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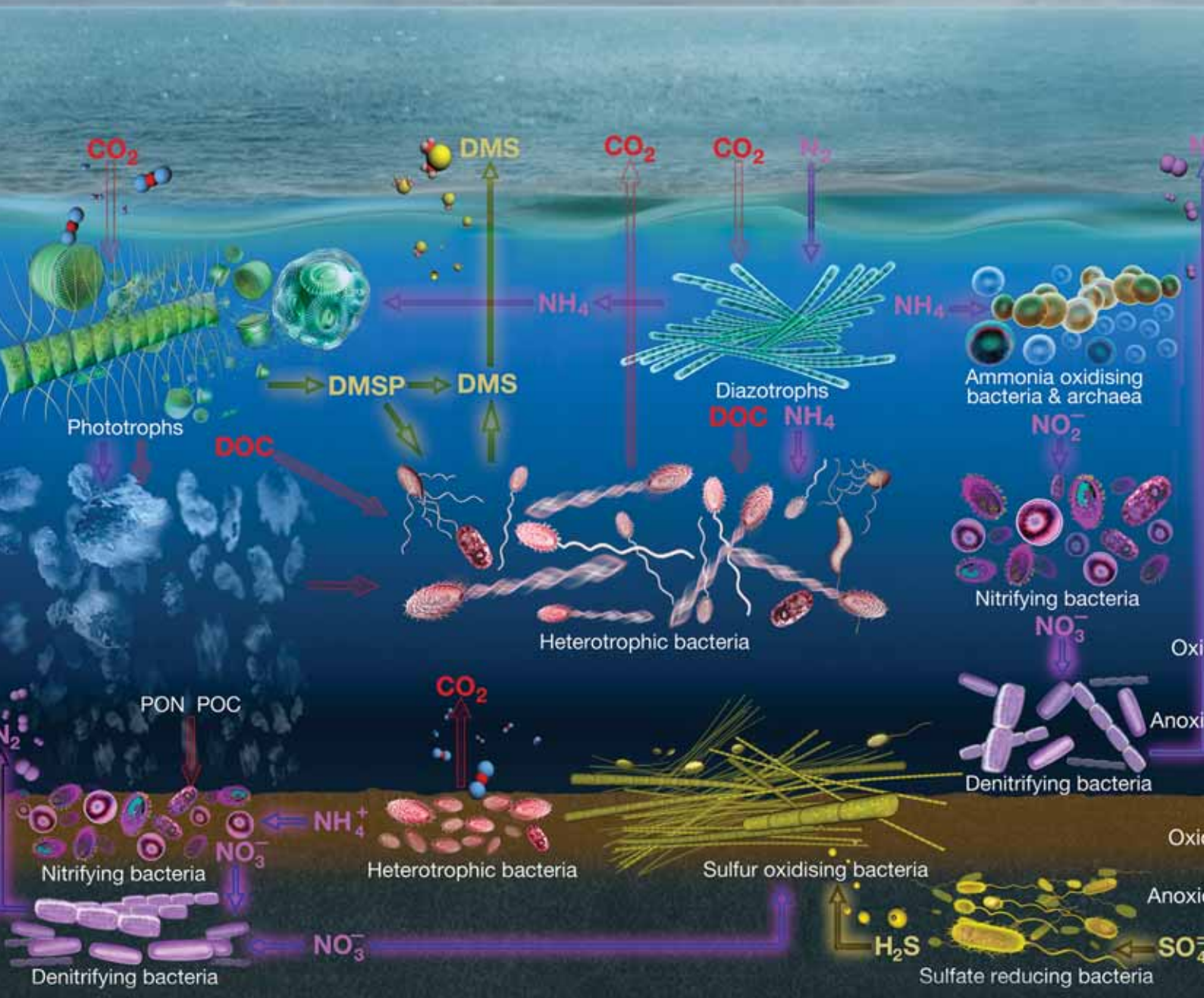

The microbial ecology of the environment

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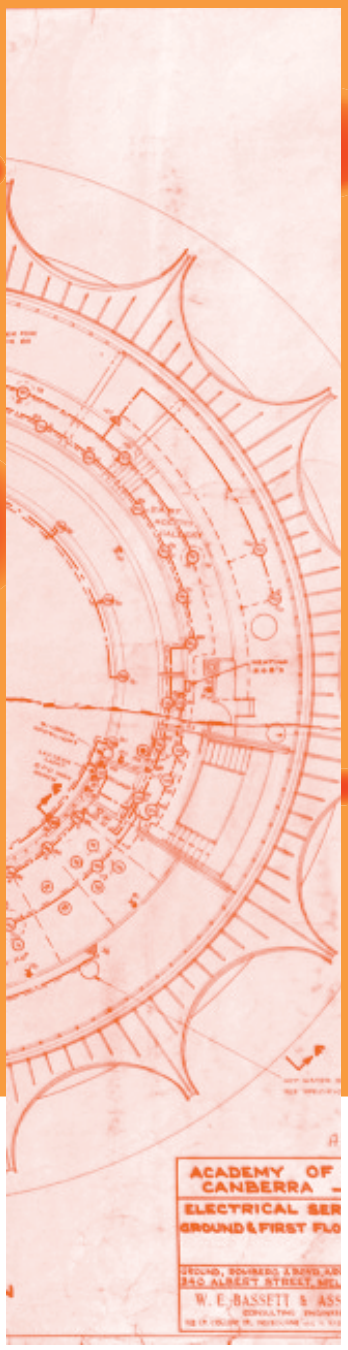

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The Australian Society for Microbiology Inc.

9/397 Smith Street
Fitzroy, Vic. 3065
Tel: 1300 656 423
Fax: 03 9329 1777
Email: admin@theasm.com.au
www.theasm.org.au
ABN 24 065 463 274

For *Microbiology Australia* correspondence, see address below.

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Editorial correspondence
Prof. Ian Macreadie/Mrs Jo Macreadie
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Email: ian.macreadie@gmail.com

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150 Oxford Street
Collingwood, Vic. 3066
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Microbial ecology of the environment

Andrew S Ball

To study microbes, is to study the biosphere. Carl Woese (15 July 1928–30 December 2012)

Microbes represent not only the most abundant but also exhibit the greatest diversity of any group of organisms on the planet. Through culture-dependent methods, the extraordinary physiological diversity of microbial life has long been recognised. However, with the application of molecular microbial techniques, estimates of microbial diversity have increased dramatically. In particular, the advent of low cost, next generation sequencing technologies has led to an explosion in sequence-based microbial community studies investigating taxon diversity and community structure (e.g. via rRNA gene analysis) and/or microbial function via metagenomics of the uncultivated majority of microorganisms present within the environment. Such approaches have revealed a diverse wealth of hitherto unknown microbial taxa and provided new understanding of the ecological and biological functions and adaptations of environmental microbes. What is required now is to link our understanding of microbial diversity and complexity to ecosystem function. In natural environments, microorganisms interact with both biotic and abiotic components of their ecosystems. These interactions are essential for ecosystem function with key specific functions including biogeochemical cycling, biodegradation of pollutants and the impacts of microbes upon the activity and health of plants and animals, including humans.

Defining the specific role of individual microorganisms in the environment is complex, due in part to the metabolic flexibility and diversity within individual species, and additionally by functional redundancy whereby diverse species can carry out the same biological activity. This is complicated further by the need to consider microbiology at different levels, namely:

- First, at the smallest scale, the activity of the single microbial cell;
- Second, at the population level, involving interactions and communication between members of the same species; and
- Third, at the community level where interactions occur between members of multiple microbial species, and often with plants and animals.

At each level, we also need to investigate the interrelationship of the microorganism(s) with environmental factors such as nutrient and water availability and temperature and pH and, in turn, how these affect ecological activity.

This 'Microbial Ecology of the Environment' Special Issue focuses on linking microbial diversity with ecosystem function. Questions to be addressed include:

- What interactions with the environment and with other organisms control microbial activity?
- How can we better understand the biotechnological potential associated with the activity and diversity of microbial communities?
- What factors influence the rate and extent of degradation of pollutants by microorganisms in the environment?

Overall, the eight 'In Focus' and 'Under the Microscope' articles offer an introduction to the importance of microbial diversity and activity to ecosystem function across diverse environments. This issue focuses on specific environments and microbial communities with an Australian focus (either of Australian environments and/or by Australian researchers) to highlight some of the key developments across the microbial ecology discipline. The first In Focus article by Justin Seymour examines our current understanding of microbial diversity and activity in the marine environment. The second In Focus article, written by Jacob Munro, Deborah Rich, Simon Dingsdag and Nick Coleman provides valuable insight into the development and use of culture-independent microbiology. In the first of the Under the Microscope articles, Eric Adetutu leads us through the microbial diversity and activity associated with caves within Australia, while Jacqueline Stroud and Mike Manefield describe the microbiology of acid sulphate soils and sulfidic sediments. The theme then focuses on applied environmental microbiology as Albert Juhasz examines the impact of bioavailability of the biodegradation of polycyclic aromatic hydrocarbons in Australian soils, while Ashley Franks and Lucie Semenec discuss the microbiology of Microbial Electrolysis Cells. Mark Osborn and Slobodanka Stojkovic continue the environmental pollution theme, with an article examining the role of microorganisms in the colonisation and degradation of plastic pollutants in marine environments. Finally, Sayali Patil, Eric Adetutu and I discuss the activity and diversity of the microbial communities in Australian contaminated groundwater.

I hope you find this Special issue both of interest and of value. I would like to thank all of the authors for their timely contributions and also the *Microbiology Australia* Editorial Team, led by Ian Macreadie for their guidance and assistance through the development of this issue.

Biography

Professor Ball is a graduate of Liverpool University in the UK (BSc, 1983; PhD, 1986). He has been working in the field of environmental microbiology since 1983 with a focus on biogeochemical cycling and the degradation of pollutants in the environment. Professor Ball currently teaches in the fields of environmental microbiology and biotechnology in the School of Applied Science, RMIT University in Melbourne, Australia. He is also Director of the Centre for Environmental Sustainability and Remediation (EnSuRe) at RMIT University.

A sea of microbes: the diversity and activity of marine microorganisms

Justin R Seymour

Plant Functional Biology & Climate Change Cluster
University of Technology Sydney
GPO Box 123, Broadway
Sydney, NSW 2007, Australia
Tel: +61 2 9514 1776
Email: Justin.Seymour@uts.edu.au

Covering 70% of the earth's surface, with an average depth of 3.6 km, the ocean's total volume of 1.3 billion cubic kilometres represents perhaps the largest inhabitable space in the biosphere. Within this vast ecosystem, 90% of all living biomass is microbial. Indeed, seawater from all marine environments, ranging from the warm and sunlit upper ocean to the cold, dark and anoxic deep sea floor, and from the tropics to the arctic, is teeming with microbial life. A single teaspoon of seawater typically contains over 50 million viruses, 5 million *Bacteria*, 100,000 *Archaea* and 50,000 eukaryotic microbes. The numerical importance of these microbes is matched only by their ecological and biogeochemical significance. By performing the bulk of oceanic primary production and mediating key chemical transformation processes, planktonic microbes form the base of the marine food-web and are the engines that drive the ocean's major biogeochemical cycles (Figure 1). While marine microbes are the dominant biological feature throughout the entire water column and within ocean sediments, as well as being important symbionts and pathogens of marine animals and plants, this review will focus on the activity and diversity of microbes inhabiting seawater in the upper sun-lit depths of the global ocean.

'Keystone microbes' in a sea of diversity

The ecological and biogeochemical importance of marine microbes is underpinned by the staggering diversity of microbial communities within the ocean. This has only become apparent relatively recently, as the advent of molecular microbiological and genomic approaches has revolutionised the field of marine microbiology. Deep 16S rRNA amplicon sequencing approaches have led to estimates that a single litre of seawater can contain tens of thousands of microbial 'species'[1]. More recent approaches using single cell genomics have revealed that even a single marine bacterial species is comprised of hundreds of discrete co-existing sub-populations, which differ in flexible gene content and exhibit different ecological characteristics and environmental tolerances and preferences[2]. These and other observations of the adaptive divergence of distinct ecotypes among marine bacterial species[3] indicate that there is likely to be a microbe to exploit every niche in the ocean. However, embedded within this striking biodiversity are some star players that can be considered 'keystone organisms' among the ocean's microbiota.

Between 25–50% of all prokaryotic cells in the ocean belong to a single clade of *Alphaproteobacteria*, known as SAR11[4]. Originally named after clone 11 from a clone library derived from seawater collected in the Sargasso Sea[5], the SAR11 clade is now recognised as perhaps the most abundant group of closely related organisms on earth. While SAR11 dominates microbial assemblages in ocean surface water across the globe[4], bacteria belonging to this clade are classic oligotrophs and are most successful in the open ocean, which from a microbe's perspective is akin to a desert, where organic resources are significantly below the levels required by most other heterotrophic bacteria. SAR11 can thrive under these conditions with the aid of genome streamlining, whereby their extremely small genomes lack many of the cellular functions thought to be required by free-living bacteria[6]. As a consequence, SAR11 bacteria utilise atypical nutrient acquisition strategies that appear to minimise the cells' requirements for organic substrates and allow replication in the most nutrient deplete conditions[6].

Not all important marine bacteria are oligotrophs like SAR11. Several copiotrophic species, which prefer the nutrient rich waters found near to the coast or in association with phytoplankton blooms, also often represent a significant fraction of marine bacterial assemblages. Genera including the *Roseobacters* and *Flavobacteria* are ecologically important groups that commonly occur in high numbers in seawater[7–9]. Unlike the highly streamlined genome of the specialist oligotroph SAR11, the genomes of these organisms are often large, providing the cells with significant metabolic flexibility underpinned by a diverse repertoire of energy and carbon acquisition strategies, which allows them to exploit a wide variety of marine niches and thrive under a range of environmental conditions[9].

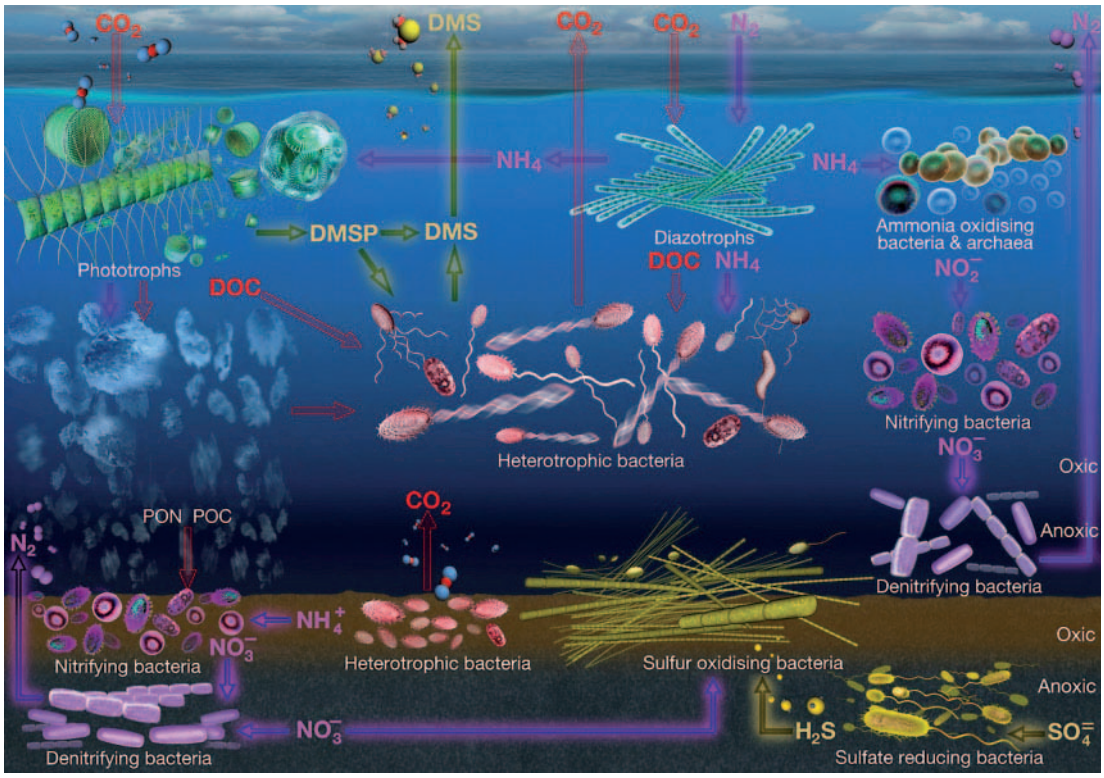

Figure 1. Diverse populations of marine microorganisms are responsible for the chemical transformations that underpin key features of ocean biogeochemistry, including the carbon (red arrows), nitrogen (purple arrows) and sulphur (yellow arrows) cycles shown here. The surface sunlit and oxic layers of the ocean occur within the navy blue region of the diagram, while the deep and anoxic ocean is characterised by the dark blue-black region. The oxic layer of the ocean sediments is denoted by the orange-brown layer, while the bottom grey layer denotes the anoxic sediments. POC = particulate organic carbon; DOC = dissolved organic carbon; PON = particulate organic nitrogen; DMSP = dimethylsulfoniopropoinate; DMS = diemthylsulfide.

While spatial partitioning of the key oligotrophic and copiotrophic bacteria occurs across geographic boundaries[10], within a given location ocean microbial communities also undergo constant substrate controlled successional shifts. During periods of low seawater nutrient concentrations and reduced primary production, oligotrophs such as SAR11 dominate the microbial community. However, following nutrient input events, like those that occur during and immediately after the spring phytoplankton bloom, bacterial communities rapidly shift to become dominated by copiototrophic organisms, including the *Roseobacters* and *Flavobacteria*, which can efficiently degrade phytoplankton derived carbohydrates[11]. These successional processes are often repeatable and predictable, with highly synchronous seasonal shifts in the composition of microbial communities observed over multiple years[12], indicating the presence of well-defined microbial niches and unique functional properties of the organisms that inhabit them[13].

Other important marine bacteria include members of the cyanobacteria. Two particularly numerically and ecologically important genera are *Prochlorococcous* and *Synechococcus*, which often comprise a significant fraction of the total bacterial community in the sunlit ocean surface waters[14]. Commonly occurring in concentrations of 10^5 cells/mL, *Prochlorococcus* is the most abundant phototrophic organism on earth and is responsible for a significant fraction of global photosynthesis[15]. While occurring in latitudes as high as 40°, *Prochlorococcus* dominates in tropical oligotrophic regions[14]. On the other hand, the closely related genus *Synechococcus* is more cosmopolitan, occurring in high abundances in temperate coastal waters as well as in the open ocean[14]. In some coastal environments *Synechococcus* can represent the most abundant bacterial genus[16].

