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Culture

Microbicides against emerging and new pathogens: a cause for concern

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Introduction

Examples of the use of microbicides can be traced back to ancient times when natural products were used to combat infection or to preserve mummies, and when metals such as silver were used to decontaminate potable water. Fumigation was introduced much later with, for example, the burning of juniper branches to decontaminate buildings where Black Death sufferers were housed, and later in the trenches during the First World War. The 'modern' use of microbicides to combat microbial infections probably dates from the 19th century, when pharmacists and medics experimented with hand hygiene and wound dressings with tremendous success. The 20th century witnessed an explosion in the diversity and use of microbicides as preservatives of pharmaceutical, medical and food products, as disinfectants and antiseptics, and in the plastics and textile industries (Figure 1). Today, public awareness of the role of microbial contamination in infection and spoilage has served as a springboard for the commercialisation of numerous products containing microbicides. As a result the market for microbicides is buoyant and competitive, although the European Scientific Committee for Emerging and Newly Identified Health Risks (SCENIHR 2009) recommended prudent use of microbicides. This is particularly relevant with the tightening control over microbicides allowed on the European market following the introduction and implementation of the Biocide Product Directive 1998 (BPD) and, more recently, the Registration, Evaluation and Authorisation of Chemicals Regulations 2006 (REACH).

Although the uses and applications of microbicides have increased tremendously over the last 10 years, their interactions with microbial cells remain poorly understood. This review addresses some of the issues in the light of emerging and re-emerging human pathogens.

Figure 1
Today's use of microbicides. Microbicides are extensively used nowadays and they can be divided into three main applications: industrial, domestic and medical/pharmaceutical. Many microbicides of the same type will be used for multiple purposes.

Activity of microbicides

Empirically, microbicides have been described to have multiple targets against microbial cells; the number of targets damaged and the severity of damage produces either a lethal or a reversible static effect (Maillard 2002). It is now thought that such an effect is concentration dependent, whereby at a low concentration more specific interactions against defined bacterial targets might occur. This has been exemplified extensively with the bisphenol triclosan, which interacts specifically with an enoyl acyl reductase protein in bacteria at a low concentration, whereas at a higher concentration, non-specific membrane damage is likely to occur. Triclosan is unique at present as it is the most widely studied microbicide. With other microbicides such as chlorhexidine and isothiazolinones, specific targets have been reported, although there is an overall dearth of information on this subject (Maillard 2002).

The concentration of a microbicide is key to delivering a microbicidal effect (McDonnell and Russell 1999), but it is not the only factor that will affect the efficacy of a given microbicide (Maillard 2005). The nature of different microorganisms also needs to be taken into consideration. This was recognised in the Spaulding's classification (Figure 2), which provide an indication of the intrinsic

resistance of a microorganism to microbicides. Among the least susceptible are prions and bacterial endospores, while the least resistant are enveloped viruses. Although there are exceptions, this classification provides useful information about the susceptibility of different microorganisms and the types of microbicides that are required to kill them. It does not, however, take into consideration the response of a given microorganism to a microbicide in terms of decreased susceptibility and it does not cover activity against microbial biofilms (Maillard and Denyer 2009).

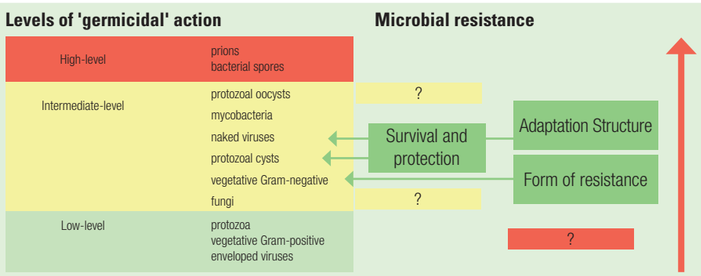

Figure 2
Spaulding's classification. The level of microbicidal action is divided into three levels of activity and it provides information on the susceptibility of microorganisms. For some microorganisms such as protozoal oocysts and fungi, information is scarce or not available. The effect of microbicide on the microbial cell or in exerting a selective pressure might alter the classification (indicated with the green boxes and the red box with a ?).

