Chapter 24
Modern Bioremediation Approaches: Use of Biosurfactants, Emulsifiers, Enzymes, Biopesticides, GMOs

Martin Halecký and Evguenii Kozliak

Abstract New trends in bioremediation are reviewed with the major focus on applications of both synthetic and biological surfactants including high molecular weight bioemulsifiers. The use of eco-friendly biosurfactants is discussed including their applications for removal of heavy metals from soil in addition to traditional organic contaminants. Both success stories and limitations of biosurfactant applications are described on the basis of current literature. Another newly developed technology, the use of enzymes (free or immobilized) instead of/in combination with microorganisms is discussed with respect to removal of both organic contaminants and metals from soil along with addressing the key disadvantage of the enzyme application, which is its high cost. Application of genetically modified organisms, i.e., microorganisms and plants, for soil bioremediation is reviewed focusing on introduction into the environment as well as contained use in closed reactors. Finally, a brief review is provided on the current research and application of biopesticides as promising agents for prevention of soil contamination.

Keywords Biosurfactants · Synthetic surfactants · Emulsifiers · Enzymes · Biopesticides · GMOs

24.1 Introduction

As mentioned in the introduction to Part V, several decontamination methods have been successfully used for soil and sediment cleaning for decades. Some of them, however, require application of drastic conditions and/or use of toxic agents causing a negative impact on the local wildlife and the environment. In addition, these traditional decontamination methods in many cases ended up incurring rather high

---

M. Halecký (✉)
Department of Biotechnology, University of Chemistry and Technology, Prague, Czech Republic
e-mail: martin.halecky@vscht.cz

E. Kozliak
Department of Chemistry, University of North Dakota, Grand Forks, ND, USA

J. Filip et al. (eds.), *Advanced Nano-Bio Technologies for Water and Soil Treatment*,
Applied Environmental Science and Engineering for a Sustainable Future,
https://doi.org/10.1007/978-3-030-29840-1_24

costs. To make the traditional methods cost-effective and eco-friendly, their optimization is required, namely, the application of new eco-friendly yet efficient agents as well as an appropriate eco-friendly enhanced environment attenuation.

As another new development, the high potential of GMOs, with either their direct introduction into the environment or the contained use for a cost-effective production of decontamination agents in closed bioreactors, is being considered but not sufficiently used at this point. Currently the emphasis is also put on preventing contamination while using Integrated Pest Management and Integrated Crop Management and favoring biopesticides over traditional pesticides in agriculture as legislatively established in the framework for Community action to achieve sustainable use of pesticides by Directive 2009/128/EC of the European Parliament. Although novel agents enhancing current technologies, e.g., biosurfactants and biopesticides have been experimentally tested for decades and are currently highly supported by the governments, their wide commercial use is limited because of their high price, fragmented (regional) market, a lack of awareness of the benefits of biosurfactants and biopesticides and, in some cases, quality fluctuations and/or poor customer support.

24.2 Perspectives of the Use of Synthetic Surfactants and Biosurfactants in Remediation Protocols

24.2.1 Surfactant Properties and Types

Generally, surfactants (either synthetic or biosurfactants) are amphiphilic compounds that lower the surface tension between two immiscible liquids or between the liquid and solid phases. Surfactants accumulate at the liquid–air, liquid–liquid, and liquid–solid interface and reduce the repulsive forces between those phases, allowing them to mix and interact more readily (Soberón-Chávez and Maier 2011). Thus, they may be used as wetting agents, detergents, foaming agents, emulsifiers, and dispersants. To have these specific abilities, surfactants always contain two domains/moieties—hydrophobic and hydrophilic.

The most important characteristics of any surfactant are critical micelle concentration (CMC), hydrophilic–lipophilic balance (HLB), chemical structure, and charge. CMC is the concentration of surfactants above which micelles start to form and the newly added surfactant molecules incorporate into the micelles instead of being dispersed throughout the solution. The surface tension strongly depends on the surfactant concentration below the CMC, then leveling off once the CMC is reached/exceeded (Christofi and Ivshina 2002). CMC characterizes the degree of influence on the surface tension; the lower the CMC value, the more efficient the surfactant. The HLB defines the extent of surfactant hydrophilicity or lipophilicity. All surfactants belong to one of two possible emulsion types. High HLB value indicates the propensity toward the formation of an oil-in-water emulsion, while low HLB indicates the likely stabilization of a water-in-oil emulsion (Christofi and Ivshina 2002; Van Hamme et al. 2006).

Synthetic surfactants are frequently used worldwide in bioremediation technologies as solvent washing enhancers and emulsifying agents for removal of organic pollutants. Those technologies have been verified in both lab-scale and field-scale studies; they have been applied in situ and ex situ (Sekhon et al. 2011). The hydrophobic moiety of synthetic surfactants is represented by alkanes (paraffins), alkenes (olefins), alkylphenols, alkylbenzenes, and alcohols. Poly(oxyethylenes), sucrose, or polypeptides are common polar domains in nonionic surfactants, while anionic surfactants contain sulfate, sulfonate, or carboxylate groups and cationic surfactants contain a quaternary ammonium group (Sekhon et al. 2011). Sodium dodecyl sulfate (SDS, anionic), Triton X-100, Tween 80, Brij 35, and Tergitol NP10 (neutral/non-ionic) are the most common synthetic surfactants successfully used in remediation technologies (Bustamante et al. 2012).

Biosurfactants are natural agents produced predominantly by microorganisms, but also by plants, e.g., fruit pericarp from *Sapindus mukurossi* (Roy et al. 1997), soapberry-derived saponin (Maity et al. 2013), and soya lecithin (Fava et al. 2004). They could also be in the form of specific natural molecules such as humic acids (Conte et al. 2005) or waste materials such as humic substrates from compost (Kulikowska et al. 2015a). As opposed to low molecular weight compounds commonly defined as biosurfactants, the amphiphilic molecules with high molecular weight are called bioemulsifiers because of their specific behavior in oil/water systems; owing to their high HLB values, biosurfactants usually make stable oil-in-water emulsions (Calvo et al. 2009).

Similarly to synthetic surfactants, biosurfactants can be classified by their charge; however, only few are cationic as anionic or neutral are the most common types. The hydrophobic domains of biosurfactants are those of long-chain fatty acids or hydroxylated fatty acids, while the hydrophilic moieties are represented by amino acids, cyclic peptides, carboxylic acids, alcohols, phosphates, or carbohydrates (Mulligan 2005). Bioemulsifiers, high-molecular-mass biosurfactants, consist of amphipathic proteins, polysaccharides, lipoproteins, lipopolysaccharides, or complexes/combinations of those biopolymers (Calvo et al. 2009).

Microbial biosurfactants can be divided into several basic groups according to their molecular structure including glycolipids (rhamnolipids, trehalolipids, and sophorolipids); fatty acids, phospholipids; and neutral lipids (corynomycolic acid, spiculisporic acid, and phosphatidylethanolamine); lipopeptides (surfactin and lichenysin); and polymeric biosurfactants (emulsan, alasan, biodispersan, liposan, and mannoprotein) (Pacwa-Płociniczak et al. 2011). Biosurfactants have a higher level of specificity than chemical surfactants, which is caused by the wide diversity of their basic chemical structures, e.g., lipids, glycolipids, phospholipids, fatty acids, or peptide structures as well as by the broad variety of their functional groups and detailed chemical structure, e.g., branching, number of functional groups or chains, and carbon chain length.

Specifically, microbial biosurfactants may also be classified by their producers. Bacteria, e.g., *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Rhodococcus*; and fungi, e.g., *Candida* and *Saccharomyces* are the most frequently mentioned biosurfactant-producing microbial strains as reviewed in detail previously (Pacwa-Płociniczak et al. 2011; de Cássia et al. 2014).

24.2.2 Synthetic Surfactants vs. Biosurfactants

The advantages of synthetic surfactants are their availability via synthesis, low costs, and precisely defined chemical composition, all leading to their predictable effects. On the other hand, their major disadvantage compared to biosurfactants is higher toxicity. Another fundamental problem of biosurfactants is the inherently low yield of bioprocesses required for their production, making their market prices relatively high (Lau et al. 2014). In addition, some of their other properties rightly mentioned above as benefits should at the same time be considered as potentially unfavorable. Namely, their biodegradability usually leads to the necessity of periodical biosurfactant additions in order to maintain their effective concentration within the non-sterile processing medium (Maslin and Maier 2000). Also, significant variability caused by rather small changes in the cultivation environment can be observed among the batch cultivations, thus making the composition of the final product and its properties less certain. However, there are many important inherent advantages of biosurfactants, e.g., low toxicity, low CMC values, ecological acceptability, biodegradability, high selectivity, high yet specific activity at extreme temperatures, pH, and salinity as reviewed elsewhere (Bustamante et al. 2012; Bezza and Chirwa 2015).

Several comparative studies stated either a minimal negative effect, if any, or even a boost in cell growth and degradation activity by biosurfactants as opposed to chemical surfactants. Solubilization and/or emulsification of biosurfactants were also similar to those of their synthetic analogs, in many cases even higher. For example, *Sphingomonas* sp. GF2B was able to mineralize up to 83.6% of phenantrene (PHE) within 10 days without the addition of surfactants. Biosurfactants facilitated PHE biodegradation to make it as high as 99.5%, whereas the addition of Tween 80, conversely, inhibited the PHE biodegradation to merely 33.5% (Pei et al. 2010). Oil dispersion by several chemical surfactants and biosurfactants with simultaneous bioremediation of a marine offshore oil spill was studied by Pi et al. (2017). The results showed that the enhancement of petroleum hydrocarbon bioremediation by rhamnolipids was twice as efficient compared to commercial chemical dispersants, GM-2, without any toxic effect on microorganisms. Moreover, when assessing the effect of surfactant combination, a consistent negative effect of GM-2 on rhamnolipids' efficiency was observed.

Because of the above-mentioned disadvantages of synthetic surfactants, especially higher toxicity, low selectivity, and higher CMC values, their effective eco-friendly replacement with biosurfactants is sought. However, synthetic surfactants remain dominant because of the disadvantages of biosurfactants, particularly their high price (Lau et al. 2014). Thus, the most feasible cost-effective alternative to synthetic surfactants may be the use of in situ biosurfactant-producing (and pollutant-biodegrading) microorganisms in bioremediation technologies (Ángeles and Refugio 2013; Hosseininoosheri et al. 2016). Another promising approach to making biosurfactants cost-competitive is the optimization of cultivation conditions (Soares dos Santos et al. 2016) or the use of improved GMO producers (Sekhon et al. 2011; Martins Das Neves et al. 2007).

24.2.3 Specific Properties of Biosurfactants

Generally, biosurfactants show very low or no toxicity, thus they can be widely used in remediation protocols. On the other hand, biosurfactants induce an increase in bioavailability and mobility of toxic compounds that can cause secondary toxic effects against some biota. For example, tests evaluating the seed germination and growth inhibition showed an increase in the phytotoxicity of diesel oil for four terrestrial plant species (alfalfa, sorghum, mustard, and cuckooflower) after the addition of rhamnolipids (Marecik et al. 2012).

Rhamnolipids, as surfactants, can negatively affect microbial cell adhesion and/or biofilm development, thus they can be used to control the microbial biofilm. On the other hand, biosurfactants may exhibit toxicity against some pathogenic microorganisms; therefore, they can counteract human (Das et al. 2008) or plant (Borah et al. 2016) pathogens.

The action of biosurfactants or bioemulsifiers on the environment intensifies the cell–pollutant interactions, thus increasing the pollutant bioavailability. Microorganisms gain direct access to water-solubilized hydrocarbons, heavy metals, or pesticides; their cells have direct contact with released oil as a separate phase in the form of large drops, pseudosolubilized, or emulsified oil (Zhong et al. 2014; Alvarez Yela et al. 2016; Yang et al. 2016). Microorganisms usually uptake exterior water-solubilized and pseudosolubilized hydrocarbon molecules; however, an alternate mechanism similar to active pinocytosis in appearance, by which small rhamnolipid-coated hexadecane droplets were transported through the membrane to cell interior, was proved to exist for *Pseudomonas* sp. (Cameotra and Singh 2009).

Incensement of pollutants' mobility and bioavailability can fundamentally increase the efficiency of remediation techniques like washing or microorganism-based remediation, e.g., bioremediation or bioaugmentation. Depending on the concentration and molecular weight, three basic modes of biosurfactant action can be distinguished, i.e., mobilization, solubilization, and emulsification (Pacwa-Płociniczak et al. 2011). Mobilization occurs under a biosurfactant concentration below CMC when the adsorbed organic compounds are desorbed from a matrix by means of biosurfactants breaking free as separate molecules to become (bio)available in the aqueous phase.

