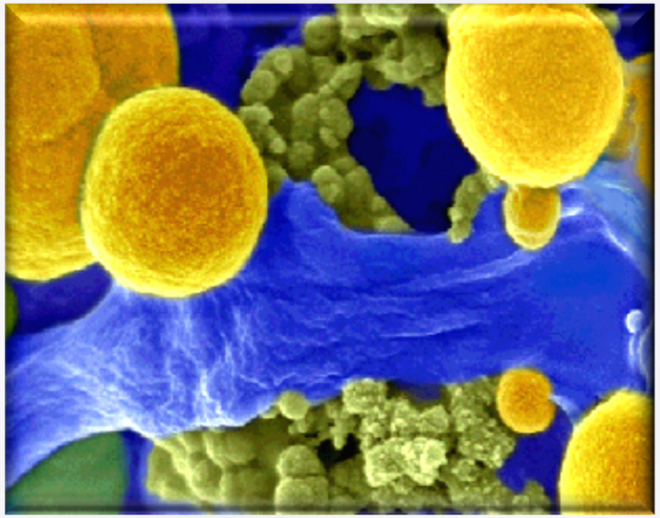


"What's that stink?"

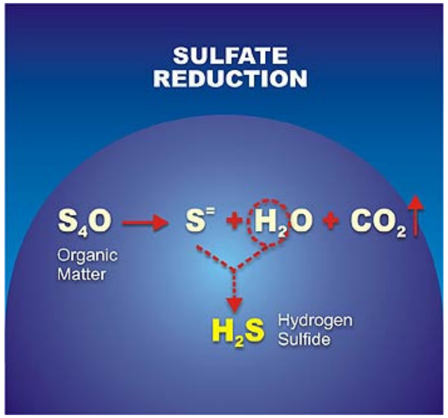
Sulfate Reducing Bacteria (SRB)

Dr. Richard Cresswell
Practice Leader: Coal Seam Gas related Groundwater

What are Sulfate Reducing Bacteria (SRB)?

- Oldest form of micro-organisms traced back 3.5 billion years
- Contributed to the sulfur cycle soon after life on Earth, and also part of the carbon cycle
- Anaerobic micro-organisms which ‘breathe’ sulfate rather than oxygen
- Produces hydrogen sulfide gas (H_2S)

  – rotten eggs smell



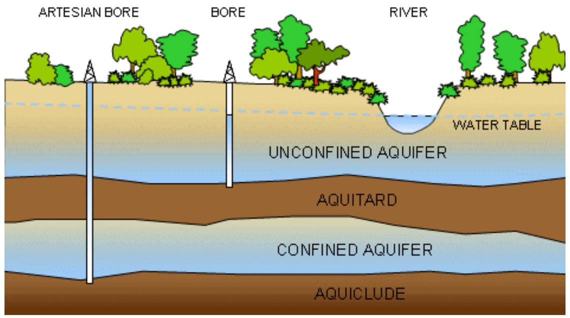
Where are SRB found?

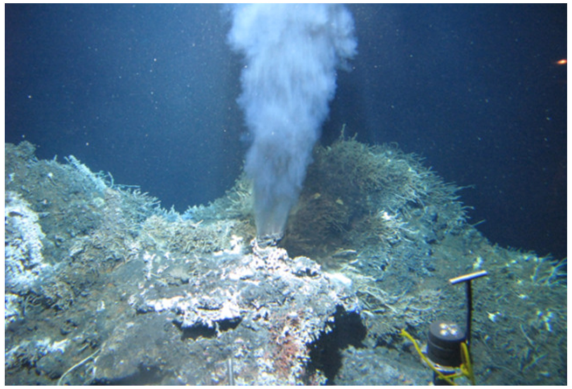
- Most commonly found in environments where no oxygen is present (anoxic conditions)

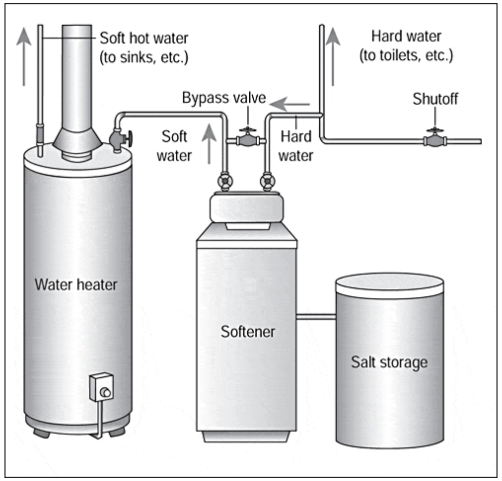
- Environments where sulfate is present

For example:

- Surface water
- Deep groundwater
- Seawater
- Plumbing systems
- Water softeners
- Water heaters
- Deep sea hydrothermal vents
- Human gut







How do they survive?

- SRB compete with similar micro-organisms (eg: methanogens, which produce methane) for organic compounds and hydrogen to produce energy.

- The presence of sulfate enables SRB to out-compete methanogens

→ As a result, sulfate enriched groundwater inhibits methane production within coal seams.

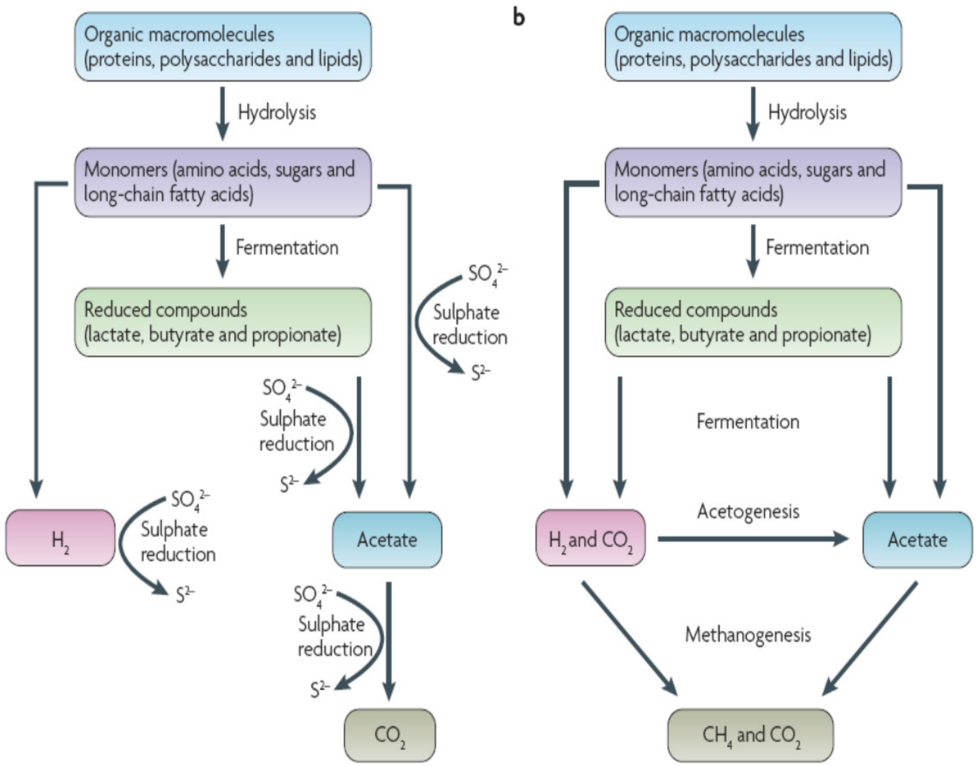


Figure 1. The sequential pattern of microbial degradation of complex organic matter in anoxic environments in the presence (a) and absence (b) of sulfate (Muyzer & Stams, 2008).

Health effects from SRB?

- No direct health implications for SRB in water

- Water alone containing H_2S (not sewage) does not cause disease

- Australian Drinking Water Guidelines (ADWG, 2011) have no guidance of acceptable levels

- Health impacts generally arise from exposure to high concentrations of H_2S gas (acceptable limit is10ppm) or prolonged exposure at low concentrations

- Rotten egg odour increases as the gas becomes more concentrated.

→ Dangerous at concentrations above 30ppm as the smell is difficult to identify.

