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Emerging technologies in bioremediation: constraints and opportunities

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Abstract Intensive industrialisation, inadequate disposal, large-scale manufacturing activities and leaks of organic compounds have resulted in long-term persistent sources of contamination of soil and groundwater. This is a major environmental, policy and health issue because of adverse effects of contaminants on humans and ecosystems. Current technologies for remediation of contaminated sites include chemical and physical remediation, incineration and bioremediation. With recent advancements, bioremediation offers an environmentally friendly, economically viable and socially acceptable option to remove contaminants from the environment. Three main approaches of bioremediation include use of microbes, plants and enzymatic remediation. All three approaches have been used with some success but are limited by various confounding factors. In this paper, we provide a brief overview on the approaches, their limitations and highlights emerging technologies that have potential to revolutionise the enzymatic and plant-based bioremediation approaches.

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Introduction

Intensive industrialisation and large-scale use of synthetic xenobiotic compounds have generated hazardous contaminants including organics, inorganics and heavy metals. These contaminants create numerous environmental problems including harmful impact on biogeochemical cycling, environmental health and toxic effects onto non-target organisms including humans (Singh 2009). Therefore, decontaminating our environment is a major policy priority in most developed countries. Current technologies for remediation of contaminated sites include solidification/stabilization, soil vapour extraction, incineration, bioremediation, solvent extraction, chemical treatments. However, these conventional physicochemical approaches are generally expensive and remediation process is often incomplete due to the conversion of the parent compound to metabolites which are more persistent and equally or more toxic to non-target organisms. On the other hand, bioremediation offers an environmentally friendly and economically feasible option to remove contaminants from the environment (Singh and Walker 2006). Because bioremediation

exploits the natural ability of plants and microbes for decontamination, organic pollutants in theory can be completely mineralised into water and carbon dioxide. Previously, such approaches have produced success (Mulbry et al. 1998; Strong et al. 2000) and failures (Blasco et al. 1995). The latter have been attributed to low competitiveness and adaptability of the microbial inocula, reduced bioavailability of the target pollutants, inappropriate inoculation procedures (Megharaj et al. 2011). Detailed and critical reviews on bioremediation have been recently published (Gulmaraes et al. 2010; Megharaj et al. 2011). Consequently the scope of this paper excludes in depth-literature reviews or methodological details. Instead, our aim is to highlight critical aspects of microbial bioremediation and novel approaches to overcome the current limitations including phytoremediation and plant–microbe combination systems. In addition, the utilization of bioremediation technologies within the wider context of 'green biotechnology' applications is discussed.

Microbial bioremediation

Microorganisms are ubiquitous, the most abundant and diverse group of organisms on Earth (Curtis et al. 2002; Singh et al. 2009). They are known to possess highly versatile and effective metabolic systems/catalytic mechanisms (Paul et al. 2005) capable of degrading and utilizing various toxic compounds as an energy source for growth (Watanabe 2001). Depending on the biochemical environment where the biodegradation of a pollutant occurs, the metabolic processes can be broadly categorised as aerobic and anaerobic. In aerobic biodegradation, micro-organisms use oxygen as the final electron acceptor, while in anaerobic degradation, the conversion of the parent compound is performed by microorganisms in the absence of oxygen. In anaerobic biotransformation, manganese and iron ions and substances such as sulphur, sulphate, nitrate and carbon dioxide can act as electron acceptors in place of oxygen. Nonetheless, some poly-halogenated organic compounds like polychlorinated biphenyls (PCBs) and dibenzodioxins, trichloroethylene and carbon tetrachloride are relatively resistant to microbial biodegradation. Additionally, some of these compounds are degraded by co-metabolism, where microorganisms transform a substance without being able to utilize the energy derived from the degradation process (Gulmaraes et al. 2010).

Bioremediation approaches can be broadly classified to *ex situ* or in situ (Hatzinger et al. 2002). In situ techniques are defined as those applied to soil and groundwater at the contaminated site with minimal disturbance. *Ex situ* techniques involve excavation or pumping in case of soil and water, respectively, of the contaminated substrate and placement in a contained area where bioremediation is employed. There have been several examples of successful utilisation of either *ex situ* or in situ microbial bioremediation of organic contaminants. In an *ex situ* application of microbial bioremediation, filter bioreactors with a consortium of microbes was developed and used successfully to degrade up to 15,000 L of coumaphos-containing cattle-dip, an organophophorus pesticide used widely for quarantine purposes in cattle industries (Mulbry et al. 1998). Two such units have been operational since 1996 in the USA. Similarly Strong et al. (2000) reported the successful bioremediation of a soil heavily contaminated with high concentrations of the herbicide atrazine (up to 29,000 ppm) using a combination of biostimulation (via soil amendment with 300 ppm of phosphate) and inoculation with killed, recombinant *E. coli* cells encapsulating atrazine chlorohydrolase ATZA. The recovery of the Gulf of Mexico by the Deepwater Horizon blowout is a recent example of in situ bioremediation based on natural attenuation. In this instance no bioaugmentation with exogenous oil-degrading bacteria was needed since the microflora of the deep sea ecosystem was quickly adapted to oil contamination and became dominated by bacteria of the order Oceanospirillales in the γ-Proteobacteria which included known psychrophilic hydrocarbon degraders and microorganisms from hydrocarbon-dominated environments (Hazen et al. 2010).

