of these methods. Today, commercial laboratories provide these services for various environmental, engineering, or generally academic purposes. For nucleic-acid methods in particular, analytical products are developing rapidly in order to provide a more cost-effective analytical product using the same base techniques.

PFLA analysis: Phospholipids are the main component of the cell membranes of all microbes. Phospholipid fatty acids with ester linkages (the subject of PLFA) are the cell membrane components of bacteria and eukaryotes. PLFA provides information about a site's microbial community in three key areas that DNA analysis does not: viable (living) biomass (how much mass is there?), a community composition or population "fingerprint" (what the organisms "do for a living," e.g. iron reducers, sulphate reducers, or fermenters), and microbial activity – who is alive and functioning? A drawback of commercial PLFA analysis is that it cannot analyze for archaeans such as methanogens, which have an alternative cell membrane architecture based on phospholipid ether lipids (PLEL). PLEL analysis is practiced but is not as readily available commercially (Gattinger et al., 2003). Lipid biomarkers are readily extracted from a variety of media (fluid, solid, or gradations in between). The extracted biomarkers are extracted in organic solvents, purified, and concentrated for GC-MS analysis. This is a robust analytical method that can provide useful "signal" even from degraded samples, and is therefore relatively forgiving of lapses in sample handling or delay in processing, although these are not recommended.

Phylogenetic methods: Although a range of phylogenetic methods have been used to study microbial populations (Reeves, 1997), the commonly used method for environmental studies at the present time is the polymerase chain reaction (PCR). Specifically, DNA fragments from a PCR reaction using a specific primer are separated to differentiate closely related microbial strains. A variation on this approach is CENSUS, as employed by Microbial Insights Inc. (USA and Australia, www.microbe.com). CENSUS employs quantitative PCR (qPCR) for enumeration of specific microorganisms or genes encoding specific biological processes, and the end result is an analysis of what microbial groups may be present and if there is sufficient DNA for further statistical community analysis. CENSUS further can be targeted to functional interests, for example, groundwater remediation or microbially influenced corrosion (MIC). CENSUS provides a functional result as with the following from aquifer and groundwater samples from Jordan (Ground Water Science client project):

Phylogenetic class	Code	DBP2 2012 as cells/mL	DBP2 2013 as cells/mL	W37 2013 as cells/mL	PZ8 400-m core sample
Eubacteria	EBAC	2.55×10^4 to 5.01×10^4	1.12×10^5	3.03×10^6	2.34×10^6
Iron- and Sulphate-reducing bacteria	APS	~1.00×10^{-1}	1.64×10^3	4.93×10^4	1.50×10^3
Nirk denitrifying bacteria	nirK	$1.96\text{-}3.98 \times 10^4$	5.72×10^5	1.21×10^6	2.00×10^3
Methanogens	MGN	$3.43\text{-}4.55 \times 10^5$	1.17×10^1	2.02×10^5	NA
Acetogens	AGN	~2.28×10^3	NA*	NA	NA
Archaeal	ARC	5.87×10^1 to 2.79×10^2	NA	NA	NA

As with PLFA, useful information can be extracted even from degraded samples. This is also an example of how biochemical methods detect microflora classifications of interest that would be overlooked using cultural methods alone. Evidence of iron- and sulphate-reducing bacteria had been ambivalent from culturing, and nitrate-reducing bacteria, acetogens (a form of methanotrophs) and methanogens entirely overlooked. The analysis also provided insight into a difficult to identify form of bore biofouling.

An example of further community analysis that is widely used in soil and groundwater analysis is terminal restriction fragment length polymorphism (TRFLP or T-RFLP) analysis. TRFLP takes the analysis beyond CENSUS to provide a profile of the microbial community and a more definitive identification of the microorganisms present. It provides results on a percentage basis to provide assessment of the microbial population that can then be compared from time to time. TRFLP analysis begins with CENSUS. In the case of the above-mentioned case study, the TRFLP results did not shed as much light as hoped, as the majority of signals analyzed were “dark matter” that did not match Microbial Insights’ library of 3 million sequences. However, the analysis did permit a review of the bacteria types that clarified probable origins of the various types.