Effects on groundwater wells

Negative:

- SRB cause corrosion of iron

- Significant sulfate levels can exist below the intake level of pumps & where water has become stagnant

- Remediate by cleaning pump and removing organic material (use chlorine and agitation)

Positive:

- SRB can remove sulfate and toxic metals from waste water streams

- Commonly used to remediate leachate water from acid waste and abandoned mines

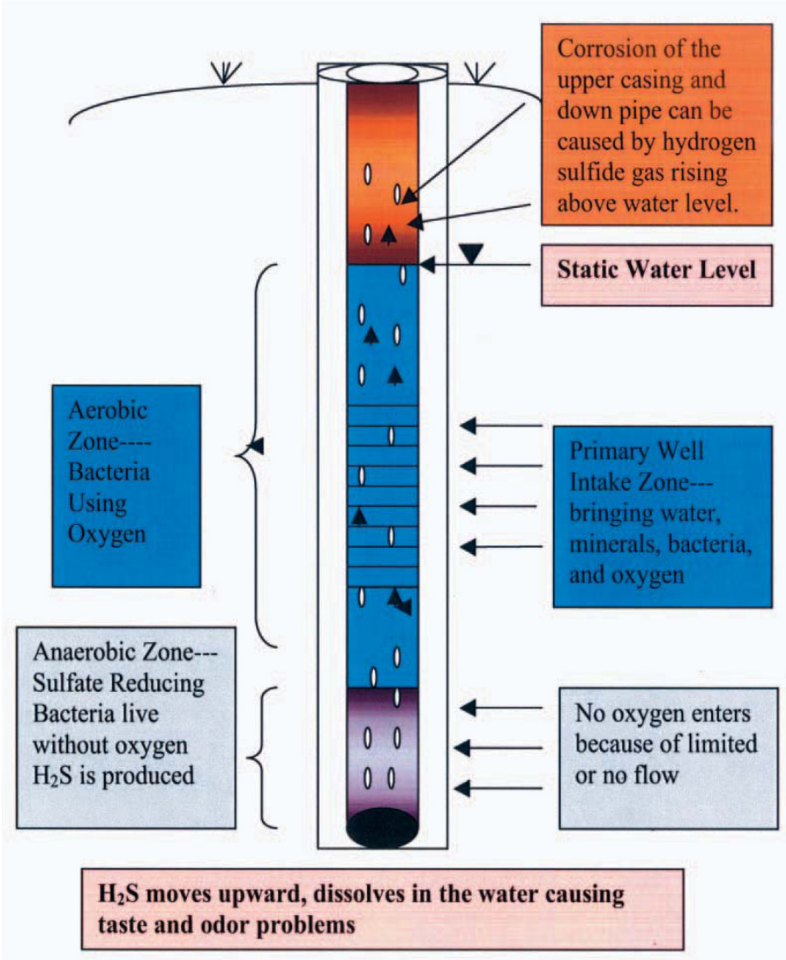


Figure 2: Typical well conditions (Schneiders, 2008)

SRB and Coal Seam Gas (CSG)

- Methane is not produced in coal seam groundwater containing sulfate concentrations above 500mg/L (Van Voast, 2003).
  H_2S gas is produced instead.

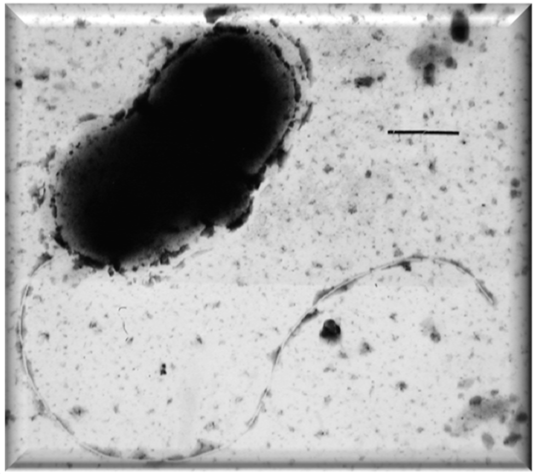
- However, natural geochemical processes remove sulfate from groundwater over time, which eventually depletes SRB.

⇩

Allows methanogenic bacteria to dominate and produce methane within the coal seam.

<u>As a result, CSG wells are generally unrelated to wells which may contain SRB</u>

Summary



Sulfate Reducing Bacteria:

- exist in anaerobic conditions where sulfate is present
- consume sulfate to produce H_2S gas
- require sulfate to survive and out-compete methanogens
- pose no direct health implications in water
  - however, H_2S produced is dangerous after prolonged exposure and exposure to high concentrations (above 10ppm)
- Negative effects: causes corrosion of iron
- Positive effects: SRB can be managed by cleaning and removing organic matter. Used to remove sulfate and toxic metals from waste water.
- Exclusivity generally exists between groundwater wells containing SRB, and CSG wells.

"Groundwater is the core issue"

SINCLAIR KNIGHT MERZ

SKM

Great Artesian Basin (GAB) in NSW

Dr. Richard Cresswell
Practice Leader: Coal Seam Gas related Groundwater

First borehole in 1878

SKM

achieve.

History of development

Pre-1960s

Pastoral water use

uncontrolled bores and pressure decline

irrigation development in some areas

1970s - present

Mining developments (Olympic Dam, etc.)

Petroleum resources (natural gas, coal)

Bore rehabilitation scheme

EPBC Act, 1999 (protection of springs and habitats)

Outlook 2010 - 2050

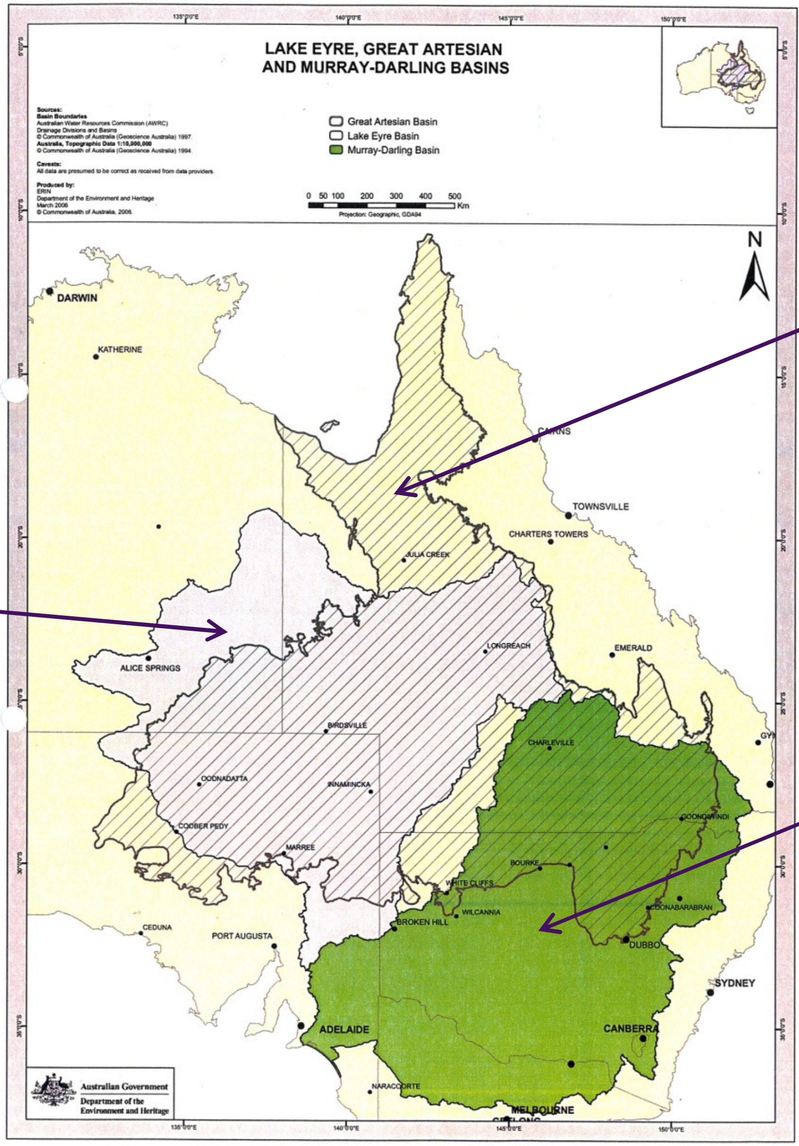
Increasing demand for coal seam gas (water extraction; disposal)