Intrinsic mechanisms conferring resistance to microbicides

The intrinsic properties of microorganisms are interesting and deserve explanation. Apart from the prions, which are proteinaceous in nature and, as such, not considered to be microorganisms but infectious agents that can self replicate, the most resistant form of a microorganism is the bacterial endospore. Sporulation is a well-described mechanism by which certain bacteria, of the *Clostridium* and *Bacillus* genera, can survive detrimental conditions (for example lack of nutrients) and detrimental physical and chemical factors, including exposure to microbicides. It is well recognised that the mechanisms of resistance of bacterial endospores reside in their structure, notably the presence of spore coats, cortex, a highly compressed inner membrane and the presence of small acid-soluble proteins that protect the spore nucleic acid in the core from oxidising damage. In addition, the spore is dehydrated and the concentration of Ca^{2+} in the core is high, conferring resistance to heat (Leggett *et al.* 2012). It should be noted that there are differences in susceptibility/resistance of endospores to microbicides (Maillard 2011).

The other forms of microbial resistance to microbicides are not that dissimilar to the formation of endospores. For example, the formation of protozoal cysts confers resistance of amoebae to microbicides and their presence, notably in water treatment, might be responsible for the seasonal variation of a number of waterborne diseases (Thomas *et al.* 2010). Amoebal cysts are also responsible for the failure of disinfection treatment of contact lenses and their survival in cleaning and disinfecting solution is responsible for diseases such as amoebic keratitis (Thomas *et al.* 2010). There is little information on the process of encystation and the resulting decrease in susceptibility to microbicides. However, it is clear that cysts possess a cell wall and are highly dehydrated (Figure 3). Unfortunately, it has now been shown that protozoa can support the growth of a number of intracellular pathogens (bacteria and viruses) and that protozoal cysts can protect bacteria from a microbicide effect (Thomas *et al.* 2010).

Interestingly, it has recently been reported that there are differences in microbicidal susceptibility of amoeba cysts depending on their origin, with cysts isolated from the hospital environment being the least susceptible. It is inferred that the decreased susceptibility of these cysts could be associated with the regular use of microbicides in such an environment (Coulon *et al.* 2010). Conversely, an increase in bacterial endospore resistance to microbicide has not been reported, probably due to the lack of a specific study.

The microbial structures (endospores and cysts) that confer resistance to microbicides have been well documented and it might be bold but interesting to draw parallels to specific structures in vegetative bacteria. In *Vibrio* spp., the formation of rugose forms, which differ markedly from the usual bacterial cell appearance (Figure 3) results from a change in environmental conditions (nutrient availability). In *V. cholerae*, rugose variants have been associated with a marked decrease in susceptibility to chlorination (Yildiz and Schoolnik 1999). The exact microbial mechanisms that confer such resistance to chlorine have not been studied, but the appearance of rugose variants, shorter, round cells, is interesting. It is worthy to note that rugose-colony variants are also better biofilm producers (Yildiz and Schoolnik 1999).

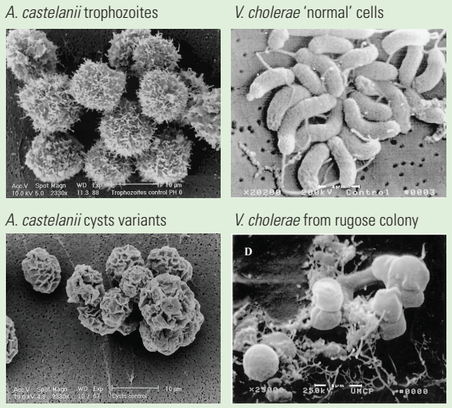

Figure 3
Morphology of *Vibrio cholerae* cells from normal colony and a rugose-colony variant (from Chaiyanan *et al.* 2001) and trophozoites and cysts of *Acanthamoeba castelanii* (from Khunkitti *et al.* 1998).

The appearance of different colony morphotypes and bacterial cell structures following changes in environmental conditions has been described in other bacterial species such as *Staphylococcus aureus, Burkholderia* spp. and *Clostridium* spp. It is also worth noting that bacterial colony appearance following microbicide exposure sometimes changes; often small colonies are observed. This phenomenon has been associated with the presence of damaged bacterial cells that are recovering from microbicide challenge. In *S. aureus*, small colony variants have been associated with decreased susceptibility to triclosan (Bayston *et al.* 2007). Although it would be tempting, at this time, we cannot draw conclusions on the association of different bacterial colony morphotypes with a change in microbicide susceptibility. A better understanding of these variants is needed, but the observations are interesting.