Schematic mechanisms of the action of both low- and high-molecular-mass biosurfactants in soil are shown in Figs. 24.1 and 24.2, respectively. By contrast, solubilization occurs under biosurfactant concentrations above CMC and leads to

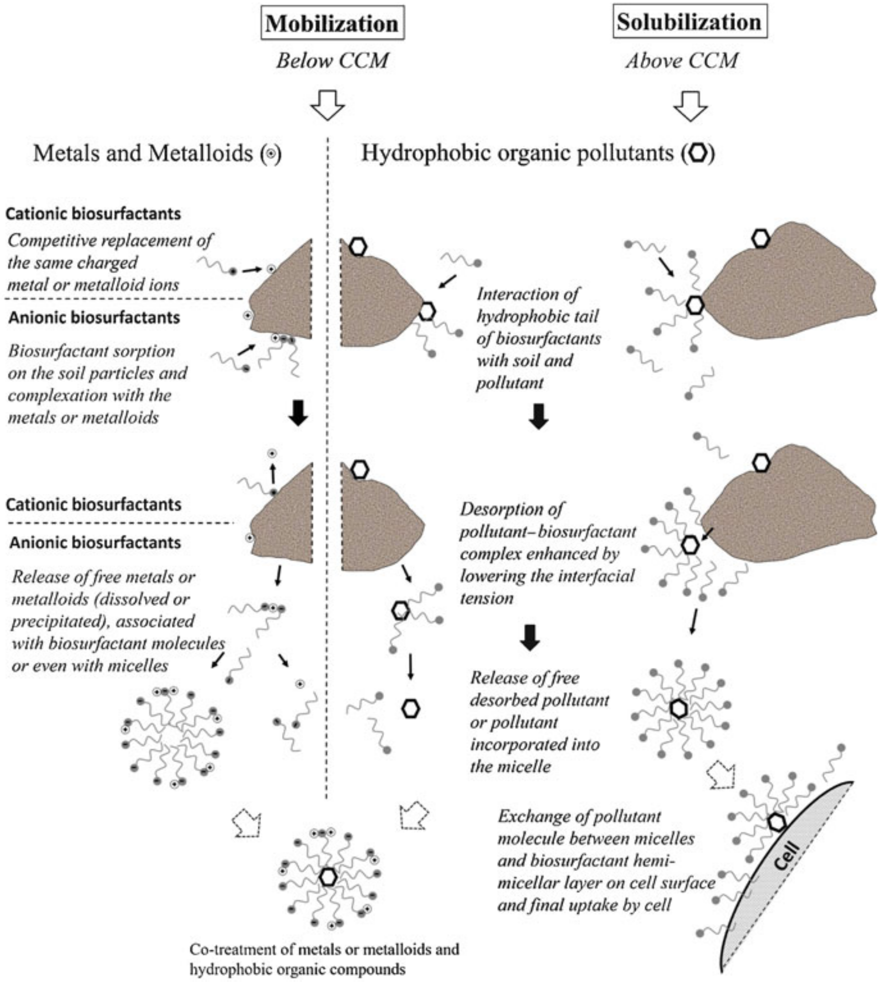

Fig. 24.1 Action of low-molecular-mass biosurfactants. Cationic biosurfactants provide competitive replacement of metal ions while anionic biosurfactants form ionic or coordination bonds with heavy metals enhanced by lowering the interfacial tension. Biosurfactants increase the solubility, bioavailability, and mobility of hydrophobic organic pollutants via their mediated desorption (mobilization) and formation of micelles (solubilization) (Mulligan 2005; Li and Chen 2009; Pacwa-Płociniczak et al. 2011)

incorporating the hydrophobic pollutants into the micelles as a microphase. In this case, the hydrophobic pollutants become protected against the water-based chemicals occurring in the aqueous phase and show an increased formal pollutant concentration in water above its normal equilibrium value (so-called pseudosolubility). Mobilization and solubilization are biosurfactant actions that increase the mobility and bioavailability of not only organic compounds present as single molecules, e.g., pesticides and PAHs or other hydrocarbons but also liquid

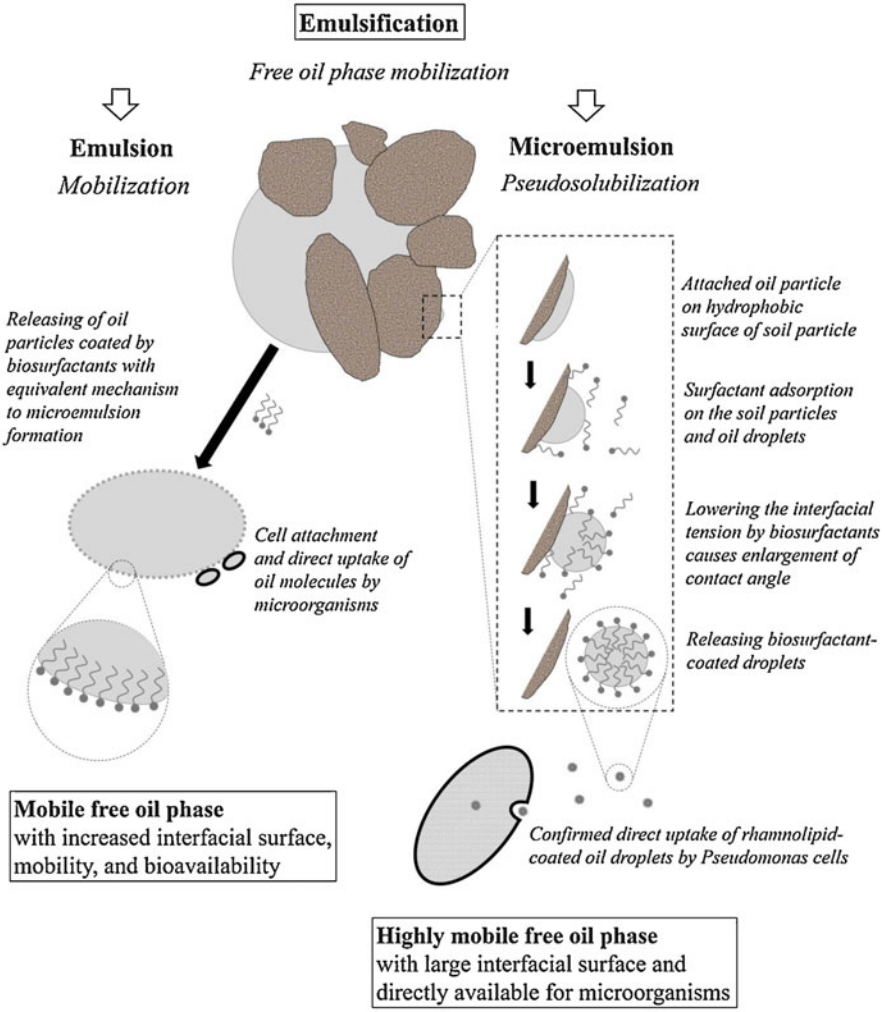

Fig. 24.2 Action of high-molecular-mass biosurfactants. Biosurfactant-coated oil particles (emulsion) or droplets (microemulsions) are released with either an increased interfacial surface, mobility, and bioavailablability or even become directly available for microorganisms (da Rosa et al. 2015; Cameotra and Singh 2009)

hydrocarbons occurring as separate phases forming emulsions or microemulsions. High molecular weight biosurfactants, i.e., bioemulsifiers physically interact with both the immiscible liquid, typically oil occurring as small droplets, and water, thus stabilizing the interface between these two phases in the suspension.

Many biosurfactants are anionic; therefore, they may form ionic or coordination bonds with heavy metals that are stronger than weak metal–soil interactions. This process is enhanced by lowering the interfacial tension. On the other hand, cationic biosurfactants can replace the same charged metal ions by competition and any types of biosurfactants can simply release metals bonded to micelles (Pacwa-Płociniczak et al. 2011).

While in the bulk aqueous phase, the above-mentioned processes are carried out as described; the sorption on particles may reduce the biosurfactant effective concentration in more complex systems with water–solid interfaces (represented by wet soil or sediment). Singh and Cameotra (2013) stated a 50% adsorption of a lipopeptide biosurfactant on soil. This phenomenon combined with the biosurfactant biodegradation should be taken into account when determining the required amount of a biosurfactant for the given task (Maslin and Maier 2000; Ochoa-Loza et al. 2007). To address this problem, a pulse strategy (with a periodic addition of biosurfactant into the system) is often used (Maslin and Maier 2000). However, whenever possible, the best strategy is an in situ (natural or enhanced) production of biosurfactants by suitable species (Hosseininoosheri et al. 2016). The other common setback is that biosurfactants accumulated at the water–gas interface significantly decrease the oxygen mass transfer from the gas to aqueous phase (Sheppard and Cooper 1990), thus potentially causing an oxygen depletion, which can negatively affect remediation bioprocesses.

The above-mentioned influence of biosurfactants on pollutant availability could be accompanied by their influence on microorganisms. The protective function of biosurfactants against toxic compounds described in several publications can be utilized in remediation techniques (Maslin and Maier 2000; Chrzanowski et al. 2009). In addition, biosurfactants can increase the cell wall hydrophobicity, thus enhancing the degradation of hydrophobic compounds by *Pseudomonas aeruginosa* strains with a low cell wall hydrophobicity. However, the action of other strains with low cell wall hydrophobicity was not enhanced (Zhang and Miller 1994). The mechanism of this process was studied in detail by Al-Tahhan et al. (2000). They found that rhamnolipids increase the cell surface hydrophobicity by releasing the genus-specific lipopolysaccharides from the outer membrane of *Pseudomonas aeruginosa*. Another mechanism for *Pseudomonas aeruginosa* was described by Sotirova et al. (2009) when the cell surface hydrophobicity was increased by changing the composition of outer membrane proteins in the presence of a rhamnolipid.

24.2.4 Application Potential of Biosurfactants

24.2.4.1 Treatment of the Sites Contaminated by Organic Pollutants

Hydrocarbon contamination is one of the major environmental problems that result from the activities related to the petrochemical industry. Small-scale field experiments conducted by Tahseen et al. (2016) showed a significant improvement in oil-contaminated soil remediation as a result of rhamnolipid addition. The addition of rhamnolipids caused a greater effect than the addition of mineral nutrients in the form of an NPK (nitrogen, phosphorus, and potassium) fertilizer (32% vs. 26% of degraded oil in 90 days, respectively). Similarly to those results, a significant improvement was observed for the rhamnolipids + bacterial consortium treatment compared to the rhamnolipids + nutrients addition (53% vs. 36% of degraded oil in 90 days, respectively). As expected, the combined rhamnolipids + nutrients + bacterial consortium treatment led to an even greater efficiency, degradation of oil by 77% in 90 days.

Besides petroleum contamination, PAHs are among the most common toxic soil contaminants. Because the environmental PAHs usually occur as a mixture, the biosurfactants' specificity must be taken into account. Portet-Koltalo et al. (2013) conducted a comparative study on the removal of seven PAHs from contaminated soil by either SDS (a synthetic surfactant) or two cyclolipopeptide-based biosurfactants (amphisin and viscosin-like mixture), both produced by two *Pseudomonas fluorescens* strains. SDS was able to release all the studied PAHs while both biosurfactants were only effective toward the desorption of the lowest molecular weight PAHs (naphthalene to fluorene). This selectivity was confirmed by Bezza and Chirwa (2015) when they reported a higher efficiency and faster kinetics of the three-ring phenanthrene desorption from soil in comparison to four-ring pyrene by a lipopeptidal biosurfactant produced by *Paenibacillus dendritiformis*.

Pesticides are widespread, dangerous, and persistent pollutants that tend to be strongly adsorbed in contaminated soils. A glucolipid-based biosurfactant produced by *Burkholderia cenocepacia* BSP3 noticeably enhanced the pesticide solubilization making it available for either washing or bioremediation treatments (Wattanaphon et al. 2008). Also an in situ release of a rhamnolipid produced by *Pseudomonas aeruginosa* CH7, a β-cypermethrin-degrading bacterium, has the potential for enhancing the degradation of pesticides by increasing their bioavailibility (Zhang et al. 2011).

24.2.4.2 Treatment of Sites Contaminated with Heavy Metals

Heavy metals can be efficiently removed from contaminated water, sludge, and soils by a biosurfactant action. Das et al. (2008) reported an efficient chelation of Pb and Cd followed by precipitation by an anionic lipopeptide biosurfactant of a marine origin. Tang et al. (2017) applied saponin, which is an anionic plant biosurfactant having carboxyl groups at the hydrophilic end of its molecule, and showed its ability to bind heavy metals (Zn, Ni, Cu, Mn, Cr, and Pb) for their ultimate removal from water sludge. A specific feature of this surfactant is that its metal binding facilitates the micelle formation by saponin molecules. It was shown that Cr exhibits the highest (65%), while Pb and Mn the lowest (25–35%) extraction efficiency.

