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Potential microbial interactions with cements and steels

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Foreword

This report has been prepared as a component of the CSIRO GISERA project 'Review of cements, steels and microbial activity for Qld CSG wells'. The scope of this report is task 5 of the project proposal, which covers a summary and analysis of the available data on subsurface microbial communities in Queensland aquifers in areas of coal seam gas activity.

A companion report, 'Cement and steel used in coal seam gas (CSG) well construction in Queensland' (Grigore et al., 2024), addresses task 2, 3 and 4 of the project proposal, which covers data collection and collation, summarising and analysing data on the steels used in well casing, and summarising and analysing data on the cements and cement additives used in well cement.

With this report, an attempt has been made to make it as accessible as possible for all readers, however, some technical knowledge is assumed.

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We also pay our respects to the cultural authority of Aboriginal and Torres Strait Islander people and their nations in Queensland, and in particular the original owners of the Western Downs Region of Queensland, the Barunggam to the west of Dalby, the Iman (Yiman) around the Wandoan area, the Bigambul around the Tara area, and the Wakka Wakka and Jarowair around the Bunya Mountains area.

Executive summary

There is increasing public concern about well integrity issues including corrosion of metals and damage to cements used in well construction. This concern is primarily centred around contamination of subsurface groundwater resources, though emissions or damage to the surface environment are also of concern. Processes that damage wells (both CSG and agricultural wells) can have abiotic (non-living) or biotic (living) mechanisms. This report focuses on microbial capability to interact with metal casings or cements used in CSG well construction.

Microbes that are capable of producing acidic products or directly interacting with various metals occur universally in the subsurface, down to a depth where the high temperatures (>120°C) essentially sterilise the rocks. Microbial acid production can take many forms: production of sulphides, organic acids, protons or (more rarely) inorganic acids. Direct interactions can also occur, that is, some microbes are capable of removing electrons directly from metals.

All of these processes, however, are highly dependent on geochemical conditions. This reliance on particular chemicals can be direct, for example, microbial production of sulphide cannot occur in the absence of organic sulphur compounds, sulphate, sulphite, thiosulphate or elemental sulphur. Conversely, the reliance on other chemicals can be indirect, for example, the absence of nitrogen or phosphorus (important macronutrients) limits the ability of microbes to grow. It should also be noted that there are other mechanisms for acid production that do not rely on the presence of sulphur, though these are less well studied.

This study sought to gather data on the types of microbes (and their habitats) that occur in subsurface environments of South East Queensland. No publicly available data are available on microbes from non-coal aquifers of the region. No publicly available data were found on microbes that adhered to surfaces (pipes or other infrastructure). All subsurface microbial data from the region are associated with water from coal resources in the Surat or Bowen basins.

Analysis of the available data from the microbes associated with the coal seams indicate approximately one fifth of the identified species of microbes likely have the potential to produce sulphide when in the presence of suitable sulphur sources. It is noteworthy that the waters of the Surat and Bowen basins are extremely deficient in phosphorus, and mostly deficient in oxidised forms of sulphur. Both deficiencies are likely to be limiting factors for microbial production of sulphides within these coal formations.

Based on the results of this study, further surveys of water chemistry and microbiomes in shallow, unconfined aquifers of South East Queensland are required for establishing representative baselines. Surveys of Queensland groundwater pipework for microbial biofilms would be valuable, as biofilm formation is likely critical to microbial interactions with infrastructure. Furthermore, studying microbially-influenced corrosion through laboratory experiments, ideally using indigenous microbial communities from Queensland aquifers or well bores, could aid in the development of strategies to minimise microbial impacts on infrastructure.

1 Background

This background section provides important context and explanation for Section 2, the research program of the project. These background topics are covered in a series of separate subsections, each with a subheading, and readers should familiarise themselves with topics of which they are unfamiliar prior to reading Sections 2 and 3.

1.1 Motivations for this study

In Australia, there is growing public concern about corrosion of coal seam gas (CSG) and other wells that are drilled in the subsurface (Labouchardiere et al., 2014; Little & Lee, 2009; McCrea et al., 2020)¹. One key concern is that, should corrosion occur, it could impact the integrity of the well, which would potentially damage important groundwater resources on which many communities rely. Corrosion can be caused by both biological (living) and abiotic (non-living) means. This report deals only with potential microbial (mainly bacterial) interactions with cement and steels that may cause potential deleterious effects. When microbes cause corrosion, the process is called Microbial Influenced Corrosion² often shortened to MIC (Jia et al., 2019; Little et al., 2020).

1.2 The study area

This report is focused on microbes in the subsurface environments in South East Queensland, with a specific emphasis on areas in which significant CSG production has been (or may be) undertaken. In this instance, this area centres mostly in the Western Downs Region, and further west towards Roma in Sout Central Queensland.

¹ Community priorities revealed in GISERA survey <https://gisera.csiro.au/community-priorities-revealed-in-gisera-survey/>

² Sometimes Microbially Influenced Corrosion is reported as Microbially Induced Corrosion, but they refer to the same process. This report uses the former.

1.3 Aquifer environments

An aquifer is an underground layer of permeable rock, sediment, or soil that permits the movement of groundwater. Groundwater is water that saturates the pore spaces within the geological formations and is both stored in and moves through the aquifer.

Aquifers may have fresh or saline water moving through them. In low-rainfall areas, freshwater aquifers are particularly valuable resources. The groundwater chemistry of the water contained in an aquifer can vary widely in terms of their composition and properties and is affected by the composition of the aquifer host rock, sediment, or soil. Some common types of aquifers include sand, gravel, limestone or sandstone aquifers. Coal seams are themselves often aquifers.

1.4 Life in the subsurface

This study primarily discusses microbes that live in the subsurface, specifically bacteria and similar looking but unrelated organisms called archaea³. Collectively, bacteria and archaea are called prokaryotes and are all single-celled microorganisms.

For terrestrial (land-associated) environments, the 'deep subsurface' refers to all subsurface regions below the soil horizon and is something of an unusual habitat. It is increasingly warm, with the temperature continuously rising with greater depths into the subsurface. The typical maximum depth for life to survive is around 3-5km below ground level, by which point the temperature is generally over 120°C, and all microbial life has perished. There is also considerable pressure in subsurface environments, and, in many rocks, water is relatively scarce. Microbial diversity and abundance⁴ in the subsurface is greatest in environments with groundwater (such as aquifers), and within a reasonable temperature range.

1.4.1 Two habitats in the subsurface

In the subsurface, organisms can live suspended in the groundwater, or attached to the rock associated with the groundwater. Microbiologists refer to the organisms that live suspended in the groundwater as planktonic, while those attached to rocks are called adherent. The main mode of life for microbes adhered to rocks is in biofilms. Biofilms are populations or communities of

³ A detailed discussion of the tree of life that includes the relationships between bacteria, archaea and the other domain of life is available in Appendix 5.1.

⁴ Diversity refers to the number of species while abundance refers to how many individuals there are of a particular species.

microorganisms that are adhered to surfaces and include a range of sticky materials called extracellular polymeric substances (often abbreviated to 'EPS'). Biofilm formation involves the initial attachment of microorganisms to a surface, followed by the production of extracellular polymeric substances (Figure 1), which in turn makes it easier for other organisms to adhere to the surface, which then also produce their own extracellular polymeric substances. Almost all biofilms are complex, multilayered structures in which a range of cells from a range of species are embedded (see Figure 2 for an example).