In addition to their intrinsic properties, microorganisms can acquire new properties enabling them to survive microbicide exposure. With the number of commercially available products or applications that make use of a low microbicide concentration, there is a concern that bacterial exposure to such a product, might promote the survival of microorganisms that have a reduced susceptibility to antimicrobials, including antibiotics [Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), 2009, 2010; Scientific Committee on Consumer Safety (SCCS, 2010)]. The ability of a microorganism to survive microbicide exposure means that disinfection, preservation or antisepsis will fail. Persistence of microorganisms in controlled environments (food factories, healthcare facilities) is likely to occur (Maillard 2011).

The importance of microbial biofilms must be mentioned here. It is recognised that bacterial biofilms occur widely in the environment. Furthermore, it is well accepted that bacteria in a biofilm (i.e. sessile bacteria) are far less susceptible to antimicrobials than planktonic bacteria. A number of mechanisms have been proposed to explain this difference in susceptibility. These mechanisms include differential diffusion, low metabolism, a "mopping up" effect, expression of detoxifying enzymes and efflux (Maillard and Denyer 2009). It is worth noting that standard microbicide efficacy tests do not include the measurement of microbicide efficacy against bacterial biofilms (Maillard and Denyer 2009).

Evidence of bacterial pathogens surviving biocidal exposure in practice

There are examples of microorganisms surviving microbicide exposure resulting in outbreaks or pseudo-outbreaks. A very useful paper is that of Rutala and Weber which reviews the scientific literature describing survival of bacteria following exposure mainly to antiseptics but also to disinfectants, and the development subsequently of infections (Weber and Rutala 2007). An earlier example of microbicide usage and survival of the target bacteria is provided with the use of silver nitrate (ionic silver) in wound dressings to combat *Pseudomonas aeruginosa* infections (Cason *et al.* 1966). Although the incorporation of silver achieved the control of pseudomonads, this paper provides information on emerging *Ps. aeruginosa* strains resistant to silver and the alteration in the commensal flora following the use of silver in the dressings. This paper provides an early example of the selective effect caused by microbicides, a concept that will be developed later. With the increasing use of silver and nano silver in numerous consumer applications, there are questions about the safety and consequences of the extensive use of such a microbicide (Maillard and Hartemann 2012).

One of the most recent examples of association between microbicide and bacterial outbreaks comes from a study from Brazil where outbreaks of *Mycobacterium massiliense* were reported in 35 hospitals (Duarte *et al.* 2009). The bacterial clone responsible for the outbreaks is resistant to glutaraldehyde, a microbicide use for the disinfection of heat-labile medical equipment, and also is resistant to frontline antimycobacterial antibiotics. Although it is unclear that the clone originated from the reprocessing of medical equipment, cross-resistance between a microbicide and antibiotics warrants further investigation.

There is a view that emerging resistance in microorganisms arises mainly from the use of less reactive microbicides such as quarternary ammonium compounds (QAC), biguanides and phenolics, compared to oxidising agents and alkylating microbicides. Although questions have been asked about the use of some microbicides such as QAC at a low concentration, emerging resistance to alkylating and oxidising microbicides has been reported, notably in microorganisms originating from the healthcare environment. For example, *Mycobacterium chelonae* resistant to glutaraldehyde but also to other unrelated microbicides such as sodium dichloroisocyanurate (NaDCC) and Virkon® has been described (Griffith *et al.* 1997). Likewise, Martin *et al.* (2008) reported the isolation of a vegetative *Bacillus subtilis* strain cross-resistant to chlorine dioxide and hydrogen peroxide at their in-use concentrations or above. This isolate was also found to be resistant to peracetic acid and to be an excellent biofilm producer.

Overall, survival of microorganisms, in particular bacteria (which have been the most studied), in microbicidal products or following microbicide exposure has not been well reported and there has been a call to set up microbicide susceptibility/resistance surveillance, at least in the healthcare environment (Cookson 2005, Maillard and Denyer 2009).

Interestingly, Ciusa *et al.* (2012) have recently reported the susceptibility/resistance profiles of 1388 environmental isolates of *Staphylococcus aureus* to triclosan. There were differences in profile between laboratory strains and the environmental isolates, but more importantly, a mutated *fabI* gene region conferring on the bacteria resistance to triclosan differed between these two types of strains. This study confirmed in a sense that the making of laboratory isolates resistant to specific microbicides is not always easy (Walsh *et al.* 2003) and might not reflect exposure to microbicides in practice (Maillard and Denyer 2009). This reinforces the concept of studying environmental isolates for their resistance mechanisms (and hence surveillance reporting) rather than laboratory strains.