Luna et al. (2016) described an efficient treatment of soil and aqueous solutions contaminated from the automotive battery industry using an anionic biosurfactant from *Candida sphaerica*. The results showed the removal rates of 95%, 90%, and 79% for Fe, Zn, and Pb, respectively. The addition of HCl increased the metal removal rate. Additionally, the re-use of a recycled biosurfactant after metal precipitation yielded a reduced but still suitable removal efficiencies of 70%, 62%, and 45% for Fe, Zn, and Pb, respectively.

A foam-enhanced washing technique has been developed to reduce the channeling effect characteristic for soil treatment, thus achieving a homogenous flow, even in a heterogeneous porous medium. Foam can be successfully created and stabilized by biosurfactants. Maity et al. (2013) demonstrated a foam-enhanced removal of Cu, Pb, and Zn from contaminated soil using a plant-based biosurfactant saponin from soapberry *Sapindus mukorossi* and surfactin from *Bacillus subtilis* (BBK006). The optimized foam-enhanced technology resulted in removing 98%, 95%, and 56% of Pb, Cu, and Zn, respectively, while the efficiency of simple washing was twice less efficient.

24.2.4.3 Treatment of Sites Co-contaminated with Heavy Metals and Hydrocarbons

Industrial areas are often co-contaminated with heavy metals and hydrocarbons. Remediation of heavy metal- and hydrocarbon-contaminated soil could be difficult because of the different chemical nature of these two contaminant types. Nevertheless, several recent studies showed the potential of biosurfactants for addressing this problem. One study on washing soil contaminated with high concentrations of heavy metals (Fe, Pb, Ni, Cd, Cu, Co, and Zn) and petroleum hydrocarbons using alipopeptide biosurfactant consisting of surfactin and fengycin originated from *Bacillus subtilis* A21 was published by Singh and Cameotra (2013). Bisurfactant-based washing removed significant amounts of both petroleum hydrocarbons (64.5%) and metals, namely Cd (44.2%), Co (35.4%), Pb (40.3%), Ni (32.2%), Co (26.2%), and Zn (32.07%). The treated soil exhibited a non-inhibited (100%) germination of brown Indian mustard (*Brassica juncea*), whereas the simply washed soil yielded 0% germination. Maslin and Maier (2000) concluded that the biodegradation of phenanthrene can be rhamnolipid-enhanced in organic-metal co-contaminated soils by masking (complexing) toxic cadmium. This protective effect was further improved during the lab-scale simulated degradation of phenanthrene in the aqueous medium and two co-contaminated soils.

24.2.4.4 Long-Term Contaminated Sites—Aged Pollutants

The sorption of pollutants often becomes nearly irreversible over time. Such “aging” of both types of pollutants, either organic compounds (Ncibi et al. 2007) or heavy metals (Liang et al. 2016) causes their low bioavailability, thus prolonging the bioremediation time and decreasing its efficiency. However, it was found that biosurfactants help to effectively release such “irreversibly” adsorbed PAHs (Bezza and Chirwa 2016, 2017; Sánchez-Trujillo et al. 2013), pesticides (Wattanaphon et al. 2008), PCBs (Fava et al. 2003), and heavy metals (Kulikowska et al. 2015b), making the remediation process shorter and more efficient.

Humic substances extracted from compost were used as a washing agent for a simultaneous removal of Cu, Cd, Zn, Pb, and Ni from artificially contaminated soils aged for 1 month, 12 months, and 24 months using both single and multiple washing. The washing efficiency was as high as 80%. Regardless of the applied washing mode, the removal of Cd and Pb was not affected by the contamination age, whereas the removal of Cu, Ni, and Zn was higher in soils that had been aged for a shorter time (Kulikowska et al. 2015b). Bezza and Chirwa (2016) investigated the biosurfactant-enhanced bioremediation of aged PAHs when an additional biosurfactant from *Pseudomonas aeruginosa* and stimulation of the biosurfactant in situ production were applied. The biosurfactant-enhanced degradation of PAHs was 86.5% and 57% in the controls with no second biosurfactant. The additional biosurfactant increased the PAHs bioavailability, thus stimulating the growth of degraders. Then, the population growth led to the depletion of bioavailable PAHs, which triggered an in situ biosurfactant production.

24.2.4.5 Biosurfactant in Prevention of Soil Contamination

As stated above, the assumed low biosurfactant toxicity is not necessarily displayed under all conditions. However, the specific toxicity of some biosurfactants can be appropriately used in many fields. Biosurfactants can actually be used as biopesticides, thus replacing chemical pesticides in order to reduce the pollution of soil, water, and sediment. Increasing amount of studies have already proved the possibility of using biosurfactants against pathogens in agriculture. For example, potato late blight disease caused by pathogen *Phytophthora infestans* was significantly reduced by biosurfactant-producing strain *Pseudomonas koreensis* and its biosurfactant (Hultberg et al. 2010). On a similar token, rhamnolipids can be effectively used against *Fusarium verticillioides* to control the stalk and ear rot disease of maize (Borah et al. 2016).

24.3 Perspectives of Enzymes in Remediation Techniques

Enzymes are naturally present in soil ecosystems. They are produced by soil microorganisms as well as by plants and soil invertebrates. Typical enzymes with potential for bioremediation are listed in Table 24.1. Many enzymes, e.g., peroxidases, laccases, tyrosinases, organophosphorous hydrolases and dehalogenases, lipases, proteases, phosphotriesterases, nitrile- and cyanide-degrading enzymes, and mono- and dioxygenases have been studied for decades to treat the targeted organic compounds, e.g., hydrocarbons; phenols; polyaromatic, nitroaromatic, and chlorinated compounds; dyes; organophosphorous pesticides; nerve/paralyzing agents; or inorganic compounds, e.g., those of metals or metalloids (Piotrowska-Długosz 2017). Fungi and bacteria are the most studied producers of these enzymes (Piotrowska-Długosz 2017); however, marine microorganisms producing enzymes with specific and promising properties are also widely tested (Sivaperumal et al. 2017).

Table 24.1 Enzymes and their actions for soil bioremediation purposes

Enzyme	Producer	Degradation/detoxication action	References
Laccase	*Coriolopsis gallica*	Conversion of bisphenol A into carboxylic acid derivatives such as tartaric acid, β-hydroxybutyric acid and pyroglutamic acid in presence of 1-hydroxybenzotriazole as a laccase mediator	Daâssi et al. (2016)
Manganese peroxidase	*Anthracophyllum discolor*	Conversion of pyrene, anthracene, fluoranthene and phenanthrene in presence of $MnSO_4$	Acevedo et al. (2010)
Cyanide hydratase	*Aspergillus niger* K10	Cyanide decomposition into formate and ammonia	Rinágelová et al. (2014)
Rhodanese (engineered)	*Pseudomonas aeruginosa* (*Escherichia coli* as producer)	Cyanide decomposition in presence of thiosulfate into thiocyanate	Cipollone et al. (2006)
Nitrilase	*Rhodococcus rhodochrous* J1	Conversion of benzonitrile into benzoic acid and acrylonitrile into acrylic acid	Nagasawa et al. (2000)
Nitrile hydratase	*Rhodococcus* sp. RHA1	Conversion of acetonitrile and propionitrile, acrylonitrile and butyronitrile into their amides	Okamoto and Eltis (2007)
Chromate reductase	*Bacillus amyloliquefaciens*	Reduction of Cr(VI) into Cr(III)	Rath et al. (2014)

24.3.1 Specifics of the Use of Enzymes for Soil Bioremediation

The efficiency of enzymes' application, similarly to biosurfactants, strongly depends on the soil composition and the physical properties. Thus the porosity and composition of both mineral and biotic fractions as well as grain fraction distribution, inhomogeneities, pH, and pollutant availability must be evaluated on the contaminated site to choose the best strategy for application of enzymes, which are still considered expensive bioagents (Tuomela and Hatakka 2011; Quiquampoix et al. 2002).

Soil is a multiphase time-variable system consisting of mineral matter, organic matter, water, soil air/gas, and organisms—both invertebrates and microorganisms. Many physical, chemical, and biological interactions occur in soil on several levels of soil structure. In addition, inhomogeneities in both structure and composition as well as local specific processes may further hinder precise soil characterization and application of enzyme-based bioremediation procedures (Zimmerman and Ahn 2010).

Enzymes as protein-based molecules are naturally susceptible to interactions with mineral and organic soil matter (Quiquampoix et al. 2002). Several mechanisms, e.g., covalent attachment, physical entrapment, nonpolar and electrostatic interactions, hydrogen and ionic bonding, result in the formation of enzyme–humus or enzyme–clay complexes (Quiquampoix et al. 2002; Zimmerman and Ahn 2010). Sorption on soil components protects enzymes against inhibitory factors in the environment, thus maintaining a suitable enzyme activity at non-ideal pH, ionic strength, and temperature as well as in the presence of light (photodegradation), proteolytic enzymes, and toxic heavy metals (Tietjen and Wetzel 2003; Quiquampoix et al. 2002; Wang et al. 2017; Wang et al. 2014).

On the other hand, many adverse effects resulting from enzyme–soil interactions decreasing enzyme activity have been described as well, namely: conformational changes, interfacial pH affection, diffusion-limiting kinetics, orientation effects (steric factors), restriction of co-enzyme availability, enzyme encapsulation or denaturation by organic matter or toxic metals, and enzymes' degradation by oxidative minerals (Quiquampoix et al. 2002; Zimmerman and Ahn 2010).

24.3.2 Evaluation of Using Enzymes for Bioremediation

Advantages and disadvantages of enzymes over microorganisms for their use in bioremediation are summarized in Table 24.2. While free enzymes tend to lower mass transfer limitations resulting in faster reactions, immobilized enzymes, either the artificially attached to suitable particles or the naturally immobilized on soil particles, show significant mass transfer limitations (Datta et al. 2017; Quiquampoix et al. 2002). Enzymes can be applied under the lack of substances or conditions essential for live cell functioning, thus eliminating the limitations of nutrient availability, cell acclimation, formation of metabolic by-products, inhibition by other present cell-toxic chemicals. This creates the possibility of their application to toxic or concentrated cell-inhibiting compounds as well as to harsh operational conditions, e.g., extreme pH, temperature, and ionic strength/salinity (Huang et al. 2009; Gianfreda et al. 2016). While the introduction of non-native microbial species is common and mostly non-controversial, yet a subject to approval by state authorities, the introduction of genetically modified microorganisms is strictly regulated and rarely publically acceptable. However, both non-native and genetically modified species can be replaced by enzymes, either native or engineered, presumably with no ecological impact.

The key disadvantage is still the high cost of enzyme application, despite the advances in enzyme production, isolation and purification, potential of the use of genetically engineered enzymes and their hyperproduction by genetically modified species (Eibes et al. 2015). Total mineralization of the majority of complex polluting molecules is undermined by the existence of complex degradation pathways involving many different enzymes. Single enzymes usually only "detoxify" a given pollutant by a single-step chemical modification and thus reduce its toxicity by cleaving a specific group (Acevedo et al. 2010; Daâssi et al. 2016). This limitation can be addressed by applying either mixtures of free enzymes or enzymatic nanoparticles (constructed or natural) that have all the necessary enzymes attached to them. However, the cost of such an advanced bioremediation application is enormous. In addition, it must be ensured that the action of each essential enzyme occurs with the same substrate molecule but at a different time.

Table 24.2 Comparison of advantages and disadvantages of enzymes application in comparison to live microorganisms (Datta et al. 2017; Zimmerman and Ahn 2010; Eibes et al. 2015; Gianfreda et al. 2016; Wang 2006; Acevedo et al. 2010; Wang et al. 2014)

Advantages	Disadvantages
Lower mass transfer limitations (for free enzymes)	Still expensive despite the advances in production
Higher availability of pollutants adsorbed in soil compared to microbial cells	Some need specific cofactors, inductors or mediators
Minimum ecological impact	Necessity of using enzyme mixtures for complex pollutants
Easily controlled process	Risk of mutual decomposition, e.g., proteolysis in enzyme mixtures
Effective in small quantity	Potentially quick decomposition in soil by microbial cells or free enzymes, e.g., peptidases
Long-term storage	Short lifetime
	Competition effects in soil (organic matter)
No DNA	Sorption on soil particles
No introduction of foreign (microbial) species at site	
Publicly acceptable even if GMO-produced	
Overcoming of microorganisms' limits:	Lack of advantageous features of live cells:
No nutrient requirements	Self-replication
No need for acclimation characteristic for whole cells	Active movement (chemotaxis)
No formation of metabolic by-products	Adaptation (changes in cell transport and metabolism)
Enabled application to toxic or concentrated (cell-inhibiting) compounds	Evolution (mutation, horizontal gene transfer)
Potential application under harsh operational conditions (pH, temperature, and ionic strength/salinity)	Production of supporting substances (biosurfactants for pollutants' release)
No inhibition by other cell-toxic chemicals present on site	

Additional compounds essential for enzymatic reactions may also be required. Mediators and/or co-factors, whether naturally present or added, are compounds with redox potential serving as redox shuttles between the enzyme and substrate. For example, laccase mediators, e.g., *p*-coumaric acid, syringaldehyde, or acetosyringone are essential for catalysis of redox reactions catalyzed by this enzyme (Ji et al. 2016). Similarly, hydrogen peroxide for peroxidases, organic hydroperoxides for peroxygenases, and Mn^{2+} for manganese peroxidase are necessary for enzyme action (Acevedo et al. 2010). Ensuring that all necessary substances are lined up at the same spot for the effective course of a given enzyme-catalyzed reaction is problematic; this problem, in particular, can be a major obstacle in static, heterogeneous systems with high and varied sorption properties such as soil (Datta et al. 2017).