Recent practical upgrades for qPCR-based analyses: The molecular microbiological laboratory Microbial Insights Inc. (www.microbe.com, Knoxville, Tennessee and Urrbrae (Adelaide), S.A.), which Ground Water Science has used for its projects involving these technologies, has recently added two versions of qPCR-based techniques that focus analysis on active groups of specific interest to the application. For the purpose of the present investigation, the MIC (microbially influenced corrosion) “track” has application, since the focus is on effects on water bores. Two versions of the analysis are available: referred to as Next-Generation Sequencing and QuantArray-MIC, which are essentially different technical approaches to provide a similar end – an analysis “good enough” to identify functional groups (e.g., SRB bacteria) and some genetic classification. Thus, these methods offer many of the advantages of PLFA analysis with some phylogenetic information. Cost-effectiveness would have to be evaluated.

The QuantArray-MIC analysis subdivides samples and analyzes for specific genetic markers, and analyzes for the following:

QuantArray-MIC qPCR Functional Analytical Targets (Microbial Insights Inc., http://www.microbe.com/quantarray-mic/)

Target	Relevance / Data Interpretation
Total Eubacteria	MIC is initiated by growth of a biofilm on the material surface. Monitoring total bacteria provides a general measure for evaluating bacterial growth.
Total Archaea	Depending upon types and environmental conditions, total archaea can outnumber total bacteria and be a more important factor in MIC.
Sulphate Reducing Bacteria	SRB consume hydrogen, produce hydrogen sulphide and are the microflora most commonly implicated in the pitting corrosion of various metals.
Sulphate Reducing Archaea	Sulfate reducing archaea (SRA) consume hydrogen, produce hydrogen sulphide and have been implicated in MIC at elevated temperatures.
Exopolysaccaride Production	Targets genes involved in the production of exopolysaccharide (EPS) and biofilm formation by some *Burkholderia* spp.
Methanogens	Methanogens utilize hydrogen and can contribute to cathodic depolarization and can cause corrosion rates comparable to SRB.
Fermenting Bacteria	Anaerobic bacteria produce organic acids and hydrogen. Acid production can lead to localized drops in pH facilitating corrosion while supporting methanogens and SRB.
Nitrate Reducing Bacteria	The qDNF assay quantifies target genes encoding enzymes responsible for a key step in biological nitrate reduction (which can result in FeII → FeIII oxidation).
Acid Producing Bacteria	Acetogenic bacteria are strict anaerobes that produce acetate from the conversion of H_2-CO_2, CO, or formate, supporting methanogens. The presence of acetic acid is known to exacerbate CO_2 corrosion of carbon steel.
Iron Oxidizing Bacteria	Iron oxidizing bacteria are a group of microorganisms commonly implicated in metal deposition and tubercle formation. FeIII oxides aggregate As.
Manganese Oxidizing Bacteria	Like iron oxidizing bacteria, manganese oxidizing bacteria are capable of making deposits of metal oxides.
Sulphur Oxidizing Bacteria	Often aerobic bacteria oxidize sulphide or elemental sulphur producing sulphuric acid and biofouling.
Iron Reducing Bacteria (three assays)	Iron reducing bacteria reduce insoluble ferric iron to soluble ferrous iron potentially facilitating the removal of protective corrosion products formed on exposed iron alloy surfaces and increasing total Fe in water. Three assays targeting 1) Deferribacter, Ferrimonas, Geopsychrobacter, Geothermobacter, Geothrix, Geovibrio, Geothermobacterium and Albidiferax, 2) Geobacter, 3) Shewanella.
Iron Reducing Archaea	Targets two genera of iron reducing archaea, Ferroglobus and Geoglobus.
Nitrogen Fixing Bacteria	Nitrogen fixation converts nitrogen gas into ammonia.
Ammonia Oxidizing Bacteria	Ammonia oxidation or nitrification produces nitric acid causing. Depending on alkalinity levels, nitrification in water systems can increase lead contamination and increase copper solubility.
Deinococcus spp.	Genus of bacteria considered very efficient primary biofilm formers and therefore have been implicated in slime formation and biofouling.
Meiothermus spp.	Like *Deinococcus* spp., *Meiothermus* spp. are efficient primary biofilm formers and frequently implicated in slime formation and biofouling.

Comparison of "signal": Much as geophysicists would run a suite of analyses to improve the resolution of a geophysical study, with each type of signal providing a piece of the picture, it is useful to run a suite of microbiological analyses to improve the information gathered. For example, it is highly useful to run both PLFA and nucleic acid focused analyses such as TRFLP, and the occasional PLFA and/or PCR-based method to refine results from culturing (such as BART). The suite gives much more information than one

analysis alone. Biochemical results can be used to “calibrate” interpretation of ongoing (and potentially less expensive) culturing analysis. The new qPCR packages may scramble this early 21st Century cost-effectiveness reasoning.