Mechanisms of bacterial response to microbicides

Empirically, bacterial mechanisms conferring resistance to microbicides have been divided into intrinsic and acquired resistances. With the increasing number of microbicide applications, notably in consumer products, such division requires refining, together with the definition of resistance. Thus, in recent years the term 'reduced susceptibility', which refers to an increase in the minimum bactericidal concentration, has been used. It is also now accepted that the use of minimum inhibitory concentration can only provide limited information towards a susceptibility trend, but does not inform the resistance of a given bacterium to a microbicide. A useful practical definition of bacterial resistance to microbicides refers to the survival of bacteria in a product containing the in-use microbicide concentration.

Over the years a number of mechanisms that decrease the susceptibility of a bacterium to a microbicide have been described (Maillard and Denyer 2009). These mechanisms aim to reduce the amount of a microbicide interacting with the cell and encompass a decrease in microbicide penetration, for example, with a change in outer cell membrane (lipopolysaccharide, porins), or a reduction of

the microbicide concentration through the expression of efflux pumps, or both, accompanied by the expression of detoxifying enzymes. More specific mechanisms have been described following the study of triclosan. Triclosan, so far, is unique amongst microbicides, since, at a low concentration, it has a specific mechanism of action targeting the enoyl acyl carrier protein that is involved in fatty acid synthesis. Changes in the triclosan target or a change in the metabolic process provides bacterial resistance to triclosan. These mechanisms have usually only been described following the use of antibiotics.

Because of their nature, microbicides exert a selective pressure on microbial cells and hence will select for the least susceptible population. This is true when pure cultures or mixed bacterial populations are concerned. There is much debate at the moment on this selective effect of microbicides and its implication in practice. The fitness of less susceptible bacteria following microbicide exposure seems to depend upon the mechanisms of resistance expressed. Some studies show that surviving microbicide exposure is costly for the bacterium, which reverts to being susceptible in the absence of microbicide. Other studies demonstrated that the mechanisms expressed are constitutive, with no detriment to bacterial competitiveness or virulence.

The real concern is the effect of microbicide exposure on the maintenance of transfer of resistance genes between bacteria (SCENIHR 2010). There is evidence that microbicides may be involved in horizontal gene transfer, but overall this has not been well studied and evidence is still lacking.

Conclusion

The use of microbicides is essential to prevent contamination and infection. They are becoming a limited resource following imposition of stricter regulations, at least in Europe. As with antibiotic misuse and abuse, there is a risk that improper and extensive use of microbicides may result in emerging resistance and cross-resistance in bacteria. There is already evidence emerging from the peer-reviewed literature that resistant bacteria have been isolated and associated with outbreaks and pseudo-outbreaks. However, examples remain anecdotal since the susceptibility of bacterial isolates is not regularly investigated. Indeed, to date, a call for a surveillance programme has been ignored, probably because of the financial resources that would be needed.

The application of microbicides at a sub-lethal concentration is of particular concern, since laboratory studies have demonstrated beyond doubt the ability of bacteria to express mechanisms enabling them to survive a microbicide insult. Mutations in bacteria (an acquired resistance mechanism) have also been under-studied over the years, although recently the association between expression of efflux and mutation rate has been reported.

In conclusion, microbicide interactions with micro-organisms remain a fascinating subject with practical applications. Although research activity has increased over the last 10 years, the subject warrants further investment considering the extensive use of microbicides today.

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Bioremediation of hydrocarbon contaminated sites: an industry-academic perspective

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Introduction

Most of us have witnessed as a result of extensive media coverage the environmental impact of petroleum hydrocarbon spills. For example, the Exxon Valdez oil spill of 1989 and the BP Deep Horizon spill of 2010, both of which are covered in a review by Atlas and Hazen (2011). Thankfully hydrocarbon spill events of this magnitude don't happen every day, however, contamination is widespread in both marine and terrestrial environments.

Terrestrial sites in particular are contaminated with hydrocarbons as a result of a wide variety of industrial uses which include metal processing works, gasworks, oil refineries, and fuel depots. An example of a contaminated gasworks site undergoing remedial works is shown opposite (Figure 1).