A different yet similarly hampering limitation of enzymatic remediation is characteristic for metal and metalloid contaminated sites. Enzymes as well as microorganisms can convert toxic metals and metalloids into their less toxic forms, e.g., reduced or insoluble, but they cannot physically remove them from a contaminated site; furthermore, such conversion is reversible (Wall and Krumholz 2006).

One more limitation is that the enzymes used for pollution bioremediation, e.g., chitinase, carboxymethyl cellulase, β-glucosidase, protease, acid phosphatase, polyphenol oxidase, laccase, and guaiacol oxidase also exhibit an undesired activity against organic soil colloids (Wang et al. 2014), thus decreasing the efficiency of the attack on the target pollutant because of competition.

Natural biodegradability of enzymes can shorten the lifetime of the applied enzymes because of their decomposition in soil by naturally occurring peptidases and microorganisms. Also the risk of mutual decomposition, e.g., proteolysis, should be taken into account when mixtures of enzymes are applied; therefore, repetitive enzyme application can be essential (Gianfreda et al. 2016). In contrast, specific features of live cells lacking in enzyme application, namely self-replication, active movement (chemotaxis), adaptation (changes in cell transport and metabolisms), evolution (mutation, horizontal gene transfer), and production of supporting substances (biosurfactants for pollutants' release) may favor their use.

24.3.3 Application Potential of Enzymes in Bioremediation

Although the list of potential disadvantages is relatively long, a number of promising lab-scale applications of enzymes, either free or immobilized, have been described, as summarized below. The current focus addressing most of the above-listed limitations is on using nanoscale carriers for bioremediation of toxic and recalcitrant pollutants.

24.3.3.1 Treatment of Organic Pollutants

As stated above, enzymatic attacks cause detoxification of complex pollutants rather than their mineralization. For example, bisphenol A was completely removed within 3 h by the action of *Coriolopsis gallica* laccase; however, GC-MS analyses revealed its conversion into carboxylic acid derivatives such as tartaric, β-hydroxybutyric, and pyroglutamic acids (Daâssi et al. 2016). Similarly, several main metabolites, e.g., 10,11-dihydro-10,11-dihydroxy-CBZ, 10,11-dihydro-10,11-epoxy-CBZ, and acridone were identified resulting from the laccase action on carbamazepine. The concomitant decrease of biotoxicity was confirmed (Ji et al. 2016). In another study, both free and immobilized laccases decreased the concentration of sulfathiazole and sulfamethoxazole as well as their biotoxicity in the presence of 1-hydroxybenzotriazole as a laccase mediator. Intermediates were not determined; however, detoxification rather than mineralization was assumed (Rahmani et al. 2015).

In these cases, the ultimate products turned out to be non-toxic, i.e., the enzyme application was justified; however, in general this may not be the case. Another way of enzymatic detoxication is the formation of polymers. For example, triclosan was converted by an integrated nano-bio redox process into non-toxic dimer and trimer products (Bokare et al. 2010).

Enzymes can also be effectively applied to detoxification of cyanide and nitriles. Although rhodanese can be used to detoxicate cyanide in the presence of thiosulfate forming less toxic thiocyanate (Cipollone et al. 2006), a more promising way is to use cyanide hydratases that detoxify cyanide in the sole presence of water. For example, cyanide hydratases convert cyanide into formamide and then cyanide dihydratase converts this intermediate into ammonia and formate. Some bacteria, e.g., *Pseudomonas stutzeri*, and many fungi, e.g., *Fusarium lateritium*, *Aspergillus niger*, *Botryotinia fuckeliana*, produce a variety of cyanide (di)hydratases that were successfully tested for cyanide detoxification (Martínková et al. 2015).

Two main pathways were described for nitrile hydrolysis, either direct hydrolysis to the corresponding carboxylic acids and ammonia by nitrilase (Nagasawa et al. 2000) or a two-step conversion into their amides by nitrile hydratase and further into their carboxylic acids and ammonia by amidase (Okamoto and Eltis 2007). The nitrilase isolated from *Rhodococcus rhodochrous* J1 was able to catalyze a quick conversion of both aromatic and aliphatic nitriles, namely benzonitrile into benzoic acid and acrylonitrile into acrylic acid (Nagasawa et al. 2000). The isolated nitrile hydratase of *Rhodococcus* sp. RHA1 effectively converted acetonitrile, propionitrile, acrylonitrile, and butyronitrile into the corresponding amides. Bacteria containing both essential enzymes to complete the nitrile group detoxification, namely both nitrile hydratase and amidase, could be isolated, too (Okamoto and Eltis 2007). For real-world field scale remediation, however, nitrilases converting nitriles directly to carboxylic acids are much more practical than nitrile hydratases, which require a second step and another enzyme to complete the conversion.

24.3.3.2 Detoxification of Heavy Metals

Detoxification of metals is based mainly on their reduction that usually lowers both their toxicity and solubility. The redox reactions catalyzed by enzymes require an electron donor, usually NAD(P)H originated from aerobic respiration. The second common metabolic pathway occurs when metals/metalloids serve as terminal electron acceptors in anaerobic respiration. In either case, the electrons for metal reduction are produced by complex metabolism. Thus, extracellular reduction by free enzymes (as opposed to extracellular reduction conducted by externally bounded membrane reductases) suffers from a deficiency of essential co-factors (Thatoi et al. 2014). Therefore, the addition of NAD(P)H, a mixture of enzymes or the use of the whole cell metabolism is essential for most cases of enzymatic metal reduction. Payne et al. (2002) explored a promising approach when a mixture of hydrogenase and cytochrome c3 from *Desulfovibrio vulgaris* was used in combination with lactate, pyruvate, and hydrogen as electron donors for the reduction of uranium(VI) to uranium(IV). Cytochrome c3 regenerates the hydrogenase using those electron donors (Payne et al. 2002). However, being merely detoxified rather than removed, metals can be reoxidized chemically by oxygen or can serve as electron (energy) sources for endogenous lithotrophic microorganisms, thus such detoxification can be viewed as only temporary (Wall and Krumholz 2006).

24.3.3.3 Enzyme-Enhanced (Bio)Remediation

Enzymes can be used for enhancing other bioremediation methods. In many cases, enzymatic attacks on toxic pollutants lead to both reduction of their toxicity and increase in their biodegradability. Thus, microbial bioremediation can be fundamentally improved, especially in the case of recalcitrant pollutants with heteroatoms, e.g., organophosphates, chlorinated and nitro compounds. For example, undefined mixtures of extracellular enzymes produced by a microbial consortium under induction by diesel as a substrate were applied during microbial degradation of hydrocarbons on three different oil-contaminated sites. A diesel hydrocarbon degradation of approximately 90% was achieved in 60 h for enzyme-enhanced tests, whereas as many as 175 h were needed to match this efficiency for the degradation tests involving only microbial cells (Jiménez-T et al. 2011).

An application of nano-bio decontamination based on sequential reduction–oxidation was reported by Bokare et al. (2010) for triclosan detoxification under anaerobic conditions (Bokare et al. 2010). A rapid reductive dechlorination by palladized zero-valent iron nanoparticles generated 2-phenoxyphenol, followed by its transformation into a non-toxic polymer using laccase derived from *Trametes versicolor* in the presence of a natural redox mediator, syringaldehyde (Bokare et al. 2010).

24.3.3.4 Enhanced Applications of Enzymes

A number of ways to immobilize/stabilize enzymes on (nano)carriers, e.g., entrapment, encapsulation, adsorption, covalent binding, and self-immobilization as well as many types of nanocarriers, both natural or artificial, e.g., mesostructured silica materials, magnetite nanoparticles, caolinite, porous carbon tubes, and alginate have been described so far (Fernández-Fernández et al. 2013). For example, the laccase of *Trametes versicolor* was immobilized with only a slight loss of activity on functionalized nanoparticles SBA-15 with the average diameter smaller than 10 nm, which are suitable for applications to soil bioremediation (Fernando Bautista et al. 2010). To decrease the anthracene toxicity by conversion to anthroquinone, the laccase from *Trametes versicolor* was immobilized on functionalized (silicated) kaolinite using glutaraldehyde. In both cases, significant enzyme stabilization was shown.

Among other applications, Hong et al. (2017) developed an enzyme-silicate conjugate material consisting of a self-assembled molecular-size thin silicate network cage encasing each individual enzyme molecule (α-chymotrypsin and lipase). Due to the near molecular size of these conjugates, both minimized substrate diffusion limitations and high enzyme stability were achieved. While the k_{cat}/K_m

ratio of α-chymotrypsin slightly decreased from 7.6 ± 0.2 to 6.0 ± 0.1 × 10^5 1/(M s) comparing the native and the coated enzymes, the stability of the coated enzymes increased drastically retaining ca 90% of its initial activity after 3 days of the use, whereas the activity of native enzymes at that point was only about 10%. A similar strong stabilization effect was observed for lipase (Hong et al. 2017).

In addition to artificial nanoparticles, Ng et al. (2015) investigated the production of natural nanoparticles with multiple enzymatic activities by algae *Shewanella* (Ng et al. 2015). *Shewanella xiamenensis* BC01 produced nanoparticles with oxidoreductases including catalase, manganese peroxidase, laccase, NADH dehydrogenase, flavin reductase, azoreductase, and Fe reductase that were generally spherical in shape with a particle size of 7–8 nm (Ng et al. 2015).

Engineered enzymes as well as their producers are a rapidly expanding field of research allowing for production of large quantities of more stable, active, and/or selective enzymes, yielding cheaper products with a higher utility value (Zhang et al. 2016b).

24.3.4 Enzymes in Prevention and Detection of Contamination

Enzymes, e.g., chitinases, proteases, and lipases, can be used as eco-friendly biopesticides because of their anti-insect and anti-fungal potential. Chitinases–bacterial, fungal, or plant–are promising agents for controlling harmful fungi and plants owing to the presence of chitin in the exoskeletal and intestinal linings of insects and in the fungal cell walls (Yan et al. 2008; Liu et al. 2002). Binod et al. (2007) reported that chitinase of *Trichoderma harzianum* negatively affects the growth and the metamorphosis of *Helicoverpa armigera* larvae, with up to 70% mortality rate. Proteases cleaving the peptide bonds of proteins are naturally produced by insect and nematode pathogens in order to penetrate cuticles made up of 70% of protein (Goettel et al. 1989). Serine proteases isolated from a nematophagous fungus, *Lecanicillium psalliotae*, were able to eradicate nematode *Panagrellus redivivus* with 81–100% efficiency (Yang et al. 2005).

On a similar token, lipases may be used for insect control by hydrolyzing ester bonds of lipoproteins, fats, and waxes in the interior parts of the insect body. A purified extracellular lipase from a fungus applied for biocontrol, *Nomuraea rileyi* MJ, was used, as a single agent, to both promote the fungus spore germination and enhance the mortality of an agricultural pest, *Spodoptera litura* (Supakdamrongkul et al. 2010).

Currently, synergistic effects of enzymes and toxin-derived biopesticides, e.g., δ-endotoxins of *Bacillus thuringiensis*, are investigated when these agents are applied in combination. Protease and chitinase of *Paecilomyces lilacinus* were studied by

Khan et al. (2004) to evaluate their activity against the plant-pathogenic nematode *Meloidogyne javanica*. The individual enzymes showed high activities against eggs and juveniles of this nematode, which were further enhanced in their mixture. Similarly, a synergistic effect was shown for chitinase-producing *B. thuringiensis* enhancing the insecticidal activity of *B. thuringiensis* strain DL5789 against agricultural pest insects *Spodoptera exigua* larvae by more than twofold (Liu et al. 2002).