Further mining development

Climate change

Compliance with EPBC Act

River basins and the GAB



Carpentaria Basin

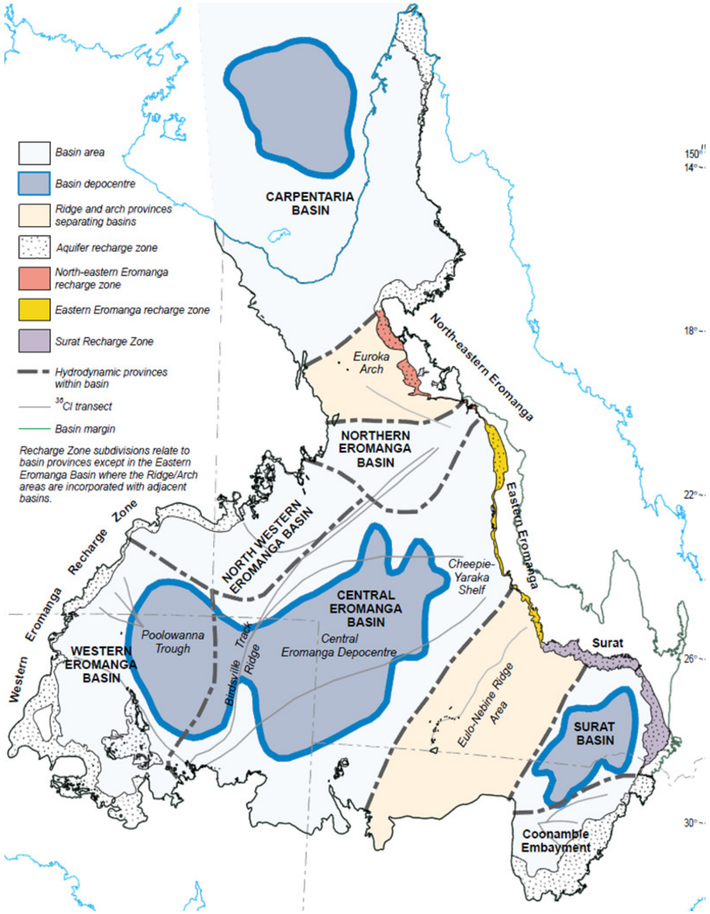
Lake Eyre Basin (LEB)

Murray-Darling Basin (MDB)

A few statistics

Area of the GAB	1.7×10^6 km^2
Max depth	3,000 m
Volume of water stored	8,700 km^3
Bore discharge (>5,000 bores)	5×10^8 m^3/yr
Total est. discharge	1.2×10^9 m^3/yr (???)
Estimate Recharge	1×10^9 m^3/yr
Water age	Up to 2 million years

Delineation of GAB and regions



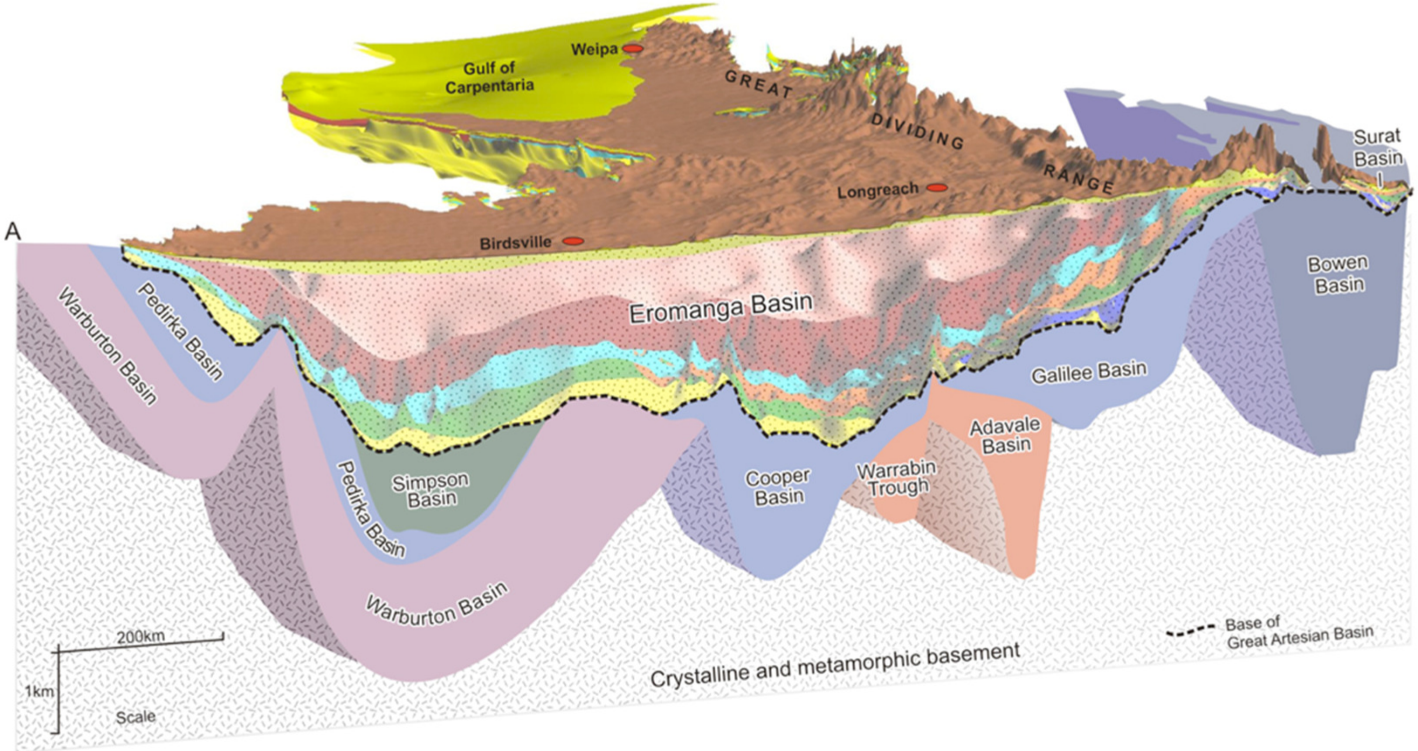
4 regions

Delineated on the basis of structural boundaries and flow systems:

- Surat (incl. Coonamble Embayment)
- Eromanga (Central)
- Eromanga (West)
- Carpentaria

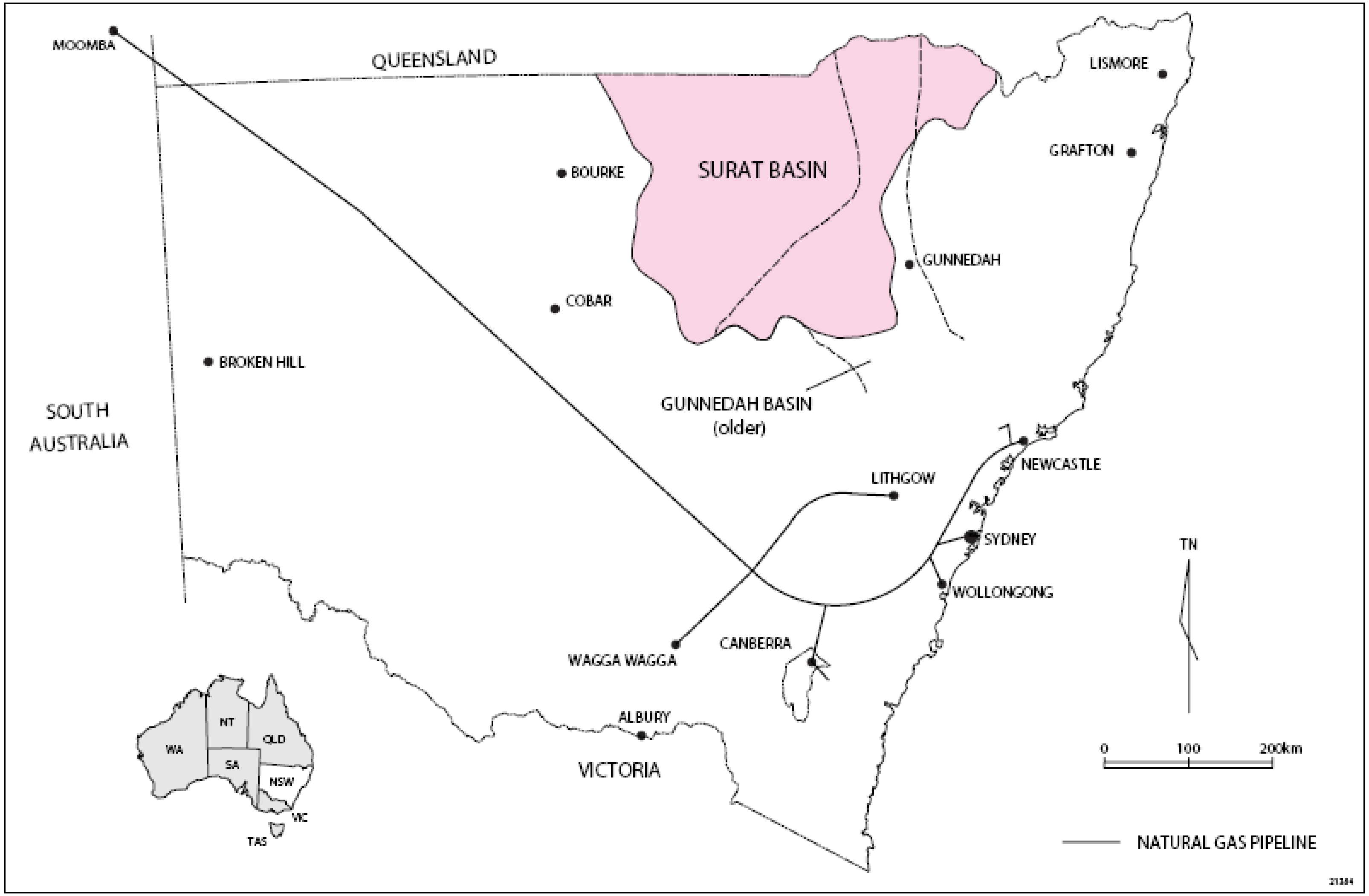
Updating the Conceptualisation of the GAB