Traditionally, disposal to landfill ('dig and dump') has been the main approach for dealing with contaminated waste material arising from brownfield sites, however, legislative and financial drivers (such as the annually-rising landfill tax) are making this an increasingly less desirable option. This article gives an overview of the current status on the use of bioremediation for treating hydrocarbon contamination in soil (and to a lesser extent groundwater with which it is usually associated) as well as some future research directions.

Bioremediation process

Bioremediation involves the use of microorganisms or plants (the latter usually specifically referred to as phytoremediation) to mitigate risk posed by contaminants to the environment and/or human health. When compared to traditional 'dig and dump', and more modern chemical and physical based remediation techniques (e.g. chemical oxidation, thermal desorption), bioremediation can be a cost effective and efficient technique. Although costs and timescales are site specific, some publicised US based cost ranges are shown (Table 1).

Microorganisms can treat a wide range of hydrocarbon contaminants through biodegradation; either mineralisation where the contaminant is used as a primary food source or, cometabolism where contaminant biodegradation is a beneficial side-effect of other metabolic processes. Hydrocarbon contaminants treatable by bioremediation include not only crude oils and refined petroleum products but also solvents (including a number of chlorinated hydrocarbons), phenols, glycols, surfactants, pesticides and explosives.

Monitored natural attenuation

Natural attenuation occurs in the environment through a number of mechanisms including biodegradation, dispersion, dilution, sorption and various chemical reactions (Declercq *et al.*, 2012). Of these mechanisms, biodegradation is considered the primary mechanism for attenuation. Therefore, natural attenuation can be a bioremediation approach when a formally managed monitoring programme is used and aligned with site remediation objectives.

Figure 1
Excavation of contaminated soil from a gasworks holder tank located in a residential setting (courtesy of VHE Construction Plc).

Table 1
Common soil remediation techniques and associated costs

Remediation technique	Cost ($/m³)	
	'Small' site	'Large' site
Dig and dump	600-1020	
Thermal desorption	81-252	44-110
Soil washing	187	90
Chemical oxidation	190-660	
Bioremediation	80-260	
Stabilisation/solidification	216-248	124-190

Adapted from information available on the US Federal Remediation Technology Roundtable (FRTR) screening matrix and reference guide website (http://www.frtr.gov/matrix2/top_page.html).

The programme will obviously rely on contaminant chemical data to monitor progress; however, supporting lines of evidence are required to demonstrate conclusively that biodegradation as a mechanism is responsible. Towards achieving this, molecular biology techniques such as quantitative polymerase chain reaction (qPCR) are increasingly being used to confirm presence/absence of specific organisms or functional traits as well as characterise differences in an indigenous microbial community with time and space within contaminated sites. The qPCR technique in particular has been applied to benzene, toluene, ethylbenzene and xylenes (BTEX) and phenol monoaromatic compounds, as well as, alkanes and low molecular weight polyaromatic hydrocarbon contaminated sites (Baldwin *et al.*, 2008; Sagarkar *et al.*, 2013). The development of molecular biology techniques such as qPCR help to minimise the cost of monitoring programmes, making monitored natural attenuation (MNA) an increasingly used technique compared to engineered

bioremediation approaches, especially in the current economic climate where brownfield sites are generally turned over more slowly.

Engineered bioremediation approaches

As stated above, passive approaches such as MNA can be slow particularly for developer-driven remediation projects. As such, human intervention in the form of engineered approaches is more often than not required to accelerate bioremediation processes in a controlled manner.

The two engineered approaches to remediate contaminated soil and groundwater are to either treat it '*in situ*' in the ground or, '*ex situ*' following excavation (soil) or pumping (groundwater). *In situ* remediation can address both unsaturated (above groundwater level) and saturated (below groundwater level) environments. *Ex situ* remediation can occur both on the site of origin or by moving contaminated soil/water to an alternative site for treatment. A summary of *in situ* and *ex situ* soil and groundwater approaches is shown in Table 2. These engineering approaches are in the main designed to introduce air (oxygen) into the contaminated matrix to promote aerobic biodegradation because although many organic contaminants can be degraded anaerobically, the rates of reaction are far less favourable. For example, the half life of benzene under aerobic conditions is a few days rather than years as is the case under anaerobic conditions. An example of soil arranged as windrows and aerated using an excavator mounted processing bucket is shown (Figure 2).