Enzymes can be used as active agents for selective biosensors detecting a wide range of pollutants, both organic and inorganic. For example, a change in conductivity of the enzyme membrane occurs in a biosensor when tyrosinase either interacts with 4-chlorophenol substrate or is inhibited by toxic compounds including diuron, atrazine, copper, lead, and zinc ions (Anh et al. 2006). Cyanide dihydratase converting cyanide into ammonia, which is then detected by an ammonia-selective electrode, was used for constructing a fast-responding and accurate biosensor (Keusgen et al. 2004).

24.4 Perspectives of Using Genetically Modified Organisms in Bioremediation Techniques

In Europe, three possible ways are distinguished for using GMOs, namely, a contained use, introduction of GMO into the environment, and placing GMO or genetically enhanced products on the market. The contained use, i.e., the GMM cultivation in closed reactor systems, is the only use of GMOs unanimously accepted by the general public. A number of studies have proved a high bioremediation potential of genetically modified microorganisms and plants. However, due to possible risks and low public acceptance, the applications of GMOs for remediation technologies are still scarce (Singh et al. 2011; Kolseth et al. 2015).

There are several ways to use GMOs in bioremediation techniques. The first and most logical approach is the introduction into the environment of modified microorganisms or plants having the ability to degrade a broad range of organic soil pollutants as well as to accumulate in biomass and/or to transform toxic heavy metals (usually via reduction). Also, a cost-effective production of eco-friendly bioremediation agents, e.g., biosurfactants and enzymes is a promising way of how genetically modified microorganisms could be used. In addition, enzymes with enhanced original or new unique properties can be created by genetic engineering and used in bioremediation. Prevention of soil contamination using cost-effectively GMO-produced biopesticides or insect-resistant and disease-resistant transgenic crops is another promising way to maintain soil and aquifer clean.

24.4.1 Use of GMO in Bioremediation (Introduction into the Environment)

24.4.1.1 Genetically Modified Microorganisms

A number of studies have been conducted on the bacterial metabolism of engineered microorganisms, e.g., *Escherichia coli*. However, for real-world bioremediation application, the strains that were isolated from the exact contaminated location are considered being more suitable, with a higher likelihood of long-term survival (Lan et al. 2006).

Natural bioremediation processes promoted by microorganisms, e.g., metal reduction, complexation, precipitation, and promotion of the plant growth on the contaminated site (phytoremediation) can be further improved by genetic engineering. For example, *Pichia pastoris* plants were genetically engineered to overexpress a metal-resistant variant of cytochrome b5 reductase to provide a high-throughput bioaccumulation and biotransformation of silver and selenium. Their ions were enzymatically reduced to form stable 70–180 nm elemental nanoparticles. These nanoparticles exhibited at least a tenfold lower cytotoxicity toward HDF, EPG85–257, and T47D cells than silver nitrate and selenium dioxide (Elahian et al. 2017).

Multipurpose approaches can be enabled by enhanced engineering. For example, a cadmium-resistant bacterium *Pseudomonas aeruginosa* was isolated from a Cd-contaminated oil field and further engineered to overexpress targeting metallothioneins on the cell surface to immobilize Cd^{2+}. Introduction of engineered bacteria with improved cadmium tolerance and accumulation enabled the growth promotion of green peas, *Pisum sativum* L., significantly elevating the shoot and root biomass production and leaf chlorophyll content (Huang et al. 2016). *Pseudomonas putida* X3 was engineered by introducing methyl parathion (MP)-degrading gene to obtain the ability to degrade methyl parathion. The application of *Pseudomonas putida* X3 into Cd-contaminated soil reduced the amount of bioavailable Cd by its conversion into a less soluble/exchangeable form and organic-bound Cd (Zhang et al. 2016a).

Enhanced bioremediation of pesticides and chlorinated organic compounds is a common target of genetic engineering. Yang et al. (2010) genetically engineered a native soil bacterium *Stenotrophomonas* sp. strain YC-1 producing methyl parathion hydrolase by a surface anchor system derived from the truncated ice nucleation protein from *Pseudomonas syringae* to possess a broader substrate specificity combined with an enhanced degradation rate of organophosphates. As a result, a mixture of six organophosphate pesticides was completely degraded within 5 h.

24.4.1.2 Genetically Modified Plants

Genetic modification can be used for either improving existing (accumulation of heavy metals) or introducing new abilities (degradation of chlorinated hydrocarbons, explosives, and pesticides). The natural ability of some plants to bioaccumulate heavy metals is used in phytoremediation of contaminated sites.

Overproduction of metal transporters (metal bioaccumulation), overproduction of the enzymes involved in glutathione synthesis and sulfur utilization (cell protection against heavy metals), overproduction of phytochelatin synthase (metal phytochelation), and enhanced phytovolatization of Hg and Se are the most common approaches to using genetically modified organisms for enhanced phytoremediation of heavy metals (Kotrba et al. 2009). For example, the cpSL transgenic Indian mustard *Brassica juncea* accumulated twice as much Se in shoots, it had a 1.8 times higher leaf Se concentration, and was more metal-tolerant (showing a faster growth) than the wild-type plants growing on selenium- and boron-contaminated saline sediments (Bañuelos et al. 2007). Transgenic *Arabidopsis thaliana* plants overexpressing yeast protein YCF1 (detoxifying cadmium by transporting it into vacuoles in yeast) showed a 2.2 times higher biomass yield and 1.5 times higher Cd and Pb accumulation in shoots as compared to wild-type plants (Song et al. 2003).

Introduction of mammalian cytochrome P450 2E1 can drastically increase the degradation of trichloroethylene, ethylene dibromide, carbon tetrachloride, chloroform, and vinyl chloride in the engineered plants (Gohel et al. 2006). The transgenic plants showed a dramatic, up to 640-fold, enhancement of the metabolism of trichloroethylene compared to null vector control plants. They also showed an increased uptake and debromination of ethylene dibromide (Doty et al. 2000). A field trial of trichloroethylene phytoremediation by transgenic poplars expressing cytochrome P450 2E1 was conducted by Legault et al. (2017). The trichloroethylene biodegradation was improved (but not as much as in the corresponding laboratory-scale study), via the evapotranspiration, i.e., evaporation facilitated by plant translocation, so the trichloroethylene content of transgenic leaves was reduced by 80% and its diffusion from transgenic stems (measured as a loss of volatiles from the stem) was reduced by 90% compared to the wild-type poplars.

Explosives and pesticides can also be effectively treated by phytoremediation using genetically engineered plants. Expression of a bacterial nitroreductase gene in *Arabidopsis thaliana* was used to enhance its tolerance to the uptake and degradation of 2,4,6-trinitrotoluene (Kurumata et al. 2005). Genetically modified *Arabidopsis thaliana* plants expressing mammalian cytochrome P450 enzyme CYP1A2 were able to efficiently degrade the herbicide chlortoluron and showed an improved herbicide resistance (Kebeish et al. 2014). Enhanced tolerance to two herbicides, atrazine and metolachlor, as well as their uptake and degradation, were described by Kawahigashi et al. (2006) for transgenic rice expressing human CYP1A1, CYP2B6, and CYP2C19.

A well-functioning rhizosphere is essential for normal plant growth and health in natural environments as well as for the efficient phytoremediation. Symbiosis between plants and microbes can be enhanced using genetic modification tools to make phytoremediation more efficient. For instance, the addition of a plant growth promoting transgenic rhizobacterium *Pseudomonas putida* to the rhizosphere of sunflower (*Helianthus annuus*), cowpea (*Vigna unguiculata*), wheat (*Triticum sativum*), and corn (*Zea* spp.) was tested. The expression of a metal-binding peptide (EC20) in the rhizosphere led to a decrease in cadmium phytotoxicity and up to 40% increase in cadmium accumulation in the root (Wu et al. 2006).

A double genetically modified symbiotic system (both rhizobacterium and plant) was used to improve the Cu phytostabilization in legume roots (Pérez-Palacios et al. 2017). *Medicago truncatula* plants expressing the metallothionein gene *mt4a* from *Arabidopsis thaliana* in roots were used in a symbiotic system to improve the plant Cu tolerance while the genetically modified rhizobacterium *Ensifer medicae*, expressing copper resistance genes *copAB* from *Pseudomonas fluorescens,* was used to improve the plant root Cu accumulation. Results suggested a reduced oxidative stress and further improved root Cu accumulation without altering the metal loading to shoots, thus leading to diminished values of the metal translocation from roots to shoots (Pérez-Palacios et al. 2017).

24.4.2 Production of Useful Chemicals for Remediation Technologies by GMOs (Contained Use)

Surfactant-producing microorganisms can be genetically modified to become hyperproducing; an unpretentious yet well-cultivable strain can be advantageously modified to become a producer. Another application may be a producer modification to enable the use of cheap waste materials as substrates.

Currently the most promising approach is probably the in situ production in a closed reactor, as it is acceptable by the general public. This production poses no risk of adverse environmental effects and may enable production of such biosurfactants that would be cost-competitive to synthetic surfactants (Sekhon et al. 2011; Rashid et al. 2015). For example, there was a twofold increase in the biosurfactant and esterase activities after a successful cloning of the biosurfactant genes from *Bacillus subtilis* SK320 into *E. coli* using olive oil as a substrate. Moreover, the obtained biosurfactant–esterase complex turned out to be a powerful emulsifier (exhibiting the reduction of the surface tension of water from 72 dyn/cm to as low as 30.7 dyn/cm), thus showing promise for bioremediation, hydrocarbon biodegradation, and pharmaceutical applications (Sekhon et al. 2011).

Not only can useful natural enzymes be overexpressed in hyperproduction strains but also modified enzymes may be obtained this way, with enhanced selectivity and/or reaction kinetics. For example, using protein engineering through combinatorial active site saturation testing (CASTing), a 5000-fold increase in $k_{\mathrm{cat}}/K_{\mathrm{M}}$

(measuring the catalytic efficiency) of a specific phosphotriesterase was achieved, enabling its application for the detoxification of an organophosphate insecticide, malathion (Naqvi et al. 2014). Brissos et al. (2015) reported the major improvement of metallo-oxidase McoA from a hyperthermophilic bacterium *Aquifex aeolicus* for aromatic compounds through direct evolution. The k_{cat}/K_M of the obtained enzyme was found to be two orders of magnitude higher than that of the wild-type enzyme for a typical laccase substrate, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), along with a higher activity for phenolics and synthetic aromatic dyes.

24.4.3 GMOs in Prevention of Contamination

To prevent or reduce the use of pesticides, genetically modified insect-resistant and disease-resistant transgenic crops have been engineered. Insect-resistant crops, e.g., maize, potato, and cotton are mostly based on a manipulation resulting in expressing the genes encoding the entomocidal δ-endotoxin from *Bacillus thuringiensis* (Bt-expressing crops). However, besides these currently successfully commercialized crops, other modification approaches, e.g., a RNAi-mediated crop protection, crops with multiple resistance genes, and crops expressing protease inhibitors were applied (Scott et al. 2013; Christou et al. 2006). In addition, many variants of disease-resistant transgenic crops (virus-, fungi-, and bacteria-resistant) have been engineered and some of them have been commercially applied (Galvez et al. 2014; Collinge et al. 2008).

Another way of applying genetic modification is enhanced high-yield production of biopesticides to obtain cost-competitive products thus replacing pesticides in agriculture (Gohel et al. 2006).

24.5 Biopesticides

Advanced production technologies, advanced waste water and waste air cleaning technologies to prevent the deposition of either soluble or volatile contaminants in soil or sediments, advanced risk management, prevention and mitigation of the consequences of environmental disasters are the most common measures taken to minimize soil contamination. However, there is one group of important soil contaminants directly applied to the agricultural land, namely pesticides. To minimize the land contamination by pesticides, the Integrated Pest Management and Integrated Crop Management strategies are preferred in the modern agriculture introducing specific crop cultivation practices, eco-friendly fertilization techniques, and favoring biopesticides over pesticides. Therefore, biopesticides protecting plants against insect pests, weeds, and pathogens as well as biostimulants enhancing nutrition efficiency, abiotic stress tolerance, and/or crop quality traits are promising agents for prevention of soil contamination (du Jardin 2015; Mishra et al. 2015).

The International Biocontrol Manufacturers' Association (IBMA) classifies biocontrol agents into four groups: (1) macrobial, (2) microbial, (3) natural products, and (4) semiochemicals. The application of genetically manipulated plants (GMPs) with enhanced resistance against pests is another promising way. van Lenteren et al. (2018) reviewed in detail the current state of the art in the area of commercially available macrobial and microbial biopesticides. Summarizing these data, microorganisms account for 63% and invertebrates add the remaining 37% of the commercially used biopesticides. Individual microorganism types are represented within the microorganism group as follows: bacteria 45%, fungi 40%, viruses 10%, yeasts 4%, and bacteriophages 1%. The target organisms of microbial biopesticides are a variety of insect pests, weeds, and pathogens. The most important microbial agent is *Bacillus thuringiensis*, which produces dozens of different biopesticides and, in fact, the first commercial biopesticide.