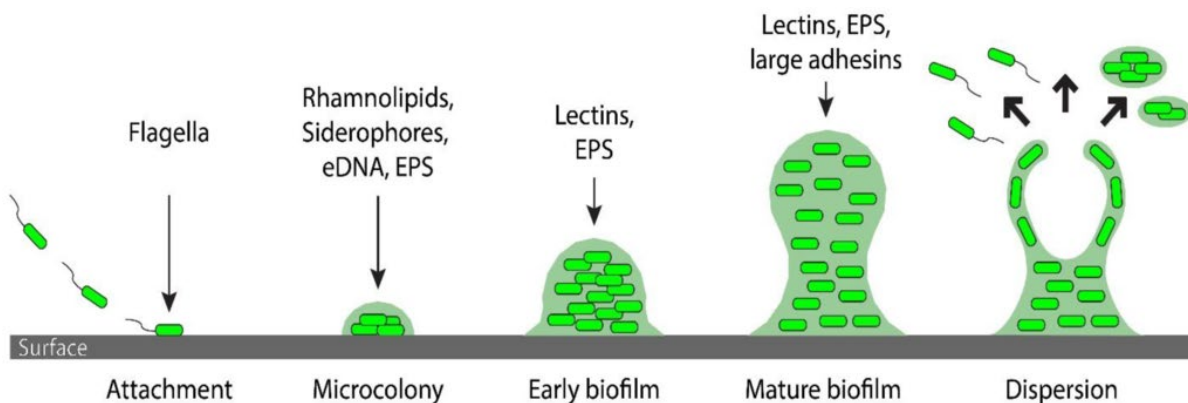


Figure 1: A schematic showing bacterial biofilm formation

Note that this schematic shows just one 'species.' Outside of laboratory settings, biofilms almost always include multiple species. (CC) 4.0. Image by Daniel Passos da Silva, Melissa C. Schofield, Matthew R. Parsek and Boo Shan Tseng. (da Silva et al., 2017)

Through this additive process biofilms grow and form a three-dimensional structure. The biofilm not only enhances cell adhesion but also shields the incorporated microorganisms from external stresses. Biofilms are naturally occurring norms of microbial life. In virtually all environments, biofilm formation is common, though the best studied examples occur in moist or aquatic environments. The biofilm matrix also allows cells to co-exist in close proximity to one another by enhancing communication and interaction between the microorganisms. The microenvironment created within a biofilm can be vastly different from aquifer conditions within well bores, this occurs because microorganisms can produce metabolites that can affect their local environmental conditions (Bjarnsholt et al., 2022; Kip & Van Veen, 2014; Sauer et al., 2022).

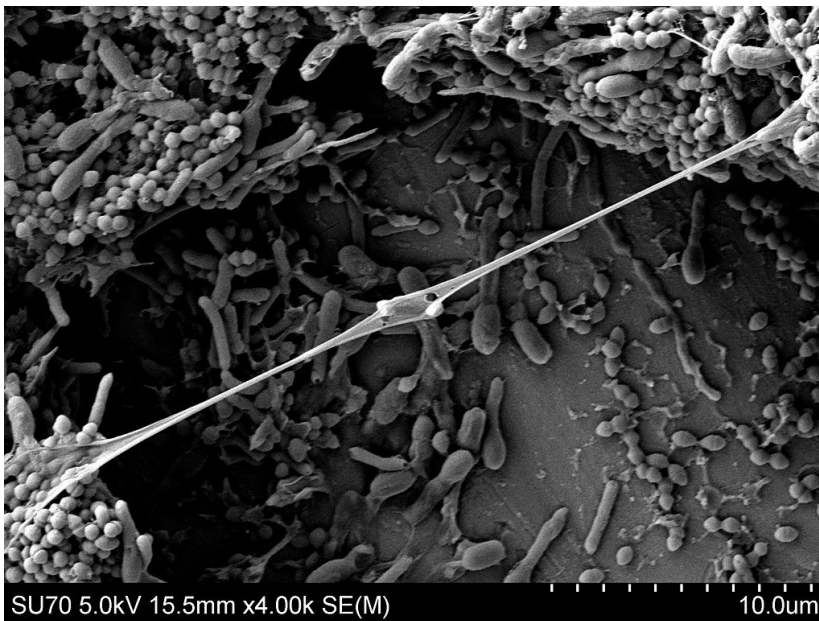


Figure 2: An example of a biofilm growing on stainless steel in a laboratory

Note the rod (long) and cocci (round) cells throughout the image, and how they are matted together and adhered to the steel. Image by: Krzysztof A. Zacharski⁵.

These microenvironments may either promote or prevent Microbial Influenced Corrosion (Kip & Van Veen, 2014). There is a substantial body of literature examining biofilms (Costerton et al., 1995; Penesyan et al., 2021; Sauer et al., 2022; Stoodley et al., 2002). It has been reported that in the subsurface, as in many aquatic habitats, microbial communities are partitioned between planktonic and adherent (in a biofilm) communities (Vick et al., 2019). This is likely due to differences in metabolic processes of microbes within the two communities (though the former must also act as a ‘seed-bank’ and dispersal medium for the latter). Attachment sites are non-random and are often associated with a requirement to obtain a particular macronutrient (often carbon, nitrogen, phosphorus or an important energy molecule). For example, bacteria have been shown to preferentially attach to mineral-rich regions in coal along with areas with significant cracking (McLeish et al., 2021). This is relevant here, as no studies have been conducted to date on adherent microbes in Queensland aquifers, and all studies to date have focused on water extracted from bores, which would represent the planktonic fraction of the community. Understanding the adherent community is particularly important for corrosion as it is the adherent microorganisms (Beech & Sunner, 2004; Wei et al., 2013), in close contact with steels and cements, that are more likely to provide insights into the potential for microbial impacts on well infrastructure.

⁵ https://commons.wikimedia.org/wiki/File:Mixed-culture_biofilm.jpg

1.5 Reduction and oxidation reactions (redox)

This report discusses the concept of microbial ‘reduction’ and ‘oxidation’. Neither of these terms are specific to microbiology, and they are general chemistry terms. Technically, reduction and oxidation (collectively called redox) are fundamental concepts in chemistry because both processes are the basis of forming chemical bonds. The formation of oxides (e.g. iron oxide or rust) are examples of redox reactions, though it is important to note that in this chemistry sense, oxidation does not necessarily involve oxides or oxygen.

One example, relevant to this report, underpins the process of microbial methanogenesis (making methane), which is an important respiration process for producing energy in the subsurface. Here, specific microbes called methanogens oxidise H_2 and reduce CO_2 to generate energy and produce methane as a byproduct of this process (Lyu et al., 2018).

1.6 Redox states of sulphur

One specific element, and a series of compounds, of note are the various forms of sulphur. This is because the best studied aspects of Microbial Influenced Corrosion are related to sulphide (a reduced form of sulphur) production by bacteria (and archaea). In the subsurface, sulphur can exist in multiple redox states including sulphate, sulphite, and elemental sulphur (Table 1). The most oxidised forms of naturally occurring sulphur are typically sulphates, and the most reduced forms of sulphur are various sulphides (either hydrogen sulphide or, more typically in the subsurface, various metal sulphides). Various microbes can chemically reduce oxidised forms of sulphur to more reduced forms. This transformation, however, is not always complete, and some microbes will only partially lower the oxidation state of sulphur (i.e. not all the way to sulphide).