Table 2
Summary of bioremediation approaches for soils and groundwater

	Soil	Groundwater
In situ	Bioventing (unsaturated) Biosparging (saturated)	Biosparging
Ex situ	Landfarming Windrow Biopile Soil slurry bioreactor	Bioreactor

Figure 2
Soil arranged in windrows being aerated using an excavator mounted processing bucket (courtesy of VHE Construction Plc).

Although *in situ* remediation is concerned solely with mitigating risk, and the protection of human or environmental receptors, there are several options available once soil is excavated, or groundwater pumped, using *ex situ* remediation. For soil, if not treated for re-use onsite to specific risk assessment criteria, it can be treated offsite at a soil treatment centre (or hub site) leading to site re-use, or treated for waste reclassification (due to becoming a waste following removal from the ground) prior to disposal. Options for groundwater include treatment for disposal to foul sewer, or tankering offsite for treatment and subsequent disposal.

Environmental factors limiting biodegradation in soils

Although the main factor limiting (aerobic) bioremediation of hydrocarbon contaminated soils is usually oxygen supply, various other factors should be considered (and where appropriate optimised) before proceeding with a bioremediation treatment programme. A number of key factors are presented below (Table 3).

Table 3
Summary of bioremediation approaches for soils and groundwater

Factor	Optimal conditions for hydrocarbon bioremediation
Soil pH	≥ 6 pH ≤ 8
Soil moisture content	40 – 85 % field capacity
Nutrient content (C:N:P)	100:10:1
Temperature	10 – 45 °C
Organic contaminants	< 50,000 ppm
Heavy metals	< 2,500 ppm
Soil texture	Minimal clay content

In the case of nutrient content especially, although hydrocarbon contamination results in an influx of carbon into the system, nitrogen is often found to be the limiting essential nutrient. Therefore, the addition of nitrogen rich nutrients is an effective way to enhance engineered bioremediation approaches (Hollender *et al.*, 2003). The need for nitrogen amendment can be estimated theoretically based on known microbial cell requirements for nitrogen relative to carbon (C:N of 10:1), however, error can be made in determining the relative biodegradability of the carbon present as well as bioavailability of both carbon and nitrogen. Such errors can lead to under performance of the system or unnecessary expense.

A more precise approach involves determining practically the requirements of the soil microbial community for optimal aerobic biodegradation by stimulating microbial activity in site derived soil samples as part of a treatability testing programme (Aspray *et al.*, 2007). The latter approach avoids uncertainty in the composition of carbon and the bioavailability of nutrients already present in the soil. Methods to assess microbial activity in soils are readily available including those based on molecular biology, culture-based and enzyme assays; however, their performance in the application to biodegradation of hydrocarbon contamination in soil is variable. Moreover, these techniques can be labour intensive and expensive in assay reagents for practitioners to determine in-house. Metabolic gas respirometry on the other hand is a straightforward technique which involves measuring either O_2 consumption or CO_2 production from soil to determine the activity of the microbial community present. Soil microbial activity can subsequently be optimised by determining the nutrient concentration which leads to significant increase in activity.

Further, by monitoring CO_2 and O_2 simultaneously it is possible to observe changes in the overall metabolic processes occurring in the soils (Aspray *et al.*, 2008).

To enhance *ex situ* soil bioremediation, exogenous nitrogen can be added as an inorganic nutrient in the form of agricultural fertilisers, or organic materials such as poultry litter, spent mushroom compost, horse manure, green and food waste derived composts (Atagana, 2004; Semple *et al.*, 2001) and even anaerobic digestate. There are numerous potential advantages of using organic materials over inorganic fertilisers for *ex situ* soil bioremediation; besides being cheap sources of nitrogen. In many cases they can enhance soil porosity and aid oxygen diffusion. In addition, as slow-release nutrient sources the possibility of external ecosystem contamination from nutrient leaching is minimal (Sarkar *et al.*, 2005). Finally, organic (waste derived) materials have enormous added potential for bioremediation, as they are capable of sustaining diverse populations of microorganisms, which themselves have the potential to degrade hydrocarbon contaminants. Although organic materials have been successfully applied for bioremediation of hydrocarbon contaminated soils, further research is needed to optimise the approach and understand the benefits of the introduced microorganisms.