Bioremediation could result in the complete decomposition of the chemicals or it can be coupled with alternative treatment technologies in cases were mixed and complex wastes should be removed (McMahon et al. 2008; Yergeau et al. 2009). For example electro-bioremediation, a hybrid technology of bioremediation and electrokinetics, have been used for the treatment of hydrophobic organic compounds (Li et al. 2010). However, the use of living microbes for bioremediation has inherent constraints, which

include the need for continuous supply of fresh inocula, aeration and nutritional supplements (Singh and Walker 2006). These limitations encouraged industries and researchers to explore the potential of using microbial enzymes instead of whole cells in bioremediation (Karns et al. 1998). For example, organophosphorus-hydrolysing enzymes isolated from a soil bacterium are commercially available and used for the detoxification of diazinon-containing sheep-dip wastes (Scott et al. 2011; Singh 2009). Potentially, degrading enzymes could be an effective tool in remediation technology, however, for large scale success, this approach needs to overcome three major constraints: (1) Lack of enzyme diversity which is a key to select the most suitable enzymes for bioremediation based on the chemical structure of pollutants and characteristics of impacted sites. Currently, the bioremediation process is carried out within constraints of available enzymes which results into suboptimal efficiency. (2) Lack of reliability and high cost associated with enzyme production making such an approach less attractive commercially. (3) Low stability of the formulated enzymes due to mechanical and biotic stresses which reduce the efficacy of the process (Singh 2010). For example, enzymes efficacy is compromised due to loss of their three-dimensional structure in bioreactors where mechanical stress condition dominate the process, while in soil/water ecosystems, their efficacy is compromised because of protease activities and non-reversal binding onto humic and clay particle in the soil.

Emerging approaches for microbial and enzymatic bioremediation

In recent years, a number of technological advancements have overcome some of the above constraints leading to improve reliability, cost efficiency and speed of bioremediation. These methods range from mere monitoring and improvement of intrinsic bioremediation to novel ideas of genetically engineering the functional genes for bioremediation application. Natural organisms exhibit evolutionary capabilities to adapt to a wide range of chemicals but natural adaptation occurs at a relatively slow rate. Also, for some xenobiotics no degradation routes have been described and for others incomplete/inefficient transformation leads to production and often accumulation of complex mixtures of contaminants that resists further degradation by existing pathways (Jones and de Voogt 1999). Given that the bioremediation rates of microbes in nature are often considerably slower mainly due to extreme environmental conditions (oligotrophy, soil structure, moisture), new engineering tools are gaining momentum in research and showing much promise to improve the performance of bioremediation process. A brief overview of such important tools follows.

Metagenomics

Environmental microbes constitute an important source of genetic material with biotechnological interest and applications across all major industries including remediation. Unfortunately, more than 99 % of microbes are uncultivable under current laboratory regime, which prevents access to the enormous variety of their products which have the potential for industrial exploitation. Metagenomics promises continuous source of novel pollutant-degrading genes for increased efficiency and utility of transgenic (microbes and plants) technologies for direct use in bioremediation sectors. Additionally, the technology can be used to mass-produce novel degrading enzymes from uncultivable bacteria for improved enzymatic remediation technology. In recent years, metagenomic approaches have started yielding some novel industrial products including bioremediation gene/enzyme from uncultivable microbes. Using such an approach, Fan et al. (2012) isolated a novel thermostable pyrethroid-hydrolysing enzyme which could be used in the detoxification of pyrethroids. Following a similar metagenomic approach in cow rumen, a novel gene responsible for the degradation of 3,5,6-trichloro-2-pyridinol, a persistent and toxic metabolite of the insecticide chlorpyrifos was isolated (Renukaradhya et al. 2010).

In the metagenomic approach, total genetic materials are harvested from environmental samples without the need for an intermediate step of cultivation. To generate, a clone library, the entire genetic material is then transferred into a surrogate host (mainly *E. coli*) (Singh 2010). Search of biotechnological products from the metagenomic clone library can be achieved by two complimentary approaches; (1) sequencing the whole metagenome; with rapid advancements in next generation sequencing technologies, this approach is

becoming more popular than previously. Protein composition and structure is inferred from DNA sequences using bioinformatic tools. If sequences of interest are found, particular genes could be PCR-amplified and cloned for expression of target genes. However, the bottleneck for this approach is (a) lack of fast bioinformatic tools which have not evolved at the same speed as next-generation sequencing technology and (b) reliance for the identification of genes/proteins of industrial interest to known sequences existing in the current gene/protein databases. (2) Functional screening of metagenomic libraries; here the clones of the surrogate host are tested for desired activities such as reaction catalysed by a particular enzymes. The bottleneck of this approach is the logistics to generate, maintain and screen clone libraries. It is estimated that, microbes are so diverse that to cover the entire metagenome from one gram of soil at least two million clones should be generated (Ginolhac et al. 2004). However, the number of clones can be reduced by combining metagenomics with stable-isotope probing (SIP). In this approach, samples are first selectively enriched for microbes utilising a particular substrate which is labelled with a stable isotope (e.g. ^{13}C-labelled compounds). The DNA of enriched microbes, which is separated from others by ultra-centrifugation, is then used for construction of a metagenomic library. Such an approach has already produced a number of novel genes and gene products for remediation including biphenyl-degrading genes (Sul et al. 2009).