Once thought to be primarily extremophiles inhabiting only harsh environments like the extremely hot and anoxic waters surrounding deep sea hydrothermal vents, the *Archaea* are now recognised as another ubiquitous and important group of marine microbes[17]. The two main groups of marine *Archaea*, the Marine Group I *Thaumarchaeota* and Marine Group II *Euryarchaeota*, are in fact estimated to account for more than 20% of all prokaryotic cells in the global ocean[18]. Indeed, below depths of 100 m, the Group I *Thaumarchaeota* are often the dominant group of microbes in seawater. A significant representative from this group, *Nitrosopumilus maritimus* is globally abundant and due to its unique mechanisms for nitrification and autotrophy is believed to play an important role in marine carbon and nitrogen biogeochemical cycling[19].

The most abundant and diverse of all marine microbes are viruses. There are 10^{31} viruses in the global ocean and it has been estimated that every second 10^{23} viral infections occur in the sea[20]. Marine viruses are known to infect most marine organisms from seaweeds to whales, but as a simple consequence of available host density, most viruses in seawater are bacteriophages[20]. By manipulating community composition through the selective killing of dominant organisms and facilitating the exchange of genetic material via horizontal gene transfer, marine viruses are a fundamental structuring agent within marine microbial foodwebs[21]. Moreover, by lysing microbial cells, viruses can strongly alter marine chemical cycling pathways, which has a substantial impact on ocean biogeochemistry[22].

Marine microbial activities and their influence on ocean biogeochemistry

Marine biogeochemical cycling processes, driven by the activities of microbes, control the rates and directions of ocean-atmosphere gas exchange, which strongly influences global climate[23]. However, marine microbial activities and growth rates are highly variable in space and time and across species. On average, marine bacterial growth rates are relatively low, with cells in the open ocean dividing only 0.2 times per day[24]. However, under optimum conditions, some marine bacteria have the capacity for staggering metabolic rates. For instance, *Pseudomonas natrigens* is capable of dividing once every 10 minutes[24], a likely adaptation for the opportunistic exploitation of intermittent and ephemeral substrate pulses in a dynamic ocean environment. The sum of this heterogeneous pool of marine microbial activities controls the turnover of labile organic substrates and inorganic nutrients in seawater, which ultimately regulates the ocean's major biogeochemical cycles[23] (Figure 1).

Within the ocean's carbon cycle, phototrophic microbes including cyanobacteria like *Prochlorococcus* and *Synechococcus* and a diverse assemblage of eukaryotic phytoplankton use sunlight to fix CO_2 into living biomass. Approximately 60 billion tonnes of carbon are fixed each year by these phototrophic microbes[25]. For perspective, this equates to 40% of total global carbon fixation, yet the biomass of marine microbial phototrophs is equivalent to only about 1% of terrestrial plant biomass[25]. The carbon fixed by marine photosynthetic microbes has several fates, which are largely determined by the activities of other microbes within seawater, and the balance of these processes profoundly influences global carbon budgets.

Some of the photosynthetically fixed carbon is transferred from phototrophic microbial biomass directly into the marine foodweb via zooplankton grazing. In addition, up to 50% of fixed carbon is exuded by phytoplankton back into the surrounding seawater in the form of dissolved organic carbon (DOC)[26]. This carbon is ultimately transferred to the higher food-web via a trophic pathway called the 'Microbial Loop', whereby heterotrophic bacteria rapidly assimilate DOC from the water column and are subsequently consumed by bacterivorous protists, which are then eaten by larger zooplankton[27].

In addition to the fixed carbon that is channeled into the foodweb either by zooplankton grazing or the microbial loop, another significant fraction of C sinks out of the photic zone as dead or senescent phytoplankton biomass, in the form of Particulate Organic Carbon (POC). This POC is transported to the deep ocean sediments and effectively removed from the carbon cycle for millennia, via a process known as the 'Biological Carbon Pump'. This downward flux of POC leads to the sequestration of up to 300 million tonnes of C to the deep sea each year. However, before all sinking POC is sequestered to the deep sea floor, a significant amount is metabolised by heterotrophic bacteria in the water column, leading to the return of the sinking C to the food web and its subsequent conversion to CO_2 through respiration[28]. Importantly though, recent evidence indicates that not all of the sinking POC that is metabolised by bacteria is respired back as CO_2. A potentially large proportion is instead converted into refractory (non-bioavailable) DOC, or RDOC, which remains in the water column. This process, known as the Microbial Carbon Pump, leads to the sequestration of biologically unavailable RDOC in the water column, where it may be stored for thousands of years[29].

The balance of these microbiologically mediated carbon cycling processes ultimately determines whether regions of the ocean act as sources or sinks of CO_2[30]. Indeed, given that marine carbon cycling processes are so tightly coupled to microbiological activity within seawater, and the oceanic pools and fluxes of carbon are among the largest on earth, even seemingly subtle changes in the composition and activity of microbial assemblages have the potential to profoundly influence the global carbon cycle.

The role of planktonic microbes in the marine sulphur cycle is largely tied to the production and transformation of an organic sulphur compound called dimethylsulphoniopropionate, or DMSP, which is produced by several species of phytoplankton. DMSP is believed to act as an antioxidant, cryoprotectant or osmolyte for phytoplankton[31], but also represents an important microbial growth resource, contributing to up to 10% of the carbon demand and over 40% of the sulphur requirements for heterotrophic bacteria in ocean environments[32]. However, not all marine bacteria use, and cycle, DMSP in the same way, and this has important implications for marine sulphur cycling.

Many marine bacteria demethylate DMSP and subsequently assimilate the sulphur into proteins[33]. However, others cleave DMSP in a manner that liberates the volatile compound dimethyl sulphide, or DMS[34]. This is significant because DMS is the major vehicle for ocean to atmosphere sulphur efflux and once in the atmosphere, DMS is rapidly oxidised into aerosol sulphates, which act as cloud condensation nuclei (CCN)[35]. The balance between the competing demethylation and cleavage pathways of DMSP degradation, which is determined by the composition and activities of marine bacterial populations, influences the amount of DMS that is released from the ocean into the atmosphere and subsequently influences regional climate.

Nitrogen is a key limiting nutrient in many parts of the ocean, so its input and cycling by microbial activity fundamentally shapes the fertility and biology of the global ocean. Diverse populations of bacteria, archaea and eukaryotic phytoplankton are responsible for performing and mediating the key nitrogen cycling steps of fixation, assimilation, nitrification and denitrification[36]. Analogous to soil environments, where discrete nitrogen cycling microbes and transformation processes are vertically partitioned across oxic niches, different depths of the ocean water column play host to specific microbiological modules of the marine nitrogen cycle[37]. Additionally, there is strong geographic partitioning of some key nitrogen cycling microbes and processes across the global ocean, with, for instance, much of cyanobacterial nitrogen fixation concentrated within the warm, well-lit oligotrophic regions of the ocean[38]. Among all of the ocean's biogeochemical cycles, our understanding of the nitrogen cycle has perhaps been most radically re-shaped with the advent of molecular microbiological approaches[36]. The resultant discoveries of new groups of nitrogen cycling microbes, including unicellular nitrogen fixing cyanobacteria and large populations of ammonia oxidising archaea, have fundamentally shifted our understanding of marine nitrogen cycling and the microbiological processes that regulate global nitrogen budgets.

New directions: dynamic microbial networks control ocean function

Our view of the identities and roles of microbes in the ocean continues to rapidly expand. Microbial oceanography is currently transitioning from a fruitful era of discovery where molecular tools allowed us to uncover the diversity of marine microbial communities and their functions, to an exciting new phase where this information is feeding sophisticated new ecological questions and concepts. For instance, recent evidence suggests that rather than consisting of a soup of loosely associated populations, the microbial communities in seawater are comprised of highly interconnected ecological units that function in tight synchrony[12,13,39,40]. It is becoming apparent that networks of key groups of marine microbes consistently co-occur non-randomly, indicating the existence of community assembly rules among the microbial plankton[41], while the occurrence of multi-species timing of gene regulation in seawater[40,42] likely permits coordination of complex metabolic processes. With the aid of a suite of new automated sampling technologies[42] and rapidly advancing ecogenomic approaches[2], exploration of these new ecological concepts promises to deliver unprecedented insights into the lives of the ocean's smallest, but arguably most important inhabitants.

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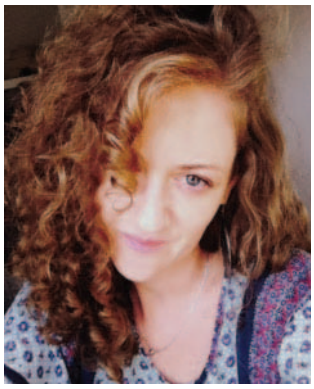
Biography

Justin Seymour is an ARC Future Fellow, based at the University of Technology, Sydney and his research interests incorporate aquatic microbial ecology and biological oceanography. His research involves examinations of the ecology of microbes across a range of marine environments, spanning tropical coral reefs to Antarctica, and a continuum of spatiotemporal scales. At the ocean-basin scale he is interested in how large-scale oceanographic processes influence microbial community dynamics and functionality, while at the scale of individual drops of seawater he studies the foraging behaviours of individual marine microbes living within a patchy chemical seascape.

Wastewater, wheat and table wipes: adventures in culture-independent microbiology

Jacob E Munro[A], *Deborah J Rich*[A], *Simon Dingsdag*[A] *and Nicholas V Coleman*[A,B]

[A]School of Molecular Bioscience, Building G08, University of Sydney, Darlington, NSW 2006, Australia.

[B]Corresponding author. Tel: +61 2 9351 6047, Fax: +61 2 9351 5858, Email: nicholas.coleman@sydney.edu.au

The sequencing of ribosomal RNA and DNA (rRNA/rDNA) from environmental samples heralded a new age in microbiology[1–3]. The advent of next-generation sequencing supercharged these methods, which now give high-resolution data sets, enabling real insights into microbial diversity and function in complex systems[4–7]. Here, three local applications of 16S rDNA pyrosequencing are described, which highlight the usefulness of this approach for addressing practical questions in diverse areas of microbiology. Limitations of the sequence-based approach will also be discussed.

Wastewater: understanding the shutdown response in biological aerated filters

An industry partner approached our lab for assistance with managing microbes in the biological aerated filter units (BAFs) in their wastewater treatment plant. The BAFs were designed to degrade volatile fatty acids (VFAs) in the wastewater stream, but were problematic after 'shutdown' events. Such events involve stopping the water flow for many days, and in some cases cleaning the BAFs; these actions change the physiology and/or community in the BAFs such that they do not readily re-start VFA oxidation.

We sampled the BAF material (zeolite+biofilm) at intervals over a time course spanning both shutdown and restart events. RNA was extracted, and reverse-transcribed to cDNA, then used for 16S and 18S rRNA gene PCRs, and tag-pyrosequencing. We used bead-beating combined with a commercial RNA extraction kit to good effect; this was an 'easy' template due to the abundance of biomass in the BAF material (Figure 1). Our rationale for using ribosomal RNA as the template was that this is a better marker for the *activity* of microbes compared to ribosomal DNA, which is better correlated to cell *abundance* (e.g. see Hunt *et al.*[8]).

Distinctive changes in the BAF community occurred during shutdown (representative data from one of three replicate BAFs are shown in Figure 2); *Arcobacter*, *Zoogloea*, and *Bdellovibrio* declined, while *Rubrivivax* and *Pedomicrobium* increased. After restart of flow, the community seemed to return to the initial structure, but this robust response to perturbation at the genus level did not correspond to success in restarting VFA oxidation (data not shown). The changes involved in the shutdown response may be occurring at finer-scale taxonomic resolution, or might not involve ribosome abundance (e.g. they may involve enzyme induction).

Bdellovibrio is a predator on other bacteria. This genus suffered dramatic declines in rRNA abundance after shutdown (50- to 150-fold). It is interesting that this 'top predator' was the most sensitive to ecosystem disturbance – this mirrors patterns seen in macro-organisms[9]. *Bdellovibrio* may be a predator of a bacterium that is inhibitory to VFA oxidation, or it may be acting here as an

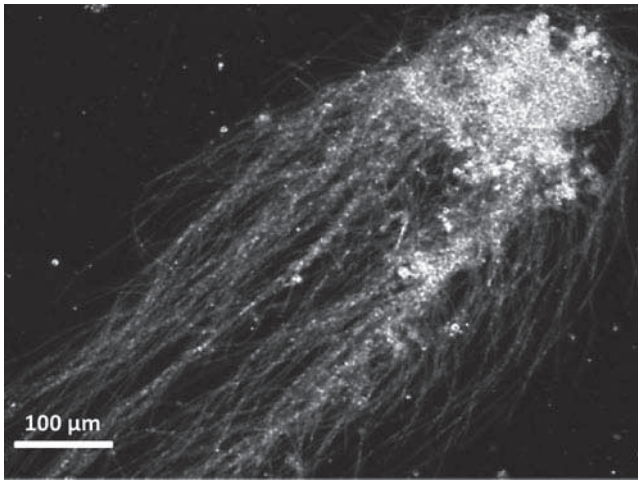


Figure 1. Microscopy of BAF biomass floc (acridine orange stain). The abundant filamentous cells that define these flocs could be *Sphaerotilus* and/or *Thiothrix*.

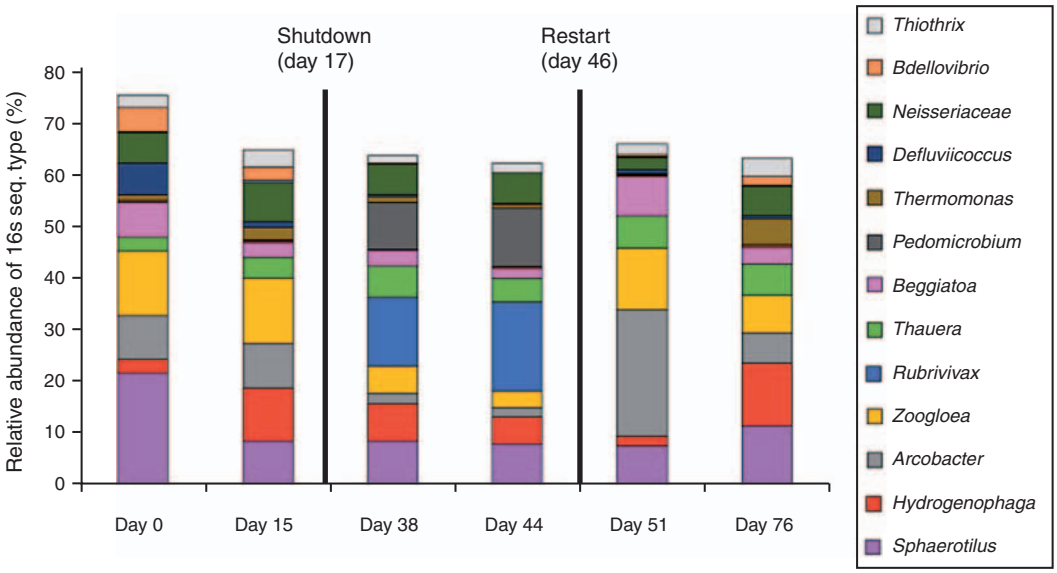


Figure 2. Bacterial community response to BAF shutdown and restart, as inferred from 16S rRNA relative sequence abundances.

indicator organism of the chemical changes in the system. Further work is needed to elucidate the microbial basis of the BAF shutdown response.

Wheat: tracking inoculant strains and discovering indigenous microbiota

Ethylene (C_2H_4) is a gaseous plant hormone[10]. Ethylene-oxidising bacteria can be readily isolated from soil[11] – do these bacteria interact with plants based on the ethylene system? Alternatively, these bacteria could be consuming ethylene made by microbial fermentation[12]. . .the jury is out. Ethylene-oxidising isolates are nearly always fast-growing *Mycobacterium* spp.[13,14] – these are a fascinating group of microbes, which are mostly non-pathogenic, but highly immunogenic[15]. They may even play a role in influencing our moods[16,17].

We have begun a study to investigate the interactions of ethylene-oxidising bacteria and plants. The 16S pyrosequencing approach was used to provide information on the persistence of the inoculant strains and to reveal which types of indigenous bacteria were present. Note that although the tag-pyrosequencing data are not quantitative in the sense that sequences do not correlate 1 : 1 with cells, the data can be used to discern trends in relative abundances, and provide a reference point for viable counts in the case of the inoculant strain.

Preliminary data from two individual wheat plants (control/inoculated) are shown in Table 1. Note that the DNA extraction method used here has captured both the surface microbiota and the endophytes in the wheat plants, as evidenced by the abundant chloroplast sequences that are recovered (green highlight). These organelles contain their own 16S rDNA, which bears testament to their cyanobacterial ancestry[18]. At this stage it is not clear which of these taxa are surface microbiota, and which are endophytes.

The tag-pyrosequencing approach easily detected our inoculated ethylene-oxidising bacterium (yellow highlight), but intriguingly, the sequence data also revealed an indigenous *Mycobacterium* species (blue highlight) – this was the 6th-most abundant bacterial sequence detected in the uninoculated plants. Closer inspection of this sequence reveals that the indigenous *Mycobacterium* was closely related to species known as ethylene-oxidisers. Is this organism involved in ethylene oxidation *in vivo* in the wheat plant?

Table 1. Relative abundance (%) of bacterial 16S rRNA gene sequences in inoculated and control wheat plants.

Control		Inoculated	
Mezorhizobium	63	*Ralstonia*	41
Ralstonia	25	*Mezorhizobium*	39
Acinetobacter	2.9	*Mycobacterium*	7.8
Xanthobacter	2.8	*Xanthobacter*	3.5
Chloroplast	1.5	*Acinetobacter*	2.3
Mycobacterium	0.4	*Acidovorax*	0.8
Variovorax	0.3	Chloroplast	0.8
Acidovorax	0.3	*Pseudomonas*	0.6
Pseudomonas	0.2	*Variovorax*	0.6
Ralstonia	0.2	*Mycobacterium*	0.5

Wipes: assessing risks from bacteria in a shopping centre food court

Our lab was contacted by a union representing cleaners to undertake an investigation into the microbiology of the shopping centre food court. There was concern from the union that the cleaners were under-resourced, based on rumours of poor practices such

as re-using cleaning cloths for excessive lengths of time, or using the same cloths in multiple places (e.g. bathrooms and food court).