Table 1: Oxidation state and structures of common forms of sulphur

| Oxidation State | Common Species |
|-----------------|---|
| S^{2-} | Sulphides: H_2S Various metal sulphides: ZnS , MnS Some thiols: methanethiol CH_3SH |
| S^{1-} | Disulphides, pyrite (FeS_2) |
| S^0 | Elemental sulphur |
| S^{2+} | Thiosulphates (e.g., $S_2O_3^{2-}$) |
| S^{4+} | Sulphite (e.g., SO_3^{2-}) |
| S^{6+} | Sulphate (e.g. Na_2SO_4 , H_2SO_4) |

1.7 Microbial respiration

It is important to have some understanding of microbial respiration for discussions of microbes involved in reduction of oxidised sulphur species. All respiration, including our own, requires a source of energy. In simple terms, human respiration uses the energy in glucose to produce various other forms of chemical energy that our cells can use. In the final step of this process, oxygen receives ‘energy’ (electrons) from this degradation of glucose. For this human example, the electron donor is glucose, and the electron acceptor is oxygen. In environments that lack oxygen, microbes employ similar strategies however using different electron acceptors. For example, in environments with nitrate, microbes can use nitrate instead of oxygen as the electron acceptor (Figure 3). Instead of producing carbon dioxide as a byproduct, as happens during human respiration, microbes respiring nitrate mostly produce nitrogen gas (Figure 3).

It is also important to note that different electron acceptors yield different amounts of energy when they are reduced. Oxygen yields the most, followed by nitrate, a range of metals, sulphate, sulphur and carbon dioxide, which yields the least energy (Figure 3). This difference results in microbial communities that tend to be dominated by specific respiratory processes. For example, in anoxic environments with sufficient nitrate, nitrate reduction tends to dominate and other respiratory processes (methanogenesis or sulphur reduction) are less common.

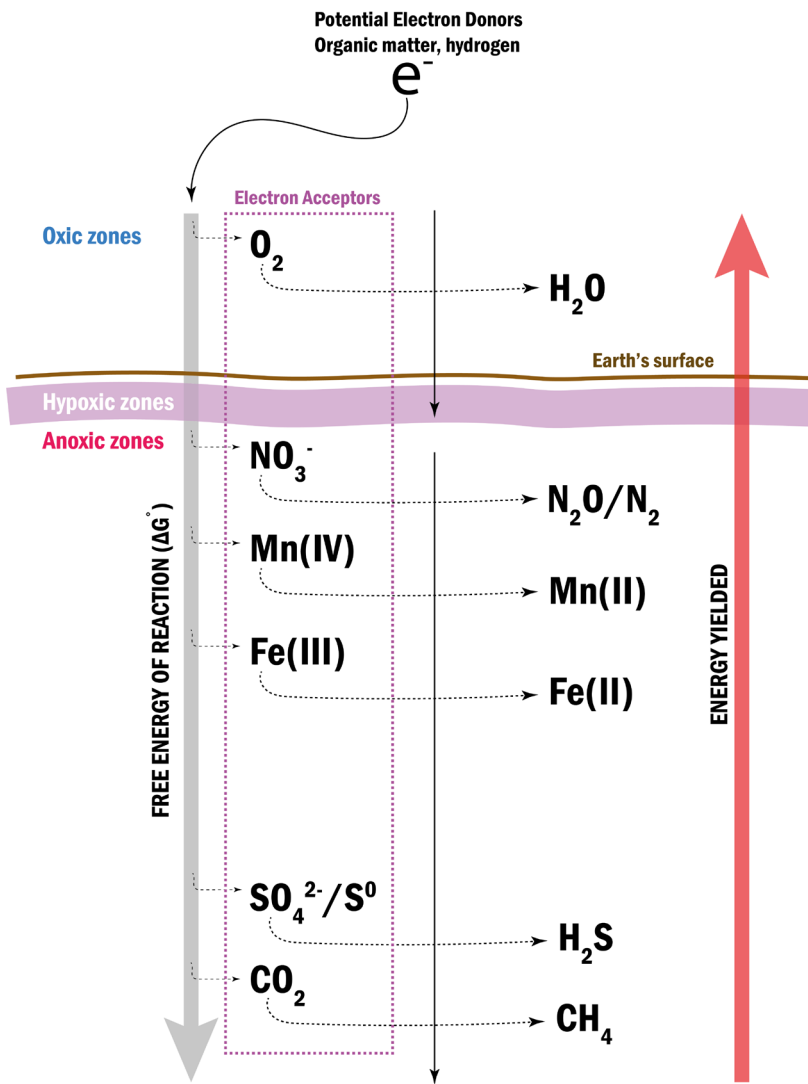


Figure 3: Potential electron donors, acceptors and their products with energy yielded through their use

1.8 Microbial nutrition

It is also noteworthy that microbial activity in aquifers (and elsewhere) is also dependent on other chemical factors, particularly the presence of important macronutrients such as nitrogen or phosphorus and a source of accessible organic carbon. Typically, if these macronutrients are scarce, this is likely to limit microbial activity. This is because without nitrogen and phosphorus, organisms cannot make important biological molecules like proteins, DNA, RNA or phospholipids (the constituents of cell membranes).

1.9 Microbial mechanisms of corrosion

Scientific literature, industry and others, often talk about sulphate reducing bacteria (SRBs) and acid producing bacteria (APBs) as a defined group of related species. This is somewhat misleading as many bacteria from a host of different lineages (evolutionary paths) are capable of some form of acid production including engaging in sulphur activity. This acid production can take at least six forms:

1.9.1 Prokaryotes that produce organic acids

The majority of prokaryotes from diverse lineages can produce acids like lactic, acetic, citric, propionic, oxoglutaric and succinic acids. The production of these acids is commonplace for bacteria and can also occur through multiple processes. For example, acetic acid can be produced through a process called acetogenesis (i.e. using substrates like CO, H₂ or CO₂) or can be the oxidation product from alcohol oxidation (e.g. ethanol is oxidised to acetaldehyde, which is in turn oxidised to acetic acid). Similarly, *Bifidobacterium* and *Lactobacillus* species both produce lactic acid but use different mechanisms and are in different phyla (i.e. they are very much unrelated species).

1.9.2 Microbial use of proton-powered transporters

Numerous bacteria use membrane bound transporters that export protons across the cell membrane to power a range of processes. This also occurs in some microbial eukaryotes⁶. For example, some fungi use H⁺-translocating enzymes called ATPases to move protons into the external environment so that they can take in important nutrients like ammonia (Roos & Luckner, 1984). Regardless of the reason, proton-powered transporters can rapidly acidify microhabitats around the organism and are widespread in terms of occurrence in many unrelated microbes.

1.9.3 Prokaryotic production of inorganic acids

Less widespread than either organic acid production or proton-powered transporters is prokaryotic production of inorganic acids. This capacity is somewhat limited as it requires the organisms involved to have significant tolerance to very low pH. Examples of this process are

⁶ An organism whose cells contain a nucleus and other membrane-bound organelles. E.g. fungi, animals and plants are all eukaryotes.

Acidithiobacillus species, which can produce sulphuric acid via various forms of sulphur oxidation (Ng et al., 2000; Sugio et al., 1985; Valdés et al., 2008).

1.9.4 Sulphur reduction and the production of H₂S

It is the acidic, corrosive nature of H₂S that underpins the role of sulphur-active species in production of acids. Most Microbial Influenced Corrosion research is therefore directed towards sulphur-active microbes and their impacts on materials. This has been described extensively below, noting that the process is dependent on the availability of oxidised forms of sulphur (e.g. sulphate, sulphite, various organic compounds that include sulphur, or elemental sulphur). In the absence of these forms of sulphur, sulphur-active species engage in other processes (Plugge et al., 2011).