Macrobial biological control agents (natural enemies) and their percentage terms are as follows: Hymenoptera 46%, Acari 18%, Coleoptera 12%, Neuroptera 10%, Hemiptera 6%, Diptera 4%, Nematode 2%, Mantodea 2%, Thysanoptera 1%. They are effective against a wide spectrum of insect pests, e.g., Aphids, Mites, Dipterans, Thrips, Pseudococcids, Lepidopterans.

Natural products/biochemicals are represented by a variety of secondary metabolites produced by plants and some microorganisms, e.g., actinomicetes, which either deter or kill microorganisms, insects, and/or plants. Semiochemicals serve as insect behavior-modifying agents, mostly insect sex pheromones, for crop protection. They are used for either monitoring or pest control by mass trapping, lure-and-kill systems, and mating disruption (Chandler et al. 2011).

Biological control is especially successful and currently plays the central role in the production of many greenhouse crops (Chandler et al. 2011). For example, the predatory mite *Amblyseius swirskii* was successfully used as a biological control agent for whitefly and thrips in sweet pepper greenhouses in Spain and was further integrated into Integrated Pest Management in commercial greenhouses (Calvo et al. 2012). Although biopesticides are known, experimentally tested, commercially used for decades, and highly supported by governments, the global market of biological control agents, e.g., invertebrates, microorganisms, and biochemicals is less than 2% of the pesticide market (van Lenteren et al. 2018).

Although biopesticides are supported by the framework for Community action to achieve the sustainable use of pesticides by Directive 2009/128/EC of the European Parliament, in Europe, unlike the USA, the registration of new biopesticides is still slow and the procedure is expensive, particularly for small manufacturers. The changes in registration procedures will result in faster registration of more microbial biological control agents and, consequently, in lower product costs (van Lenteren et al. 2018). Generally, the wide use of biopesticides is limited because of their high price, expensive and long research, expensive and slow registration, fragmented (regional) market, and lack of awareness about their benefits, poor customer support, and, in some cases, poor and/or fluctuating quality and shelf life, and inconsistent field performance (Mishra et al. 2015). Recently, several company mergers and acquisitions that may facilitate a healthy and sustainable development of the biopesticide industry have occurred. In addition, the largest agrochemical firms, e.g., Bayer CropScience and Syngenta have turned to the biopesticide market investing into the corresponding research, production capacities, and distribution as well as the acquisitions of smaller producers of biopestides.

This biopesticide industry consolidation could lead to a new round of commercial and academic research on novel biopesticides and biostimulants directly connected to cost-effective production, established worldwide distribution and market as well as full customer support, which are the essential prerequisites for a significant increase of the share of these eco-friendly biological agents at the expense of chemical agents.

References

Acevedo F, Pizzul L, Castillo MP, González ME, Cea M, Gianfreda L, Diez MC (2010) Degradation of polycyclic aromatic hydrocarbons by free and nanoclay-immobilized manganese peroxidase from *Anthracophyllum discolor*. Chemosphere 80(3):271–278. https://doi.org/10.1016/j.chemosphere.2010.04.022

Al-Tahhan RA, Sandrin TR, Bodour AA, Maier RM (2000) Rhamnolipid-induced removal of lipopolysaccharide from *Pseudomonas aeruginosa*: effect on cell surface properties and interaction with hydrophobic substrates. Appl Environ Microbiol 66(8):3262–3268. https://doi.org/10.1128/AEM.66.8.3262-3268.2000

Alvarez Yela AC, Tibaquirá Martínez MA, Rangel Piñeros GA, López VC, Villamizar SH, Núñez Vélez VL, Abraham W-R, Vives Flórez MJ, González Barrios AF (2016) A comparison between conventional *Pseudomonas aeruginosa* rhamnolipids and *Escherichia coli* transmembrane proteins for oil recovery enhancing. Int Biodeterior Biodegrad 112:59–65. https://doi.org/10.1016/j.ibiod.2016.04.033

Ángeles M-T, Refugio R-V (2013) *In situ* biosurfactant production and hydrocarbon removal by *Pseudomonas putida* CB-100 in bioaugmented and biostimulated oil-contaminated soil. Braz J Microbiol 44(2):595–605. https://doi.org/10.1590/S1517-83822013000200040

Anh TM, Dzyadevych SV, Prieur N, Duc CN, Pham TD, Jaffrezic Renault N, Chovelon J-M (2006) Detection of toxic compounds in real water samples using a conductometric tyrosinase biosensor. Mater Sci Eng C 26(2–3):453–456. https://doi.org/10.1016/j.msec.2005.10.025

Bañuelos G, LeDuc DL, Pilon-Smits EAH, Terry N (2007) Transgenic Indian mustard overexpressing selenocysteine lyase or selenocysteine methyltransferase exhibit enhanced potential for selenium phytoremediation under field conditions. Environ Sci Technol 41(2):599–605. https://doi.org/10.1021/es061152i

Bezza FA, Chirwa EMN (2015) Production and applications of lipopeptide biosurfactant for bioremediation and oil recovery by *Bacillus subtilis* CN2. Biochem Eng J 101:168–178. https://doi.org/10.1016/j.bej.2015.05.007

Bezza FA, Chirwa EMN (2016) Biosurfactant-enhanced bioremediation of aged polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated soil. Chemosphere 144:635–644. https://doi.org/10.1016/j.chemosphere.2015.08.027

Bezza FA, Chirwa EMN (2017) The role of lipopeptide biosurfactant on microbial remediation of aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil. Chem Eng J 309:563–576. https://doi.org/10.1016/j.cej.2016.10.055

Binod P, Sukumaran RK, Shirke SV, Rajput JC, Pandey A (2007) Evaluation of fungal culture filtrate containing chitinase as a biocontrol agent against *Helicoverpa armigera*. J Appl Microbiol 103(5):1845–1852. https://doi.org/10.1111/j.1365-2672.2007.03428.x

Bokare V, Murugesan K, Kim Y-M, Jeon J-R, Kim E-J, Chang YS (2010) Degradation of triclosan by an integrated nano-bio redox process. Bioresour Technol 101(16):6354–6360. https://doi.org/10.1016/j.biortech.2010.03.062

Borah SN, Goswami D, Sarma HK, Cameotra SS, Deka S (2016) Rhamnolipid biosurfactant against *Fusarium verticillioides* to control stalk and ear rot disease of maize. Front Microbiol 7:1505. https://doi.org/10.3389/fmicb.2016.01505

Brissos V, Ferreira M, Grass G, Martins LO (2015) Turning a hyperthermostable metallo-oxidase into a laccase by directed evolution. ACS Catal 5(8):4932–4941. https://doi.org/10.1021/acscatal.5b00771

Bustamante M, Durán N, Diez MC (2012) Biosurfactants are useful tools for the bioremediation of contaminated soil: a review. J Soil Sci Plant Nutr 12(4):667–687. https://doi.org/10.4067/S0718-95162012005000024

Calvo C, Manzanera M, Silva-Castro GA, Uad I, González-López J (2009) Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. Sci Total Environ 407 (12):3634–3640. https://doi.org/10.1016/j.scitotenv.2008.07.008

Calvo FJ, Bolckmans K, Belda JE (2012) Biological control-based IPM in sweet pepper greenhouses using *Amblyseius swirskii* (Acari: Phytoseiidae). Biocontrol Sci Tech 22 (12):1398–1416. https://doi.org/10.1080/09583157.2012.731494

Cameotra SS, Singh P (2009) Synthesis of rhamnolipid biosurfactant and mode of hexadecane uptake by *Pseudomonas* species. Microb Cell Factories 8:16. https://doi.org/10.1186/1475-2859-8-16

Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP (2011) The development, regulation and use of biopesticides for integrated pest management. Philos Trans R Soc B 366 (1573):1987–1998. https://doi.org/10.1098/rstb.2010.0390

Christofi N, Ivshina IB (2002) Microbial surfactants and their use in field studies of soil remediation. J Appl Microbiol 93(6):915–929. https://doi.org/10.1046/j.1365-2672.2002.01774.x

Christou P, Capell T, Kohli A, Gatehouse JA, Gatehouse AMR (2006) Recent developments and future prospects in insect pest control in transgenic crops. Trends Plant Sci 11(6):302–308. https://doi.org/10.1016/j.tplants.2006.04.001

Chrzanowski Ł, Wick LY, Meulenkamp R, Kaestner M, Heipieper HJ (2009) Rhamnolipid biosurfactants decrease the toxicity of chlorinated phenols to *Pseudomonas putida* DOT-T1E. Lett Appl Microbiol 48(6):756–762. https://doi.org/10.1111/j.1472-765X.2009.02611.x

Cipollone R, Ascenzi P, Frangipani E, Visca P (2006) Cyanide detoxification by recombinant bacterial rhodanese. Chemosphere 63(6):942–949. https://doi.org/10.1016/j.chemosphere.2005.09.048

Collinge DB, Søgaard Lund O, Thordal-Christensen H (2008) What are the prospects for genetically engineered, disease resistant plants? Eur J Plant Pathol 121(3):217–231. https://doi.org/10.1007/s10658-007-9229-2

Conte P, Agretto A, Spaccini R, Piccolo A (2005) Soil remediation: humic acids as natural surfactants in the washings of highly contaminated soils. Environ Pollut 135(3):515–522. https://doi.org/10.1016/j.envpol.2004.10.006

da Rosa CFC, Freire DMG, Ferraz HC (2015) Biosurfactant microfoam: application in the removal of pollutants from soil. J Environ Chem Eng 3(1):89–94. https://doi.org/10.1016/j.jece.2014.12.008

Daâssi D, Prieto A, Zouari-Mechichi H, Martínez MJ, Nasri M, Mechichi T (2016) Degradation of bisphenol A by different fungal laccases and identification of its degradation products. Int Biodeterior Biodegrad 110:181–188. https://doi.org/10.1016/j.ibiod.2016.03.017

Das P, Mukherjee S, Sen R (2008) Antimicrobial potential of a lipopeptide biosurfactant derived from a marine *Bacillus circulans*. J Appl Microbiol 104(6):1675–1684. https://doi.org/10.1111/j.1365-2672.2007.03701.x

Datta R, Anand S, Moulick A, Baraniya D, Pathan SI, Rejsek K, Vranova V, Sharma M, Sharma D, Kelkar A, Formanek P (2017) How enzymes are adsorbed on soil solid phase and factors limiting its activity: a review. Int Agrophys 31(2):287–302. https://doi.org/10.1515/intag-2016-0049 de Cássia FS Silva R, Almeida DG, Rufino RD, Luna JM, Santos VA, Sarubbo LA (2014) Applications of biosurfactants in the petroleum industry and the remediation of oil spills. Int J Mol Sci 15(7):12523–12542. https://doi.org/10.3390/ijms150712523

Doty SL, Shang TQ, Wilson AM, Tangen J, Westergreen AD, Newman LA, Strand SE, Gordon MP (2000) Enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2E1. Proc Natl Acad Sci U S A 97(12):6287–6291. https://doi.org/10.1073/pnas.97.12.6287

du Jardin P (2015) Plant biostimulants: definition, concept, main categories and regulation. Sci Hortic 196:3–14. https://doi.org/10.1016/j.scienta.2015.09.021

Eibes G, Arca-Ramos A, Feijoo G, Lema JM, Moreira MT (2015) Enzymatic technologies for remediation of hydrophobic organic pollutants in soil. Appl Microbiol Biotechnol 99 (21):8815–8829. https://doi.org/10.1007/s00253-015-6872-y

Elahian F, Reiisi S, Shahidi A, Mirzaei SA (2017) High-throughput bioaccumulation, biotransformation, and production of silver and selenium nanoparticles using genetically engineered *Pichia pastoris*. Nanomedicine 13(3):853–861. https://doi.org/10.1016/j.nano.2016.10.009

Fava F, Bertin L, Fedi S, Zannoni D (2003) Methyl-β-cyclodextrin-enhanced solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-contaminated soils. Biotechnol Bioeng 81(4):381–390. https://doi.org/10.1002/bit.10579

Fava F, Berselli S, Conte P, Piccolo A, Marchetti L (2004) Effects of humic substances and soya lecithin on the aerobic bioremediation of a soil historically contaminated by polycyclic aromatic hydrocarbons (PAHs). Biotechnol Bioeng 88(2):214–223. https://doi.org/10.1002/bit.20225