Information for resources invested: Biochemical methods are relatively expensive on a per sample basis (perhaps epending on how time analyzing physiological samples is valued), but they provide a relatively large amount of information per sample. Using a culture-based approach to a microbial ecology problem requires settling for a limited amount of cultural diversity. If only interested in certain processes, this is reasonable. If a broad reconnaissance is required, biochemical methods become more cost-effective. In the case of difficult-to-culture microorganisms, such as methanogens or methanotrophs and their syntrophic consortia, biochemical methods are likely more cost-effective.

6.7 Coordination with physical-chemical analyses

Water quality: For water samples, it is helpful to combine microbiological analysis information with related information. A variety of water quality parameters provide indication of deterioration mechanisms active in bores and water systems, and are useful in coordination with microbiological methods to diagnose bore problems and in monitoring microbial changes in groundwater (Smith 1992, Smith-Comeskey Ground Water Science, 2000 and 2012; Smith and Comeskey, 2009). Understanding the hydrogeochemical environment helps in planning material choices for well components and potential clogging mechanisms. Water quality histories permit the identification of change in parameters that can be attributed to mechanisms such as biofouling. Analyzing physical-chemical water quality data can also provide insight into aquifer and bore redox potential, and by extension, biogeochemical activities within the capture zone of the bore of interest (Jurgens et al., 2009). In the case of the present study, sampling and analyzing gas and volatiles in bore water is of particular interest.

Biofouling and organic solids: Analyzing the nonmicrobial components of biofilm samples (e.g., deposits on pumps or pipes) is highly useful for identifying the biofouling and biocorrosion processes at work in and around a bore of interest. Where biofouling samples containing solids are collected and analyzed, it is useful to examine these by light microscopy, for Loss on Ignition (LOI), a surrogate for organic matrix (extracellular polymer or slime), and inorganic materials of interest, typically carbonate, sulphate, and metal oxides and sulphides.

Summary of relevant physico-chemical parameters in bore microbial ecology analysis

Fe (total, Fe^{2+}/Fe^{3+}, Fe minerals and complexes), Mn (total, Mn^{4+}/Mn^{2+}), minerals and complexes:	Indications of clogging potential, presence of biofouling, Eh shifts. Fe and Mn transformations are the most common among redox-sensitive metals in the environment.
S (total, $S^{2-}/S^{0}/SO_4^{2-}$, S minerals and complexes):	Indications of corrosion and clogging potential, presence of biofouling, Eh shifts.
Ammonia and nitrate-nitrite	Indications of organic clog build up, changing WQ conditions
Eh (ORP or redox potential):	Direct indication of probable metallic ion states, microbial activity. Usually bulk Eh, which is a composite of microenvironments.
pH:	Indication of acidity/basicity and likelihood of corrosion and/or mineral encrustation. Combined with Eh to determine likely metallic mineral states present.
Conductivity:	Indication of TDS content and a component of corrosivity assessment.
Major ions:	Carbonate minerals, F, Ca, Mg, Na, Cl determine the types of encrusting minerals that may be present and are used in saturation indices. One surrogate for many cations is total hardness and alkalinity for anions.
Turbidity:	Indication of suspended particles content, suitable for assessment of relative changes indicating changes in particle pumping or biofouling.
Sand/silt content (v/v, w/v):	Indication of success of development/redevelopment, potential for abrasion and clogging.

6.8 Sampling issues

The overriding issue is what are we sampling? In studying the geomicrobiology or microbial ecology of an aquifer, samples could be solids such as drive or drill cores or drill cuttings, or water from finished bores. The assumption made here is that sampling from bores is the major focus. Thus sampling would largely be focused on obtaining water samples, biofilm samples from water-affected systems, and the occasional more massive solids sample. Most of the analyses discussed are focused on water, but can be used for solids analysis. The following sample descriptions likewise focus on water.

(1) Time-series sampling: Cullimore (2008) describes a time-series pumped-sampling program that attempts to overcome the uncertainties of collecting particulates (biofilm components) from water samples by grab sampling. Cullimore's procedures involve taking advantage of the phenomenon that biofilm detachment occurs preferentially on start-up after a period of rest, in which the pump is allowed to shut down for a period of time from 2 hours to several days. This approach, which includes taking replicates of samples at each sample event, helps to overcome the statistical limitations of pumped grab sampling for cultural analysis.

