PERSPECTIVE Enhanced in situ bioremediation comes of age in Australia

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Introduction In 2004 I took up a research associate position at the University of NSW (UNSW) leading a project for the Environmental Biotechnology Cooperative Research Centre (EBCRC) on the in situ enhancement of biodegradation of chlorinated organics. The research area was new to me so I did my due diligence not just with the scientific literature and international scientific community but also through discussions with industry partners, government regulators and community groups.

Whilst I was encouraged by these interactions I had a sense that within Australia people had trouble genuinely believing that bioremediation was a feasible solution to the clean-up of some of our organochlorine or hydrocarbon contaminated sites. Perhaps with our mining and agriculture heritage it's hard to imagine that if something doesn't involve large machinery then it isn't going to work.

This healthy skepticism encountered in Australia was in stark contrast to the thriving bioremediation market in North America as I observed in attendance of industry based conferences organized by Battelle such as the *International Symposium on Bioremediation and Sustainable Environmental Technologies* and the *International Conference on Remediation of Chlorinated and Recalictrant Compounds*. As I listened to presentations, attended workshops and interacted with participants at these conferences I got to wondering what was missing in Australia that would enable biological remediation practices to gain traction. The potential advantages with respect to cost, energy, infrastructure and sustainability seemed irresistible in my mind.

I ended up identifying three key factors limiting the application of bioremediation to the clean-up of contaminated sites in Australia. Firstly, we didn't have the sufficient academic biodegradation expertise that North America has for remediation practitioners to consult. It is broadly acknowledged that in situ bioremediation applications require the input of expertise that 'pump and treat' or 'dig and dump' practices do not. Secondly, we didn't have a local commercial molecular diagnostics service available. Consequently, our capacity to find out if the right microorganisms with the right genes were present at a particular contaminated site, was limited. Thirdly, we didn't have a local commercial vendor of bacterial cultures for chlorinated aliphatic hydrocarbon degradation. This in combination with the strict quarantine laws of our island nation prohibited access to the bacterial cultures in widespread use across North America.

Academic expertise

Decades of federal, state and industry research funding investment in the US and Canada into the area of biodegradation and environmental microbiology has established an internationally respected community of scientists and engineers. This community supports remediation practitioners, government regulators and the public with expert opinion. Prior development and proliferation of environmental science and environmental engineering undergraduate programs across universities in the US had laid a foundation for this research and these expertise to flourish. Australia belatedly followed suit. I was in the first cohort of students through UNSW's environmental science degree graduating in 1995.

Indeed North America has dominated research into biological degradation processes, both aerobic and anaerobic, for compound classes from oil derived hydrocarbons (alkanes, alkenes, aromatics, polyaromatics) to industrial organohalides (chlorinated aliphatic hydrocarbons, chlorinated aromatics, dioxins). This is not to take away from the excellent research output in this area from Europe and Asia, but it is telling that industry in Australia generally turn to North American academics and regulators for expert opinion on bioremediation. In contrast, Australia has devoted funding more towards medical microbiology (infectious disease and antibiotic resistance) and cancer research, with a (lazy) eye on cures rather than prevention. This possibly reflects a lack of appreciation for the fact that environmental health underpins human health.

Over the past decade Australian industry and government has begun to nurture the development of expertise in this area. In my experience this has been led by industry and academia more so than government. My research team and its work on anaerobic organochlorine biodegradation at UNSW is an example of this. We have received funding from industry and government sources to sustain professional researchers in biodegradation and train students at undergraduate (honours) and postgraduate (PhD) level that are now taking up employment opportunities in the environmental consulting industry. The sustained funding and patience of investors is essential. It takes time to develop expertise and making mistakes and having failures along the way are key to the developmental process. Other examples include Professor Andrew Ball's group at RMIT University, which conducts research across a range of aerobic and anaerobic bioremediation applications, and Dr. Nicolas Coleman's group at the University

Culture delivery of organochlorine degrading bacteria at Dow Chemicals' Altona Chemical Complex, UNSW.

of Sydney, which specializes in the aerobic biodegradation chlorinated aliphatic hydrocarbons. Further expansion of these expertise within the academic community driven by industry and government funding and continuing interaction between academia, industry, regulators and the public will auger well for the future. We are all on the same side.

Molecular diagnostics

Chlorinated aliphatic hydrocarbons are a major class of semi volatile organic compounds contaminating sites worldwide. Australia has its fair share of sites contaminated with chemicals such as perchloroethene, dichloroethane, carbon tetrachloride and chloroform. The anaerobic biodegradation of chlorinated aliphatic hydrocarbons (chlorinated ethenes, ethanes and methanes) is carried out by a select few species of bacteria. The usual suspect genera are *Dehalococcoides, Dehalobacter, Desulfitoacterium* and *Geobacter*. These bacteria produce enzymes known as reductive dehalogenases enabling them to dechlorinate chlorinated aliphatic hydrocarbons and harvest energy at the same time. The pollutant degradation underpins proliferation of the bacteria but there is a very specific relationship between the pollutant and the 'right' bacteria.

Knowing that the right bacteria are present in the first place and that they produce the right enzymes and that their abundance is substantial is essential for establishing and monitoring the bioremediation of chlorinated aliphatic hydrocarbon contaminated sites. This is where molecular diagnostics comes in. DNA can be extracted from environmental samples (usually groundwater) and a technology known as quantitative PCR (polymerase chain reaction) can be used to quantify specific DNA sequences that serve as nametags for specific bacteria and their enzymes. This technique can be used to determine if the right bacteria are present or if a site would benefit from the addition of the right bacteria.