1.9.5 Nitrogen-active species: species that oxidise or reduce nitrogen forms

There are also indications that nitrite and some of the interspecies signalling that occurs during microbial activity against nitrite (either oxidation or reduction) can enhance rates of corrosion. For example, in a microbial community capable of nitrate reduction, nitrite was shown to be corrosive especially within certain ranges of concentration (B. Liu et al., 2021). Like sulphur-active species, many nitrogen-active microbes are capable of other metabolic activities in the absence of suitable nitrogen species.

1.9.6 Direct metal oxidation by microorganisms

Some organisms can directly attack steels and cause corrosion by removing electrons from the steel. For example, a strain of *Methanococcus maripaludis* has been shown to remove electrons from higher strength structural steel (EH40) (Chen et al., 2023). Similarly, iron (III) reducing bacteria accelerate the corrosion of steel via attacks on the passivating (protective) layer of Fe(III) oxide (Hernandez-Santana et al., 2022).

1.10 Sulphur-active bacteria

Microbes that are involved in dissimilatory sulphur cycling are commonly known as Sulphate Reducing Bacteria (SRBs). Sulphur-active bacteria, however, are a diverse group of microorganisms that are, somewhat arbitrarily, grouped together based on their ability to reduce various oxidised forms of sulphur (Table A 1). In the literature, the term Sulphate Reducing Bacteria has been used

interchangeably for both sulphur and sulphate reducing bacteria. The name is a misnomer and misleads the reader into believing (a) that these organisms only reduce sulphur (or sulphate, or sulphite); (b) that this process of sulphur species reduction is the only metabolic activity these microbes undertake in the subsurface; (c) implies that Sulphate Reducing Bacteria are related to each other; and (d) implies that all dissimilatory sulphur cycling microbes are bacteria, when sulphur-active archaeal lineages are known (L. J. Liu et al., 2021; Liu et al., 2012). For these reasons, the term Sulphate Reducing Bacteria is sparingly used in the present study and when used, it is clearly defined.

1.11 Metabolic flexibility in sulphur-active bacteria, and why their presence does not necessarily equate to sulphide production

Metabolic flexibility refers to a microorganism's ability to switch between different metabolic pathways depending on the availability of nutrients in its surrounding environment. Microbes that are involved in dissimilatory sulphur cycling have been shown to have remarkable metabolic flexibility in response to different environmental conditions (Plugge et al., 2011).

This flexibility can result in microorganisms being able to use a range of different forms of sulphur, for example, some sulphur-active anaerobes can use sulphate, sulphite or thiosulphate, while other species are more particular about which sulphur compound they use. For example, a strain of *Desulfobulbus elongatus* was shown to grow using sulphate, sulphite, thiosulphate and elemental sulphur as electron acceptors (Samain et al., 1984). In comparison, a related microbe, *Desulfobulbus oligotrophicus*, was unable to use elemental sulphur as an electron acceptor (El Houari et al., 2017).

Further, sulphur-active microbes are able to survive using this flexibility in the absence of any meaningful concentrations of sulphur. The best studied example of this is likely the co-culture⁷ of *Desulfovibrio vulgaris* 'Hildenborough' with *Methanococcus maripaludis* 'S2' (Hillesland et al., 2014). In this co-culture without sulphate, the species 'feed together' in a process known as syntrophy. Here the *Desulfovibrio* ferments lactate to acetate in the presence of water. The products of this fermentation are acetate, carbon dioxide and hydrogen, however, this is not energetically practical when hydrogen concentrations are high, and so the *Methanococcus* actively consumes the carbon dioxide and hydrogen (and some acetate) in these cultures, producing

⁷ Co-culture is where the two strains of microbes are grown together under otherwise sterile conditions in a flask.

methane. This kind of syntrophy is very common in subsurface environments that lack sulphur-compound electron acceptors. In such situations, it is important not to equate the presence of a microbe capable of sulphur-cycling with the presence of sulphur-cycling processes (and in turn H_2S production) as this is not a given, or even the norm in many situations. Equally, an absence of important macronutrients (particularly phosphorus but also nitrogen) will limit microbial sulphur-cycling activity (Figure 4).

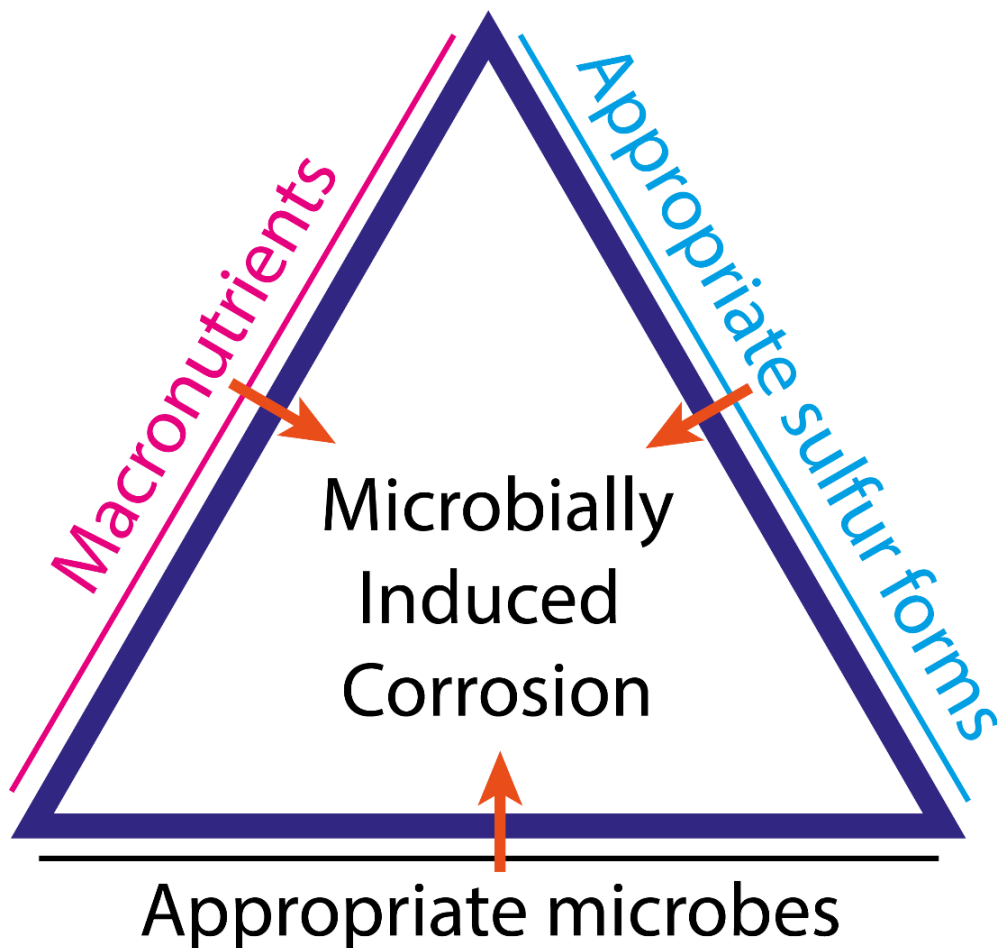


Figure 4: Numerous conditions all need to be met to produce acidic H_2S *in situ*

Similar to the Fire Triangle for understanding the necessary ingredients for most fires to start, numerous conditions all need to be met to produce acidic H_2S *in situ*. In the absence of appropriate microbes, there is no mechanism for reduction of oxidised sulphur compounds. In the absence of phosphorus and other macronutrients, microbial activity is low, inhibiting Microbial Influenced Corrosion. And in the absence of suitable oxidised sulphur species, organisms that are sulphur-active often engage in other processes (e.g. syntrophy with methanogens; or other fermentative heterotrophy).