We obtained samples of a cleaning cloth used in the food court, and also table-wipes from many individual table surfaces. Our aims were to determine the total bacterial numbers (plate count), to determine if pathogenic bacterial types were present, and to determine if faecal indicator organisms (*E. coli*) were present. These data would allow us to estimate firstly the level of public health risk, and secondly, to see if there was evidence for cross-area usage of cloths between food court and bathroom (for full study details, see Dingsdag[19]).

As part of this study, we wanted to get a sense of the relationship between the bacterial types growing on the agar used (R2A), and the total community in the cleaning cloth – are the isolates grown on agar really representative of the major types present in this environment? This once intractable-question is now easy to answer by doing a 16S PCR on DNA extracted direct from the cloth, and another PCR on DNA extracted from the pooled colonies grown on agar, and pyrosequencing both PCR products (Figure 3).

The majority of bacterial types detected on agar plates were consistent with those detected by direct DNA extraction from the cleaning cloth, although their relative order changed. *Aeromonas* was the exception; this was third-most abundant genus in the cloth, but did not grow at all on R2A. This could be due to inhibition by other faster-growing bacteria, since aeromonads can certainly be cultivated on R2A[20].

The ease of culturability of most bacteria in the cloth could be because this is a eutrophic environment (like culture media), or perhaps this habitat selects stress-resistant types (detergents, heat); these may also resist the stress of isolation on agar.

The most abundant sequences detected in the cleaning cloth were from genera that include human pathogens, such as *Stenotrophomonas*, *Acinetobacter*, and *Aeromonas*. While tag-pyrosequencing (~400 bp sequence) cannot reliably identify bacteria to the species level, the closest sequence matches in many cases (>99%) were to pathogens such as *S. maltophilia*, *Ac. baumannii* and *Ae. hydrophila*. Both coliforms (*Enterobacter, Citrobacter*) and faecal coliforms (*E.coli*) were detected, with *E.coli* at 0.3–0.5% of sequences – this may indicate the use of this cloth in the bathroom, but could also be due to poor hygiene of the general public, who contribute to the bacterial load here.

Limitations of the tag-pyrosequencing approach

Any type of PCR will be limited by the primers used. The 'universal' primers for targeting 16S and 18S rDNA are not identical to all-prokaryote or all-eukaryote ribosomal sequences, respectively, and they cannot amplify all of the sequence types in a complex habitat[21]. Further, different sequence types will be amplified with differing efficacy, if present in a mixed DNA template. The latter effect is marked, and can be demonstrated by PCR and sequencing of defined mixtures of a few dozen ribosomal sequences[22]. Another serious problem with PCRs from mixed templates is the generation of chimeric sequences, which need to be specifically detected and removed[23].

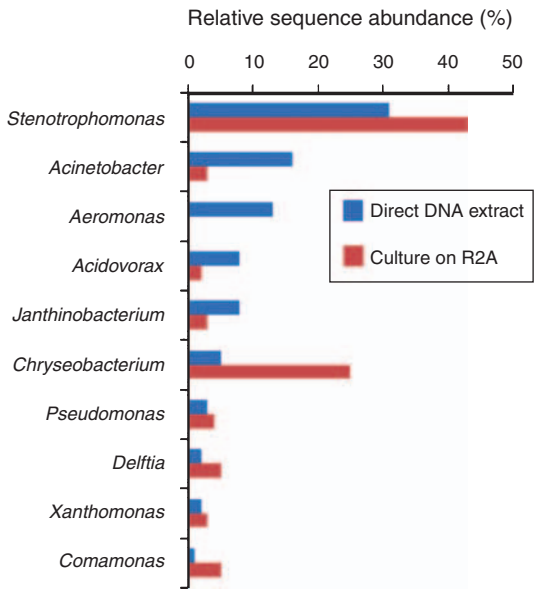


Figure 3. Comparison of culturable vs total bacterial communities in a cleaning cloth, based on 16S rDNA sequence abundance. Sequences are ranked based on abundance in the direct DNA extract (blue bars).

Biases also arise from nucleic acid extraction, since different microbes are lysed with different efficacy. Physical disruption (e.g. bead-beating) is often the method of choice, since it is rapid, and it can lyse both bacteria and eukaryotes, but neither this method or the alternatives are guaranteed to lyse all microbial types, which leads to a bias towards the more easily lysed types in sequence libraries[24,25]. The peculiarities of different environmental matrices (e.g. soils vs. clinical samples) impact strongly on the yield and purity of extracted DNA, and its usefulness for downstream amplification steps[26]. This is a particular challenge for forensic use of tag-pyrosequencing, where legal decisions are made based on sequence data[27].

Pyrosequencing is more error-prone than Sanger sequencing, and the level of errors generated can be sufficient to yield false operational taxonomic units (OTUs) if rigorous sequence quality control is not employed. This means that unique clones in sequence data may be genuine members of the rare biosphere, or they may be sequencing errors[28,29]. Another pyrosequencing artefact is the generation of false clusters of identical or closely related sequences – these are present at up to 35% in some metagenomic datasets[30].

Many traps in tag-pyrosequencing analysis relate to over-interpretation of the data[31] – this could include extrapolating cell numbers from numbers of rDNA sequences (rRNA gene copy number varies in different phyla), postulating physiological functions based on ribosome sequences (most bacteria have highly variable metabolism and physiology), or mistaking statistical correlation s between sequences as causal linkages (an error in logic).

Concluding remarks

Environmental microbiologists have an important role to play in addressing humanity's major challenges in the 21st Century. Our technical ability to attack these problems is more powerful than ever, but our efforts locally are constrained by a lack of funding and a

lack of vision from our large institutions and governments. As a Society, and as individual microbiologists, we need to push harder for recognition of the reality and seriousness of environmental problems, and the importance of microbiologists in solving these problems.

Acknowledgements

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Biographies

Nick Coleman gained a PhD (Microbiology) from USyd (2000), and worked as a postdoc at Tyndall Air Force Base (USA, 2000–2003) and at USyd (2003–2006), then began as a Lecturer at USyd in 2006. Nick is a member of the ASM NSW Branch Committee and the ASM2015 Canberra Local Organising Committee.

Jacob Munro completed his BSc(Adv) at USyd in 2011, graduating with Honours in Microbiology. He is currently studying for a MSc (IT-Bioinformatics) degree at UNSW. Jake's research interests include bioinformatics, biotechnology and microbial ecology.

Deborah Rich studied a BSc(Adv) at USyd, graduating in 2014 with Honours in Microbiology. Deb is starting a PhD in the Coleman lab, studying the interactions of plants and ethylene-oxidising-bacteria, and the potential uses of these bacteria for delaying ripening and spoilage.

Simon Dingsdag did his BSc at USyd, graduating in 2010 with Honours in Microbiology. Simon is currently studying for his PhD (USyd) at the Westmead Centre for Dental Research. His research interests include microbial ecology and oral microbiology.

Microbial diversity and activity in caves

Eric M Adetutu and Andrew S Ball

School of Applied Sciences
RMIT University
PO Box 71
Vic 3083, Australia
Tel: +61 3 9925 7122
Fax: +61 3 9925 7110
Email: eric.adetutu@rmit.edu.au

In recent times, there have been renewed interests in cave ecosystems for both economic and scientific reasons. This is because caves can contain fossils, artifacts, Palaeolithic paintings, ancient markings in form of finger flutings and beautiful speleothems (mineral deposits). These features are attractive and their presence has led to an increase in the number of people visiting caves (tourism) with associated economic benefits to the cave management authorities and the communities in which these caves are located. Unfortunately some of these cave features are susceptible to microbial damage by indigenous and foreign microorganisms, with this risk being exacerbated by unregulated human visitation. Therefore understanding microbial diversity and activities in caves is essential for cave conservation, restoration, safe and sustainable cave tourism.

Why study cave microorganisms?

Different groups of microorganisms such as bacteria, archaea, viruses and fungi are found in caves. However, increased human access (tourism) and cave modifications for tourism purposes (pavements and lighting systems' installation) can alter the natural microbial dynamics, introduce new microorganisms and change the caves' microclimatic conditions[1,2]. These changes can result in extensive damage of cave features such as Palaeolithic paintings and finger flutings over time. High numbers of human visitations can lead to increased health risks to cave visitors and workers via increased microbial load and exposure to opportunistic cave pathogens[3]. Caves can also be sources of novel microorganisms and biomolecules such as enzymes and antibiotics that may be suitable for biotechnological purposes.

Tools for studying cave microorganisms

Different culture dependent and independent methods have been used to study cave microorganisms. Culture dependent methods involve the use of either normal or oligotrophic or specialised culture media. Samples obtained from sediments, walls, atmosphere and other cave surfaces can be plated directly, or from diluents, on oligotrophic media such as 1/100 strength nutrient agar (bacteria) or media such as Potato Dextrose Agar (fungi)[4,5]. Counting, purification and identification of microbial isolates can then be carried out. Direct counting of microorganisms without plating is also possible using microscopic techniques.

Culture independent tools used for cave microbiology (taxonomy and metabolism) include polymerase chain reaction (PCR) based fingerprinting methods (DGGE and T-RFLP), clone library construction, quantitative PCR assays (including those targeting functional genes of interest), sequencing and the use of stable isotope probing methods[4–6]. In recent times, next generation sequencing tools (NGS) on a variety of platforms such as Illumina, SOLiD, Ion Torrent PGM and Roche FLX 454 and associated bioinformatics have been applied to the study of cave microorganisms[7]. NGS Data are of greater depth and higher quality than those obtained with other methods, although database limitations (poorly curated and annotated with regards to cave microorganisms such as fungi) may limit their usefulness.

Microbial diversity of caves

Caves can be terrestrial or aquatic and are usually oligotrophic in nature (nutrient limited) although some may be rich in specific minerals naturally or due to exposure to nutrient-laden sources. Therefore, different caves will have different groups of microorganisms occupying varying ecological niches and alongside cave fauna and environmental factors such as CO_2, temperature and organic matter content, define caves' biotic activities (formation/alteration of cave structures and nutrient cycling) (Figure 1). Microorganisms found in caves can be indigenous to the caves or introduced by humans, animals, water flow and wind action.

Bacteria in caves

Caves contain a broad variety of bacteria belonging to the Proteobacteria, Firmicutes, Actinobacteria and Acidobacteria. Proteobacteria appeared to be the major group detected through the use of PCR based molecular and NGS tools while most isolates from culture dependent assays belonged to Actinobacteria[3,7,8]. In open caves such as show caves, bacteria belonging to different genera such as *Cyanobacter*, *Pseudomonas, Bacillus, Micrococcus, Arthrobacter,*

Staphylococcus and *Mycobacterium* have been identified[4]. Some, like *Cyanobacter* are photoautotrophs found at the cave entrance or around light installations[9]. Others such as *Pseudomonas* and *Bacillus* are heterotrophs, degrading organic matter in the form of insects and animal droppings and extraneous matter. While these heterotrophic activities contribute to the biogeochemical cycle in caves, they can be a disadvantage in caves with Palaeolithic paintings (Figure 2). For example, the growth of bacterial species from genera such as *Aminobacter, Erythrobacter and Norcardioides*[10] on pigments from Palaeolithic paintings and cave walls may damage these paintings over time.

In flooded or underwater caves, many bacterial groups playing different ecological roles have been detected. Bacterial activities in such caves range from organotrophic to chemolithotrophic activities. For example, in aquatic caves such as Nullarbor Caves (Australia) different bacteria genera such as *Pseudomonas, Nitrospira, Cytophaga, Thioalcalovibrio* and *Flavobacterium* have been detected[11]. Some of these microorganisms (*Pseudomonas, Cytophaga* and *Flavobacterium* spp) are organotrophs while others such as *Thioalcalovibrio* and *Nitrospira* spp are chemolithotrophs[11]. Chemosynthesis is especially prevalent in sealed caves with chemotrophs such as methanotrophs, methylotrophs and metal (iron, manganese and sulphur) oxidisers or reducers with species belonging to genera such as *Thiobacillus, Sulfurospirillum, Methylomonas*, *Pantoea* and *Hyphomicrobium* being detected[12].

Fungi in caves

Although cave systems such as terrestrial caves are usually nutrient poor biotopes, they contain different groups of heterotrophic fungi that exist in the form of mycelia or spores. Over 500 genera of fungi, slime moulds and fungus-like taxa have been reported in caves worldwide[13]. These belong to different taxa such as Ascomycota, Basidiomycota, Zygomycota, Mycetozoa, Oomycota and Chytridiomycota[13]. Ascomycota appears to be the most dominant group irrespective of whether culture dependent or independent tools have been used[5,7]. Commonly encountered genera include *Aspergillus, Penicillium, Mucor, Fusarium* and *Cladosporium*. In terms of pathogens, *Histoplasma capsulatum* (causes histoplasmosis in cavers) and *Pseudogymoascus destructans*[14], which was formerly known as *Geomyces destructans* (causes the devastating white nose disease in bats) are famous examples although other opportunistic pathogens such as *Trichosporon* spp. and *Microsporum gypseum (*dermatophytes) are known[3].

Cave fungi such as *Trichurus, Fusarium* and *Cladosporium* can function as decomposers of dead cave insects, fauna, animal, droppings and extraneous organic matter[5,15]. Some fungi such as *Isaria*

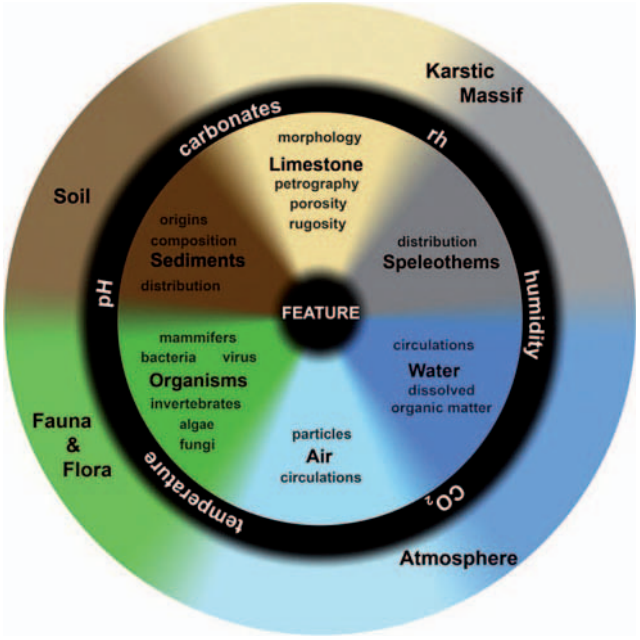

Figure 1. Ecosystem sketch of the evolution of a cave wall. Note that the inner zone refers to the endokarst, the outer zone to the karstic massif and exokarst, and the black circle to the shared parameters[22].

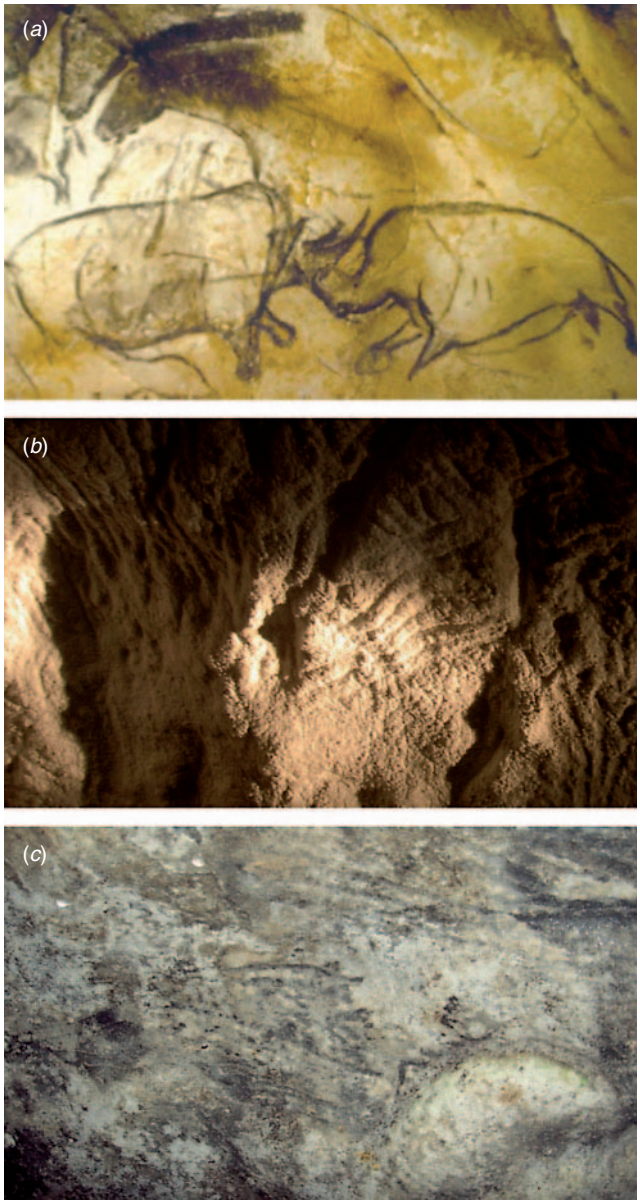

Figure 2. Horse panel from the Hillaire chamber of the Chauvet Cave in Vallon-Pont-d'Arc, France showing a rhinoceros drawn 30,000 years ago (*a*)[23], intact (*b*) and faded finger flutings (*c*) in Australian caves.

farinosa are parasites of cave insects[16] while others are food sources to cave invertebrates and protozoa. Fungi growing on cave surfaces alongside bacteria and archaea may be involved in speleothem formation[13]. Fungal solubilisation of the rocky substrata contributes to the caves' inorganic nutrient pool[17] and this process can severely damage rock art or Palaeolithic paintings. Fungal species such as *Fusarium solani* and *Ochroconis lascauxensis*[18] have being implicated in rock art damage; *F. solani*, colonisation of the famous Lascaux Cave Palaeolithic art being a good example[9,17].