Metabolic engineering

Metabolic engineering combines systematic analysis of metabolic and other pathways with molecular biological techniques to improve cellular properties by designing and implementing rational genetic modifications (Koffas et al. 1999). Knowledge of microbial physiology is important to understand the metabolic capability of an organism. Other relevant physiological characteristics like microbial fitness and robustness are more complex and difficult to acquire during evolution. If the host microbial cells are robust and fit the engineering strategy, then the focus should be on introducing the target metabolic capabilities (including necessary pathways, efficient transport and cofactor regeneration systems) into the host strain, lacking the intended metabolic activity for effective transformation. As a result, metabolic/pathway engineering is fast becoming one of the central aspects of bioremediation. For effective remediation, a complete metabolic pathway is required ensuring that the metabolites produced through biotransformation are non toxic or reactive metabolites whose release does not entail further risk for the environment or human health. Metabolic engineering has provided some promising results to overcome this constraint. For example, a stable *Pseudomonas putida* strain (that recruits enzymes from different organisms) able to degrade chloro- as well as methylo-aromatics was constructed (Rojo et al. 1987). In another example, combination of *tod* and *tol* pathways in another *P. putida* strain resulted in an increase in the biodegradation rate of benzene, toluene and *p*-xylene (Lee et al. 1995). Current efforts are devoted to accelerate the existing pathways or design a 'new' effective pathway/hybrid pathway with superior catalytic abilities on recalcitrant pollutants (Erb et al. 1997). This would require the combination of determinants for complementary pathway segments in order to form a complete pathway sequence for a target substrate, thus generating new or improved transformation products. For example, complete degradation of organophosphorus compounds was achieved by transferring two plasmids in the same host; one containing an OP degrading gene and a second harbouring the genes controlling the degradation of the metabolite produced (Walker and Keasling 2002). Metabolic pathway engineering can also be used for the development of engineered microorganisms which possess improved catalytic activity combined with (i) advanced capacity to survive under extreme environmental conditions (Marconi et al. 1997) or (ii) ability to produce suitable biosurfactants (e.g. rhamnolipids), a trait particularly useful in cases where the limited bioavailability of organic pollutants constitute an obstacle for effective biodegradation (Pei et al. 2010). The metabolic engineering approach also involves deletion strategies for eliminating competitive reaction pathways (Kind et al. 2010). For example, co-metabolism, in the microbial transformation of trichloroethylene (TCE), the use of artificial regulatory systems or constitutive expression signalling might be helpful. With continuous supply of novel pollutant-degrading genes from metagenomics, metabolic engineering can significantly improve the efficiency of bioremediation using transgenic technology. Nonetheless, despite their several advantages, the application of genetically modified organism/

microbes (GEM) in situ is still limited by the risk associated with horizontal gene transfer, uncontrolled proliferation of the introduced GEMs, inconsistencies in risk assessment procedures and low public acceptability (Velkov 2001; Singh 2010).

Protein/enzyme engineering

Proteins/enzymes can be engineered to improve stability, substrate specificity and kinetic properties. Control mechanisms and enzyme properties can be tailored by irrational approaches such as DNA shuffling (Kuchner and Arnold 1997) random priming (Shao et al. 1998) and staggered extension process (Zhao et al. 1998) or by rational design of proteins performed by site-directed mutagenesis (Ju and Parales 2006). Engineering of the enzyme can be done to fine-tune enzymes for desired substrate specificities and stereo-selectivity. For example, site-directed mutagenesis approach to engineer active site volume and topology of cytochrome $P450_{cam}$ enhanced the catalytic activity of the enzyme (Holloway et al. 1998). Another possible approach is to combine the best attributes of related enzymes and exchange subunits or subunit sequences resulting in chimeric enzymes that are superior to the parent enzymes (Beil et al. 1998) Also, incorporation of multiple binding sites within a single peptide, for binding of co-factors and other small molecules, can enhance the catalytic power of the enzyme. This may also prove to be a versatile strategy for the removal of metal wastes (Pazirandeh et al. 1998). However, only few small alterations can be made at a time in a given enzyme as multiple alterations may interfere with its three-dimension folding and catalytic activity. It has been shown that each new mutation typically inactivates between 30 and 40 % of the remaining active protein (Guo et al. 2004).