- Knowledge of the GAB is continually advancing

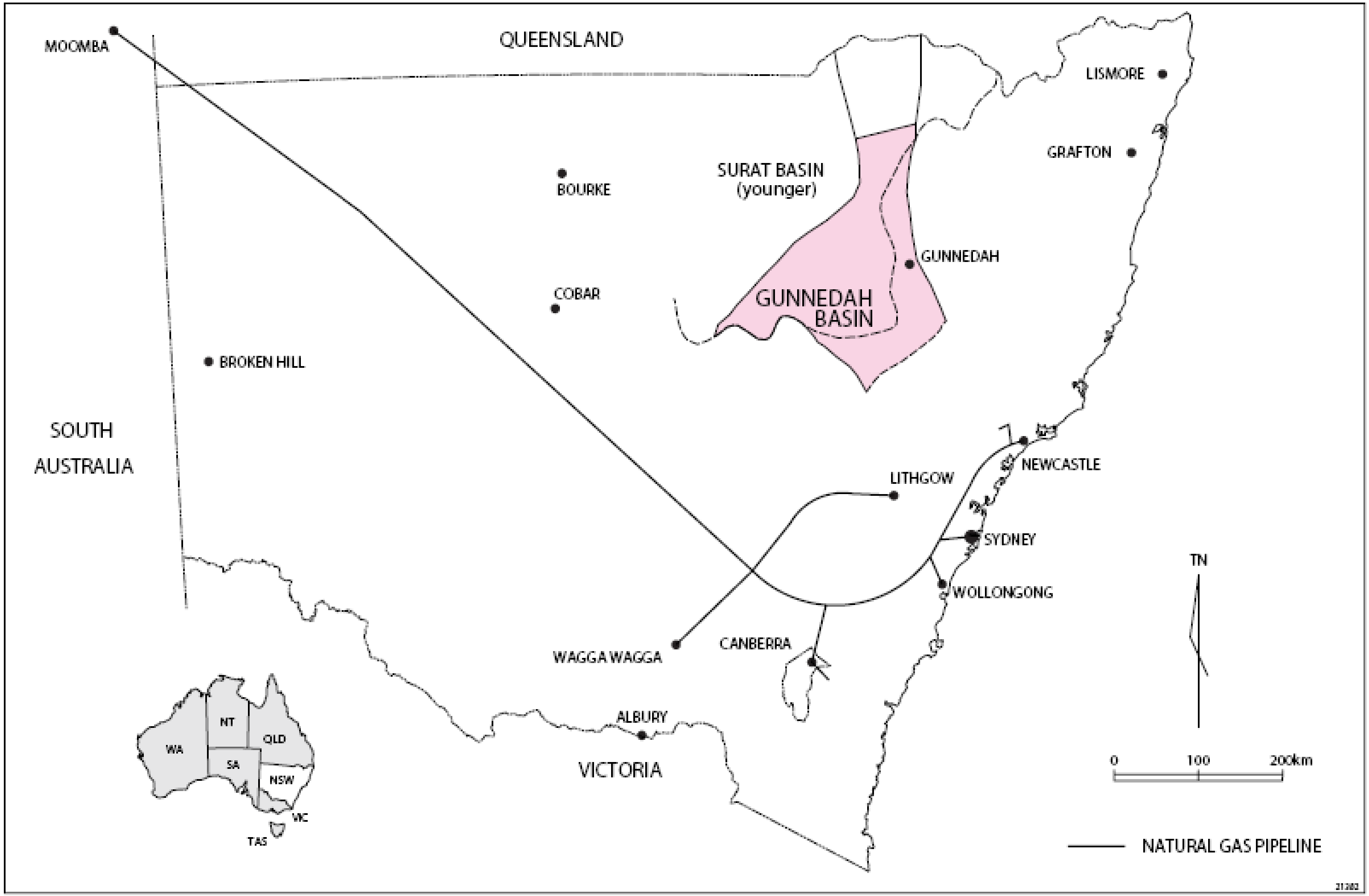


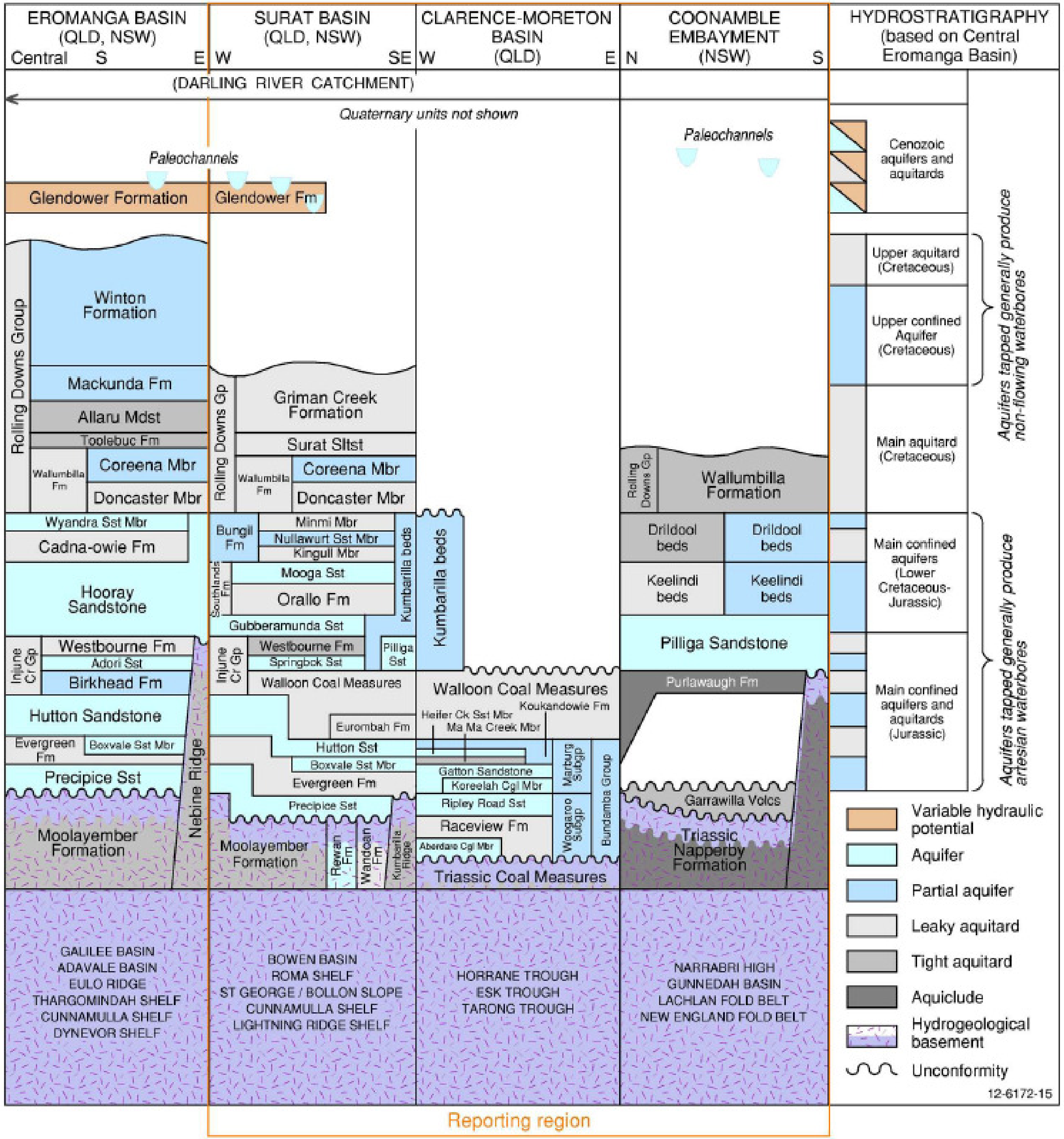
- New research provides a better understanding of the GAB
  - Enables future management to consider the complexity where appropriate
- The complex structure of the GAB governs groundwater conditions
  - Importance of potential vertical movement across layers