Other cave microbial groups

Archaea are also found in caves (although in lesser numbers) with members of the Euryarchaeota, Crenarchaeota, Thaumarchaeota, Korachaeota and Nanoarchaeota being detected. Either Euryarchaeota or Crenarchaeota appear to be the most dominant phyla in molecular (DGGE and NGS) assay results[19,20]. Some members of the Euryarchaeota and Crenarchaeota groups are heterotrophs while others are thought to be chemolithotrophs involved in the formation of iron and manganese oxides in mineral rich caves[20]. Some members of these groups alongside with bacteria and fungi are also involved in speleothem formation[19].

Viruses are also found in caves and have become important given the recent outbreak of Ebola virus in some parts of the world. Most cave viruses of health concern are borne by bats (as reservoir hosts) from which these viruses can spread to cave visitors (animals and humans). Fruit bats are natural hosts of Marburg viruses (deadly haemorrhagic fever) while some African bats are hosts of the lethal Ebola virus with no known cure. Bat guano is rich in other viruses such as Adenoviruses, Astroviruses and herpesviruses[21].

In conclusion, cave microorganisms are metabolically versatile and are able to acquire energy independently through photo- and chemo-autotrophic activities or through heterotrophic activities. Different microbial groups also interact or work co-operatively in the formation of cave features and as part of the biogeochemical cycle. Understanding these interactions in terms of microbial diversity and function is important for the maintenance of this unique ecosystem especially those that contain features of scientific, archaeological and tourist values. This will allow for sound assessment of the impact of human access on caves and health risks associated with cave visitations and is crucial for sustainable management of cave resources.

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Biographies

Dr Eric Adetutu is a Research Fellow at RMIT, University. He obtained his PhD from the University of Essex, UK. His research interests include microbiology of extreme environments (including caves), rumen microbiology, bioremediation and the application of next generation sequencing tools to the study of pristine and disturbed environments.

The biography for **Professor Ball** is on page 182.

The microbiology of acid sulfate soils and sulfidic sediments

Jacqueline L Stroud

Water Research Centre
School of Civil and Environmental Engineering
University of New South Wales
Sydney, NSW 2052, Australia
Tel: +61 2 9385 5604
Fax: +61 2 9313 8624 469036
Email: jacstroud@gmail.com

Mike Manefield

School of Biotechnology and Biomolecular Sciences
University of New South Wales
Sydney, NSW 2052, Australia

Acid sulfate soils and their associated sulfidic sediments present a major hazard to sustainable farming, water security and urban infrastructure. Traditionally these soils are limed in order to neutralise the 'leachate' that is a public health hazard and toxic to aquatic organisms. It may be more sustainable to exploit the soil microorganisms capable of sequestering metals to remediate these soils. Until recently, little was known about the microbial ecology of these environments. The soils have a moderately acidic (pH 4) chemistry and a unique ecosystem where the microbial community composition is correlated to bioaccessible metal concentrations. These environments have the potential to provide novel insights into how environmental conditions shape the microbiome that can be exploited for biotechnologies.

The formation and management of acid sulfate soils

Worldwide, the pressure for land to meet food security and urban development needs can lead to the disturbance of waterlogged soils containing iron-sulfide minerals. These minerals are oxidised to form acid sulfate soils (Figure 1); a toxic legacy of rain-induced acidic and metalliferous groundwaters that lead to the corrosion of infrastructure and the severe deterioration of water quality. The associated formation of monosulfides in affected waterways is equally problematic, capable of de-oxygenating the aquatic ecosystem in seconds if disturbed.

Current management and treatment practices of acid sulfate soils include minimising the formation of acidity; neutralising acidity and/or turning land back to wetlands. The ongoing cost of

Figure 1. Actual acid sulfate soils and associated sulfidic drain sediments.

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these disturbed environments in Queensland alone is estimated at $180 million per year[1].

Up until recently, little was known about the microbial community in coastal acid sulfate soils. Nonetheless; rates of mineral transformation[2] and mineral transformation products[3,4] cannot be fully explained by abiotic processes, indicating that soil bacteria play an essential, but unknown role in element cycling in this environment. Furthermore, under biotic, redox cycling conditions, the rapid (days to months) transformation of meta-stable minerals to stable minerals, which concomitantly sequesters trace contaminants could be possible[3]. Thus, understanding the microbial ecology, and particularly the identification of bacteria capable of iron-sulphur transformations, is an essential step towards exploiting the microbial community to remediate these environments.

Microbial ecology of acid sulfate soils and sulfidic drain sediments

The microbial ecology was determined using pyrosequencing from samples collected from the model coastal acid sulfate soil site at Blacks Drain, Tweed Valley, NSW, Australia. The sampling strategy is fully detailed elsewhere[5]. This site is considered to be a model setting as it is a pollution hotspot (contaminating adjacent waterways with acidity, Al, Mn and Fe) has undergone detailed geochemical characterisations of the soils and sediments over the past decade[5].

The acid sulfate soils and sulfidic drain sediments contained an average 186 phylotypes per sample in comparison to non-pyritic soils, which contain 5-fold higher ecosystem complexity, with an average 1,017 phylotypes per sample[6]. Ecosystem complexity is controlled by selection pressures that reduce species diversity[7]. Acid sulfate soils contain a number of selection pressures associated with the seasonal oxidation of the unstable iron minerals; however, in comparison to acid mine drainage environments (pH 2–4) that contain an average of 61 phylotypes per sample[8], these soils have a 3-fold higher ecosystem complexity.

A total of 23 phyla were identified, of which five phyla dominate (>90%) the community composition, with four common to all environments (*Proteobacteria*, *Acidobacteria*, *Firmicutes* and *Chloroflexi*), and *Bacteriodetes* as a major component of sulfidic sediments and *Actinobacteria* a major component of acid sulfate soil field horizons.

The subdivision of the phyla totalled 48 classes. Focusing on the dominant phyla, drain sediments had two-fold higher abundance of *Proteobacteria*; and were dominated by δ-*proteobacteria*. All soil horizons contained δ-*proteobacteria* but were dominated by *β-proteobacteria* and α-proteobacteria. Splitting the phylum *Chloroflexi* into classes revealed that drain sediments were dominated by *Anaerolineae* in comparison to the soils that were dominated by the *Chloroflexi* class. The phylum *Acidobacteria*, also showed that the drain sediments were dominated by the *Acidobacteria class* and soil horizons dominated by *Holophagae*. The phylum *Firmicutes* was dominated by the classes *Bacilli* and *Clostridia* across both settings.

Interestingly, there was a low abundance of the class *Nitrospira,* a group that contains acidophilic Fe(II) oxidising bacteria (*Leptospirillum* sp.). This is the most important phylotype in acid mine biofilms[7]; is moderately abundant (average 12%) in acid mine drainage sites[8], but has a low abundance in acid sulfate soils at <3%. The abundance of this group may be determined by pH due to their limited metabolic capacity (derive energy solely from Fe(II) oxidation) thus are adapted to extremely acidic environments (pH <4)[8] where competing abiotic Fe(II) oxidation kinetics are very slow.

Iron and sulphur cycling bacteria in acid sulfate soils

A patchy species distribution and a high proportion of unclassified bacteria characterised acid sulfate soils. Interestingly, these soils lacked an abundance of known acid tolerant Fe(II) oxidisers, of which there are 22 known acidophilic Fe(II) oxidising species across four phyla[9]. Only the *Firmicutes*, *Alicyclobacillus tolerans* was detected in these soils. Instead, the microbial community associated with Fe(II) oxidation was the *Betaproteobacteria, Sideroxydans lithotrophicus* and *Sideroxydans paludicola*, with a known ability for Fe(II) oxidation over neutrophilic pH range (4–7.5) under micro-aerobic conditions. Another interesting finding was that *Chloroflexus* was highly abundant in the acid sulfate soil horizons. Little is known about the role of *Chloroflexus* in iron cycling, but research from microbial mat zones indicate a positive relationship to zones of enriched Fe(II) oxidation[10] and the fully sequenced *Chloroflexus aurantiacus* contains a candidate Fe(II) trafficking protein (ATCC strain 29364).

Iron reduction is a ubiquitous microbiological mechanism[11]; but only the neutrophile *Anaeromyxobacter dehalogenans* was abundant in the acid sulfate soil horizon. Substrate based studies offer the potential to discover the largely unknown Fe(III) reducing the community in these soils as carbon substrates differ between acidophiles and neutrophiles. For example, acetate is key to *Geobacter* sp.[12]; however, acetate inhibits Fe(III) reduction in acidic conditions and sugars are a key substrate that stimulates Fe(III) reduction by acidophiles[13].

Few S-cycling bacteria were found in the acid sulfate soils; furthermore, considering there are 220 bacterial species known to be involved in sulfate reduction[14], it was surprising that no sulfate-reducing species were identified at this taxonomic level. This suggests that there is a significant repository of unknown S-cycling bacteria present in these environments.

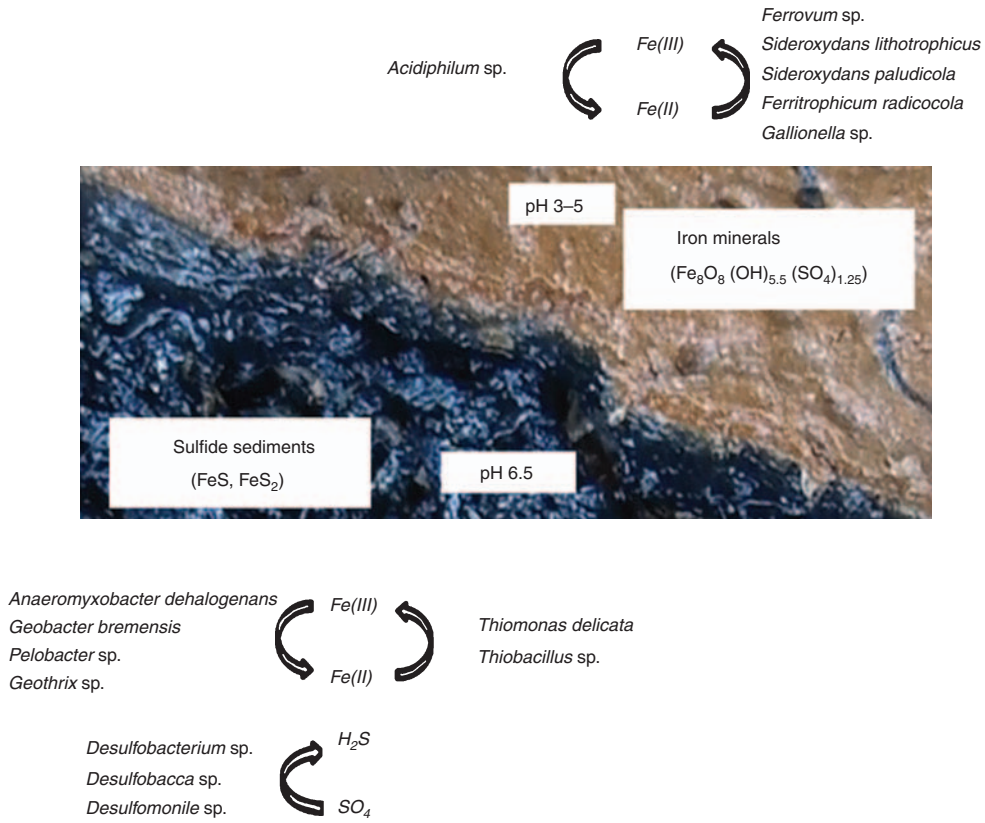

Figure 2. Biogeochemistry of sulfidic drain sediments impacted by acid sulfate soil leachates.

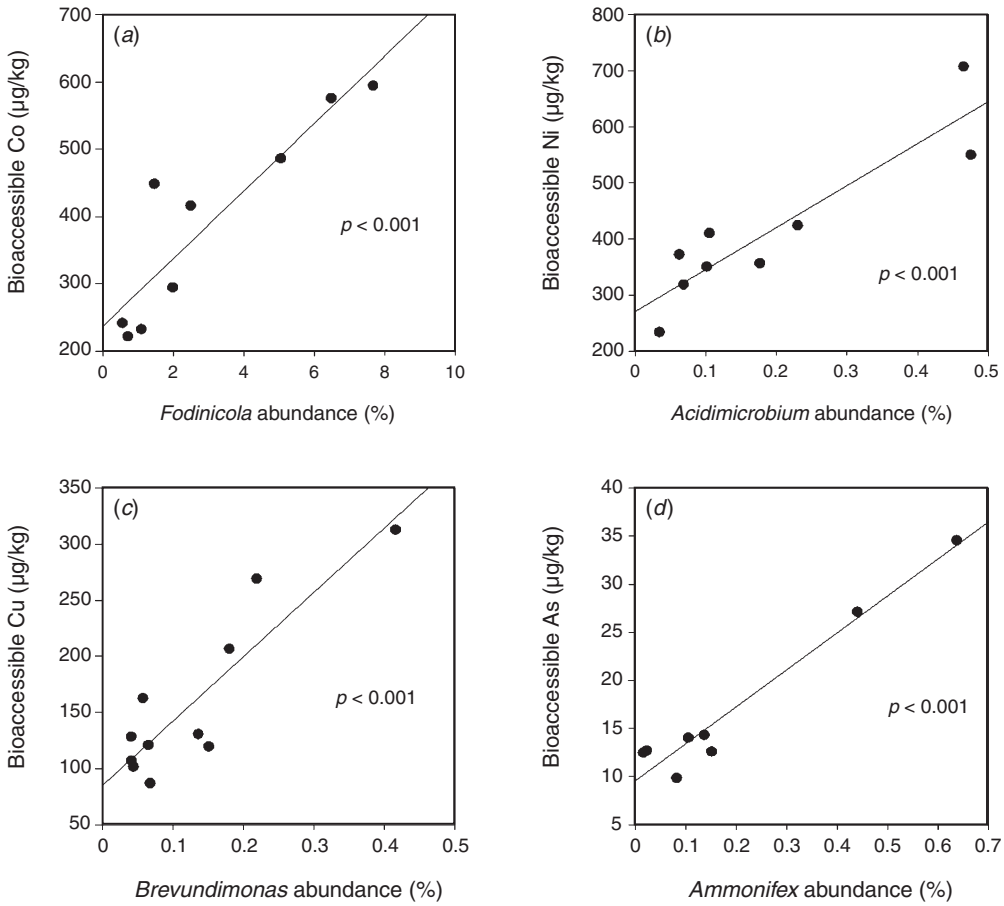

Figure 3. Correlations between genera abundance and bioaccessible metal(loid)s including (*a*) cobalt, (*b*) nickel, (*c*) copper and (*d*) arsenic.

Iron and sulphur cycling bacteria in sulfidic sediments

Unlike the acid sulfate soils, the sulfidic sediments were reasonably well resolved and a conceptual model of microbially mediated iron cycling and sulfate reduction in the sulfidic drain sediments was developed (Figure 2).

The sulfidic drain sediments contained an abundance of Fe(II) oxidising bacteria, for example, *Ferrovum.* sp; that oxidises Fe(II) aerobically using CO_2. Further, there was an abundance of microbes with capabilities to reduce Fe(III) in the sulfidic sediments which included the acidophile *Acidobacterium capsulatum* that reduces Fe(III) between pH 2–5, and the neutrophiles *Anaeromyxobacter dehalogenans* and *Geobacter* sp. The sulfidic drain sediments contained an abundance of S-compound oxidising bacteria, dominated by *Thiomonas delicata*. However, similar to the soils, no sulfate reducing species were detected, suggesting that there are many unknown S-cycling bacteria in these environments.

Metal bioaccessibility is correlated to genera abundances

Acid sulfate soil and sulfidic sediments contain highly elevated bioavailable metal(loid) concentrations that are taken up by plants and can be used as biomonitors to isolate pollution hotspots[15]. A suite of metal(loid)s (Al, As, Cr, Co, Cu, Fe, Mn, Ni and Zn) were positively correlated to genera abundance (Figure 3), indicating that generally genera in acid sulfate environments have high tolerance capabilities to high metal(loid) bioaccessibility, which shape microbial community composition.

A key finding was the abundance characteristics of the *Acidobacteria* and *Crenarcheota* to bioaccessible Manganese concentrations. Recently, these phyla were found to be enriched in Mn-stimulated microcosms and linked to Mn-dependent organic compound oxidation[16]. Thus, this demonstrates the need for high phylogenetic resolution to investigate environmental factors; because only genus level resolution identified this relationship to soil chemistry.

Conclusion

Understanding the biogeochemistry of acid sulfate soils and sulfidic sediments would transform the management and remediation of these environmentally deleterious sites. The microbial ecology has few parallels to geochemically similar environments, with a microbial community composition including both acidophiles and neutrophiles associated with iron and sulphur cycling. Furthermore, acid sulfate soils and sulfidic sediments house a repository of uncharacterised microbes but abundance patterns correlated to soil chemistry. Taken together, acid sulfate soils may be a model environment that can be used to understand the role environmental conditions play on microbial community compositions, and this information could be used to develop novel biotechnologies.

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Biographies

Jackie Stroud was a post-doc at UNSW. Her research interests are environmental soil science and its applications to sustainable agriculture and bioremediation.

Mike Manefield is an Associate Professor at UNSW. His interests are in environmental microbiology and biotechnology development.

Bioavailability and biodegradation of polycyclic aromatic hydrocarbons

Albert L Juhasz

Centre for Environmental Risk Assessment and Remediation (CERAR)
University of South Australia and Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE)
Mawson Lakes, Adelaide
SA 5095, Australia
Tel: +61 8 8302 5045
Fax: +61 8 8302 3057
Email: Albert.Juhasz@unisa.edu.au

Contaminant bioavailability plays an influential role in the efficacy of polycyclic aromatic hydrocarbon biodegradation.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants arising from the incomplete combustion of organic material. Both anthropogenic (e.g. processing and combustion of fossil fuels, waste incineration) and geogenic processes (e.g. fires, volcanoes) contribute to the burden of PAHs in environmental matrices[1]. The concern regarding the presence of PAHs in the environment and their potential to exert toxic, mutagenic and carcinogenic effects[2] has led to the development of a number of physical, chemical and biological techniques for the remediation of PAH-contaminated soil. Bioremediation is considered a 'green' technology for the remediation of PAH-contaminated soil; however, its efficacy is dependent on a number of variables including the presence and activity of PAH degrading microorganisms (e.g. genera of Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas, Stenotrophomonas), physico-chemical properties of the PAHs (which will influence their biodegradability) and environmental parameters including the availability of essential nutrients and oxygen, soil pH, moisture and temperature[3]. Another parameter that is influential for bioremediation success is PAH bioavailability. In the context of PAH biodegradation, bioavailability refers to the fraction of the total soil-bound PAH concentration that is desorbable from the soil matrix and is therefore potentially available for biodegradation[4].