2 Research Program

2.1 Research aims

The aims of the research program were to:

- 1) Determine what information was available on microbes from subsurface (aquifer) environments in South East Queensland.
- 2) Using the discovered data, determine the number of microbial species from these environments that are potentially capable of causing deleterious impacts on steels or cements.

2.2 State of knowledge for microbiology of subsurface environments in South East Queensland

Both the scientific literature and online databases were searched for data on subsurface microbiology in Queensland. Almost all microbial data in Queensland related to aquifer microbiology comes from studies of the coal seams, which focuses on produced water⁸ from coals. By comparison, no data were available on the microbiology of agricultural or domestic aquifers. No data were available from pipework from the subsurface, whether it be from aquifers related to coal seams or agricultural aquifers. All data were representative of planktonic communities only (see Section 1.4). Some data were available for agriculturally important aquifers in north Queensland (Stanley, 2019; Wakelin et al., 2011), however, these were excluded from this study due to the significant distance, climatic differences and geological differences from South East Queensland.

⁸ Produced water is water that comes to the surface through the action of a CSG well, along with the gas. In this sense, the water and gas are 'co-produced'. This water is, in some senses, indicative of the microbiology of the subsurface coal seam.

2.3 Microbiology of the produced water from coals subsurface environments in South East Queensland

The data source for the research program of this study was thus data pre-collated through the Coal-Seam Microbiome dataset (CSMB) (Vick et al., 2018). The CSMB dataset includes microbiology data from other states in Australia, as well as other countries. For the program of work, the dataset was reduced in scope to only those data that related to South East Queensland, specifically in association with the Surat and Bowen Basins.

The CSMB reference set uses Operational Taxonomic Unit (OTUs) as a model of a bacterial or archaeal 'species'. While OTUs are not exactly 'species', the concepts are similar and readers can assume for the purposes of this report that an OTU is approximately equal to a microbial species.

In total, 18 samples were included in this study; six samples were from wells that intersect the Bowen Basin, while 12 were from wells that intersect the Surat Basin. Across these bores, 1,230 OTUs were detected. Species richness (the number of different species) was similar between those bores that intersected the Bowen and Surat basins, with an average of approximately 400 OTUs per well.

2.4 Determination of which organisms are capable of acid-production in Queensland subsurface habitats

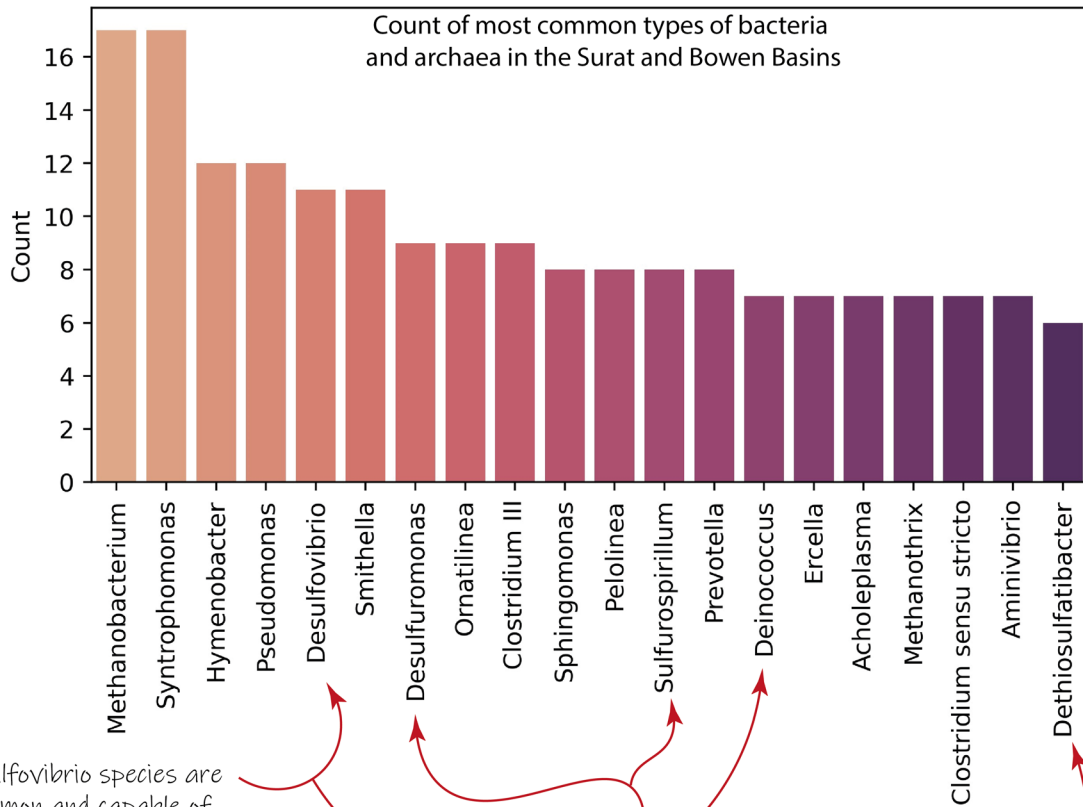
As described in Sections 1.9– 1.10, microbes are genetically capable of producing various acidic products that may adversely interact with cements and steels. Whether they actually undertake these activities in Queensland is currently unknown, and is dependent on groundwater chemistry and availability of important macronutrients (see Section 1.11).

Examining the most abundant types of microbes observed in the Surat and Bowen Basins revealed that the most common organisms included some species that could produce sulphides under the correct conditions (Figure 5). The reason to stress these constraints on sulphur activity are that in general, the waters of the Surat and Bowen Basins are very deficient in sulphur compounds of any kind. A study of 150 CSG wells found sulphate concentrations ranged between 1- 48 ppm with an average of 5.2 ppm (Rebello et al., 2017). For comparison, the Australian drinking water guidelines

specify a maximum of 250 ppm sulphate⁹, and seawater sulphate content is usually greater than 2500 ppm (Millero et al., 2008). The coals themselves do contain some organic sulphur compounds bound in the organic matter in the coal, however, breakdown of the organic matter in coals is generally limited by availability of macronutrients, particularly phosphorus and nitrogen. Low total phosphorus and nitrogen levels in Surat Basin CSG waters have been reported, and are in the range 0.01 - 1.48 ppm, and 0.5 - 0.61 ppm, respectively (Rebello et al., 2017).

To examine the metabolic capabilities of the microorganisms identified in Surat and Bowen Basins, a software approach was undertaken using PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Langille et al., 2013). PICRUSt examined the closest known relatives of each of the ~1200 OTUs observed and inferred the potential metabolic ability of each one based on information about their closest relatives.

⁹ https://www.nhmrc.gov.au/sites/default/files/documents/attachments/publications/Australian_Drinking_Water_Guidelines_ADWG_V3-8_Sep2022.pdf



Desulfovibrio species are common and capable of sulphate reduction to sulphide, but (generally) there's little sulphate in the groundwaters of the Surat and Bowen Basin - when this happens, this microbe probably engages in partnership with methane making archaea (e.g. Methanobacterium)

Desulfuromonas & Sulfurospirillum species can reduce sulphur (but not sulphate) to sulphide. Like sulphate, there's little sulphur in the environment here, so this taxon probably (mostly) engages in other interactions with species

This species can convert thiosulphates (& elemental sulphur) to sulphide.