NSW Surat Basin



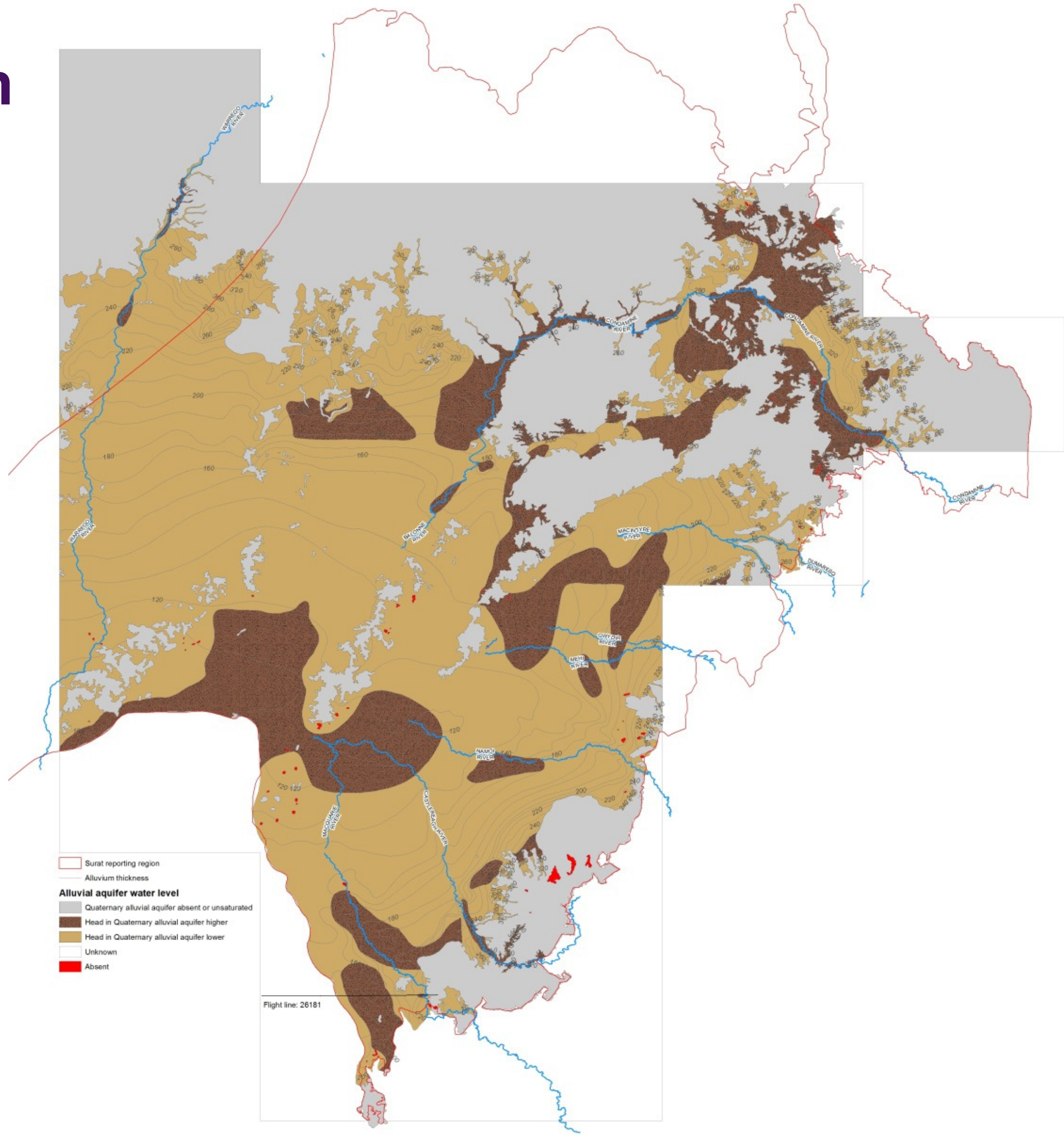
Gunnedah Basin




EROMANGA BASIN (QLD, NSW)
Central S E
SURAT BASIN (QLD, NSW)
W SE
CLARENCE-MORETON BASIN (QLD)
W E
COONAMBLE EMBAYMENT (NSW)
N S
HYDROSTRATIGRAPHY (based on Central Eromanga Basin)
(DARLING RIVER CATCHMENT)
Quaternary units not shown
Paleochannels
Paleochannels
Glendower Formation
Glendower Fm
Rolling Downs Group
Winton Formation
Mackunda Fm
Allaru Mdst
Toolebuc Fm
Wallumbilla Fm
Coreena Mbr
Doncaster Mbr
Wyandra Sst Mbr
Cadna-owie Fm
Hooray Sandstone
Injune Cr Gp
Westbourne Fm
Adori Sst
Birkhead Fm
Hutton Sandstone
Evergreen Fm
Boxvale Sst Mbr
Precipice Sst
Moolayember Formation
Nebine Ridge
Rolling Downs Gp
Griman Creek Formation
Surat Sltst
Wallumbilla Fm
Coreena Mbr
Doncaster Mbr
Bungil Fm
Minmi Mbr
Nullawurt Sst Mbr
Kingull Mbr
Southlands Fm
Mooga Sst
Orallo Fm
Kumbarilla beds
Gubberamunda Sst
Westbourne Fm
Springbok Sst
Pilliga Sst
Injune Cr Gp
Walloon Coal Measures
Eurombah Fm
Hutton Sst
Boxvale Sst Mbr
Evergreen Fm
Precipice Sst
Moolayember Formation
Rewan Fm
Wandoan Fm
Kumbarilla Ridge
Kumbarilla beds
Walloon Coal Measures
Koukandowie Fm
Heifer Ck Sst Mbr
Ma Ma Creek Mbr
Gatton Sandstone
Koreelah Cgl Mbr
Marburg Subgp
Ripley Road Sst
Raceview Fm
Aberdare Cgl Mbr
Woogaroo Subgp
Bundamba Group
Triassic Coal Measures
Rolling Downs Gp
Wallumbilla Formation
Drildool beds
Drildool beds
Keelindi beds
Keelindi beds
Pilliga Sandstone
Purlawaugh Fm
Garrawilla Volcs
Triassic Napperby Formation
GALILEE BASIN
ADAVALE BASIN
EULO RIDGE
THARGOMINDAH SHELF
CUNNAMULLA SHELF
DYNEVOR SHELF
BOWEN BASIN
ROMA SHELF
ST GEORGE / BOLLON SLOPE
CUNNAMULLA SHELF
LIGHTNING RIDGE SHELF
HORRANE TROUGH
ESK TROUGH
TARONG TROUGH
NARRABRI HIGH
GUNNEDAH BASIN
LACHLAN FOLD BELT
NEW ENGLAND FOLD BELT
Cenozoic aquifers and aquitards
Upper aquitard (Cretaceous)
Upper confined Aquifer (Cretaceous)
Main aquitard (Cretaceous)
Aquifers tapped generally produce non-flowing waterbores
Main confined aquifers (Lower Cretaceous-Jurassic)
Main confined aquifers and aquitards (Jurassic)
Aquifers tapped generally produce artesian waterbores
Variable hydraulic potential
Aquifer
Partial aquifer
Leaky aquitard
Tight aquitard
Aquiclude
Hydrogeological basement
Unconformity
12-6172-15
Reporting region