Following their entry into the soil environment, PAHs may diffuse and be occluded in soil micropores[4], which limits their propensity for desorption and therefore their bioavailability for biodegradation. However, the predominant mechanism for PAH retention within the soil matrix, and the reduction in PAH bioavailability, is via sorption to organic carbon (OC)[5]. PAH retention is influenced by the nature of the OC (i.e. amorphous or rubbery versus condensed or glassy) in addition to the octanol-water partitioning co-efficient (K_{ow}) of the PAH. The interaction between PAHs and amorphous domains constitutes a rapid sorption phase but is susceptible to rapid desorption while association with condensed OC results in slow sorption-desorption behaviour[5]. As suggested by Semple *et al.*[4], PAH bioavailability may be estimated using methodologies that quantify the rapidly and slowly desorbable domains (e.g. non-exhaustive extraction methods) as these fractions represent PAHs that have the potential to partition into soil solution and be available for biodegradation (Figure 1).

A non-exhaustive extraction method, utilising hydroxypropyl-β-cyclodextrin (HP-β-CD), has been shown to provide an estimate of the desorbable PAH fraction from contaminated soil that may therefore be available for biodegradation[6–9]. The methodology, albeit simplistic, involves extraction of PAH-contaminated soil with HP-β-CD (40 mM) at a soil:solution ratio of 1:20 for 20 h. Following extraction, soils are retrieved via centrifugation, dried and the residual PAH concentration determined following 'exhaustive' extraction and GC/HPLC analysis. The desorbable fraction is calculated by the difference between the initial PAH concentration and the residual PAH concentration following HP-β-CD extraction. A number of studies have shown the correlation between PAH bioavailability estimates, utilising HP-β-CD extraction, and PAH biodegradation/mineralisation, utilising soil microcosms[6–9]. This suggests that non-exhaustive extraction methods, such as HP-β-CD, may be utilised to predict the endpoints of PAH biodegradation based on bioavailability.

The slope of the bioavailability-biodegradability relationship may, however, vary for individual PAHs due to differences in their physico-chemical properties which will influence desorption and biodegradability (Figure 2). For example, a number of researchers have determined that the slope of the bioavailability-biodegradability relationship for three-ring PAHs is close to 1 (0.85–1.01), indicating the similarity of HP-β-CD extraction to remove low molecular weight PAHs to the same extent as biodegradation. However, for five-ring compounds, the slope of the bioavailability-biodegradability relationship may range up to 1.60 indicating that larger residual PAH fractions remain in the soil following biodegradation compared with HP-β-CD extraction. Although a disparity exists between PAH bioavailability and biodegradability, as a consequence high molecular weight (HMW) PAH biodegradation limitations (i.e. high activation energy, unfavourable Gibbs free energy and slow transport across cell membranes), the linearity of the bioavailability-biodegradability relationship suggests that it may be utilised for predicting the extent of HMW PAH biodegradation. As a consequence, the assessment of PAH bioavailability (using HP-β-CD extraction) and the prediction of PAH bioremediation efficacy, has the potential to be used to screen the appropriateness of bioremediation strategies prior to on-site implementation. This would benefit industry by

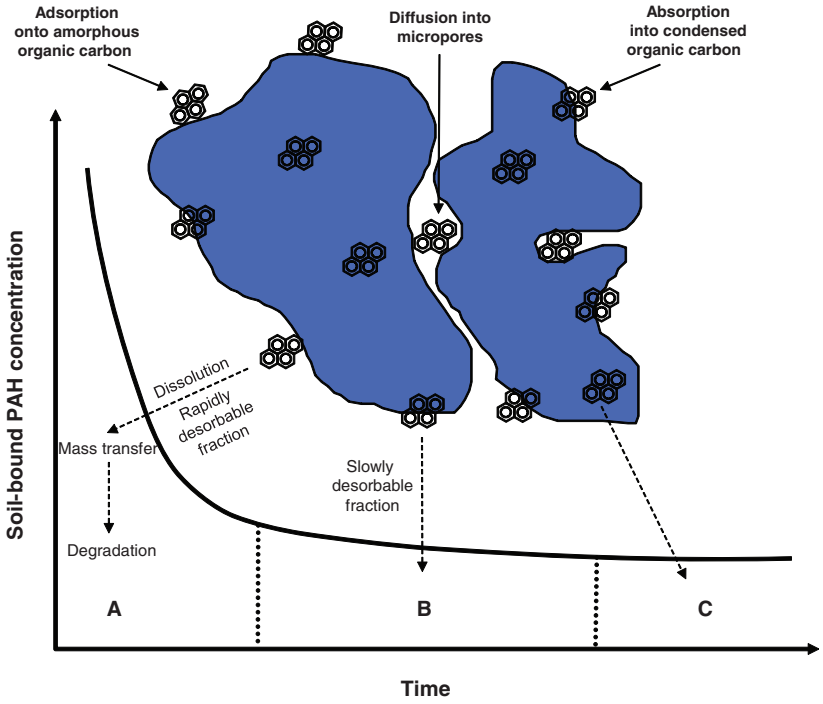

Figure 1. Schematic diagram detailing the relationship between the rates of PAH desorption and biodegradation. Phase A represents rapid PAH biodegradation where PAH removal is limited by microbial degradation kinetics and not the desorption of PAHs from the soil matrix (i.e. the rapidly desorbable PAH fraction from amorphous organic carbon). In phase, B, the rate of PAH biodegradation is limited by the slow desorption of PAHs from the soil matrix (i.e. PAH desorption from condensed organic carbon) while Phase C represents the sequestered or unavailable PAH fraction.

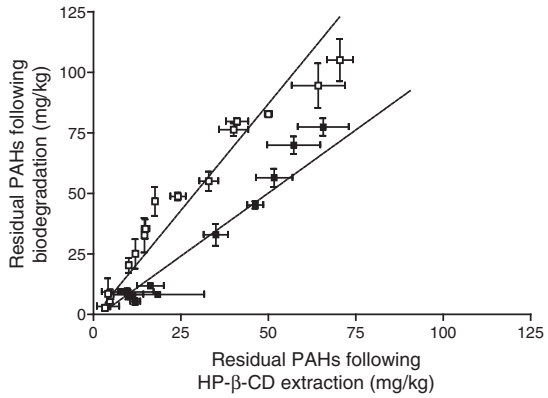

Figure 2. Relationship between the residual phenanthrene (■) and benzo[a]pyrene (□) concentration following 3 months of enhanced natural attenuation of PAH-contaminated soil ($n = 15$) and the residual concentration following HP-β-CD extraction.

providing a simple, rapid and inexpensive assay for determining the endpoints of PAH bioremediation.

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Biography

Albert Juhasz is an Associate Professor at the Centre for Environmental Risk Assessment and Remediation, University of South Australia. His research interests include the development of remediation strategies for contaminated soils and the assessment of contaminant bioavailability and bioaccessibility for refining human health exposure assessment.

The microbiology of microbial electrolysis cells

Lucie Semenec and Ashley E Franks

Department of Microbiology – Environmental Microbiology
Faculty of Science, Technology and Engineering
La Trobe University
Melbourne, Vic. 3086, Australia
Tel: +61 3 9479 2206
Email: a.franks@latrobe.edu.au

Electromicrobiology is a new discipline that investigates the ability of microbial species to interact with insoluble external electron acceptors and donors. This ability has most commonly been studied through microbial communities found in association with electrodes as part of a microbial electrolysis cell (MEC). MECs are devices that employ bacteria capable of utilising either an anode as an electron acceptor or a cathode as an electron donor to carry out biologically driven processes. In effect, these devices make use of microbes that are eating and breathing electricity. Potential applications for MECs are ever expanding and currently include bioremediation, biosensing, biofuel production and power generation. MECs that produce overall net power are referred to as microbial fuel cells (MFCs) and have helped to generate much of our initial knowledge regarding electroactive bacteria. Energy consuming MECs have more recently expanded our knowledge on microbial electrosynthesis pathways, whereby microbes reduce CO_2 using electrons provided by an electrode. Furthering of our knowledge on electrode-associated microbes has in turn led us to an increased understanding of how microbes in the environment have been developing, powering and utilising their own electricity grids all along. These electrical interactions, between microbes and components of their living and non-living environment, are potentially very important but have been overlooked until very recently.

> *nota notae est nota rei ipsius – in as much as chemical change being a sign of life, and electrical change a sign of chemical change, it follows that electrical change is a sign of life*. Waller[1]

Electron transfer from microbes to electrodes

The electrical nature of living organisms was eloquently summarised in lectures by Augustus Waller in 1903[1] and the ability to use an anode to detect an electrical current in a microbial culture during the decomposition of organic compounds was demonstrated by Potter in 1911[2]. However, it was not until half a century later that this knowledge was implemented into the first reported studies using a MFC. In the past 10 years research into electric bacteria has exponentially expanded.

Defining microbial fuel cells

A MFC is typically a two-chambered system containing an anaerobic anode chamber and an oxic cathode chamber, separated by an ion permeable membrane, and is capable of utilising electrons from microbial central metabolism for a net energy gain. Electric microbes in the anode chamber utilise the anode as a final electron acceptor for the anaerobic respiration of organic electron donors such as acetate. Electrons donated to the anode flow to the cathode through electrical wires, where they are reunited with the protons generated in the anode chamber and combine with oxygen or other electron acceptors to form reduced products. The reported anodic power density has increased from initial power outputs of 0.1 W/m^2 to more recent reports of 6.9 W/m^2 of anode surface area. Despite the many improvements made to MFC electrical current production, these systems do not yet produce enough power for commercially viable large-scale power production applications, but are able to reduce the energy demands of wastewater treatment as well as provide small scale power outputs to power remote sensing devices. Many physical, chemical and biological discoveries remain

to be unveiled in order to make the power generation application of MFCs more feasible.

Electricigens: anode respiring bacteria

The anode-associated microbial communities are dominated by electricigens, microbes capable of completely oxidising organic carbon while utilising the anode as the final electron acceptor. While research had been initially built on findings from studies based on dissimilatory metal reduction pathways of metals like Fe(III) and Mn(IV), it was found that dissimilatory metal reduction was not always indicative of anode reduction and vice versa. Mixed communities often form in association with an anode when complex organic matter is used as an energy source, microbial fermentation first reduces the compounds to simple carbohydrates such as acetate and then the fermentation end products serve as electron donors for the anode respiring microbial community members, typically found in an anode-associated biofilm.

The dominance of the *Geobacteraceae* in anode-associated communities was first reported in studies of sediment MFCs. Sediment MFCs utilise aquatic sediments for inocula, carbon sources and as a proton exchange medium. Through 16S ribosomal DNA (rDNA) analysis, it was found that bacteria from the family *Geobacteraceae* were enriched at the anode, as compared to a control, along with several other Delta-proteobacteria. It has been shown that *Geobacteraceae* dominate anode-associated biofilms from a wide range of environmental inocula such as sewage waste and rice paddy soil[3].

The number of known electricigens has been increasing and includes species of *Geobacter*, *Shewanella, Rhodoferax*, *Pseudomonas, Geothrix*, *Ochrobactrum, Clostridium*, *Desulfuromonas*, *Aeromonas*, *Desulfobulbus*, *Geopsychrobacter*, *Escherichia*, *Rhodopseudomonas*, *Desulfovibrio*, *Acidiphilium*, *Klebsiella*, *Thermincola*, and *Pichia*. Of these microbes, *Geobacter sulfurreducens* and *Shewanella oneidensis* are the most extensively studied in terms of their mechanisms of extracellular electron transfer (EET) to insoluble electron acceptors.

Electrotrophs: cathode-associated microorganisms

Microorganisms can also utilise the cathode as an electron donor in a MEC, in effect consuming electrical current as an energy source. This process requires an input of current, as the electrode often needs to be held electronically at a specific potential to make the redox reactions favourable[4]. Microorganisms that receive electrons directly from electrodes are referred to as electrotrophs and, if carbon dioxide is fixed for organic synthesis, the process is known as electrosynthesis; named due to similarities to photosynthesis[5]. Cathodes were initially demonstrated to act as electron donors for microbial metabolism through pure culture studies with *Geobacter* spp. Only a limited number of known electrotrophs that are capable of utilising a cathode in pure culture have been described thus far, including *Sporomusa ovata*, *S. sphaeroides*, *Morella thermoacetica*, *Clostridium ljungdahlii*, *C. aceticum, G. metallireducens* and *G. sulfurreducens*[5]. In pure cultures, the cathode-associated biofilms have been found to be only sparse or single layer biofilms. However, cathodes in the environment will often attract a mixed community of electrotrophic bacteria with the capability of improving bioremediation, biosensing and biosynthesis. It has also recently been demonstrated that community population dynamics, and hence electrosynthetic outcomes, can change in response to current supply fluctuations to the cathode. These results highlight the importance of understanding interspecies microbial interactions within MECs to better predict and control products of electrosynthesis under varying environmental conditions especially when the production of specific organic compounds is desired.

Electrotrophic bacteria such as *Geobacter* play an important role in cathode-assisted bioremediation. Energy from the cathode enables the reduction of nitrates, chlorinated solvents and soluble U(VI) to insoluble U(IV) in the subsurface. When compared with common biomass strategies, the use of renewable energy sources like sunlight for the production of valuable commercial synthetic compounds and transportation fuels such as acetone and butanol has large potential efficient gains without consuming land available for food production.

Unlike the thick biofilms that form on anode surfaces, only sparse or single layer biofilms are usually observed on cathode surfaces in pure cultures. Investigations into relatively thick naturally occurring cathode-associated mixed species biofilms may lead to new insights and improvements. Several avenues of research are now investigating mechanisms by which the cathode-associated biomass and production rates can be increased.

Extracellular electron transfer mechanisms

Electrogens are a more general term for electricigens and encompass all microbes that can interact in a community via extracellular electron transport using any suitable extracellular electron acceptor; whereas electricigens are microbes that specifically use an electrode as the final electron acceptor for extracellular electron transport. Initial studies of electrogenic bacteria focused mainly on *Geobacter* and *Shewanella* species. These species had been extensively studied in relation to iron reduction and were known to have different EET mechanisms (Table 1). Currently there are three known

Table 1. Summary of electron transport mechanisms in known electrogens and endogenous proteins and electron shuttles involved.

Microorganism	Anode	Cathode	Fe(III) oxide	Fe(III) citrate	Other bacteria
G. sulfurreducens	DEET[6], OmcZ[7] and PEET[A,7,8], PilA[7]	DEET[9]	PEET[10], PilA[10], OmcS[11], OmcE[11], OmcB[B,12]	DEET, OmcS[11], OmcE[11]	DIET[13]
G. metallireducens	DEET[6]	DEET[9]	PEET[14,15] PilA[14,15], FliC[15]	PilA[14]	DIET[13,16]
S. oneidensis	SEET[17,18], flavin[18], MtrC[17], OmcA[17]	N/A	SEET[19,20], flavin[20], quinone[20], MtrC[19], OmcA[19]	DEET[21], MtrA and MtrB[21], flavin[20]	N/A
Pseudomonas spp.	SEET[22], pyocyanin[14]	N/A	SEET[23], phenazine[C,23]	N/A	N/A
Clostridium spp.	DEET[24]	SEET[25]	N/A	N/A	N/A

[A]It is not yet known whether conductive pili on outer surface cells of biofilms transfer electrons directly to anodes or whether they transfer electrons to cells closer to the anode surface, which subsequently reduce the anode by DEET.
[B]OmcB is not required but deletion of it greatly impairs reduction so it plays a major role.
[C]Fe(III) hydroxide reduction.
NOTE: blue font represents proteins and mediators that are essential and red font represents proteins and mediators that are not essential.
EET, extracellular electron transport; SEET, electron shuttle mediated EET; DEET, direct EET; DIET, direct interspecies EET; PEET, pilin mediated EET.

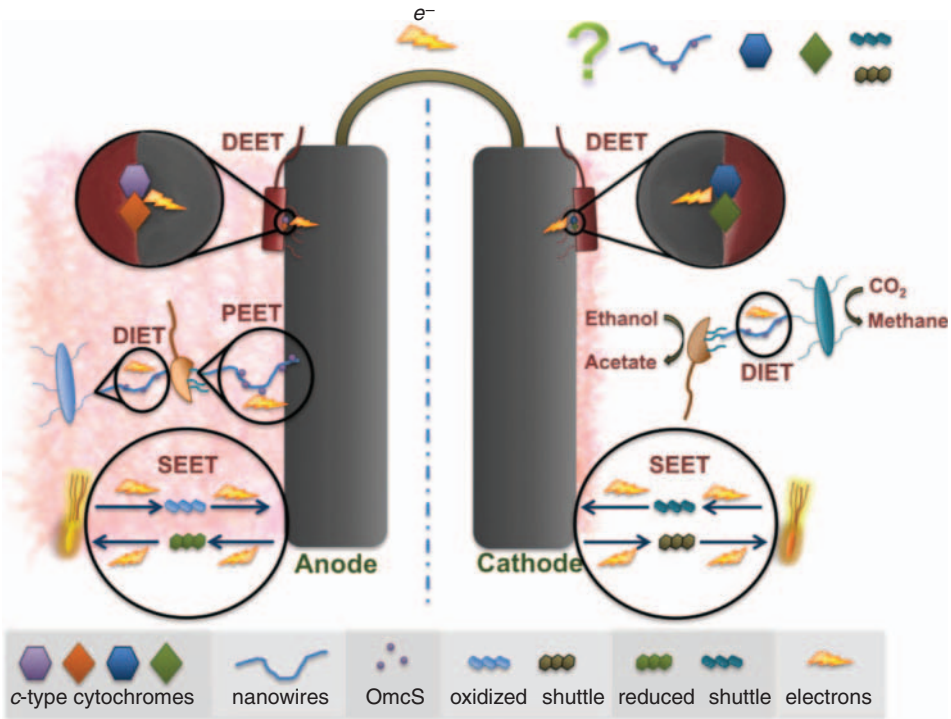

Figure 1. Schematic representation of extracellular electron transport (EET) mechanisms including electron shuttle mediated EET (SEET), direct EET (DEET), direct interspecies EET (DIET) and pilin mediated EET (PEET) at the anode. The mechanisms of EET at the cathode are not yet determined but may be similar to SEET, DEET, DIET and PEET at the anode. Biofilm formation, depicted as a pink layer on the electrodes, is thicker on the anode than biofilm formation on the cathode, which is often limited to a single cell layer biofilm in pure cultures.

mechanisms of EET that were recently reviewed in great detail[26]: (1) electron shuttle mediated EET (SEET); (2) direct EET (DEET); and (3) pilin mediated EET (PEET). These mechanisms are discussed below in relation to anode reduction (Figure 1).