Perspective for enzymatic bioremediation

Metagenomics offer an excellent opportunity to obtain novel genes and enzymes which could be used in bioremediation strategies. Genes and the corresponding enzymes extracted by this approach could be further used for complete degradation of pollutants either intact or after metabolic or enzymatic engineering, respectively. Together these approaches can overcome the constraints of the limited enzymes diversity. However, two bottlenecks need to be overcome to fully exploit the potential of metagenomics; (a) the delayed development of appropriate bioinformatic tools in order to keep up with the pace of the production of enormous amounts of information obtained through -omics technologies (b) the limited availability of high-throughput technologies for functional screening of metagenomic libraries.

Nanotechnology offers promise to stabilise and protect enzymes against mechanical and biotic degradation and therefore increases their half-life and enables recirculation in their use while reducing the cost of bioremediation strategies. Encapsulation of xenobiotic-degrading enzymes in nano-particles (1–100 nm) improves both stability and protection against degradation. Enzymes that bind to nanoparticles are more stable and, therefore, less vulnerable to mechanical shearing and loss of three-dimensional structure. At the same time, because enzymes are encapsulated inside the nano-structure, protease attack can be prevented. As a result, enzymes remain stable and can be reused several times. The utility of this approach was demonstrated in 100-day experiment where a nano-fibre-esterase enzyme complex remained functional in both repeated batch and continuous long-term operation (Lee et al. 2007). Immobilisation of enzymes using such approaches provides an excellent opportunity to extend the half-life and reusability of enzymes and therefore reduce the cost of operation. However, the true progress of emerging technologies could be realised only if all above discussed approaches are integrated at conceptual stage (Fig. 1).

Plant-assisted remediation/phytoremediation

Instead of relying on microbes and their versatility in accomplishing bioremediation of polluted ecosystems, plants either alone (Gerhardt et al. 2009) or in combination with microbes (Ramos et al. 2005) have been utilized for this purpose. The concept of using plants to clean up contaminated environments is not new. About 300 years ago, plants were proposed for use in the treatment of wastewater (Hartman Jr 1975). Vegetation-based bioremediation shows potential for accumulating, immobilising and transforming a low level of persistent contaminants (Pulford and Watson 2003). Plants facilitate remediation of the

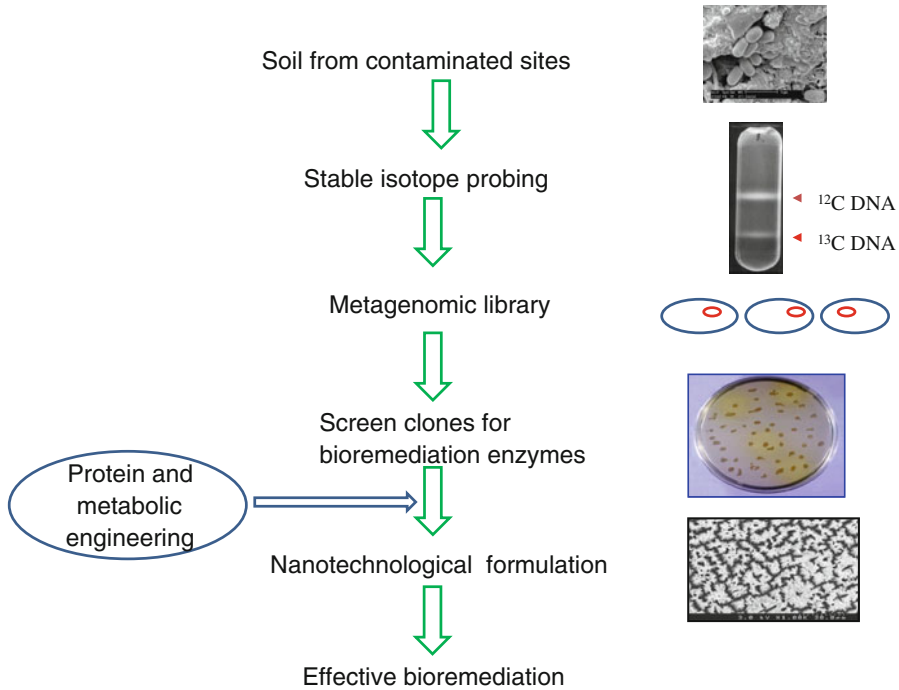

Fig. 1 Framework to isolate bioremedial enzymes from uncultivable microorganism and further modification for improved bioremediation efficiency

contaminated soils and ground-water via several mechanisms that are listed in Table 1.

The phytoremediation approach has several advantages including reduced-cost, public acceptance and most importantly the capacity of simultaneous removal of organic and inorganic contaminants. Metals cannot be chemically transformed and they can be toxic to microorganisms. Phytoremediation technologies exploit the trait of the natural ability of some plants to accumulate (hyper-accumulate) essential heavy metals in their tissues (Fe, Mn, Zn, Cu, Mg, Mo and Ni) (Brar et al. 2006; Rascio and Navari-Izzo 2011). Such an approach could also be used for the remediation of soil and groundwater contaminated with radionuclides such as uranium, organic contaminants such as chlorinated solvents, BTEX compounds, non-aromatic petroleum hydrocarbons, nitro-toluene ammunition wastes, and excess of nutrients (Schnoor et al. 1995). Additionally, plants produce many secondary metabolites (Hadacek 2002) which are believed to be pollutant analogues within the network of suprametabolism, having implications for predicting the fate of pollutants (Singer et al. 2004). Secondary metabolites such as limonene, cymene, carvone and pinene have shown to induce the expression of catabolic genes by the rhizosphere or plant-colonising bacteria resulting in enhanced biodegradation of PCBs (Singer et al. 2003). Phytoremediation may also be used as a final polishing step in combination with other microbe-driven treatment technologies, a strategy called microbe-assisted phytoremediation.