Figure 5: Annotated bar chart showing some of the common organisms from the Surat and Bowen basins in Queensland, and with notes on their activity towards various forms of sulphur

Count of wells with each type of common organisms detected.

It is worth noting that the PICRUSt analyses are reliant on close relatives of observed species being present and, often, these data are lacking, so these results should be taken as indicative rather than definitive. From these (indicative) PICRUSt data, the current study looked for those genes involved in reducing oxidisable sulphur forms (sulphate, sulphite, thiosulphate and elemental sulphur) to sulphide (Kanehisa et al., 2016; Ogata et al., 1999). The full PICRUSt dataset, including all the genes predicted for the 1,230 OTUs observed in the Surat and Bowen Basins, is available through the GISERA website.

Of the 1,230 OTUs observed across the 18 wells and analysed with PICRUSt, 198 OTUs (~16%) were predicted to have genes capable of reducing sulphate, sulphite, thiosulphate and elemental sulphur to sulphide. This is almost certainly an underestimate as it is apparent that PICRUSt fails to identify some known (and abundant) organisms that have this capacity. For example, the most widespread and abundant bacterial species in Australian subsurface coal seams is a known sulphur-reducing *Desulfuromonas* species designated CSMB_57 (McLeish et al., 2022) but is lacking from the PICRUSt analysis. Nevertheless, it highlights that a large proportion of subsurface microorganisms carry genetic potential for making H₂S. Unsurprisingly, the majority of organisms detected were from microbial families known to engage in activity against oxidised forms of sulphur. For those readers interested, taxonomic details are in Appendix A 1.

It should be noted that most Microbial Influenced Corrosion research has focused on H₂S production (to the detriment of other types of acid production). This is because the genetic method for making H₂S from oxidised sulphur forms tends to be conserved¹⁰ and is thus easier to examine. The very diverse types and methods for producing organic acids means that using a similar method to identify other acid production is problematic. Instead, what is required is an understanding of, should corrosion occur, which processes predominate on well-related infrastructure. Below, the authors have outlined a range of options for future work to help improve our understanding of the existence of, and should it exist, underpinnings of Microbial Influenced Corrosion and cement-related damage caused by microbes in Queensland.

¹⁰ That is, the genes that organisms need to reduce sulphur to sulphide are the same or quite similar across many organisms.

2.5 Conclusion

This report details the microbial-related research task of the GISERA Other 3 project, that aimed to collate microbial information from subsurface (aquifer) environments from South East Queensland, and to determine the potential of these microbes to cause deleterious impacts on infrastructure (steels or cements). The key findings and learnings from this project are summarised below:

- Microbial activity has the potential to positively or negatively impact corrosion and cement performance.
- Microbial biofilm formation in Queensland CSG and shallow aquifers pipework is unknown. No data exist on biofilms in pipes associated with South East Queensland groundwater.
- Microbial metabolic processes that affect steels or cements (acid production or electron uptake) exist commonly in microbes from the subsurface in the region, but it is unknown whether groundwater or ecological conditions favour the expression of these activities.
- Sulphur-active microbes are the best studied organisms in corrosion research; however, these organisms are metabolically flexible and their presence alone does not necessarily indicate H₂S production. Instead, H₂S production is contingent on both sufficient sulphur in oxidised forms, and sufficient essential macronutrients for microbial growth.

3 Options for future work

It seems likely that shallow, unconfined aquifers are more susceptible to Microbial Influenced Corrosion and microbial damage to cements than deeper components of wells. This is due to oxygen and nutrient ingress into these environments. At present, however, data on either microbiomes in such aquifers or their chemistry have not been collected or examined, and in many cases are not available. Work to redress this shortfall and establish representative baselines would be valuable.

Currently, there are no data on whether biofilms colonise groundwater pipework in Queensland. As biofilms can have both positive and negative effects on infrastructure, and as water-only sampling is not representative of biofilms, direct sampling of the pipe internal surfaces through swabbing or baiting would be valuable.

Laboratory experimentation could be used to study corrosion, using indigenous microbial communities from Queensland aquifers or well bores. This could help determine which microbes are involved in Microbial Influenced Corrosion, what detrimental impacts they may have on cements, and what further strategies (e.g. biocide use or different materials) need to be developed to minimise the impact of such processes.

4 Glossary

| Term | Definition |
|---|---|
| ADP | Adenosine diphosphate: by-product of the release of energy from ATP, during which one phosphate group is removed. |
| Archaea | A lineage of life distinct from bacteria and eukaryotes. |
| ATP | Adenosine triphosphate: the source of stored energy for many cellular processes. |
| ATPases | Enzymes involved in energy metabolism, which yield energy by converting ATP to ADP; i.e., removing phosphate. |
| Community | Refers to a group of microorganisms consisting of multiple populations living together. |
| Eukaryotes | An organism whose cells contain a nucleus and other membrane-bound organelles. E.g. fungi, animals and plants are all eukaryotes. |
| Extracellular polymeric substances (EPS) | Microbially produced organic polymers that are involved in bacterial cell interactions with their environment, including cell cohesion for biofilm formation. |
| MIC | Microbial Influenced Corrosion is the corrosion of metals and non-metallic materials resulting from microbial activity. |
| Microbiome | The community of microorganisms that occur together in a defined environment. |
| OTU | Operational Taxonomic Unit is a basic unit used in numerical taxonomy based on sequence similarity clustering. |
| Order | A level of taxonomic classification ranking lower than phylum but above family. For |

| | |
|--------------------------------|---|
| | example, all rodents belong to the order Rodentia. |
| Passivation | Passivation is used to finish metal surfaces to prevent corrosion. |
| Phylum/phyla | (singular/plural) A level of taxonomic classification ranking below domain. For example, all vertebrate animals (fish, mammals and reptiles) belong to a single phylum, Chordata. |
| Prokaryote | An organism whose cells do not contain a nucleus and other membrane-bound organelles. All bacteria and archaea are prokaryotes. |
| Population | Refers to a group of individual organisms belong to the same species. |
| Species richness | The number of species within a defined environment. |
| Sulphur-active microbes | Microorganisms able to reduce one or more of the various sulphur species e.g., sulphur, sulphite, sulphide, sulphate reduction. |
| Syntrophy | Between different microorganisms that have obligate interdependence and mutualistic metabolism. |

5 Appendix

This appendix contains more technical information. It is provided for readers who would like more detail on various aspects of the background and findings of this study.

5.1 Major groupings of living organisms

Bacteria are unicellular microorganisms characterised by their lack of a nucleus and membrane-bound organelles. They are classified as prokaryotes, which means their genetic material is not enclosed within a membrane-bound nucleus and they lack other membrane-bound organelles. Bacteria exhibit diverse shapes, sizes, and metabolic abilities. They are abundant in almost all environments, playing crucial roles in processes such as global geochemical cycles and decomposition.

Archaea, like bacteria, are prokaryotic microorganisms, however, they differ in certain fundamental aspects of their biochemistry and genetics. In the past, archaea were thought to be specialists in extreme environments (e.g. high temperatures, acidity, salinity) but more recent microbiological studies have found them living in most environments alongside bacteria. In some of these environments (e.g. the subsurface) they can be more numerically dominant than bacteria. Archaea are considered a distinct domain of life, separate from bacteria and more closely related to eukaryotes.