In contrast, the biological degradation of oil derived hydrocarbons (BTEX, oil, oil sludge) can be carried out by a variety of different microorganisms. These hydrocarbons are, after all, natural products, the likes of which microorganisms have been consuming as a 'food source' for millions of years. Such microorganisms are everywhere and wherever there is a contamination event, the right microorganisms are likely present. For this reason, bioaugmentation (the addition of microbes) and the prior determination of the presence or absence of the right microbes, is generally unnecessary. But this doesn't mean molecular diagnostics have no role to play in the bioremediation of hydrocarbon contaminated sites. Molecular diagnostics can be used to distinguish between the microbial equivalent of a desert or a rainforest (by quantifying the number of bacterial cells per unit volume of groundwater), and by characterizing microbial communities to determine if the hydrocarbon degrading bacteria are aerobic oranaerobic and with the latter whether they have a preference for respiration of nitrate or sulfate. These determinations can underpin a management strategy to facilitate the microbiology in its effort to clean up the environment. Microorganisms equating to the liver of the Earth.

In North America there are a number of companies and universities offering molecular diagnostic services to quantify so called organohalide respiring bacteria and the genes encoding the relevant enzymes per unit volume of groundwater. The most established of these is a company called Microbial Insights, which has been offering quality services internationally for over 20

Organohalide respiring bacteria (ORB) bioaugmentation driven bioremediation rollout at Deer Park, Victoria.. Photos provided courtesy of Orica Australia and Golder Associates.

years. In 2005 I reasoned that it would assist local remediation practitioners interested in exploring the utility of bioremediation to have a local service equivalent to Microbial Insights. For this reason in 2011 I established an Australian business (Micronovo) offering equivalent services with the same technology and the same commitment to scientific rigor. The principal differences were that there was no need to transport samples on ice to the US and that I could share pleasure or displeasure about the state of Australian cricket. Microbial Insights have since established an Australian office and the two companies enjoy healthy competition and often collaboration all to the benefit of the Australian remediation community. This has led to broader application of bioremediation in Australia and all the associated benefits such as reduced site remediation costs.

Bioaugmentation cultures

The biodegradation of chlorinated aliphatic hydrocarbons in the subsurface environment is dependent on having the right environmental conditions (low redox, neutral pH), plenty of reduced organic carbon to supply hydrogen and acetate through fermentation and the right bacteria. If the right bacteria are undetectable or at low abundance, the biodegradation process can

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be given a giddy-up by injecting laboratory grown bacteria into the subsurface. The practice is relatively simple. Bacterial cultures (100 L) are delivered to site in 20 L stainless steel kegs after 1-2 months in production and mixed in an IBC with 900 L of groundwater extracted from a contaminated area. The mixture is then gravity fed back into the aquifer. If the conditions suit the bacteria, they will proliferate and this biologically active groundwater can be moved to other locations on site.

In North America cultures such a KB-1 supplied by the Canadian company Sirem have been applied to chlorinated ethene contaminated sites far and wide. The Australian Quarantine and Inspection Service (AQIS) police the importation of microorganisms into Australia. In academia we acquire import permits for pure bacterial strains for research purposes and avoid the importation of mixed species samples of microorganisms (such as bioaugmentation cultures) owing to the strict requirements of AQIS, including making a guarantee that the imported organisms will not leave controlled quarantine laboratories.

To negotiate this limitation my research team at UNSW have developed chlorinated ethene, chlorinated ethane, and chlorinated methane degrading bacterial cultures from local environmental samples enabling application without the international quarantine wrangling. These cultures are commercially available through Micronovo in a business model that represents a hybrid between Sirem and Microbial Insights. To date, the vast majority of applications have been with the chlorinated ethene degrading culture (AusPCE) which can convert perchloroethene, trichloroethene, 1,2-cis-dichloroethene and vinyl chloride to ethene under anaerobic conditions. These applications have predominantly taken place in Victoria under regulatory guidance from the Victorian EPA. The first of these was at an Orica legacy site in Deer Park, representing the first commercial deployment of organochlorine respiring bacteria on the Australian continent. In 2017 Micronovo will, for the first time, deliver culture to Tasmania. The availability of these cultures has further enabled application of *in situ* bioremediation in Australia.

Closing remarks

How any organization or community practices anything evolves over time partly out of necessity and partly taking inspiration from others. My impression is that remediation practitioners and regulators in Australia, though initially cautious, are developing an appetite to explore in situ bioremediation as an option for contaminated site remediation owing to the obvious benefits with respect to cost and sustainability. Of course, bioremediation will not always be applicable. Complex sites with hostile conditions such as extremes of pH or otherworldly contaminant mass loads tend to be off limits. But where it can be applied, it should be applied. For the sake of the environment and the competitiveness of what Australian industry remains on shore. ■

Conflict of interest declaration: Associate Professor Mike Manefield is the founder and former director of Micronovo Pty Ltd. Micronovo Pty Ltd was incubated as a biotech start-up at the University of NSW, Sydney, Australia (2012-2016) and now trades independently as Novorem Pty Ltd.