Current limitations in phytoremediation and approaches to overcome those limitations

Despite its positive aspects, the industrial application of phytoremediation is slow. This is mainly due to the following constraints: (1) Lack or slow growth of plants at contaminated sites which results into low efficacy of phytoremediation. Most of the contaminated sites are nutrient-limited and lack soil structure to hold nutrients and water for plant growth. Additionally both organic and inorganic contaminants have direct toxic effects which prevent the proper establishment and good growth of plants. (2) The efficiency of phytoremediation is occasionally limited by the reduced capacity of plants to penetrate in the soil through their root system and access the pollutants (Juwarkar et al. 2010); (3) The time required for

Table 1 A list of different types of phytoremediation

Technique	Mechanism	Plant parts	Surface medium	References
Phytoextraction	Metal uptake and accumulation into the plant tissue with subsequent removal of the plants	Roots Shoots Leaves	Soils	Tu et al. (2003) Wei and Zhou (2006) Zhao et al. (2000)
Phytodegradation/ rhizodegradation	Enzyme-catalysed metabolism of organic contaminants into simpler molecules by rhizosphere-dwelling microorganisms	Roots Leaves	Surface water Ground water	Black (1995) Chaudhry et al. (1998)
Phytostabilization	Reduces the mobility and migration of contaminated soil	Roots	Soils Groundwater Mine tailing	Salt et al. (1995)
Rhizofiltration	Uptake of metals into plant roots	Roots	Surface water Water pumped	Kumar et al. (1995)
Phytovolatilization	Removal of pollutants like selenium, mercury, volatile hydrocarbons via evapotranspiration processes	Roots Leaves	Soils Groundwater	Gerhardt et al. (2009) McCutcheon and Schnoor (2003)
Phytostimulation	Phytostimulation (a symbiotic relationship that exists between plants and several soil microorganisms) is developed for the remediation of PCBs	Roots	Soil	Cluis (2004)

effective phytoremediation is often long relative to other conventional treatments thus making it less attractive in cases where relatively rapid decontamination is needed and finally, (4) potential for contaminants to enter food chain through animal consumption of plants accumulating organic and inorganic pollutants.

Such constraints can be overcome by combining plant–microbial remediation strategies. Plants secrete a range of sugar and other metabolites through their roots which soil microbes utilize for their growth. In return, rhizosphere microbes provide to the plants nitrogen, phosphorus and other nutrients. Additionally microbes can degrade a large number of organic contaminants before entering into plant tissues. Contaminated sites concurrently planted with remediation-capable plants and inoculated with beneficial microbes have been successfully recovered (Juwarkar et al. 2010). Alternatively, transgenic technology has been used to express bacterial genes responsible for xenobiotic degradation in plant tissues with some success. For example, transgenic plants were constructed using microbial pollutant-degrading genes for decontamination of explosives (TNT and RDX) (Kurumata et al. 2005; Rylott et al. 2006), organophosphates (Wang et al. 2008). Despite this, some organic contaminants could enter plant tissues and result not only in limited plant growth but preclude the use of the plant biomass for other purposes due to associated toxicity of the organic contaminants. This scenario is more problematic for sites contaminated with a cocktail of pollutants such as waste from oil industries containing a mixture of heavy metals, surfactants, emulsifiers and other complex inorganic/organic pollutants and wastes produced by mining industries. A novel remediation technology termed 'designer' plants, has been proposed as a possible solution to this particularly challenging remediation problem. This technology harnesses the combined capability of plants (removal of inorganic pollutant) and soil or endophytic microbes (for removal of organic contaminants) (Abhilash et al. 2012). Following this approach customised plant systems could be produced, whose rhizosphere/rhizoplane is colonized with microbes (fungi and bacteria) able to degrade complex organic contaminants while the same plants