Eukaryotes, including all plants, animals, fungi, algae and other microeukaryotes, are characterized by cells containing a membrane-bound nucleus and various organelles. Molecular analyses have revealed that archaea are more closely related to eukaryotes than to bacteria. This relationship challenges the traditional view that prokaryotes (bacteria and archaea) form a monophyletic group. Instead, it suggests a two-domain system, with bacteria diverging earlier, and archaea and eukaryotes sharing a more recent common ancestor (Figure A 1).

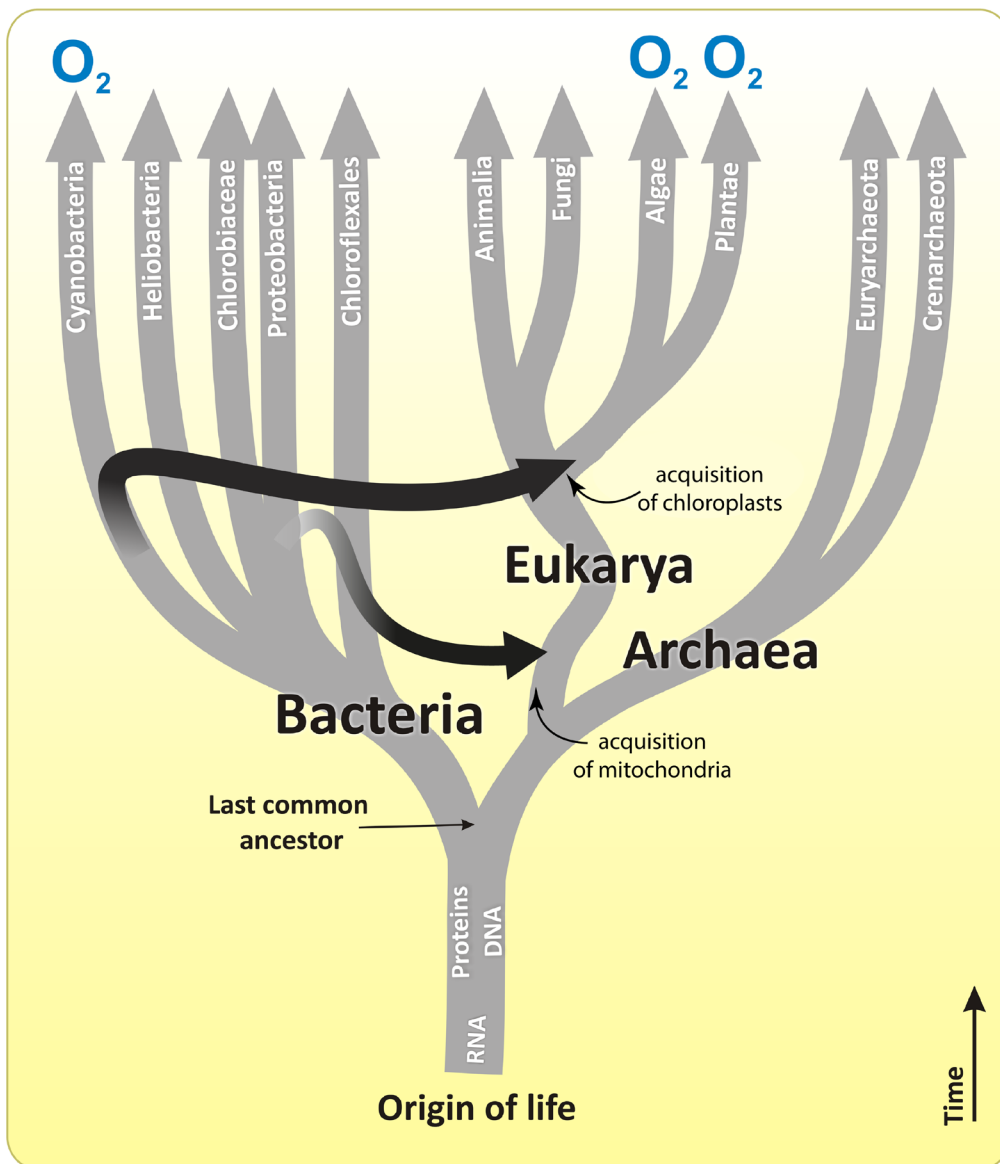


Figure A 1: Tree of life, showing the major domains of life (bacteria, archaea and eukarya)

Figure modified from an original image by Dmitriy Shevela Govindjee, modified from (Blankenship, 2002; Blankenship et al., 2007).

5.2 Sulphur reducing microbial phyla

The diversity of anaerobic sulphur-reducing organisms is extensive, and there are numerous bacterial and archaeal lineages with distinct metabolic pathways tailored to specific sulphur compounds. The major groups (phyla) of sulphur-active microbes and their sulphur cycling activity are shown in Table A 1 and Figure A 2. The Proteobacteria and the Firmicutes were the most frequently observed phyla in the wells that this study analysed, though other known sulphur-active lineages (for example, the Chloroflexi or Ignavibacteria) were also detected (Table A 1 and Figure A 2). The orders that contain these taxa are also shown in Figure A 3: Desulfobacteriales, Desulfovibrionales, Desulfuromonadales and Clostridiales were common. Analyses at order level also showed a host of non-proteobacterial and non-Firmicutes lineages including various actinomyceteous and Bacteroidetes-related orders.

Table A 1: Phyla known to engage in dissimilatory sulphur-cycling

| Phylum-level lineage | Types of sulphur-cycling |
|---|-----------------------------|
| Acidobacteria | Sulphate/sulphite reduction |
| Actinobacteria | Sulphate/sulphite reduction |
| Armatimonadetes | Sulphate/sulphite reduction |
| <i>Candidatus</i> Desantisbacteria | Sulphate/sulphite reduction |
| <i>Candidatus</i> Falkowbacteria | Unknown |
| <i>Candidatus</i> Hydrothermarchaeota | Sulphate/sulphite reduction |
| <i>Candidatus</i> Lambdaproteobacteria | Sulphite reduction |
| <i>Candidatus</i> Omnitrophica | Sulphite reduction |
| <i>Candidatus</i> Riflebacteria | Sulphite reduction |
| <i>Candidatus</i> Schekmanbacteria | Sulphate/sulphite reduction |
| <i>Candidatus</i> Zixibacteria | Sulphate/sulphite reduction |
| Chloroflexi | Sulphate/sulphite reduction |
| Proteobacteria | Sulphate/sulphite reduction |
| Ignavibacteria | Sulphate/sulphite reduction |
| Nitrospirae | Sulphate/sulphite reduction |
| Planctomycetes | Sulphate/sulphite reduction |
| Verrucomicrobia | Sulphate/sulphite reduction |

Modified from (Anantharaman et al., 2018).