Soil biostimulation and bioaugmentation

As discussed, biodegradation of hydrocarbon contaminants in soil and groundwater can usually be enhanced by addition of nutrients, a process termed biostimulation. If the indigenous microorganisms do not have the appropriate metabolic capability, then microbial cultures can be added (bioaugmentation). Although there have been many studies comparing biostimulation and bioaugmentation for petroleum hydrocarbon contaminated soils (Margesin and Schinner, 2001; Evans *et al.*, 2004; Bento *et al.*, 2005), many bioremediation practitioners would argue that bioaugmentation is not needed for these contaminants because metabolic capability to degrade them is ubiquitously present. Furthermore, the culturing of microorganisms on such a large scale may become cost prohibitive making this a less attractive option. However, there is good evidence for continuing research on bioaugmentation strategies in other areas such as for the bioremediation of xenobiotic contaminants (those synthesised by humans) including (chlorinated) solvents, pesticides and explosives where metabolic capability may be limited or lacking in the environment. Crucially the nature of contaminants such as these is that they are generally bioavailable and hence bioaugmentation in such cases would enhance bioremediation. Some examples of component degraders of solvents, pesticides and explosives are shown (Table 4).

Table 4
Example contaminant degrader microorganisms

Microorganism	Contaminant type	Example
Pseudomonas sp. strain ADP	Nitroaromatic	Atrazine
Wautersia eutropha JMP134 (pJP4)	Chloroaromatic	2,4-dichlorophenoxyacetic acid
Dehalococcoides sp. strain BAV1	Chlorinated solvent	Tetrachloroethene (PCE)
Phanerochaete chrysosporium	Nitroaromatic	2,4,6-trinitrotoluene (TNT)
Rhodococcus sp. NJUST16	Nitroaromatic	2,4,6-trinitrophenol

Contaminant bioavailability in soils

Bioavailability is a term becoming increasingly important for bioremediation practitioners, as it relates to the ability to be able to predict soil bioremediation endpoints. In short, if hydrocarbon contaminants are not bioavailable, microorganisms (whether indigenous or introduced) will not be able to degrade them. Therefore, the development of assays which can determine the bioavailable fraction of particular contaminants, rather than the total amount present (exhaustively extracted with harsh solvents), are potentially valuable. This is especially the case for polyaromatic hydrocarbons (PAHs) which often require their own separate human health risk assessment remediation criteria from petroleum hydrocarbon fractions. Towards this end, significant research has been made for PAH contaminants in developing cyclodextrin based (non-exhaustive) extraction assays. Results of this work have been shown to correlate with biodegradable fractions (Hickman *et al.*, 2008). However, as PAH contaminated sites usually also require clean up of petroleum hydrocarbon contaminants, further work is needed in this area for remediation practitioners to be able to use such tests as an indicator of the suitability for using bioremediation at a particular site.

Hydrocarbon contaminated soil function; soil quality and treatability testing

The ability of soils to provide ecosystem and social services can be affected by hydrocarbon contamination. As such, there is currently increasing interest in the remediation of contaminated soils to not only reduce pollution but also restore soil quality (function). Given the aggressive nature of many chemical and physical based remediation techniques, this is another potential advantage of bioremediation.

In terms of monitoring soil quality, a number of biological indicators have been proposed and applied to agricultural, forestry and to a lesser extent contaminated soils (Pietravalle and Aspray, 2013). One popular biological indicator method is multiple substrate induced respiration (MSIR) where soil functional diversity is assessed by measuring the response of a soil to a number of individual carbon sources (substrates) and monitoring respiration response. In addition to assessing the restoration of contaminated soils, it has been suggested that MSIR assays may provide useful information prior to starting bioremediation projects (Shi *et al.*, 2005). At this time, further research is needed on such assays for soil quality determination and treatability testing.

Conclusions

Although there remains a degree of uncertainty for remediation practitioners in the decision to adopt bioremediation over traditional dig and dump, fundamental research on hydrocarbon biodegradation and more recent development of bespoke assays (respiration and bioavailability) has helped in managing the risk. With alternatives requiring use of harsh and costly chemicals (chemical oxidation), energy (thermal desorption) or generating waste residue (soil washing) the future remains bright for bioremediation. The main challenge for bioremediation in the future will be to apply the processes to increasingly complex contaminants as well as higher concentrations and mixed contaminant scenarios.

About the author

Thomas has more than 10 years' academic/applied research and industrial experience in the environmental sector. An environmental microbiologist by formal training he has expertise in general microbiology, molecular biology and analytical chemistry. Thomas

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