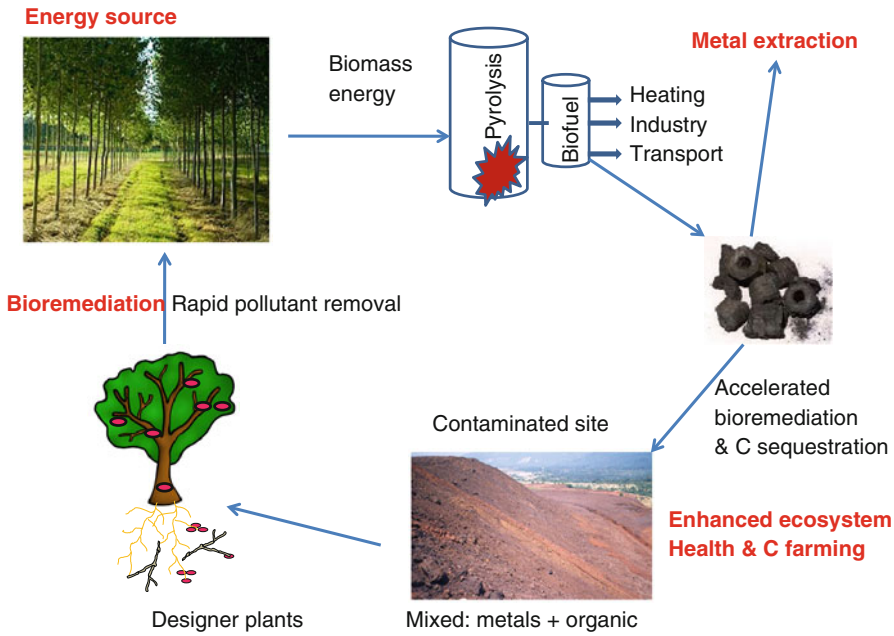

Fig. 2 A conceptual framework for environmental, economic and social benefits of 'designer' plant technology

already contain endophytic pollutant-degrading microbes with the capacity to degrade other organic pollutants. This approach not only leads to simultaneous removal of complex and simple organic plus inorganic contaminants (heavy metals) but also improves soil and groundwater quality at faster rates than conventional bioremediation strategies. This approach could provide additional benefits if plants with commercial importance (e.g. used for biofuel production) could be used for phytoremediation. Fast-growing and high biomass yielding plant species such as willow (*Salix* sp.), poplar (*Populous* sp., or jatropha (*Jatropha* sp.) could be used for both phytoremediation and energy production. If successfully replicated in field settings, this technology could revolutionise the remediation industry. The biochar produced from the energy generation can be first used to commercially extract metals from it providing additional economic benefit. Further biochar could be applied back to soils offering enhanced soil quality and higher carbon sequestration, which will in turn expedite remediation process (Fig. 2).

Conclusions

The recent advancements in bioremediation technologies have provided an exciting opportunity to unravel its full potential. For example, a combination of metagenomics with protein/metabolic engineering and nanotechnology could overcome current main challenges of remediation and increase the efficacy of enzymes in bioreactors and contaminated environments. However, to achieve this, these approaches need to be mapped and integrated from a conceptual stage. If such integration is achieved, bioremediation based on the above framework is poised to harness the full bioremediation potential of uncultivable microbes.

Multi-purpose phytoremediation (combining phytoremediation with biofuel generation and carbon sequestration) holds a great promise for future mainly due to its ability to bring economic benefits along with environmental and social benefits of afforestation. These can be achieved by transgenic or 'designer' plant approaches. In several countries, the use of transgenic organisms remains unacceptable due to policy decision and public perceptions. Until, this battle is won, non-transgenic approaches such as 'designer' plants could be main source of multi-purpose phytoremediation.

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References

Abhilash PC, Singh HB, Powell JR, Singh BK (2012) Plant–microbe interactions: novel applications for exploitation in multi-purpose remediation technologies. Trend Biotechnol 30:416–420

Beil S, Mason JR, Timmis KN, Pieper DH (1998) Identification of chlorobenzene dioxygenase sequence elements involved in dechlorination of 1,2,4,5-tetrachlorobenzene. J Bacteriol 180:5520–5528

Black H (1995) Absorbing possibilities: phytoremediation. Environ Health Perspect 103:1106

Blasco R, Wittich RM, Megharaj M, Timmis KN, Pieper DH (1995) From xenobiotic to antibiotic: formation of protoanemonin from 4-chlorocatechol by enzymes of the 3-oxoadipate pathway. J Biol Chem 270:29229–29235

Brar SK, Verma M, Surampalli RY, Misra K, Tyagi RD, Meunier N, Blais JF (2006) Bioremediation of hazardous wastes—a review. Pract Period Hazard Toxic Radioact Waste Manag 10:59

Chaudhry T, Hayes W, Khan A, Khoo C (1998) Phytoremediation—focusing on accumulator plants that remediate metal-contaminated soils. Australas J Ecotoxicol 4:37–51

Cluis C (2004) Junk-greedy greens: phytoremediation as a new option for soil decontamination. BioTeach J 2:61–67

Curtis TP, Sloan WT, Scannell JW (2002) Estimating prokaryotic diversity and its limits. Proc Nat Acad Sci USA 99:10494–10497

Erb RW, Eichner CA, Wagner-Döbler I, Timmis KN (1997) Bioprotection of microbial communities from toxic phenol mixtures by a genetically designed pseudomonad. Nat Biotechnol 15:378–382

Fan X, Liu X, Huang R, Liu Y (2012) Identification and characterization of a novel thermostable pyrethroid-hydrolyzing enzyme isolated through metagenomic approach. Microb Cell Fact 11:33–37

Gerhardt KE, Huang X-D, Glick BR, Greenberg BM (2009) Phytoremediation and rhizoremediation of organic soil contaminants: potential and challenges. Plant Sci 176: 20–30