(1) Electron shuttle mediated EET

SEET utilises extracellular electron shuttles to act as a carrier between the cell and the electrode surface. Shuttles may be biotic or abiotic compounds. If available in the environment, many

microbial species are capable of utilising exogenous electron shuttles but not all microbes produce them. *S. oneidensis* has been studied extensively due to flavin production that cycles electrons from the outer membrane cytochrome MtrC[19]. Likewise, pyocyanin production by *Pseudomonas aeruginosa* enables electrode interaction[22]. Abiotic shuttles include humic substances and sulfur compounds amongst others. The use of solid-phase humic substances as electron shuttles to reduce Fe(III) oxide has been demonstrated to occur in *G. sulfurreducens* and *Shewanella putrefaciens* despite the ability of these organisms to reduce Fe(III) oxide via other EET mechanisms (Table 1).

(2) Direct EET

EET is the direct microbial transfer of electrons to an electrode without the use of an electron shuttle and commonly utilises outer membrane cytochromes. This typically occurs over short distances and requires cells to be in close proximity to the electrode. *G. sulfurreducens* is able to reduce an electrode in pure culture even though it does not produce an electron shuttle but instead utilises outer membrane *c*-type cytochromes for DEET. The genome of *G. sulfurreducens* encodes for more than 100 *c*-type cytochromes, which may help explain this microorganism's versatility in MECs. During electrode reduction OmcZ has been shown to be essential for thick biofilm formation and it is likely that OmcS plays some role as well[7]. Studies of insoluble Fe(III) oxide reduction found differences in the electron pathways as compared to those associated with the anode, with OmcS, which is localised on pili, to be essential and OmcE to be also important for DEET to insoluble Fe (III) oxide[11]. The Mtr cytochrome system of *Shewanella* likewise transfers electrons from central metabolism to the electrode surface. Interestingly the Mtr system is reversible and capable of accepting electrons from the electrode surface where the systems in *Geobacter* are not. OmcS and OmcZ do not affect electrode oxidation whereas GSU3274, a heme containing cytochrome does[27]. Furthermore, direct electron transfer differs when the extracellular electron acceptor is soluble. For instance, OmcS and pili are not required for reduction of Fe(III) citrate by *Geobacter* whereas OmcZ is[11,28].

(3) Pilin mediated EET

G. sulfurreducens is capable of producing a multicellular thick biofilm (>50 µm) on anode surfaces that is dependent on the expression of pili[7,8,29]. The entire biofilm is metabolically active and contributes to power production and, unusual for a biofilm, is transcriptionally homologous throughout[8,30]. The PilA mutant of *G. sulfurreducens*, defective in pilin production, is incapable of insoluble Fe(III) oxide reduction but is still able to reduce soluble Fe(III) citrate[10]. This mutant also does not produce the characteristically thick anode-associated biofilms. The results of recent studies on *Geobacter* strongly suggest that electrons do not travel along the pili by the conventional mechanism of electron hopping between redox proteins. Instead, the movement of electrons along the pili appears to be occurring via metallic-like conduction through the pili outer surface via specific amino acid side chains[31]. In contrast to *Geobacter* nanowires, it has been recently demonstrated that the molecular composition of *S. oneidensis* nanowires are actually protrusions of outer membrane and periplasm. They are thus comprised of membrane material, including cytochromes involved in EET rather than being pilin based like those found in *Geobacter*. These differences in long-range EET further highlight the importance of understanding the different EET mechanisms between various electrogens and the electrode. With knowledge gained in this field, more tools will become apparent on how to increase the efficiency of EET and hence increase the bioremediative and energy production capacity of MFCs.

Community dynamics of electrogenic biofilms

It is now becoming apparent the microbes in the environment can directly transfer electrons to each other within an electrically conductive biofilm. Most practical applications of MECs involve a mixed microbial community from an environmental inoculum that is capable of interactions via extracellular electron transport. Although various studies on mixed species biofilms have been performed, the structural organisation and electron transfer mechanisms of mixed species electrogenic biofilms still needs further study. Some predictions however can be made based on the existing studies (Figure 2).

Depending on the environmental conditions, the interactions between electrogens may be either syntrophic or competitive. Syntrophic aggregates of *G. metallireducens* and *G. sulfurreducens*, utilising ethanol as an electron donor and fumarate as an electron acceptor, have been shown to exchange electrons through an electrically conductive network comprised of pilin and OmcS[13]. This interaction is known as direct interspecies electron transfer (DIET). Conversely, in environments where both microorganisms can oxidise the same electron donors for respiration, competition for this electron source ensues.

Microbial culturing studies from aquatic sediments have revealed that electrodes selectively enrich for bacteria from the *Geobacteraceae*. Despite the competitive advantage that *Geobacter* hold in these conditions, it has been demonstrated that having a mixed species biofilm on the anode also produces high electrical current

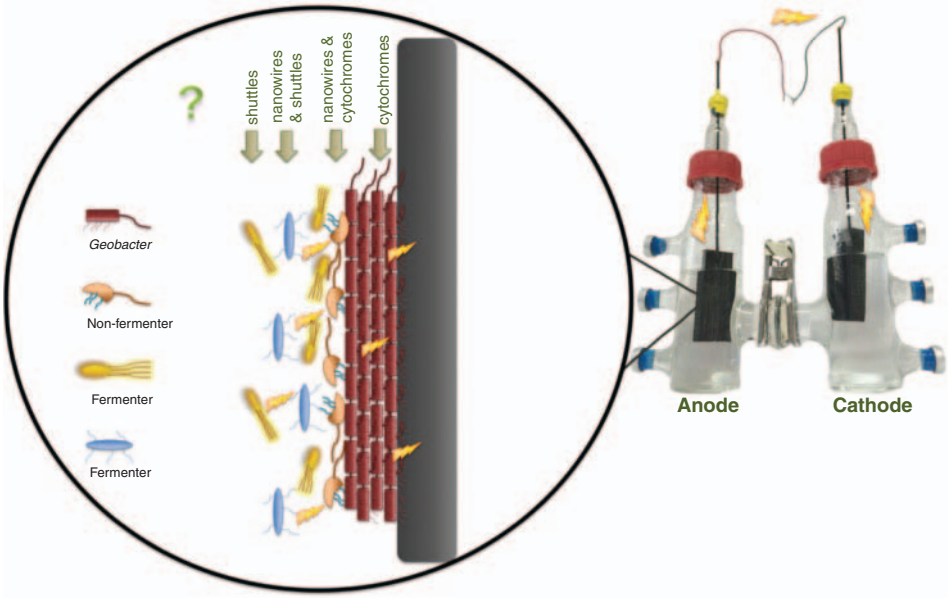

Figure 2. Potential model for mixed species electrogenic biofilm. *Geobacter* predominate near surface of anode while non-fermentative bacteria form layer above *Geobacter*. Conduction through inner biofilm layer involves nanowires, electron shuttles and cytochromes. Fermentative bacteria should prevail at the outer surface of the biofilm, where the mechanism of electron transport to the inside of the biofilm is likely through electron shuttle mediated mechanisms.

and efficient oxidation of the electron donor. It is important to understand the community dynamics that occur in these mixed species biofilms in order to optimise their functionality for future applications.

Therefore, by introducing other species that can interact directly with the electric *Geobacter* biofilm, it should be possible to increase biofilm thickness and thus electrical current production. However, increasing biofilm thickness does not come without its limitations such as accumulation of protons leading to lower pH, build up of metabolic waste products and reduced penetration of substrate into the biofilm.

An understanding of the electric community also has benefits in other systems. Direct interspecies electron transfer in anaerobic granules used in anaerobic digesters has been improved through the addition of electron conductive material such as biochar or granulated carbon[32]. An understanding for the ability of bacteria to utilise electrons in syntrophic interactions will have many potential applications in the future[32].

Conclusion

Although MEC technology has seen the proposal of a wide range of technological applications, real world applications have only begun to appear recently. The MudWatt™ is a small sediment MFC that powers an LED and has increased interest in electric microbiology through its educational applications. The US navy has developed benthic unattended generators (BUGs) to power remote sensing devices using MEC technology and the efficiency of upflow anaerobic sludge blankets (UASBs) has also been improved through the application of lessons learnt from MECs. The first industrial application of MECs has been the EcoVolt®, developed by Cambrian Innovation to improve treatment of large-scale wastewater treatment while producing methane 'biogas'. Various applications of MECs are also being actively pursued by NASA for the powering of space robots, wastewater treatment in space and air revitalisation systems. Australia has seen a large-scale pilot MEC at Foster's brewery in Queensland to determine if brewery wastewater could be more sustainably treated. Further long term plans are emerging for low power applications such as using saliva powered micro-MFCs for portable point-of-care diagnostics or Lab-on-a-Chip devices.

Many refinements have been made to improve the physical engineering of MFCs by experimenting with different electrode materials and mechanical and structural orientations. However, learning more about the biological phenomena of MECs and electrogens deserves special attention to maximally optimise the capabilities of these systems in various applications. Insights gained through the application and study in these systems is also providing information on the microbial ecology of electrogenic bacteria and their processes in the environment.

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Biographies

Lucie Semenec is a PhD candidate at Dr Ashley Franks laboratory in the Faculty of Science, Technology and Engineering at La Trobe University. Her research focuses on the community dynamics of electrogenic biofilms and the underlying mechanisms of syntrophic and competitive microbial interactions.

Ashley Edwin Franks is a senior lecturer in the Department of Microbiology at La Trobe University and head of the Applied and Environmental Microbiology research laboratory. He conducted his doctorate research as part of the Centre of Marine Biofouling and Bioinnovation at the University of New South Wales by investigating antifungal compounds produced by marine bacteria in biofilms. During his PhD he spent 4 months at the University of Exeter in the UK on an Adrian Lee Fellowship to develop dual bacterial/yeast biofilm systems. On graduating he moved to the Biomerit Research Centre in Cork, Ireland to work on bacterial plant interactions as a Government of Ireland Fellow in Science Technology and Engineering. This research looked at how to use bacteria to help plant growth. He then took a position as a Senior Scientist and Research Professor within the Geobacter Project at the University of Massachusetts Amherst in the USA where he worked on microbes that make electricity.

Marine microbes in the Plastic Age

A Mark Osborn and Slobodanka Stojkovic

School of Applied Sciences
RMIT University
Bundoora, Vic. 3083, Australia
Tel: +61 3 9925 7126
Email: mark.osborn@rmit.edu.au

We are living in the 'Plastic Age', but unfortunately our non-human relatives with whom we share our planet are not adapted to cope with the thousands of tons of plastic waste entering rivers, seas and oceans each year. Plastic poses both physical and chemical threats to aquatic life. It leads to damage or death of animals following plastic entanglement or ingestion and/or can lead to bioaccumulation of co-pollutants absorbed on plastic surfaces. Once ingested, co-pollutants can be absorbed into tissues and accumulated in the food chain. As nature's biodegraders and recyclers, microorganisms may play a role in mitigating the impact of our disposable plastic lifestyle, or alternatively, plastic may serve as a vector for transport of pathogenic microorganisms into marine fauna. Here, we review current understanding of the microbiology of marine plastics and highlight future challenges for this emerging research discipline.

The dominance of plastic across human society is a recent phenomenon, with petroleum oil-derived synthetic plastic polymers only finding widespread usage during the second half of the last century. We, alongside all other organisms, now live in the 'Plastic Age', with plastic infrastructure and industrial and consumer products now prevalent and playing a critically important role across every aspect of our lives[1]. However, the essential qualities of plastics, namely, resilience, durability, light weight, flexibility and resistance to degradation that have driven the adoption of plastics as materials of choice has also lead to the cosmopolitan distribution of plastic waste across the planet, and especially within marine environments. Initially, environmental plastic litter was considered primarily as an aesthetic issue, but the United Nations Environment Programme (UNEP) has now identified plastic pollution as a global environmental threat[2] with a proposal that plastic be designated as a hazardous waste product[3].

Our plastic world

Global plastic production increased from 1.7 million tons in 1950 to 288 million tons in 2012[4], representing an 8.7% year-on-year increase[5]. Plastic consumption in Australia alone exceeded an average of over 1.5 million tons per annum between 2007 and 2012[6]. The five major classes of plastic polymers, comprising ~90% of polymer production, are: polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), and polyethylene terephthalate (PET)[1]. The ever-increasing production of industrial and consumer plastics, the latter which includes a substantial proportion of single-use disposable plastics, results in a plastic deluge into marine environments. A short walk along the tideline of any beach quickly highlights the pervasive presence of plastic litter in our marine environments, but a closer look also reveals the abundance of so-called microplastics (defined as plastics $\leq$5 mm in diameter; Figure 1A).

The presence of microplastics in our oceans was first reported in the Sargasso Sea in 1972[8] with initial estimates of particle distribution of 50–12,000 per km^2. Later that year, PS spherules carrying adsorbed co-pollutants (polychlorinated biphenyl) were reported in coastal American waters[9], foreshadowing the subsequent identification of a much greater problem of adsorbed co-pollutants on plastic surfaces, now threatening marine fauna[10]. Intriguingly, both of these pioneer studies also noted the presence of microorganisms on the surface of plastics, with diatoms (and also hydroids) identified on pellets from the Sargasso Sea[8] and of rod-shaped Gram negative bacteria on PS spherules[9]. A subsequent study of American offshore waters demonstrated the widespread distribution of plastic fragments in oceanic waters[11], but for many years, interest in the environmental distribution and ecological impact of plastic particles in marine environments remained limited. In 2004, Richard Thompson and colleagues published a paper in *Science* (Lost at Sea: Where is all the Plastic?)[12] revisiting the issue of microplastics in marine environments. They demonstrated both widespread occurrence of microplastic fragments and fibres in both pelagic and benthic systems and highlighted increasing accumulation of microplastics between the 1960/70s and 1980/90s. The accumulation and fragmentation of plastics into microplastics[13] has now led to the global dispersal of plastic across marine environments[14], and in particular within the

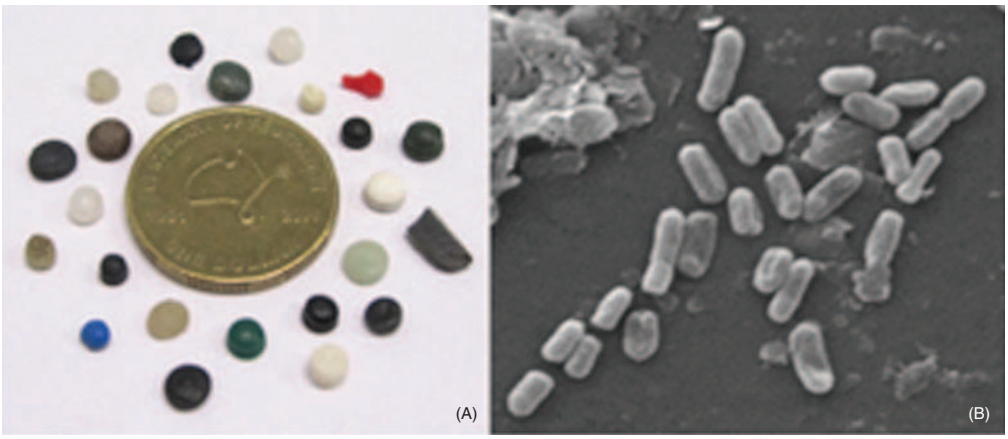

Figure 1. Microplastics and the Microbial Plastisphere. (A) Microplastics (plastic production pellets and fragments) recovered from Sandridge Beach, Port Phillip Bay, Victoria, Australia (credit: Taylor Gundry, RMIT University). Size comparison to Australian $1 coin (diameter: 25 mm). (B) Bacterial cells (~1.5 µm long) attached to and undergoing cell division on polyethylene microplastics in U.K. coastal marine sediments (Scanning Electron Microscopy image modified from Harrison *et al.*[7]).

gyres (or 'Garbage Patches') of the Atlantic[15] and Pacific[16] (but also Indian) oceans[14]. Estimates of surface plastics, alone, are as high as 5×10^5 pieces per km^2 of ocean[15], with plastic identified as the most abundant component of litter within marine environments[17]. A recent study[18] of Australian coastal waters also reported high average sea surface plastic concentrations exceeding 4,000 plastic pieces per km^2.

The microbial 'plastisphere'

As nature's biodegraders, microorganisms may already be ameliorating the accumulation of plastic and/or their associated co-pollutants within marine environments. However, hard evidence for biodegradation, especially over ecologically-relevant timescales is lacking. Indeed, our understanding of the marine microbial plastisphere is still in its infancy, with initial studies just beginning to characterise the structure and taxonomic diversity of plastisphere microbial communities.

Following the initial reports of microorganisms on plastic fragments in the North Atlantic in the early 1970s[8,9], a 25 year hiatus followed until Dang and Lovell[19] explored initial stages of biofilm formation (24–72 hours) on plastic plates in marine waters. These biofilms were dominated by *alphaproteobacteria*, in particular *Roseobacter* spp. Similar short-term exposure experiments (up to 36 hours) were then undertaken in Korean harbour waters[20], comparing communities present on acryl with those on glass and steel coupons. Molecular analysis of bacterial 16S rRNA genes suggested successional changes in community structure, with some taxa common across multiple surfaces, whilst some taxa were found only on one substrate. A third exposure experiment in surface waters in the China Sea[21], compared differences in microbial communities on PVC with those on glass and Plexiglass after 24- and 72-hour exposures. Sequencing of bacterial 16S rRNA genes showed primary clustering of communities with time rather than surface type, and identified seven bacterial phyla, with *alphaproteobacteria* (including *Roseobacteria*) and *gammaproteobacteria* most abundant. These three early studies highlighted that plastic, as with any other available substrate, in the marine environment will be colonised by diverse bacterial taxa. Furthermore, they suggest that plastic biofilm communities will not solely be comprised of bacteria (and other microorganisms) that are specific to plastic alone.