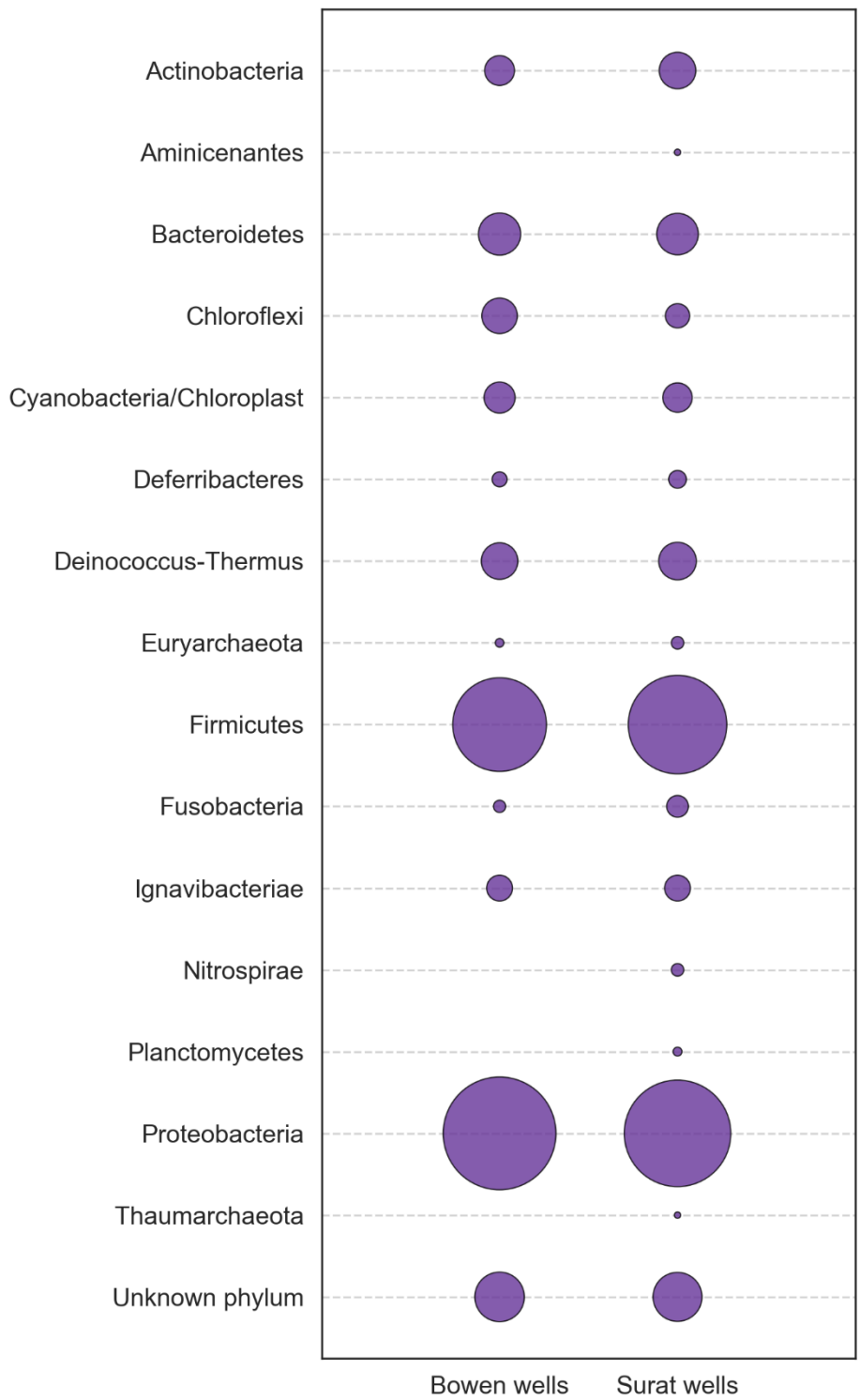


Figure A 2: Bubble plot of frequency of observation of the various phyla from the 198 OTUs with sulphur-active gene pathways examined in the CSMB database

Bubble size represents the frequency of observation.

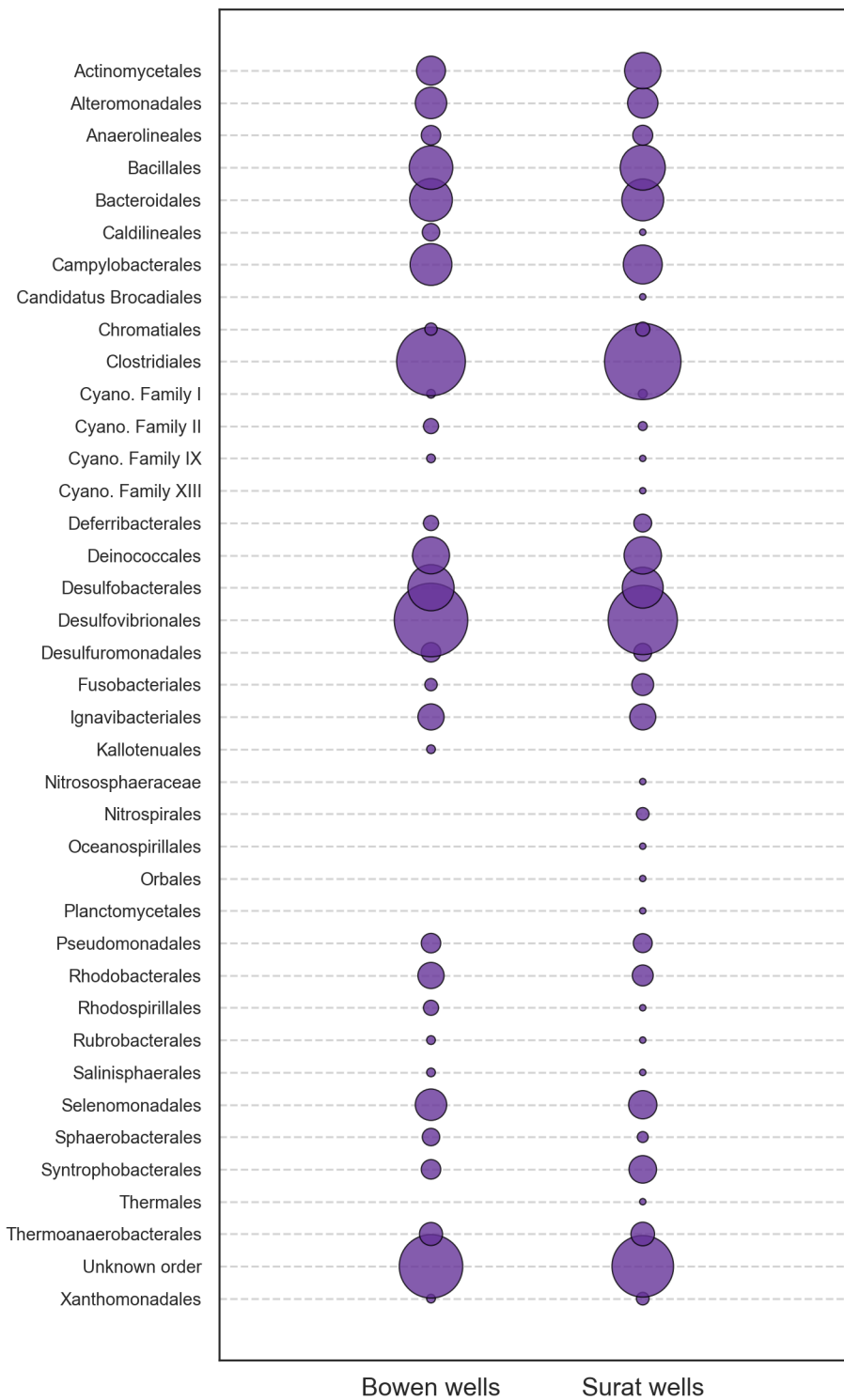


Figure A 3: Bubble plot of frequency of observation of the various orders from the 198 OTUs with sulphur-active gene pathways examined in the CSMB database

Bubble size represents frequency of observation. Note: Cyanobacterial families, rather than orders, are shown due to difference in Cyanobacterial taxonomy.

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