(2) Surface collection: While the time-series procedure improves results, grab samples remain unreliable for microscopic and biofilm-solids analysis (Smith, 1992; Tuhela et al., 1993). For this purpose, some method is needed to provide enough sample to view or otherwise analyze mineralogically or chemically. Methods for collection of biofilm on immersed surfaces can provide essentially intact biofilms for analysis. These methods are also adaptable for collection of samples of inorganic encrustations and evaluating microbially influenced corrosion (MIC) or biocorrosion effects (e.g., McLaughlan, 1996). The flow cell system in Smith (1992), illustrated in Figure 15 – since modified and simplified – has been successful in practical use for such biofilm collecting. Sample collection using this method is described in Section 9240 in the 22nd Edition of Standard Methods. Coupon sampling apparatus developed for MIC evaluation may also be used.

Fig. 15: Flow-through apparatus collecting iron-oxidizing biofilm, municipal water bore, Ohio USA

(3) Necessary adaptation: While either sampling protocol 1 or 2 is preferred for microbiological and biofouling analyses, for it to be most useful for a diagnostic and ongoing monitoring approach, a testing program should be able to gain information from less-than-ideal sampling. For example, in 2013, Ground Water Science was faced with gaining biofouling-potential information from a 600-m well without a pump. The available sampling tool was a 2-L capacity wireline sampling tool. In this case, with one sampling opportunity and a small sample, biochemical (in this case, CENSUS and TRFLP) methods were selected to gain the most information from the limited sampling opportunity.

6.9 Minimum data elements

A one-time diagnostic sampling and analysis program may involve testing for a range of microflora likely to be involved in causing reported symptoms. These may be decided on a case-by-case basis. These are best candidates for the full redox-range testing and biochemical samples where difficult or unusual microbial conditions are suspected. Sample collection should follow the procedures of Cullimore (2008) for BART grab sample collection. Biofilm collection can follow the protocol outlined in Smith and Comeskey (2009) and Smith-Comeskey Ground Water Science (2012).

At a minimum, an ongoing monitoring program for wells should include a one-time use of a relevant biochemical suite, and the use of tests kits (BART or MAG or custom) and other self-monitoring (biofilm collection and visual inspection of components). BART or MAG testing should be relatively frequent (quarterly to monthly). Biofilm collecting can be conducted in a baseline troubleshooting role and then annually or at observed changes.

A quality assurance and quality control (QA/QC) programme meeting the standards of the DNRM, Australian standards organizations, and the microbial ecology community should be in place. The QA/QC procedures and standards of APHA, AWWA, WEF (2012), including Sections 9020 to 9060, necessarily adapted for the present purpose, can provide guidance (among other references).

7.0 Conclusions and Recommendations

Mixed sedimentary lithologies including methane-producing coals also feature abundant reactive surfaces and compounds that microorganisms have adapted to exploit over earth history.

Microbial consortia and biogeochemical cycles have developed to exploit a wide-range of conditions and environment – as far as can be ascertained, the entire accessible planet – and microbial processes should be assumed to be active wherever they are possible.

Geomicrobiological processes have various consequences in freshwater systems, including oxidation and reduction of iron, manganese, nitrogen and sulfur species, arsenic, uranium, and other metals, and production of gases, including methane and carbon dioxide. These can cause clogging, corrosion, aesthetically undesirable water quality, and water quality changes of health concern.

Monitoring for microbiological processes and microflora that are involved in bore water quality and bore equipment degradation, as well as processes that impair gas wells and associated systems, is a responsible component of a larger programme to monitor coal seam gas development.

Microbiological monitoring should be focused on practical, applied methods that address the questions for which answers are needed, and not necessarily the most academically advanced. Sampling and analytical methods should be demonstrated to be effective for the purpose. At this stage in microbial ecology and industrial microbiological development, many tools and methods exist and are well-defined for the purpose.

Although advancing the frontiers of science is not the goal, advanced methods such as biochemical analysis should not be avoided where they are the most effective or cost-effective, and monitoring complex geotechnical systems may require innovation.

The microbiological programme should be an integral part of the overall monitoring programme, with data from physical-chemical, hydrogeological, engineering, and microbiological components informing the interpretation of the others.

Quality assurance and quality control should be integral to the microbiological program and data managed in a transparent fashion.

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