



Research review paper

## Bioremediation 3.0: Engineering pollutant-removing bacteria in the times of systemic biology

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## ARTICLE INFO

## Keywords:

Bioremediation  
Biodegradation pathway engineering  
Emerging pollutants  
Environmental biotechnology  
Systemic biology  
Metabolic engineering  
Systems biology  
Synthetic biology

## ABSTRACT

Elimination or mitigation of the toxic effects of chemical waste released to the environment by industrial and urban activities relies largely on the catalytic activities of microorganisms—specifically bacteria. Given their capacity to evolve rapidly, they have the biochemical power to tackle a large number of molecules mobilized from their geological repositories through human action (e.g., hydrocarbons, heavy metals) or generated through chemical synthesis (e.g., xenobiotic compounds). Whereas naturally occurring microbes already have considerable ability to remove many environmental pollutants with no external intervention, the onset of genetic engineering in the 1980s allowed the possibility of rational design of bacteria to catabolize specific compounds, which could eventually be released into the environment as bioremediation agents. The complexity of this endeavour and the lack of fundamental knowledge nonetheless led to the virtual abandonment of such a recombinant DNA-based bioremediation only a decade later. In a twist of events, the last few years have witnessed the emergence of new *systemic* fields (including systems and synthetic biology, and metabolic engineering) that allow revisiting the same environmental pollution challenges through fresh and far more powerful approaches. The focus on contaminated sites and chemicals has been broadened by the phenomenal problems of anthropogenic emissions of greenhouse gases and the accumulation of plastic waste on a global scale. In this article, we analyze how contemporary systemic biology is helping to take the design of bioremediation agents back to the core of environmental biotechnology. We inspect a number of recent strategies for catabolic pathway construction and optimization and we bring them together by proposing an engineering workflow.

## 1. Introduction

Increasing pollution of air, soils, ground and surface waters constitutes a major threat to public health both in developing countries as well as in industrialized countries including EU states, the USA, India and China. The majority of contaminants that affect soils and waters are heavy metals and organic compounds such as mineral oil hydrocarbons, polyaromatic hydrocarbons, benzene derivatives, and halogenated hydrocarbons. Many of organic polluting compounds for agricultural (the pesticides dichlorodiphenyltrichloroethane, atrazine, and pentachlorophenol), industrial (solvents such as dichloroethane or dielectric fluids such as polychlorinated biphenyls) or military use (explosives such as 2,4,6-trinitrotoluene) are xenobiotics of anthropogenic origin. There is also a spectrum of so-called *emerging contaminants* (Table 1), i.e., substances long present in the environment whose presence and

negative effects have only recently been recognized (Petrie et al., 2015). The list can be further broadened with petroleum-derived plastics and some chemicals originally considered to be *green*, including certain types of bioplastics or ionic liquids (Amde et al., 2015). Despite the recalcitrant nature of some of these polluting compounds, many are more or less susceptible to biodegradation (Alexander, 1999). In addition to these traditional causes of environmental deterioration, the recent decades have witnessed the onset of ramped-up levels of anthropogenic emissions of CO<sub>2</sub> and other greenhouse gases and their ensuing impact on climatic change. Whereas the chemicals themselves are simple (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O), the challenge here is less their biodegradation than their recapture in a non-gaseous form.

The major entity that causes large-scale transformations in the biosphere are microorganisms and their metabolic pathways. Microbes degrade toxic chemicals *via* complete mineralization or co-metabolism,

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**Table 1**

Emerging contaminants.

Sources: <http://toxics.usgs.gov>, <http://www.eugris.info> (Petrie et al., 2015).

Groups of products	Classes of chemicals	Examples
Human and veterinary pharmaceuticals	Antibiotics, anti-parasitic agents, ionophores	Amoxicillin, erythromycin, metronidazol, tetracycline, lincomycin, sulfathiazole
	Stimulants and drugs including anti-inflammatory, anti-diabetic, anti-epileptic, anti-hypertensive, or anti-cancer drugs, anticoagulants, hallucinogens, analgesics, $\beta$ -blockers, anti-depressants, lipid regulators, or erectile dysfunction drugs Hormones including natural and synthetic estrogens, androgens	Amphetamine, cocaine, caffeine, nicotine, propranolol, ibuprofen, codeine, carbamazepine, bezafibrate, metformin, fluoxetine, warfarine, valsartan, tramadol, morphine, methadone, diazepam, ephedrine, tamoxifen
Industrial and household wastewater products	Insecticides, plasticizers, detergents, flame retardants, polycyclic aromatic hydrocarbons, antioxidants, solvents, disinfectants, fumigants, fragrances, preservatives	Estrone, estriol, testosterone, progesterone, mestranol (ovulation inhibitor), cholesterol Carbaryl, chlorpyrifos, diethylphthalate, <i>p</i> -nonylphenol, tri(2-chloroethyl)phosphate, naphthalene, anthracene, 2,6-di- <i>tert</i> -butylphenol, 1,2,3-trichloropropane, phenol, 1,4-dichlorobenzene, acetophenone
Personal care products	Insect repellents, polycyclic musks, sunscreen agents, fragrances, antiseptics	Bisphenol A, 1-benzophenone, methylparaben, <i>N,N</i> -diethyltoluamide, triclosan
Nanomaterials	Miscellaneous	Nanosilver, alumina nanoparticles, titanium dioxide, fullerenes, carbon black