Ginolhac A, Jarrin C, Gillet B, Robe P, Pujic P, Tuphile K, Bertrand H, Vogel TM, Perrière G, Simonet P, Nalin R (2004) Phylogenetic analysis of polyketide synthase I domains from soil metagenomic libraries allows selection of promising clones. Appl Environ Microbiol 70:5522–5527

Gulmaraes BCM, Arends JBA, Van der Ha D, Boon N, Verstraetes W (2010) Microbial service and their management: recent progress in soil bioremediation technologies. Appl Soil Ecol 46:157–167

Guo HH, Choe J, Loeb LA (2004) Protein tolerance to random amino acid change. Proc Natl Acad Sci USA 101:9205

Hadacek F (2002) Secondary metabolites as plant traits: current assessment and future perspectives. Crit Rev Plant Sci 21:273–322

Hartman Jr WJ (1975) An evaluation of land treatment of municipal wastewater and physical siting of facility installations. DTIC Document

Hatzinger PB, Whittier MC, Arkins MD, Bryan CW, Guarini WJ (2002) In situ and ex-situ bioremediation options for treating perchlorate in groundwater. Remediat J 12:69–86

Hazen TC, Dubinsky EA, DeSantis TZ et al (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. Science 330:204–208

Holloway P, Knoke KL, Trevors JT, Lee H (1998) Alteration of the substrate range of haloalkane dehalogenase by site directed mutagenesis. Biotechnol Bioeng 59:520–523

Jones KC, de Voogt P (1999) Persistent organic pollutants (POPs): state of the science. Environ Pollut 100:209–222

Ju KS, Parales RE (2006) Control of substrate specificity by active-site residues in nitrobenzene dioxygenase. Appl Environ Microbiol 72:1817–1824

Juwarkar A, Singh S, Mudhoo A (2010) A comprehensive overview of elements in bioremediation. Rev Environ Sci Biotechnol 9:215–288

Karns JS, Hapeman CJ, Mulbry WW, Ahrens EH, Shelton DR (1998) Biotechnology for the elimination of agrochemical wastes. HortScience 33:626–631

Kind S, Jeong WK, Schröder H, Zelder O, Wittmann C (2010) Identification and elimination of the competing *N*-acetyldiaminopentane pathway for improved production of diaminopentane by *Corynebacterium glutamicum*. Appl Environ Microbiol 76:5175–5180

Koffas M, Roberge C, Lee K, Stephanopoulos G (1999) Metabolic engineering. Annu Rev Biomed Eng 1:535–557

Kuchner O, Arnold FH (1997) Directed evolution of enzyme catalysts. Trend Biotechnol 15:523–530

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238

Kurumata M, Takahashi M, Sakamotoa A, Ramos JL, Nepovim A, Vanek T, Hirata T, Morikawa H (2005) Tolerance to, and uptake and degradation of 2,4,6-trinitrotoluene (TNT) are enhanced by the expression of a bacterial nitroreductase gene in *Arabidopsis thaliana*. Z Naturforsch 60:272–278

Lee JY, Jung KH, Choi SH, Kim HS (1995) Combination of the tod and the tol pathways in redesigning a metabolic route of *Pseudomonas putida* for the mineralization of a benzene, toluene, and *p*-xylene mixture. Appl Environ Microbiol 61:2211–2217

Lee JH, Hwang ET, Kim BC, Lee SM, Sang BI, Choi YS, Kim J, Gu MB (2007) Stable and continuous long-term enzymatic reaction using an enzyme—nanofiber composite. Appl Microbiol Biotechnol 75:1301–1307

Li T, Guo S, Wu B, Li F, Niu Z (2010) Effect of electric intensity on the microbial degradation of petroleum pollutants in soil. J Environ Sci 22:1381–1386

Marconi AM, Kieboom J, De Bont JAM (1997) Improving the catabolic functions in the toluene-resistant strain *Pseudomonas putida* s12. Biotechnol Lett 19:603–606

McCutcheon SC, Schnoor JL (2003) Phytoremediation: transformation and control of contaminants, vol 118. LibreDigital

McMahon V, Garg A, Aldred D, Hobbs G, Smith R, Tothill I (2008) Composting and bioremediation process evaluation

of wood waste materials generated from the construction and demolition industry. Chemosphere 71:1617–1628

Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R (2011) Bioremediation approaches for organic pollutants: a critical perspective. Environ Int 37:1362–1375

Mulbry W, Ahrens E, Karns J (1998) Use of a field scale biofilter for the degradation of the organophosphate insecticide coumaphos in cattle dip wastes. Pestic Sci 52:268–274

Paul D, Pandey G, Pandey J, Jain RK (2005) Accessing microbial diversity for bioremediation and environmental restoration. Trend Biotechnol 23:135–142

Pazirandeh M, Wells BM, Ryan RL (1998) Development of bacterium-based heavy metal biosorbents: enhanced uptake of cadmium and mercury by *Escherichia coli* expressing a metal binding motif. Appl Environ Microbiol 64:4068–4072