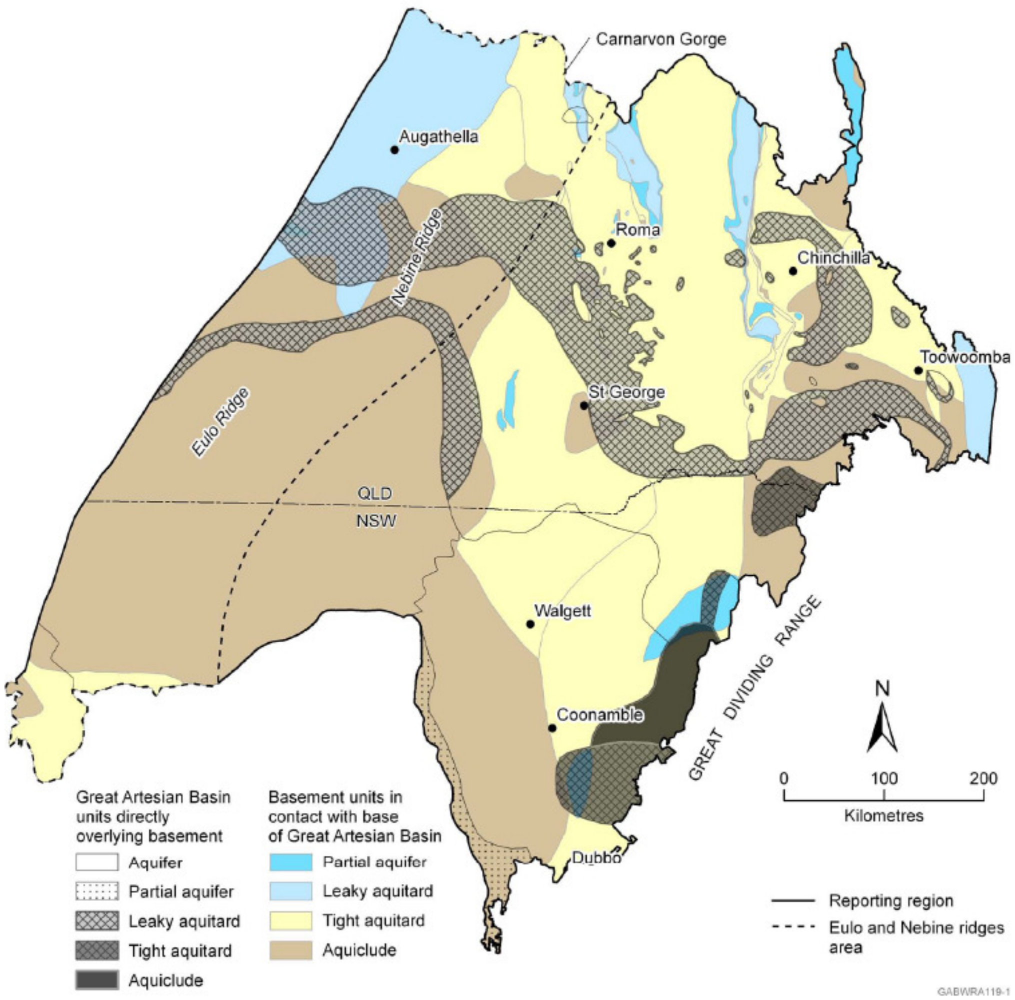
Connection between Surat Basin and alluvium

➔Likely to be both recharge into and discharge from the GAB to overlying alluvial aquifers

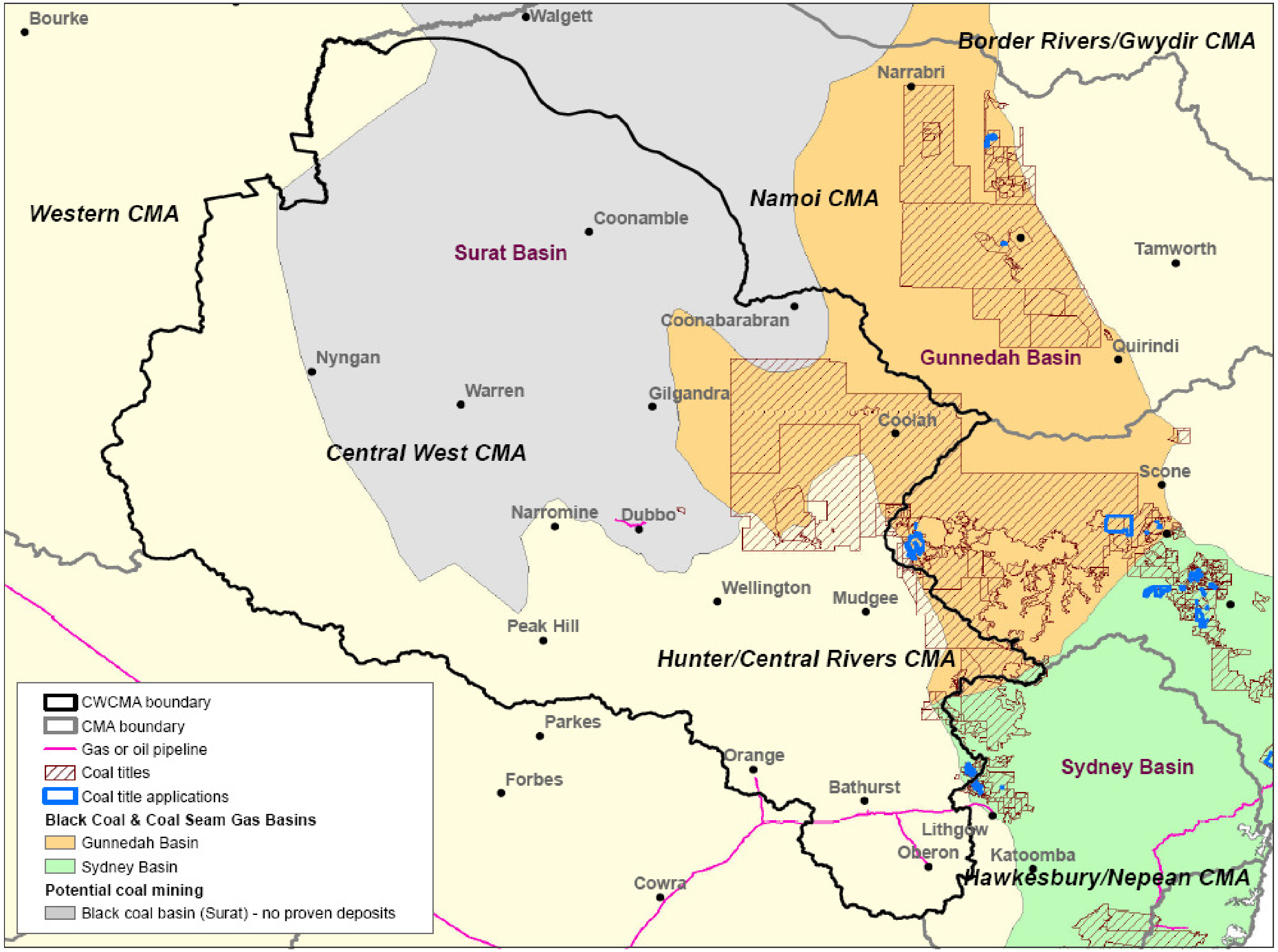


Connection between Surat Basin and underlying rocks

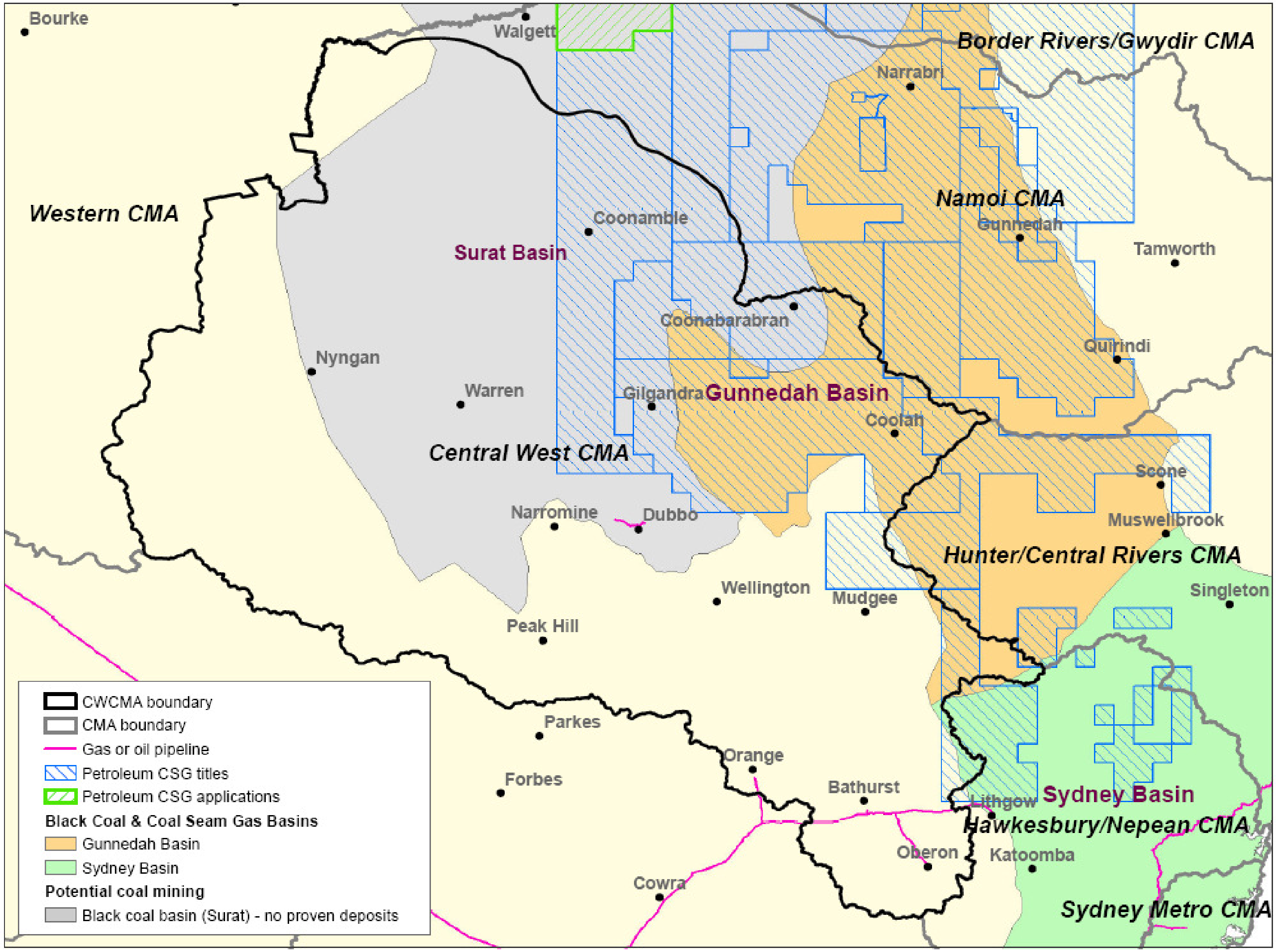
➔Some areas leak, others do not



Location of Coal Mining Activities



Location of Coal Seam Gas Titles



Dr Richard Cresswell

rcresswell@globalskm.com
+61 417 063 993
Visit: **www.globalskm.com**

achieve outstanding client success

Biography

Santos
We have the energy.