in aerobic or anaerobic conditions. Advantageous properties such as small genome size, relative simplicity of the cell, short replication times, rapid evolution and adaptation to the new environmental conditions made microbes, and particularly bacteria, favourable candidates for bioremediation technologies, that is *in situ* or *ex situ* removal of polluting chemicals from the environment using biological agents. The removal of environmental pollution caused by the extensive activities of industrial society is a serious topic that draws the attention of biotechnologists. This is because beyond the medical and environmental consequences, the situation signs considerable potential for growth of eco-industry focused on clean-up technologies and removal of environmental contaminants. In fact, valorization of waste chemicals accumulating in industry is one of the pillars of the circular economy and the 4th Industrial Revolution (Schmidt, 2012; Wigginton et al., 2012).

The earliest attempts at directed bioremediation, although not formalized as such at the time, dated back to the late 19th century with the origins of the first wastewater treatment plants (Litchfield, 2005). Bioremediation began in earnest some 45 years ago with the isolation of culturable bacteria from contaminated sites and studying their degradation pathways. The first report on enhanced *in situ* bioremediation of soil contaminated with petroleum-derived hydrocarbons was published in 1975 by Raymond et al. (1975). Natural microbial degraders were later applied with success in world-wide and local biotechnological processes including large-scale wastewater denitrification, uranium removal, and degradation of 1,2-dichloroethane from groundwater or the organophosphorus pesticide coumaphos from cattle-dip waste (Francis and Mankin, 1977; Lovley et al., 1991; Mulbry et al., 1998; Stucki and Thuerer, 1995). The advent of technologies for pollutant removal using naturally emerging microorganisms could be called the era of *Bioremediation 1.0*. Even so, a number of specific chemicals, especially of anthropogenic origin, including persistent organic pollutants such as dichlorodiphenyltrichloroethane (DDT), trichloroethylene, 1,2,3-trichloropropane, some polychlorinated biphenyls (PCB) or dioxins continued to be resistant to natural biodegradation due to lack of efficient microbial catabolic traits whose evolution was not sufficiently rapid or ended in a deadlock (Janssen et al., 2005).

Initial discoveries in molecular biology and progress in biological engineering disciplines seemed to provide a partial solution for such challenges through rational interventions in the metabolic networks of selected microbial hosts. The rise of recombinant DNA technology allowed the transformation of bioremediation from empirical practice into an exercise in genetic engineering, giving rise to what we might term *Bioremediation 2.0*. The goal of the new field was to engineer whole microbes, their biodegradation pathways, and the corresponding enzymes towards *in situ* mineralization of target pollutants. Such

*superbugs* were expected to provide an economically feasible, environmentally friendly alternative to the costly conventional technologies for pollutant removal available at the time (Ramos et al., 2011). The late 1980s and early 1990s represented the golden era of biodegradation research, with numerous engineering attempts following the pioneering work by Chakrabarty and co-workers (Kellogg et al., 1981). They described the preparation of recombinant *Pseudomonas putida* strains able to break down crude oil by the *plasmid-assisted molecular breeding*, that is, propagation of novel catabolic capabilities through directed bacterial conjugation and plasmid transfer. The persistence of many xenobiotics was attributed mainly to the absence of complete degradative pathways in a single organism (Brenner et al., 1994; Reineke and Knackmuss, 1979). Recruitment of complementary enzyme sequences by conjugative gene transfer and so called *patchwork assembly* of several existing natural pathways in a suitable host was believed to generate functional synthetic routes that would allow for the complete mineralization of persistent target compounds such as PCB (Lehrbach et al., 1984; Ramos et al., 1987; Rojo et al., 1987).

Despite some success with the *patchwork* strategy and engineering of *superbugs* with extended substrate scope in laboratory conditions, this initial and rather naive approach led to many disappointments as well (Cases and de Lorenzo, 2005; de Lorenzo, 2009). A prominent example was the case of engineered *Pseudomonas* strains that did not grow on 2-chlorotoluene as the only carbon source, even though they possessed all the genetic components presumed necessary for substrate mineralization (Haro and de Lorenzo, 2001). From a contemporary perspective, such failures can be explained by lack of insight into important factors such as: (i) thermodynamic feasibility of assembled catabolic networks, (ii) kinetic characteristics of enzymes and physicochemical properties of metabolites, (iii) expression levels of pathway modules, (iv) cross-talk between exogenous and endogenous metabolic routes, and (v) stress responses and changes in overall host cell physiology after introduction of new metabolic modules and exposure to toxic substrates and metabolites (de Lorenzo, 2009; Ramos et al., 2011).

Fortunately, the last decade has witnessed the onset of what can be called *systemic biology*, which merges different approaches of systems biology, metabolic engineering, and synthetic biology, for the sake of understanding and reprogramming biological systems. Systemic biology has the potential to remove the unknowns and bottlenecks encountered in past trials and paves the way towards the era of *Bioremediation 3.0*. The joint power of the systemic biology disciplines can ensure that biodegradation and bioremediation using genetically modified microorganisms will remain a vital concept deserving of the full attention of new generations of bioengineers.

In this article we review the applications of novel engineering strategies to the design and evolution of microbial biodegradation

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