Pei X-H, Zhan X-H, Wang S-M, Lin Y-S, Zhou L-X (2010) Effects of a biosurfactant and a synthetic surfactant on phenanthrene degradation by a *Sphingomonas* strain. Pedosphere 20:771–779

Pulford I, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29: 529–540

Ramos JL, Gonzalez-Perez MM, Caballero A, van Dillewijn P (2005) Bioremediation of polynitrated aromatic compounds: plants and microbes put up a fight. Curr Opin Biotechnol 16:275–281

Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci 180:169–181

Renukaradhya M, Shah AI et al (2010) Isolation of a novel gene encoding a 3,5,6-trichloro-2-pyridinol degrading enzyme from a cow rumen metagenomic library. Biodegradation 21:565–573

Rojo F, Pieper DH, Engesser KH, Knackmuss HJ, Timmis KN (1987) Assemblage of ortho cleavage route for simultaneous degradation of chloro- and methylaromatics. Science 238:1395–1398

Rylott EL, Jackson RG, Edwards J, Womack GL, Seth-Smith HMB, Rathbone DA, Strand SE, Bruce NC (2006) An explosive-degrading cytochrome P450 activity and its targeted application for the phytoremediation of RDX. Nat Biotechnol 24:216–219

Salt DE, Blaylock M, Kumar NPBA, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Nat Biotechnol 13:468–474

Schnoor JL, Light LA, McCutcheon SC, Wolfe NL, Carreia LH (1995) Phytoremediation of organic and nutrient contaminants. Environ Sci Technol 29:318–323

Scott C, Begley C, Taylor MJ, Pandey G, Momiroski V, French N, Brearley B, Kotsonis SE, Selleck MJ, Carino FA, Bajet CM, Clarke C, Oakeshott JG, Russell RJ (2011) Free enzyme bioremediation of pesticides. ACS symposium series book 1075:155–174

Shao Z, Zhao H, Giver L, Arnold FH (1998) Random-priming in vitro recombination: an effective tool for directed evolution. Nucleic Acids Res 26:681–683

Singer AC, Crowley DE, Thompson IP (2003) Secondary plant metabolites in phytoremediation and biotransformation. Trend Biotechnol 21:123–130

Singer AC, Thompson IP, Bailey MJ (2004) The tritrophic trinity: a source of pollutant-degrading enzymes and its implications for phytoremediation. Curr Opin Microbiol 7:239–244

Singh BK (2009) Organophosphorus-degrading bacteria: ecology and industrial applications. Nat Rev Microbiol 7:156–164

Singh BK (2010) Exploring microbial diversity for biotechnology: the way forward. Trend Biotechnol 28:111–116

Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30:428–471

Singh BK, Campbell C, Sorensen SJ, Zhou J (2009) Soil genomics is the way forward. Nat Rev Microbiol 7:756

Strong LC, McTavish H, Sadowsky MJ, Wackett LP (2000) Field-scale remediation of atrazine-contaminated soil using recombinant *Escherichia coli* expressing atrazine chlorohydrolase. Environ Microbiol 2:91–98

Sul WJ, Park J, Quensen JF III, Rodrigues JLM, Seliger L, Tsoi TV, Zylstra GJ, Tiedje JM (2009) DNA-stable isotope probing integrated with metagenomics for retrieval of biphenyl dioxygenase genes from polychlorinated biphenyl-contaminated river sediment. Appl Environ Microbiol 75:5501–5506

Tu C, Ma LQ, Zhang W, Cai Y, Harris WG (2003) Arsenic species and leachability in the fronds of the hyperaccumulator Chinese brake (*Pteris vittata* L.). Environ Pollut 124:223–230

Velkov VV (2001) Stress-induced evolution and the biosafety of genetically modified microorganisms released into the environment. J Biosci 26:667–683

Walker AW, Keasling JD (2002) Metabolic engineering of *Pseudomonas putida* for the utilization of parathion as a carbon and energy source. Biotechnol Bioeng 78:715–721

Wang XX et al (2008) Phytodegradation of organophosphorus compounds by transgenic plants expressing a bacterial organophosphorus hydrolase. Biochem Biophys Res Commun 365:453–458

Watanabe K (2001) Microorganisms relevant to bioremediation. Curr Opin Biotechnol 12:237–241

Wei S, Zhou QX (2006) Phytoremediation of cadmium-contaminated soils by *Rorippa globosa* using two-phase planting. Environ Sci Pollut Res 13:151–155

Yergeau E, Arbour M, Brousseau R, Juck D, Lawrence JR, Masson L, Whyte LG, Greer CW (2009) Microarray and real-time PCR analyses of the responses of high-arctic soil bacteria to hydrocarbon pollution and bioremediation treatments. Appl Environ Microbiol 75:6258–6267

Zhao H, Giver L, Shao Z, Affholter JA, Arnold FH (1998) Molecular evolution by staggered extension process (StEP) in vitro recombination. Nat Biotechnol 16:258–261

Zhao F, Lombi E, Breedon T (2000) Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. Plant Cell Environ 23:507–514