Fernández-Fernández M, Sanromán MÁ, Moldes D (2013) Recent developments and applications of immobilized laccase. Biotechnol Adv 31(8):1808–1825. https://doi.org/10.1016/j.biotechadv.2012.02.013

Fernando Bautista L, Morales G, Sanz R (2010) Immobilization strategies for laccase from *Trametes versicolor* on mesostructured silica materials and the application to the degradation of naphthalene. Bioresour Technol 101(22):8541–8548. https://doi.org/10.1016/j.biortech.2010.06.042

Galvez LC, Banerjee J, Pinar H, Mitra A (2014) Engineered plant virus resistance. Plant Sci 228:11–25. https://doi.org/10.1016/j.plantsci.2014.07.006

Gianfreda L, Rao MA, Scelza R, de la Luz Mora M (2016) Chapter 6: Role of enzymes in environment cleanup/remediation. In: Dhillon GS, Kaur S (eds) Agro-industrial wastes as feedstock for enzyme production. Academic, San Diego, p 133–155. doi:https://doi.org/10.1016/B978-0-12-802392-1.00006-X

Goettel MS, St Leger RJ, Rizzo NW, Staples RC, Roberts DW (1989) Ultrastructural localization of a cuticle-degrading protease produced by the entomopathogenic fungus *Metarhizium anisopliae* during penetration of host (*Manduca sexta*) cuticle. J Gen Microbiol 135:2233–2239

Gohel V, Singh A, Vimal M, Ashwini P, Chhatpar HS (2006) Bioprospecting and antifungal potential of chitinolytic microorganisms. Afr J Biotechnol 5(2):54–72

Hong S-G, Kim BC, Na HB, Lee J, Youn J, Chung S-W, Lee C-W, Lee B, Kim HS, Hsiao E, Kim SH, Kim B-G, Park HG, Chang HN, Hyeon T, Dordick JS, Grate JW, Kim J (2017) Single enzyme nanoparticles armored by a thin silicate network: single enzyme caged nanoparticles. Chem Eng J 322:510–515. https://doi.org/10.1016/j.cej.2017.04.022

Hosseininoosheri P, Lashgari HR, Sepehrnoori K (2016) A novel method to model and characterize in-situ bio-surfactant production in microbial enhanced oil recovery. Fuel 183:501–511. https://doi.org/10.1016/j.fuel.2016.06.035

Huang Q, Zhu J, Qiao X, Cai P, Rong X, Liang W, Chen W (2009) Conformation, activity and proteolytic stability of acid phosphatase on clay minerals and soil colloids from an Alfisol. Colloids Surf B Biointerfaces 74(1):279–283. https://doi.org/10.1016/j.colsurfb.2009.07.031

Huang JL, Liu ZB, Li SY, Xu B, Gong YH, Yang Y, Sun HX (2016) Isolation and engineering of plant growth promoting rhizobacteria *Pseudomonas aeruginosa* for enhanced cadmium bioremediation. J Gen Appl Microbiol 62(5):258–265. https://doi.org/10.2323/jgam.2016.04.007

Hultberg M, Bengtsson T, Liljeroth E (2010) Late blight on potato is suppressed by the biosurfactant-producing strain *Pseudomonas koreensis* 2.74 and its biosurfactant. BioControl 55(4):543–550. https://doi.org/10.1007/s10526-010-9289-7

Ji C, Hou JW, Wang K, Zhang YT, Chen V (2016) Biocatalytic degradation of carbamazepine with immobilized laccase-mediator membrane hybrid reactor. J Membr Sci 502:11–20. https://doi.org/10.1016/j.memsci.2015.12.043

Jiménez-T RG, Moliterni E, Rodríguez L, Fernández FJ, Villaseñor J (2011) Feasibility of mixed enzymatic complexes to enhanced soil bioremediation processes. Procedia Environ Sci 9:54–59. https://doi.org/10.1016/j.proenv.2011.11.010

Kawahigashi H, Hirose S, Ohkawa H, Ohkawa Y (2006) Phytoremediation of the herbicides atrazine and metolachlor by transgenic rice plants expressing human *CYP1A1*, *CYP2B6*, and *CYP2C19*. J Agric Food Chem 54(8):2985–2991. https://doi.org/10.1021/jf052610u

Kebeish R, Azab E, Peterhaensel C, El-Basheer R (2014) Engineering the metabolism of the phenylurea herbicide chlortoluron in genetically modified *Arabidopsis thaliana* plants expressing the mammalian cytochrome P450 enzyme CYP1A2. Environ Sci Pollut Res 21 (13):8224–8232. https://doi.org/10.1007/s11356-014-2710-5

Keusgen M, Kloock JP, Knobbe D-T, Jünger M, Krest I, Goldbach M, Klein W, Schöning MJ (2004) Direct determination of cyanides by potentiometric biosensors. Sensors Actuators B Chem 103(1–2):380–385. https://doi.org/10.1016/j.snb.2004.04.067

Khan A, Williams KL, Nevalainen HKM (2004) Effects of *Paecilomyces lilacinus* protease and chitinase on the eggshell structures and hatching of *Meloidogyne javanica* juveniles. Biol Control 31(3):346–352. https://doi.org/10.1016/j.biocontrol.2004.07.011

Kolseth A-K, D'Hertefeldt T, Emmerich M, Forabosco F, Marklund S, Cheeke TE, Hallin S, Weih M (2015) Influence of genetically modified organisms on agro-ecosystem processes. Agric Ecosyst Environ 214:96–106. https://doi.org/10.1016/j.agee.2015.08.021

Kotrba P, Najmanova J, Macek T, Ruml T, Mackova M (2009) Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. Biotechnol Adv 27 (6):799–810. https://doi.org/10.1016/j.biotechadv.2009.06.003

Kulikowska D, Gusiatin ZM, Bułkowska K, Kierklo K (2015a) Humic substances from sewage sludge compost as washing agent effectively remove Cu and Cd from soil. Chemosphere 136:42–49. https://doi.org/10.1016/j.chemosphere.2015.03.083

Kulikowska D, Gusiatin ZM, Bułkowska K, Klik B (2015b) Feasibility of using humic substances from compost to remove heavy metals (Cd, Cu, Ni, Pb, Zn) from contaminated soil aged for different periods of time. J Hazard Mater 300:882–891. https://doi.org/10.1016/j.jhazmat.2015.08.022

Kurumata M, Takahashi M, Sakamoto 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 C 60 (3–4):272–278. https://doi.org/10.1515/znc-2005-3-412

Lan WS, Gu JD, Zhang JL, Shen BC, Jiang H, Mulchandani A, Chen W, Qiao CL (2006) Coexpression of two detoxifying pesticide-degrading enzymes in a genetically engineered bacterium. Int Biodeterior Biodegrad 58(2):70–76. https://doi.org/10.1016/j.ibiod.2006.07.008

Lau EV, Gan S, Ng HK, Poh PE (2014) Extraction agents for the removal of polycyclic aromatic hydrocarbons (PAHs) from soil in soil washing technologies. Environ Pollut 184:640–649. https://doi.org/10.1016/j.envpol.2013.09.010

Legault EK, James CA, Stewart K, Muiznieks I, Doty SL, Strand SE (2017) A field trial of TCE phytoremediation by genetically modified poplars expressing cytochrome P450 2E1. Environ Sci Technol 51(11):6090–6099. https://doi.org/10.1021/acs.est.5b04758

Li J-L, Chen B-H (2009) Surfactant-mediated biodegradation of polycyclic aromatic hydrocarbons. Materials 2(1):76–94. https://doi.org/10.3390/ma2010076

Liang S, Guan D-X, Li J, Zhou C-Y, Luo J, Ma LQ (2016) Effect of aging on bioaccessibility of arsenic and lead in soils. Chemosphere 151:94–100. https://doi.org/10.1016/j.chemosphere.2016.02.070

Liu M, Cai QX, Liu HZ, Zhang BH, Yan JP, Yuan ZM (2002) Chitinolytic activities in *Bacillus thuringiensis* and their synergistic effects on larvicidal activity. J Appl Microbiol 93 (3):374–379. https://doi.org/10.1046/j.1365-2672.2002.01693.x

Luna JM, Rufino RD, Sarubbo LA (2016) Biosurfactant from *Candida sphaerica* UCP0995 exhibiting heavy metal remediation properties. Process Saf Environ Prot 102:558–566. https://doi.org/10.1016/j.psep.2016.05.010

Maity JP, Huang YM, Hsu C-M, Wu C-I, Chen C-C, Li C-Y, Jean J-S, Chang Y-F, Chen C-Y (2013) Removal of Cu, Pb and Zn by foam fractionation and a soil washing process from contaminated industrial soils using soapberry-derived saponin: a comparative effectiveness assessment. Chemosphere 92(10):1286–1293. https://doi.org/10.1016/j.chemosphere.2013.04.060

Marecik R, Wojtera-Kwiczor J, Ławniczak Ł, Cyplik P, Szulc A, Piotrowska-Cyplik A, Chrzanowski Ł (2012) Rhamnolipids increase the phytotoxicity of diesel oil towards four common plant species in a terrestrial environment. Water Air Soil Pollut 223(7):4275–4282. https://doi.org/10.1007/s11270-012-1190-9

Martínková L, Veselá AB, Rinágelová A, Chmátal M (2015) Cyanide hydratases and cyanide dihydratases: emerging tools in the biodegradation and biodetection of cyanide. Appl Microbiol Biotechnol 99(21):8875–8882. https://doi.org/10.1007/s00253-015-6899-0

Martins Das Neves LC, Miazaki Ohara Miyamura TT, Junji Kobayashi M, Vessoni Penna TC, Converti A (2007) Production of biosurfactant by a genetically-modified strain of *Bacillus subtilis* expressing green fluorescent protein. Ann Microbiol 57(3):377–381. https://doi.org/10.1007/BF03175076

Maslin P, Maier RM (2000) Rhamnolipid-enhanced mineralization of phenanthrene in organic-metal co-contaminated soils. Biorem J 4(4):295–308. https://doi.org/10.1080/10889860091114266

Mishra J, Tewari S, Singh S, Arora NK (2015) Biopesticides: where we stand? In: Arora NK (ed) Plant microbes Symbiosis: applied facets. Springer, New Delhi, pp 37–75. https://doi.org/10.1007/978-81-322-2068-8_2

Mulligan CN (2005) Environmental applications for biosurfactants. Environ Pollut 133 (2):183–198. https://doi.org/10.1016/j.envpol.2004.06.009

Nagasawa T, Wieser M, Nakamura T, Iwahara H, Yoshida T, Gekko K (2000) Nitrilase of *Rhodococcus rhodochrous* J1. Eur J Biochem 267(1):138–144. https://doi.org/10.1046/j.1432-1327.2000.00983.x

Naqvi T, Warden AC, French N, Sugrue E, Carr PD, Jackson CJ, Scott C (2014) A 5000-fold increase in the specificity of a bacterial phosphotriesterase for malathion through combinatorial active site mutagenesis. PLoS One 9(4):e94177. https://doi.org/10.1371/journal.pone.0094177

Ncibi MC, Mahjoub B, Gourdon R (2007) Effects of aging on the extractability of naphthalene and phenanthrene from Mediterranean soils. J Hazard Mater 146(1–2):378–384. https://doi.org/10.1016/j.jhazmat.2006.12.032

Ng I-S, Xu F, Zhang X, Ye C (2015) Enzymatic exploration of catalase from a nanoparticle producing and biodecolorizing algae *Shewanella xiamenensis* BC01. Bioresour Technol 184:429–435. https://doi.org/10.1016/j.biortech.2014.09.079

Ochoa-Loza FJ, Noordman WH, Jannsen DB, Brusseau ML, Maier RM (2007) Effect of clays, metal oxides, and organic matter on rhamnolipid biosurfactant sorption by soil. Chemosphere 66 (9):1634–1642. https://doi.org/10.1016/j.chemosphere.2006.07.068

Okamoto S, Eltis LD (2007) Purification and characterization of a novel nitrile hydratase from *Rhodococcus* sp. RHA1. Mol Microbiol 65(3):828–838. https://doi.org/10.1111/j.1365-2958.2007.05834.x

Pacwa-Płociniczak M, Płaza GA, Piotrowska-Seget Z, Cameotra SS (2011) Environmental applications of biosurfactants: recent advances. Int J Mol Sci 12(1):633–654. https://doi.org/10.3390/ijms12010633

Payne RB, Gentry DM, Rapp-Giles BJ, Casalot L, Wall JD (2002) Uranium reduction by *Desulfovibrio desulfuricans* strain G20 and a cytochrome c_3 mutant. Appl Environ Microbiol 68(6):3129–3132. https://doi.org/10.1128/aem.68.6.3129-3132.2002