Dr Richard Cresswell 	Richard is Practice Leader for Coal Seam Gas related Groundwater at Sinclair Knight Merz (SKM) and is a member of the federal Environment Minister's Expert Panel for Large Coal Seam Gas Projects. He also leads significant groundwater impact assessments for the coal industry in NSW. Richard is the senior hydrogeologist in SKM's Sydney office. Richard has over 25 years experience in geochemistry and the use of isotopes to study natural and anthropogenic processes, ranging from archaeology, meteoritics, geomorphology, biomedicine and, for the last 15 years, water dynamics. He was team leader within the Great Artesian Basin Water Resource Assessment run by CSIRO and Geoscience Australia and previously, whilst at CSIRO, he led the Northern Australia Sustainable Yields Project was part of the team that delivered the Murray-Darling Basin Sustainable Yields Project. He has worked on projects across Australia and internationally, with an emphasis of water resources, water quality and quantifying surface-groundwater dynamics. Born and raised in NW London, Richard earned his degree in geology from Sheffield University then achieved a Master's and Doctorate in geology from the University of Toronto, Canada. Following post-doctoral research in the Department Of Nuclear Physics, Australian National University in Canberra, Richard joined the Bureau of Rural Sciences to work on salinity issues under the National Action Plan for Salinity and Water Quality. He moved to Brisbane in 2004 to work with CSIRO and recently moved to Sydney and joined the Natural Resource Management Sector of SKM. Richard lives on 5 acres of temperate rainforest on the Central Coast and has a keen interest in aikido and horses, though seems to spend most of his spare time in the garden and renovating!

Internal Memo

To	Glenn Toogood Team Leader Water and Environment Santos - Energy NSW	Date	28 November 2012
From	Richard Cresswell	Project No	GENV17062
Subject	**Sulphate-reducing bacteria**		

What are sulfate-reducing bacteria?

Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that can obtain energy by oxidizing organic compounds (CH_2O) or molecular hydrogen (H_2) in the presence of sulfate (SO_2^{-4}). This process generates dissolved sulfide (HS-) at pH > 7, or hydrogen sulfide gas (H_2S) at pH < 7and bicarbonate (HCO_3^-). These organisms may be thought of as "breathing" sulfate rather than oxygen, in a form of anaerobic respiration.

Sulfate-reducing bacteria can be traced back over 3.5 billion years and are considered to be among the oldest forms of microorganisms, having contributed to the sulfur cycle soon after life emerged on Earth. It is now appreciated that SRBs also play a significant role in the carbon cycle.

Many bacteria will reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction. In contrast, the sulfate-reducing bacteria reduce sulfate in large amounts to obtain energy and they expel the resulting sulfide as waste; this is known as dissimilatory sulfate reduction. Most of them are anaerobes, that is, they live in oxygen-deficient environments, though a few can tolerate oxic (oxygen-rich) conditions and even thrive long enough to generate an anoxic (oxygen-deficient) environment for other SRBs to thrive. Some SRBs can also use nitrate, iron and other compounds (including oxygen) instead of, or as well as, sulfate to generate the energy they need to survive. There are over 220 species of SRB and the ecology of most is still poorly known or unknown (e.g. Muyzer & Stams, 2008).

Health effects

The Australian Drinking Water Guidelines (ADWG, 2011) do not have guidance on acceptable levels of SRB as there are no known direct health implications, though the presence may be indicative of faecal contamination. The US EPA also states that SRB pose no known health risks. Based on aesthetic considerations the ADWG suggest the concentration of H_2S should not exceed 0.05 mg/L.

SRB generate hydrogen sulfide (H_2S) (rotten egg smell) as a product of the sulfate reduction process, although the extent is dependent on the pH of the system. In NSW the EPA set odour impact assessment criteria for H_2S in air, ranging between 1 ppb and 3 ppb with the lower value applying in densely populated areas and the higher value applying for a single receiver, for example and isolated rural property. Health impacts from exposure to H_2S in air occur at much higher concentrations, with permissible exposure levels typically set at 10 ppm. H_2S odour increases as the gas becomes more concentrated, with the strong rotten egg smell recognisable up to 30 ppm. Above this level, the gas has a sickeningly sweet odour up to around 100 ppm. However, at concentrations above 100 ppm, a person's ability to detect the gas is affected by rapid temporary paralysis of the olfactory nerves in the nose, leading to a loss of the sense of smell. This means that the gas can be present at dangerously high concentrations, with no perceivable odour. Prolonged exposure to lower concentrations can also result in similar effects of olfactory fatigue. This unusual property of H_2S makes it extremely dangerous to rely totally on the sense of smell to warn of the presence of the gas.

SRB may be present in the human gut and elevated levels (>1,000,000 per g of faeces) has been reported in some individuals. While this does not, in itself, have health effects, the production of excess H_2S may have links to gastrointestinal diseases, but this does not affect all individuals (Christophersen, et al., 2011).

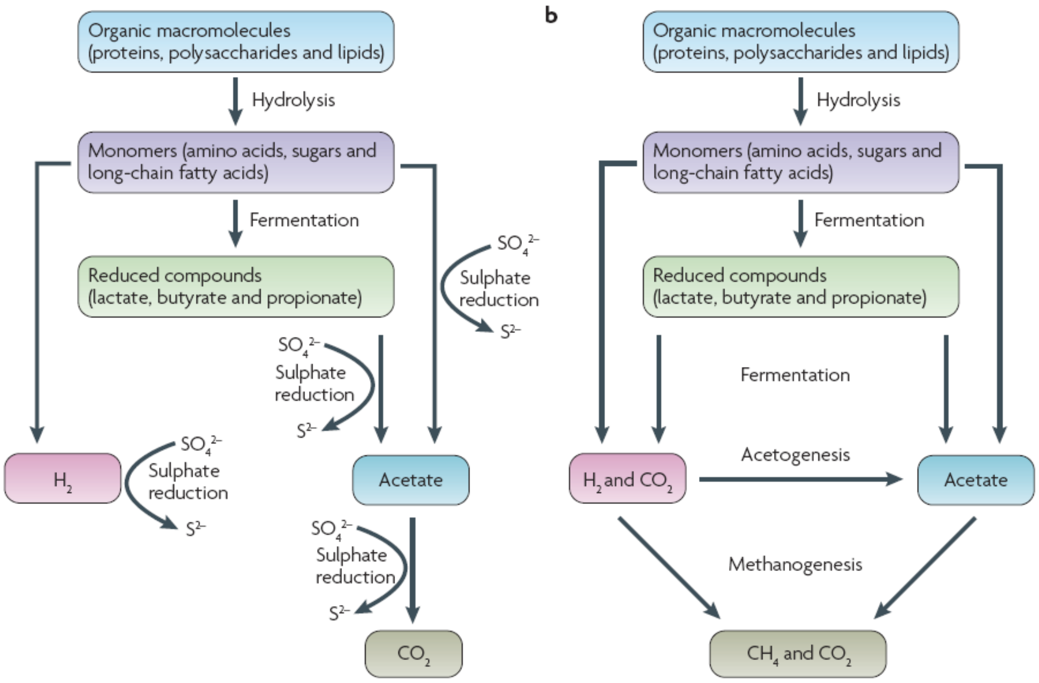
Water with hydrogen sulfide alone (not sewage) does not cause disease; however, sulfate-reducing bacteria can negatively impact the water industry because of their primary role in the anaerobic corrosion of iron in pipelines, heating systems, and other structures. However, these organisms can also be beneficial by removing sulfate and heavy metals from waste streams and are commonly used in the coal and metal mine industry to remediate leachate from acid-waste tailings and abandoned mines

Where do you find SRBs?