Two exposure experiments explored colonisation of environmentally-abundant plastics, namely PET (synonymous with plastic bottled drinks) and with the most abundant marine plastic: PE (used for production of plastic bags and food packaging). A six-month exposure experiment using PET in seawater[22] yielded biofilms up to 90 µm thick and demonstrated a capacity for longer-term microbial survival on marine plastics. Culture-based analysis of PE-food bags submerged for 3 weeks below the seawater surface[23] showed significant increases in heterotrophic bacterial numbers on PE bags over time, accompanied by corresponding decreases in PE buoyancy. This study suggests that microbial colonisation (biofouling) of PE could contribute towards transport of previously buoyant plastic from surface into deeper waters. As microbial colonisation of plastics will be widespread in marine environments, this mechanism may partly explain the recent and perhaps surprising finding that global loads of buoyant plastic (especially PE, PP and PS) currently present at the ocean surface are estimated to be ten of thousands of tons lower than expected from estimates of plastic loads released into open oceans[14]. This raises a number of intriguing questions concerning plastic-microbial interactions in marine systems, in particular, as to whether microbial biofouling contributes to plastic transport to deeper waters and sediments, analogous to the concept of marine snow[24], in addition, as to whether microorganisms may degrade either the plastics and/or plastic-adsorbed co-pollutants, as we have hypothesised previously[25].

Following these earlier studies, there is now considerable interest in characterising the microbial communities present on marine plastic surfaces. In the first study exploring microbial community

composition on plastic fragments recovered from the open ocean, Zettler and colleagues[26] coined the term 'plastisphere' to define communities of microorganisms colonising plastic in the environment. They used 454-pyrosequencing of bacterial 16S rRNA genes amplified from plastic fragments from the Atlantic Ocean and showed that the plastisphere of just six different fragments (3 each of PE and PP) were comprised of over 1,000 different operational taxonomic units (OTU, analogous to species). Comparing these communities with those in the seawater from which the plastics were recovered, identified a number of species detected only on the plastic surface, including the cyanobacterium *Phormidium*; *Pseudoalteromonas* spp., often associated with marine algae and also members of the *Hyphomonodaceae*, which possess prosthecate filaments facilitating surficial attachment. It is unknown whether abundance of these taxa reflects a 'preference' for plastic as a substrate, or alternatively whether they would colonise other substrates in marine waters. Intriguingly, the authors highlighted the presence of cells in 'pits' in the plastic, using electron microscopy speculating this is suggestive of microbial degradation of plastic surfaces.

Two other recent studies have utilised electron microscopy to investigate microbial diversity on marine plastics. Firstly, rod-shaped bacteria and pennate diatoms were shown to be most prevalent on plastic fragments from the North Pacific gyre[27]. Analysis of plastic fragments recovered from seawater around Australia[28] similarly revealed a morphologically diverse array of microorganisms, especially of diatoms, but also of other microbial eukaryotes, including coccolithophores, dinoflagellates and fungi. Assorted marine invertebrates were also identified suggesting plastics may serve as a 'raft' for complex multitrophic communities. This study also identified the presence of 'pits' and 'grooves' in plastic surfaces, again highlighting an urgent need for research to provide definitive evidence of marine plastic biodegradation.

We recently identified several further challenges as we investigate plastisphere microbial ecology. Firstly, we showed that the structure and composition of plastisphere microbial communities varies both seasonally and with geographical location[29]. In this research, PET drinking water bottles were attached onto buoys at three locations in the North Sea in winter, spring and summer. Seasonal differences in plastisphere communities were observed, with higher relative abundance of photosynthetic brown algae and cyanobacteria on bottles exposed during summer months, while winter communities were dominated by heterotrophic bacteria, including *Bacteroidetes* and *gammaproteobacteria*, in addition to photosynthetic diatoms (*Synedra* spp). Comparison of communities on plastic fragments from offshore waters around Northern Europe additionally demonstrated that plastisphere communities varied both with polymer type and the geographical location from which fragments were recovered. We also explored early stage microbial biofilm formation on PE microplastics (Figure 1B) within sediment (rather than pelagic) systems across sediment types[7]. These experiments revealed rapid successional changes in bacterial community structure on microplastics, with communities at 14 days dominated by *Arcobacter* and *Colwellia* spp. Interestingly, we observed convergence in the structure and composition of these plastisphere communities, while the structure of the communities in the different sediment types remained different, suggesting possible selection for these two genera in the PE plastisphere. While both *Arcobacter* and *Colwellia* have been associated with hydrocarbon degradation, we can, at this stage, only speculate on whether these bacteria are involved in PE biodegradation.

Much of the research undertaken thus far has been partly motivated by an interest in identifying evidence of biodegradation of marine plastics or, at least, has discussed its potential. However, an alternative impact of microbial plastic colonisation has also been highlighted by the observation of a high relative abundance of *Vibrio* spp. on plastic fragments recovered from the North Atlantic[26]. This observation, together with a report of *Escherichia coli* on plastic (and also seaweed) in beach waters suggests that plastic could serve as a vector for the transport of pathogenic microorganisms into marine fauna[30].

Outlook

To understand the diversity and ecology of the microbial plastisphere, we will need to consider the likelihood that each individual plastic fragment present within the marine environment will have been subject to complex dynamic changes in its biofilm community structure and ecology, during the myriad of divergent routes, transitioning across and between the terrestrial, freshwater and marine environment. Along that journey, each plastic fragment may develop into a unique environmental microhabitat, shaped by both travel through differing physical–chemical environments, but additionally, due to adsorption of organic and inorganic chemicals and by the colonisation of diverse microorganisms. We conclude by highlighting five key questions and challenges for this emergent research topic:

(1) Do plastic surfaces select specifically for particular microbial species and/or alternatively, are plastic surfaces just primarily a convenient substrate for colonisation of microbial phototrophs driving development of multi-trophic complex biofilm assemblages?
(2) Does microbial biofilm formation (biofouling) drive reductions in plastic buoyancy leading to plastic transport to the deeper ocean and into sediments?
(3) How do the structure and function of plastisphere microbial communities change during transport from terrestrial environments, via freshwater, into marine waters and additionally into benthic environments?
(4) Does microbial degradation of plastic (and bioplastic) and of adsorbed co-pollutants occur in marine environments and if so over what timescales? What are the ecological constraints upon plastic and co-pollutant degradation?
(5) Are plastic surfaces a potential site for accumulation of pathogenic microorganisms that can be ingested by and impact upon marine fauna?

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Biographies

Mark Osborn is an Environmental Microbiologist and Associate Professor in the School of Applied Sciences at RMIT University, Melbourne, Australia studying the ecology, diversity, function and biotechnological capabilities of microorganisms within natural and urban environments. His research investigates how microorganisms respond to and mitigate against the impact of anthropogenically sourced environmental pollutants, including hydrocarbons, plastics, heavy metals, nitrogenous fertilisers and greenhouse gases. Mark has published over 70 peer-reviewed articles and reviews and is the editor of a textbook entitled *Molecular Microbial Ecology*. He is the Section Editor (for Microbiology) of the Open Access journal *Cogent Biology* and a member of the editorial board of the *ISME Journal*. Mark also tweets (@MicrobialLife) on eclectic aspects of current microbiology research.

Slobodanka Stojkovic is an Algal Ecophysiologist, Microbial Ecologist and Postdoctoral Fellow in the School of Applied Sciences at RMIT University. Her research studies the ecology and functioning of aquatic microorganisms. Her research focuses on impacts of pollutants and environmental factors especially in relation to climate change impacts on the performance of aquatic organisms, and phototrophs, in particular. Slobodanka has worked on a range of aquatic ecosystems, both nationally and internationally, resulting in over 15 peer-reviewed publications, and a number of industry reports.

Microbiology of chloroethene degradation in groundwater



Sayali S Patil[A], *Eric M Adetutu*[A] *and Andrew S Ball*[A,B]

[A]School of Applied Sciences, RMIT University, PO Box 71, Vic. 3083, Australia.

[B]Corresponding author. Tel: +61 3 9925 7122, Fax: +61 3 9925 7110, Email: andy.ball@rmit.edu.au

Industrial development, population growth and urbanisation have all contributed to an increase in the release of chemical pollutants into the environment. Consequently, many natural resources show some degree of anthropogenic impact, including the widespread contamination of groundwater aquifers by hazardous wastes[1]. This is particularly significant because groundwater represents about 98% of the available freshwater on the planet. The fact that we are already using approximately 50% of readily available freshwater makes groundwater protection and clean-up of paramount importance. Increasing incidences of aquifer contamination by chloroethene solvents is of current concern throughout Australia. Further, due to the adverse effects of chloroethene contaminants to environmental and human well-being, it is of upmost importance to understand the potential for the natural microbial population within the groundwater to degrade the chloroethene to innocuous byproducts.

Introduction to chlorinated hydrocarbons

Chlorinated ethenes or chloroethene are common groundwater pollutants; in 1995 trichloroethene (TCE) was found to be the second most common hazardous waste in the US[2]. TCE does not occur in the environment naturally, it is an anthropogenic chemical[3]. It was widely used as a metal degreaser, in paints, dry cleaning, paint strippers and carpet shampoos. Improper handling and disposal of these compounds has led to severe soil and groundwater contamination[4]. TCE is a clear liquid, denser than water, which allows it to sink forming a dense-non-aqueous-phase-liquid (DNAPL). Since the partition coefficient (Log Kow) is 2.61, TCE is hydrophobic and so is only slightly soluble in water, aiding the formation of the DNAPL.

TCE has been found to be a carcinogen in model animals such as rats and mice, causing kidney, lung and liver cancers[5]. It has been found to strongly correlate with a higher incidence of renal cancer in humans when exposed to TCE as metal degreasers[6]. People exposed to TCE due to their occupation were also found to experience an increase in the prevalence of non-Hodgkin's lymphoma[6].

Environmental fate and prevalence of chlorinated solvents

TCEs degrade slowly with half-lives in the natural environment of 80–800 days[7], and due to the formation of DNAPLs at the bottom of aquifers, TCE exhibits sustained release over time into groundwater[8]. TCE was found in breast milk in Arizona, USA at concentrations of 6 µg/L, which is above the EPA maximum contaminant level (MCL) of 5 µg/L. The MCL for cis-DCE and VC are 70 µg/L and 1.2 µg/L respectively. TCE had also been found in foods such as decaffeinated coffee and eggs, demonstrating the scope of products that can lead to TCE ingestion[9].

Chloroethene contaminant detoxification: the microbiology

The discovery of microorganisms in the mid-1990s that gain energy from the process called reductive dechlorination of chloroethene

led to a turning point from a predominantly co-metabolic view of chloroethene biodegradation to the concept of chloroethenes serving as primary substrates for microbial metabolism[10]. Most of the chlorinated compounds have a synthetic origin and have not been in contact with microorganisms through evolutionary periods of time[11]. As a result, chlorinated solvents are not frequently metabolised by indigenous organisms. Nevertheless, several biotransformation mechanisms have been identified that could be exploited for degradation.

The main biotransformation pathways for chlorinated ethenes are explained below[12]:

(1) Aerobic oxidation: the pollutant serves as the primary substrate for growth. Oxygen serves as the electron acceptor. Aerobic metabolism is limited to the less chlorinated compounds such as chloromethane, dichloromethane, chloroethane, 1, 2-DCA and VC.
(2) Aerobic co-metabolism: in addition to oxygen, an electron donor must also be present. In general, the fewer the Cl atoms, the better the co-metabolic process will work. Toluene, methane, propane, butane and phenol have all been used as primary substrates to support such co-metabolic transformation.
(3) Anaerobic oxidation: in this mechanism, the chlorinated organic serves as an electron donor for growth. Only a few chlorinated aliphatics are amenable to this treatment (i.e. dichloromethane; 1, 2-dichloroethene; cis- and trans-DCE and VC). Nitrate and sulphate can serve as electron acceptors in such cases and dichloromethane can also be fermented. Nevertheless, degradation rates are relatively slow and this process has not yet been demonstrated or exploited for site remediation.
(4) Anaerobic reductive dechlorination: in this process, the compound serves as an electron acceptor. All chlorinated aliphatics are susceptible to anaerobic, co-metabolic, reductive dechlorination. This requires a suitable electron donor and it works mainly under sulphate-reducing or methanogenic conditions. An exception is carbon tetrachloride, which can also be dechlorinated under denitrifying conditions.

Current research activity is focused on dehalorespiration, where PCE, TCE, DCE and VC serve as terminal electron acceptors in support of microorganism growth. There are two reductive dehalogenation mechanisms. The first is hydrogenolysis (hydrodehalogenation), which involves replacing halogen atoms such as Cl, Br and F by a hydrogen atom. This is illustrated in Figure 1 for the stepwise reduction of TCE via DCE to VC and ultimately to ethene. The other reductive dehalogenation mechanisms are dihaloelimination, which involves the simultaneous removal of two halogen atoms after two electrons are transferred. Reductive dechlorination generally decreases the toxicity and enhances the solubility (bioavailability) of the pollutant, but there are exceptions where the toxicity can be accentuated (e.g. TCE reduction to VC). Reductive dechlorination is often a co-metabolic reaction since the microorganisms that catalyse it cannot harvest the energy released by the redox process. Recently however, many bacterial strains have been found that can utilise PCE and TCE as a terminal electron acceptor during respiration using H_2, formate, acetate and pyruvate as electron donor. This process is known as halorespiration and it can be mediated by species such as *Desulfomonile tiedjei*, *Dehalobacter restrictus*, *Desulfitobacterium* and *Dehalococcoidese thenogenes*[11]. PCE and TCE readily undergo reductive dechlorination but the efficiency of the reaction decreases with decreasing degree of chlorination. Some dechlorinators sequentially dechlorinate PCE to TCE, some preferentially to cis-DCE and some to VC. However, the conversion of DCE and VC as electron acceptor to non-toxic ethene is principally mediated by *Dehalococcoides* species-affiliated bacteria. Conversely, the tendency for aerobic oxidation of chlorinated ethenes increases with decreasing number of chlorine atoms of the molecule. Both metabolic and cometabolic oxidation of lower chlorinated ethenes have been reported. However, mineralisation of DCE and VC tends to increase with higher reduction potential.

Dehalorespiring bacteria

All of the known dehalorespiring microorganisms are bacteria and their dehalogenation capacities are highly strain dependent[13]. Anaerobic bacteria that grow with chloroethenes as final electron acceptors include *Dehalobacter, Dehalococcoides, Desulfitobacterium, Desulfuromonas, Geaobacter* and *Sulfurospirillum*. The well-studied organisms, *Sulfurospirillum multivorans* and *Dehalobacter restrictus* PER-K23 dechlorinate PCE to *cis*-DCE[11]. *S. multivorans* is a Gram-negative anaerobic spirilum, which belongs to the ε- subdivision of Proteobacteria. The *Dehalobacter* genus belongs to *Firmicutes* and is allied with the genus *Desulfitobacterium.* However, dehalorespiration is the sole system of energy production in the genus *Dehalobacter.* Although these strains can utilise PCE or TCE as electron acceptors, they cannot completely dechlorinated cis-DCE or VC to ethene. One genus of particular interest for such bioremediation is '*Dehalococcoides*' *(Dhc)*, obligate anaerobes that cannot use oxygen, nitrate or sulphate as electron acceptors. They are Gram-positive, coccoid cells closely related to a member of the *Chloreflexi* phylum (green non-sulphur bacteria), which possess diverse dehalogenation ability, grow robustly in mixed cultures and are present globally in microbial populations[14]. *Dhc* species are of particular interest as members of the genus are the only known bacteria capable of complete reduction of chlorinated ethenes (PCE and TCE) to ethene (Figure 1). *Dehalococcoides ethenogenes* 195 and *Dhc*sp.FL2 respectively dechlorinated PCE and TCE to ethene[15]. However, these two strains are unable to use VC as an electron acceptor. Thus, the slow dechlorination of VC to ethene is considered to proceed in a co-metabolic fashion uncoupled to energy production[16]. In contrast, four other *Dhc* strains, BAV1, VS, GT and KB1/VC can use VC as the electron acceptor in their dehalorespiration and can dechlorinated VC to ethene efficiently[17]. In the genus *Dhc*, dehalorespiration is solely an energy preservation system. These isolates exhibit a metabolic specialisation, using only H_2 as an electron donor and chlorinated compounds as electron acceptors to support growth.

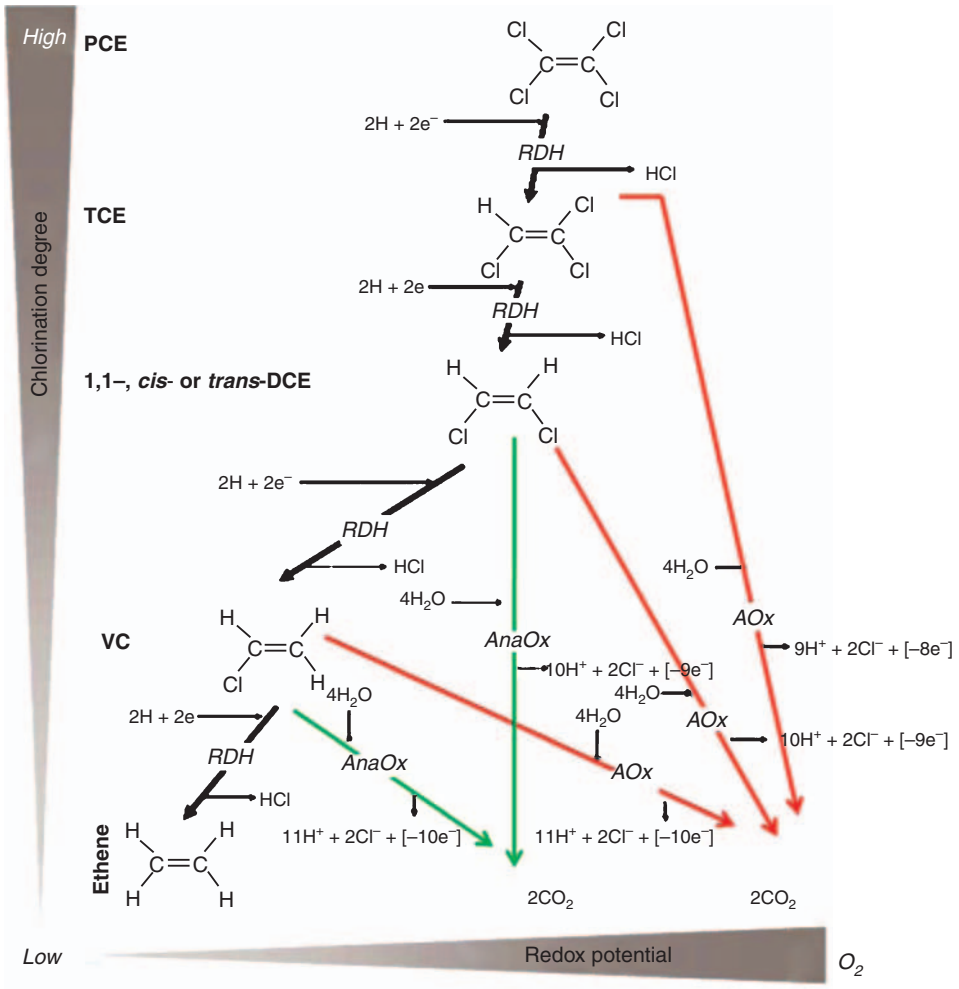

Figure 1. Pathways for the degradation of chlorinated ethene. Black, green and red arrows indicate different pathways such as reductive dechlorination (RDH); anaerobic oxidation (AnaOx) and aerobic oxidation (AOx) respectively[12].