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 (6):771–779. https://doi.org/10.1016/s1002-0160(10)60067-7

Pérez-Palacios P, Romero-Aguilar A, Delgadillo J, Doukkali B, Caviedes MA, Rodríguez-Llorente ID, Pajuelo E (2017) Double genetically modified symbiotic system for improved Cu phytostabilization in legume roots. Environ Sci Pollut Res 24(17):14910–14923. https://doi.org/10.1007/s11356-017-9092-4

Pi Y, Chen B, Bao M, Fan F, Cai Q, Ze L, Zhang B (2017) Microbial degradation of four crude oil by biosurfactant producing strain *Rhodococcus* sp. Bioresour Technol 232:263–269. https://doi.org/10.1016/j.biortech.2017.02.007

Piotrowska-Długosz A (2017) The use of enzymes in bioremediation of soil xenobiotics. In: Hashmi MZ, Kumar V, Varma A (eds) Xenobiotics in the soil environment: monitoring, toxicity and management. Springer, Cham, pp 243–265. https://doi.org/10.1007/978-3-319-47744-2_17

Portet-Koltalo F, Ammami MT, Benamar A, Wang H, Le Derf F, Duclairoir-Poc C (2013) Investigation of the release of PAHs from artificially contaminated sediments using cyclolipopeptidic biosurfactants. J Hazard Mater 261:593–601. https://doi.org/10.1016/j.jhazmat.2013.07.062

Quiquampoix H, Servagent-Noinville S, Baron M-H (2002) Enzyme adsorption on soil mineral surfaces and consequences for the catalytic activity. In: Burns RG, Dick RP (eds) Enzymes in the environment: activity, ecology, and applications. CRC Press, Boca Raton, pp 285–306

Rahmani K, Faramarzi MA, Mahvi AH, Gholami M, Esrafili A, Forootanfar H, Farzadkia M (2015) Elimination and detoxification of sulfathiazole and sulfamethoxazole assisted by laccase immobilized on porous silica beads. Int Biodeter Biodegrad 97:107–114. https://doi.org/10.1016/j.ibiod.2014.10.018

Rashid N-FM, Azemi M-AFM, Amirul A-AA, Wahid MEA, Bhubalan K (2015) Simultaneous production of biopolymer and biosurfactant by genetically modified *Pseudomonas Aeruginosa* UMTKB-5. Int Proc Chem, Biol Environ Eng 90(3):16–21. https://doi.org/10.7763/ipcbee

Rath BP, Das S, Mohapatra PKD, Thatoi H (2014) Optimization of extracellular chromate reductase production by *Bacillus amyloliquefaciens* (CSB 9) isolated from chromite mine environment. Biocatal Agric Biotechnol 3(3):35–41. https://doi.org/10.1016/j.bcab.2014.01.004

Rinágelová A, Kaplan O, Veselá AB, Chmátal M, Křenková A, Plíhal O, Pasquarelli F, Cantarella M, Martínková L (2014) Cyanide hydratase from *Aspergillus niger* K10: overproduction in *Escherichia coli*, purification, characterization and use in continuous cyanide degradation. Process Biochem 49(3):445–450. https://doi.org/10.1016/j.procbio.2013.12.008

Roy D, Kommalapati RR, Mandava SS, Valsaraj KT, Constant WD (1997) Soil washing potential of a natural surfactant. Environ Sci Technol 31(3):670–675. https://doi.org/10.1021/es960181y

Sánchez-Trujillo MA, Morillo E, Villaverde J, Lacorte S (2013) Comparative effects of several cyclodextrins on the extraction of PAHs from an aged contaminated soil. Environ Pollut 178:52–58. https://doi.org/10.1016/j.envpol.2013.02.029

Scott JG, Michel K, Bartholomay LC, Siegfried BD, Hunter WB, Smagghe G, Zhu KY, Douglas AE (2013) Towards the elements of successful insect RNAi. J Insect Physiol 59 (12):1212–1221. https://doi.org/10.1016/j.jinsphys.2013.08.014

Sekhon KK, Khanna S, Cameotra SS (2011) Enhanced biosurfactant production through cloning of three genes and role of esterase in biosurfactant release. Microb Cell Factories 10(1):49. https://doi.org/10.1186/1475-2859-10-49

Sheppard JD, Cooper DG (1990) The effects of a biosurfactant on oxygen transfer in a cyclone column reactor. J Chem Technol Biotechnol 48(3):325–336. https://doi.org/10.1002/jctb.280480308

Singh AK, Cameotra SS (2013) Efficiency of lipopeptide biosurfactants in removal of petroleum hydrocarbons and heavy metals from contaminated soil. Environ Sci Pollut Res 20 (10):7367–7376. https://doi.org/10.1007/s11356-013-1752-4

Singh JS, Abhilash PC, Singh HB, Singh RP, Singh DP (2011) Genetically engineered bacteria: an emerging tool for environmental remediation and future research perspectives. Gene 480 (1–2):1–9. https://doi.org/10.1016/j.gene.2011.03.001

Sivaperumal P, Kamala K, Rajaram R (2017) Chapter 8: Bioremediation of industrial waste through enzyme producing marine microorganisms. Adv Food Nutr Res 80:165–179. https://doi.org/10.1016/bs.afnr.2016.10.006

Soares dos Santos A, Pereira N Jr, Freire DMG (2016) Strategies for improved rhamnolipid production by *Pseudomonas aeruginosa* PA1. PeerJ 4:e2078. https://doi.org/10.7717/peerj.2078

Soberón-Chávez G, Maier RM (2011) Biosurfactants: a general overview. In: Soberón-Chávez G (ed) Biosurfactants: from genes to applications. Springer, Berlin/Heidelberg, pp 1–11. https://doi.org/10.1007/978-3-642-14490-5_1

Song W-Y, Ju Sohn E, Martinoia E, Jik Lee Y, Yang Y-Y, Jasinski M, Forestier C, Hwang I, Lee Y (2003) Engineering tolerance and accumulation of lead and cadmium in transgenic plants. Nat Biotechnol 21(8):914–919. https://doi.org/10.1038/nbt850

Sotirova A, Spasova D, Vasileva-Tonkova E, Galabova D (2009) Effects of rhamnolipid-biosurfactant on cell surface of *Pseudomonas aeruginosa*. Microbiol Res 164(3):297–303. https://doi.org/10.1016/j.micres.2007.01.005

Supakdamrongkul P, Bhumiratana A, Wiwat C (2010) Characterization of an extracellular lipase from the biocontrol fungus, *Nomuraea rileyi* MJ, and its toxicity toward *Spodoptera litura*. J Invertebr Pathol 105(3):228–235. https://doi.org/10.1016/j.jip.2010.06.011

Tahseen R, Afzal M, Iqbal S, Shabir G, Khan QM, Khalid ZM, Banat IM (2016) Rhamnolipids and nutrients boost remediation of crude oil-contaminated soil by enhancing bacterial colonization and metabolic activities. Int Biodeterior Biodegrad 115:192–198. https://doi.org/10.1016/j.ibiod.2016.08.010

Tang J, He J, Liu T, Xin X (2017) Removal of heavy metals with sequential sludge washing techniques using saponin: optimization conditions, kinetics, removal effectiveness, binding intensity, mobility and mechanism. RSC Adv 7(53):33385–33401. https://doi.org/10.1039/C7RA04284A

Thatoi H, Das S, Mishra J, Rath BP, Das N (2014) Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: a review. J Environ Manag 146:383–399. https://doi.org/10.1016/j.jenvman.2014.07.014

Tietjen T, Wetzel RG (2003) Extracellular enzyme-clay mineral complexes: enzyme adsorption, alteration of enzyme activity, and protection from photodegradation. Aquat Ecol 37 (4):331–339. https://doi.org/10.1023/B:AECO.0000007044.52801.6b

Tuomela M, Hatakka A (2011) 6.16 – oxidative fungal enzymes for bioremediation. In: Moo-Young M (ed) Comprehensive biotechnology, 2nd edn. Academic, Burlington, pp 183–196. https://doi.org/10.1016/B978-0-08-088504-9.00370-6

Van Hamme JD, Singh A, Ward OP (2006) Physiological aspects. Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnol Adv 24(6):604–620. https://doi.org/10.1016/j.biotechadv.2006.08.001

van Lenteren JC, Bolckmans K, Köhl J, Ravensberg WJ, Urbaneja A (2018) Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63(1):39–59. https://doi.org/10.1007/s10526-017-9801-4

Wall JD, Krumholz LR (2006) Uranium reduction. Annu Rev Microbiol 60:149–166. https://doi.org/10.1146/annurev.micro.59.030804.121357

Wang P (2006) Nanoscale biocatalyst systems. Curr Opin Biotechnol 17(6):574–579. https://doi.org/10.1016/j.copbio.2006.10.009

Wang WJ, Li YH, Wang HM, Zu YG (2014) Differences in the activities of eight enzymes from ten soil fungi and their possible influences on the surface structure, functional groups, and element composition of soil colloids. PLoS One 9(11):e111740. https://doi.org/10.1371/journal.pone.0111740

Wang ZQ, Li YB, Tan XP, He WX, Xie W, Megharaj M, Wei GH (2017) Effect of arsenate contamination on free, immobilized and soil alkaline phosphatases: activity, kinetics and thermodynamics. Eur J Soil Sci 68(1):126–135. https://doi.org/10.1111/ejss.12397

Wattanaphon HT, Kerdsin A, Thammacharoen C, Sangvanich P, Vangnai AS (2008) A biosurfactant from *Burkholderia cenocepacia* BSP3 and its enhancement of pesticide solubilization. J Appl Microbiol 105(2):416–423. https://doi.org/10.1111/j.1365-2672.2008.03755.x

Wu CH, Wood TK, Mulchandani A, Chen W (2006) Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. Appl Environ Microbiol 72(2):1129–1134. https://doi.org/10.1128/AEM.72.2.1129-1134.2006

Yan RX, Hou JH, Ding DF, Guan WQ, Wang CY, Wu ZQ, Li MG (2008) *In vitro* antifungal activity and mechanism of action of chitinase against four plant pathogenic fungi. J Basic Microbiol 48(4):293–301. https://doi.org/10.1002/jobm.200700392

Yang J, Huang X, Tian B, Wang M, Niu Q, Zhang K (2005) Isolation and characterization of a serine protease from the nematophagous fungus, *Lecanicillium psalliotae*, displaying nematicidal activity. Biotechnol Lett 27(15):1123–1128. https://doi.org/10.1007/s10529-005-8461-0

Yang C, Song C, Mulchandani A, Qiao C (2010) Genetic engineering of *Stenotrophomonas* strain YC-1 to possess a broader substrate range for organophosphates. J Agric Food Chem 58 (11):6762–6766. https://doi.org/10.1021/jf101105s

Yang Z, Zhang Z, Chai L, Wang Y, Liu Y, Xiao R (2016) Bioleaching remediation of heavy metal-contaminated soils using *Burkholderia* sp. Z-90. J Hazard Mater 301:145–152. https://doi.org/10.1016/j.jhazmat.2015.08.047

Zhang YM, Miller RM (1994) Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. Appl Environ Microbiol 60(6):2101–2106

Zhang C, Wang S, Yan Y (2011) Isomerization and biodegradation of beta-cypermethrin by *Pseudomonas aeruginosa* CH7 with biosurfactant production. Bioresour Technol 102 (14):7139–7146. https://doi.org/10.1016/j.biortech.2011.03.086

Zhang R, Xu XJ, Chen WL, Huang QY (2016a) Genetically engineered *Pseudomonas putida* X3 strain and its potential ability to bioremediate soil microcosms contaminated with methyl parathion and cadmium. Appl Microbiol Biotechnol 100(4):1987–1997. https://doi.org/10.1007/s00253-015-7099-7

Zhang X, Ng I-S, Chang J-S (2016b) Cloning and characterization of a robust recombinant azoreductase from *Shewanella xiamenensis* BC01. J Taiwan Inst Chem Eng 61:97–105. https://doi.org/10.1016/j.jtice.2016.01.002

Zhong H, Liu Y, Liu Z, Jiang Y, Tan F, Zeng G, Yuan X, Yan M, Niu Q, Liang Y (2014) Degradation of pseudo-solubilized and mass hexadecane by a *Pseudomonas aeruginosa* with treatment of rhamnolipid biosurfactant. Int Biodeterior Biodegrad 94:152–159. https://doi.org/10.1016/j.ibiod.2014.07.012

Zimmerman AR, Ahn M-Y (2010) Organo-mineral–enzyme interaction and soil enzyme activity. In: Shukla G, Varma A (eds) Soil enzymology. Springer, Berlin/Heidelberg, pp 271–292. https://doi.org/10.1007/978-3-642-14225-3_15