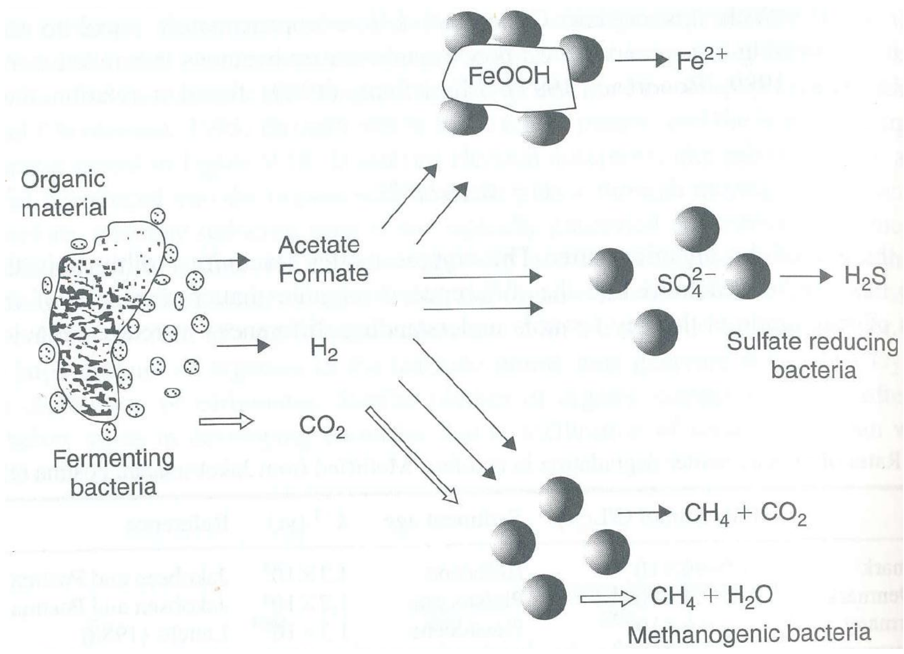
SRB are ubiquitous and can be found in many natural and engineered environments where sulfate is present. Most commonly where anoxic conditions exist, but they have been found in oxic environments. SRB live in environments such as deep wells, plumbing systems, water softeners, and water heaters. These bacteria usually flourish on the hot water side of a water distribution system. Sulfate reduction can occur over a wide range of pH, pressure, temperature, and salinity conditions. Indeed, SRBs are commonly found in surface waters and seawater. Often sulfate and sulfur reduction is apparent from the smell of hydrogen sulfide (similar to rotten eggs) and the blackening of water and sediment by iron sulfide. Scrape the heel of your shoe along the sand at the waters edged of the beach front and the blackened sand is a result of naturally occurring SRB activity.

Organic degradation pathways in anoxic environments

The commonest occurrence of SRB is in organic, anoxic environments. In these environments a distinction can be made between those that also have sulfate present and those that do not. Where sulfate is present, SRB thrive and the degradation process results in generation of H_2S together with carbon dioxide and bicarbonate. Where sulfate is absent the methanogenic bacteria dominate and methane and carbon dioxide is produced. This is outlined in Figure 1 and shown schematically in Figure 2.



- **Figure 1. The sequential pattern of microbial degradation of complex organic matter in anoxic environments in the presence (a) and absence (b) of sulfate (Muyzer & Stams, 2008).**

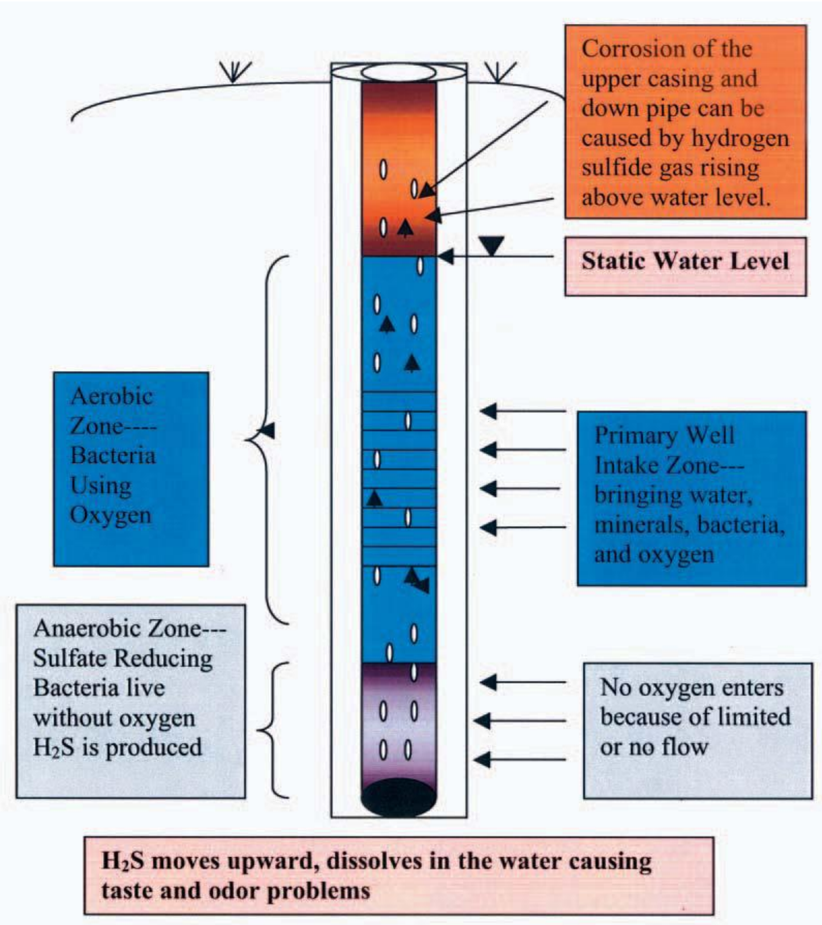


- **Figure 2. Schematic pathway of organic matter decomposition under anaerobic conditions (Appelo & Postma, 2005).**

SRB in water wells

SRB occur in any environment where oxygen levels are low and sulfate levels are significant (not necessarily high). Such conditions invariably exist in wells below the intake zone for pumps or where the water in the well has been allowed to stand for a period of time (Figure 3).

Bacteria reproduce rapidly, doubling every 20 minutes. Wells left standing for even a month will develop communities of anaerobic bacteria and, if there is any sulfate dissolved in the groundwater, then SRB will flourish.



- **Figure 3. Typical water well conditions (Schneiders, 2008)**

In wells that are in constant use, the oxidising effect of continual flushing reduces the levels of SRB as they do not survive in highly oxic environments. There is usually a sump beneath the intake slots, however, and this bottom zone may remain stagnant and therefore provide good conditions for bacterial growth.

SRB generate H_2S, and this is a corrosive gas that will pit and corrode any steel-cased well. These corrosion zones may also provide anoxic conditions (behind the scale) for bacteria to thrive.

Remediation involves thorough cleaning of the pump and removal of any organic material in the bottom of the well and oxidation of the well using chlorine and agitation (Schneiders, 2005). Maintaining a non-acidic environment in the well is also important, particularly in regions where groundwaters have a naturally low pH.

SRBs and CSG

SRB will out-compete methanogenic bacteria if any sulfate is present in the groundwater system. Methane is therefore not produced with coalbed waters that contain significant concentrations of sulfate (>500 mg/L)(e.g. Van Voast, 2003). Fortunately, the geochemical evolution of groundwaters recharged in coal-bearing beds results in rapid biochemical reduction of any sulfate present, precipitating sulfides, thereby depleting the sulfate. Methanogenic bacteria are then free to consume the organic material and produce methane.

There is thus generally exclusivity between CSG wells and wells where SRB may exist.

Yours sincerely

Dr Richard Cresswell
Senior Hydrogeologist
Phone: (02) 9032 1700
Fax: (02) 9928 2224
E-mail: rcresswell@globalskm.com

References:

Appelo, CAJ & Postma, D (2005) Geochemistry, groundwater and pollution (2nd edition). A.A. Balkema publishers, Leiden. 649 pp.

Christophersen, CT, Morrison, M & Conlon, MA (2011) Overestimation of the Abundance of Sulfate-Reducing Bacteria in Human Feces Quantitative PCR Targeting the *Desulfovibrio* 16S rRNA Gene. Appl. Environ. Microbiol. **77**, 3544-3546 doi: 10.1128/AEM.02851-10

Muyzer, G & Stams, AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nature Review: Microbiology **6**, 441-454

Schneiders, J (2008) Sulfate-reducing bacteria. Water Well Journal, November 2005. National Groundwater Association

Van Voast, WA (2003) Geochemical signature of formation waters associated with coalbed methane. AAPG Bulletin **87**, 667-676