Reductive dehalogenases (RDases)

Reductive dechlorination reactions are catalysed by the reductive dehalogenases (RDases). RDases are a class of enzymes found mostly in *Dhc* species and other dechlorinating organisms that catalyse the following reaction[13]:

$$R{-}Cl + 2[H] \rightarrow R{-}H^{+}H^{+} + Cl^{-}$$

Hydrogenases are a crucial part of the reaction mechanism because they supply electrons to the reaction from H_2. In anoxic environments, the above reaction is thermodynamically favourable and chlorinated compounds can act as electron acceptors. However, it has been observed previously that hydrogenases are oxygen sensitive, whereas RDases may retain some activity following exposure to oxygen[18]. 'Dehalorespiration' is defined as the process whereby energy from the above reaction is conserved and coupled to ATP synthesis in a chemo-osmotic mechanism[11]. Dechlorinating organisms obtain energy from the process and in many cases dechlorination activity can be linked to growth[19]. The hydrogenases spilt hydrogen into protons, driving the proton gradient that is utilised for ATP synthesis; and electrons (e^-) are carried through the electron transport chain to the dechlorination reaction, where the chlorinated substrate acts as a terminal electron acceptor. Reactions are proposed to take place with a coronoid co-factor and 2 Fe-S clusters.

Although many putative dehalogenases exist, few have been purified and characterised. Those relevant to TCE dechlorination are listed in Table 1 along with their distribution among *Dhc* isolates. A TCE dehalogenase, encoded by the *tceA* gene was first discovered in *Dehalococcoides ethenogenes* strain 195 and is thought to be co-transcribed with the *tceB* gene encoding a small membrane anchor. This gene has a wide distribution among a range of environmental samples and those that contain *tceA* can degrade TCE, although not all TCE-degrading organisms contain *tceA*. Two VC-RDases have been discovered, originating from two different isolates –*vcrA* from strain VS and *bvcA* from strain BAV1[20]. These are believed to be the distinguishing feature of *Dhc* from other dechlorinating organisms.

In conclusion, chloroethenes represent a serious threat to both human health and the environment. Microbial communities naturally present in contaminated groundwater have been found that are

Table 1. Distribution of characterised RDases involved in the dechlorination of TCE to ethene in Dhc isolates[19].

Dhc strains	Known expression of RDase genes	Reaction catalysed	Molecular mass (Da)
195[A]	*pceA*	PCE → TCE	50,800
	tceA	TCE → VC	57,700
VS	*vcrA*	DCEs, VC → ethene	53,100
BAV1[B]	*bvcA*	VC → ethene	52,800
FL2	*tceA*	TCE → VC	–
CBDB1	*pceA*	None, respires other chlorinated compounds	–
GT	–	TCE, cDCE, VC	–

[A]Dhc strain 195 co-metabolises VC at a slow rate.
[B]BAV1 only co-metabolises PCE and TCE in the presence of DCE and VC.

capable of the complete mineralisation of chloroethenes using a variety of mechanisms of which anaerobic reductive dechlorination represents the most ecologically significant process. Recent advances in molecular microbial ecology have led to greater understanding of the mechanisms underpinning the degradation process. This will lead to improvements in the management and remediation of contaminated groundwater.

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Biographies

Dr Sayali Patil received her PhD degree in Environmental Biotechnology from Flinders University in South Australia, Adelaide. Dr Patil has produced publications in the field of Environmental Microbiology and Biotechnology and given proffered presentations at several international conferences. Dr Patil has worked as a Project Manager at the South Australian Research and Development Institute (SARDI) for two years.

Dr Eric Adetutu is a Research Fellow at RMIT, University. He obtained his PhD from the University of Essex, UK. His research interests include microbiology of extreme environments (including caves), rumen microbiology, bioremediation and the application of next generation sequencing tools to the study of pristine and disturbed environments.

The biography for **Professor Ball** is on page 182.

Report from ASM 2014: Solving the Puzzles

Enzo Palombo
Melbourne 2014 LOC Chair

The recent ASM 2014 Annual Scientific Meeting and Exhibition was a wonderful event that attracted almost 700 delegates from across Australia and the world over four days (6–9 July) at the Pullman Albert Park. The venue provided an intimate environment which was conducive to networking, socialising and greater interaction between all delegates.

The meeting theme was 'Solving the Puzzles', which reflected the ever-increasing need for collaboration across the diverse areas of microbiology and other disciplines to address the microbiological issues facing our society. Indeed, the general atmosphere of the meeting showed how much microbiologists enjoy working and socialising together!

The meeting brought together international leaders complemented by the best of the local microbiology community. The inaugural pre-conference EduCon meeting, modelled after the American ASM Annual Educators Conference (ASMCUE), was an outstanding success. Topics explored included inquiry-orientated learning, employer expectations of graduates, innovations in the teaching lab and microbiology curricula. The workshop program was held on the weekend and was well supported by delegates. The conference kicked off on Sunday afternoon with an incredibly stimulating, engaging and informative public lecture delivered by Sir Gustav Nossal on the topic of vaccination. The Bazeley Oration (Linfa Wang) and the Fenner Lecture (Ben Howden) set the standard for quality presentations for the remainder of the meeting and no-one was disappointed. The poster sessions (held every evening) gave every delegate plenty to see while enjoying refreshments which were appreciated following days of busy schedules. A new initiative launched this year was the Nancy Millis Mentoring Program designed for our student members. A number of networking and social events were organised and the response by students was well above our expectations with more than 50 postgraduate students taking part in the program.

The scientific program contained many highlights, especially with the calibre of international invited speakers who were able to attend. Not only were their plenary and symposium talks motivating and thought-provoking, the speakers engaged with other delegates and were available to share a coffee and a chat. For those who are avid listeners to Vincent Racaniello's podcasts, look out for special ASM

ASM2014 Local Organising Committee. Back row: Rob Moore, Tony Marshall, Libby Grabsch, Priscilla Johanesen, Jacob Amy, Estee Madaschi, Danilla Grando, Dena Lyras, Narelle Fegan, Enzo Palombo. Front row: Catherine Butler, Mary Valcanis, Karena Waller, Christine Seers, Helen Stefanatos. Absent: Susan Cornish, Carl Kirkwood, John Boyce.

2014 editions (TWiM #84: Microbiology Down Under, TWiV #293: Virology Down Under and TWiV #296: The real Batman, Linfa Wang). The Rubbo Oration delivered this year by Roy Robins-Browne was a fitting tribute to a much loved and admired Melbourne microbiologist.

I wish to express my gratitude to the extremely hard-working and dedicated members of the Local Organising Committee and Scientific Committee. You should all be proud of your achievements in bringing ASM 2014 together. Thank you also the ASN Events team whose great work in the background meant that everything went smoothly and efficiently at the meeting.

We now look forward to Canberra in 2015!

New Fellows of the Australian Society for Microbiology

New Fellows of ASM were presented with their certificates: Steven Giglio, Healthscope Pathology; Karena Waller, University of Melbourne, Yogitha Srikhanta, University of Melbourne, Kate Sieb, Griffith University with Paul Young (centre).

Burnet Hayes Postgraduate Award: Erica Kintz

Erica Kintz is a Postdoctoral research associate at the Centre for Immunology and Infection, University of York. The Burnet Hayes Postgraduate Award enabled her participation at the ASM2014. She presented her work entitled 'Addition of glucose to O antigen subunit by glycosyltransferase operons of *Salmonella Typhi* results in increased serum survival'.

David White Excellence in Teaching Award: Priscilla Johansen

Priscilla Johansen and Paul Young.

Priscilla works in the Faculty of Medicine, Nursing & Health Sciences at Monash University as a Lecturer. She began teaching as a practical demonstrator in second and third year undergraduate Microbiology units whilst undertaking her PhD at Monash University. In her career to date she has taught various facets of Microbiology in different degree programs including Biomedical Sciences, Science and Medicine (undergraduate and graduate entry).

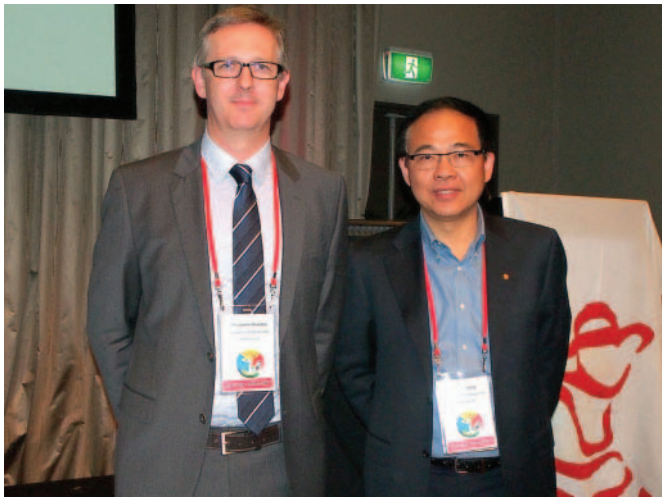
bioMérieux ASM Identifying Resistance Award: Denis Spelman

Denis Spelman is an Infectious Diseases Physician and Medical Microbiologist. He is currently the Deputy Director of the Clinical Infectious Diseases Unit and Head of the Microbiology Department. He is the Head of the Spleen Registry and has an interest in infection control, multi-resistant organisms and their management.

Denis Spelman, Helen Stefanatos (bioMérieux) and Paul Young.

ASM Frank Fenner Award: Ben Howden

Linfa Wang presented the 2014 ASM Frank Fenner Award to Professor Benjamin Howden. Ben is the recently appointed Director of the Microbiological Diagnostic Unit in the Department of Microbiology and Immunology at the University of Melbourne, within the Peter Doherty Institute for Infection and Immunity. He is also an Infectious Diseases Physician at Austin Hospital. His research interests have been driven by working in the hospital system where antibiotic resistant pathogens such as methicillin-resistant *S. aureus* and vancomycin-resistant Enterococci are a major problem. During his doctoral studies at Monash University (2004–2008) he used emerging genomic technologies to explore the molecular determinants of reduced vancomycin susceptibility in *S. aureus*. His current research activities include the application of genomics to understand the emergence, spread and pathogenesis of antimicrobial resistance bacterial pathogens; understanding the mechanisms and impacts of antimicrobial resistance, microbial adaptation, and changes in host-pathogen interactions during persistent *S. aureus* infection; and exploring the role of non-coding (small) RNAs in the *S. aureus* response to antimicrobials. His research is funded by the National Health and Medical Research Council (NHMRC), Australia, and he is and NHMRC Career Development Fellow.



Ben Howden and Linfa Wang.

BD ASM Student Travel Awardees

The BD ASM Student Travel Awards were presented by Estee Madaschi (Product Specialist, BD), and Michael Wawrzyniak (Clinical Sales Manager, BD) in a session chaired by Cheryl Power.

The **BD ASM Student Travel Awardees** and their presentations are:

New South Wales

Robert Moran, University of Sydney.

Bowel movement: resistance plasmid transfer in the gut.

Victoria

Danielle Ingle, University of Melbourne.

Phylogeny and virulence of atypical enteropathogenic *Escherichia coli*.

Queensland

Ashleigh Shannon, University of Queensland.

Defining interactions between the dengue virus NS3 protease and its cofactor NS2B by site-directed mutagenesis.

South Australia and Northern Territory

Evan McRobb, Menzies School of Health.

Identification, characterisation and treatment of *B. pseudomallei* in bore water supplies associated with melioidosis cases in northern Australia.

Tasmania

Kamarul Zaman Zarkasi, Tasmanian Institute of Agriculture.

Pyrosequencing-based characterisation of the gastrointestinal microbiota of commercially farmed Atlantic salmon (*Salmo salar* L.) in south-eastern Tasmania.

Western Australia

Amir Ariff, University of Western Australia.

Whole genome sequencing and bacteriophages of *Moraxella catarrhalis*.

Estee Madaschi, Robert Moran, Danielle Ingle, Ashleigh Shannon, Cheryl Power Evan McRobb,; Kamarul Zaman Zarkasi, Amir Shariff, Michael Wawrzyniak.

ASM Student Poster Prize award winners

Jacqueline Heath, The University of Melbourne: 'PG1058 in the Type IX Secretion System of *Porphyromonas gigivalis*'.

Carla Giles, University of South Australia: 'Development of an adenoviral rector vaccines for *Rhodococcus equi* infections in foals'.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Dr Paul Kenneth Priscott 1950–2014

Hilary Fong MASM on behalf of CAPSIG (NSW)

Dr Paul Kenneth Priscott (photo courtesy of **ams** Laboratories P/L) grew up and was educated in the UK, earned his MSc in Virology from the University of Portsmouth, Reading and PhD in Microbiology/Development Biology from the University of London. From 1967 to 1976, he held various positions in government institutions and laboratory positions in the UK. In 1976, he became a research scholar with the Medical Research Council Clinical Research Centre, Harrow, UK and University of London, from where he was awarded his PhD in 1980. His research involved numerous techniques for the growth, assay of mycoplasmas, viruses, and cell and embryo cultures. His research work in this area was first published in 1982 in the Archives of Virology. In 1981, he became the Raine Research Fellow with the University of Western Australia involving collaborative and independent research into normal and abnormal development using *in vitro* culture techniques; thus opening a new phase of Paul's life in Australia.

After four years as the Raine Research Fellow at University of WA, Paul took up a new position as Research Officer with the WA Department of Agriculture responsible for the virology laboratory and involved in various aspects of regulatory testing services and research ethics. In 1988, Paul made the move to the eastern states when he took up a position as the Operations Manager at the Glenorie facility of Arthur Webster Pty Ltd before moving on as the Director of Microbiology, Stanford Consulting Laboratories.

In 1996, Paul set up his own consulting business, **ams** Consulting Laboratories, which fast became the 'household name' in pharmaceutical and cosmetic manufacturers. From the humble beginning with a small laboratory of four staff in Rockdale, **ams** expanded to a much bigger facility with 40 staff and 900 square metre of laboratory space in Silverwater, as well as branches in Brisbane and China, which is under construction. The laboratories within **ams** at Silverwater are well-equipped providing services for a wide range of contract microbiological testings.

Paul had a vision for his company dedicated to professional consulting and testing in pharmaceutical and cosmetic microbiology. Under his direction, **ams** quickly extended their services to other therapeutic areas including tissue banking, biotherapy and other high end applications. As a person committed to excellence and compliance, Paul ensured **ams** gaining registration with various regulatory instrumentalities including the local TGA, FDA(US), NATA and ISO Standards by SAI Global.

In 1992, Paul joined the Committee of CAPSIG (NSW) and since then worked tirelessly as a very active member on the committee and later took up a dual role including the National Convenor of CAPSIG. As a Committee member, Paul had contributed significantly to organising technical seminars, CAPSIG scientific seminars at the Annual ASM Conferences and other activities. As CAPSIG National Convenor, Paul was our liaison officer between state branches of CAPSIG and ASM nationally. Paul had always taken active part in the organising committee of the annual ASM National Conference and bringing notable speakers from overseas. Under the directorship of Paul, **ams** Laboratories P/L has been one of the major sponsors for CAPSIG (NSW) for many years. More recently, Paul was instrumental to the re-establishment of CAPSIG (QLD).

Concomitant with all these voluntary activities, Paul had found time to become an ardent industry advocate, sitting on many sub-committees. He was on the Editorial Board of the PMF Newsletter, an international publication for pharmaceutical microbiologists, member of the council of NATA, member of Standards Australia sub-committee CH/34, TGA – member of the Disinfectants Working Group and ACCORD – member of industry working party on Disinfectants and Antibacterial Products. His publications, *inter alia*, include chapter in *Pharmaceutical Quality, PDA 2004* and *Microbiology in Pharmaceutical Manufacturing, 2nd Edition, PDA 2008.*

Over the past two decades, Paul has presented numerous seminars on microbiological aspects and developments in the pharmaceutical and cosmetic industries organised by CAPSIG; as well as the RACI Pharmaceutical Sciences Group, Australian Cosmetic Chemists Society, among others. Indeed, his latest and last presentation under CAPSIG was delivered on 22 July, less than four weeks before he succumbed to complications of his illness on 19 August 2014.

Paul was diagnosed with cancer quite a number of years ago; however, he always remained positive, confident and active. He never allowed his chemotherapy, stem-cell transplant and all other medical intervention to stand in his way of pursuing his dreams. His courage and dedication to moving forward was inspirational and admired by all those who were close and associated with him in any way.

Paul, a true scholar, scientist and gentleman was popular and highly regarded in the cosmetic and pharmaceutical technical community. Perhaps the feelings of all those surrounded him is best summed by the Executive Director of Accord: 'Paul was highly respected very well regarded, not only for his expertise and experience, but also his engaging style and sense of humour. . . He will be sincerely missed by all who had the privilege to have known him. . .'.

CAPSIG (NSW) would like to take this opportunity to offer our deepest sympathy to Paul's family for their loss of a beloved husband, father and grandfather, and to the staff of **ams**, their loss of a great leader and manager.

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