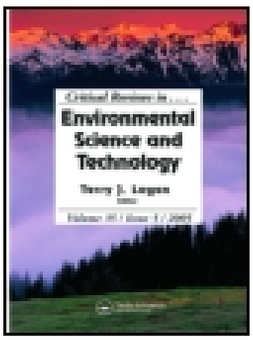

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Streptomyces genus as biotechnological tool for pesticide degradation in polluted systems

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ABSTRACT

General awareness exists regarding the detrimental effects of pesticides use and management on the environment; therefore, their removal from contaminated matrices has become a major concern in the field of environmental engineering and science. Nowadays, biological methods have attracted considerable attention and many microorganisms have been isolated, characterized and recognized as responsible for pesticide degradation. This review shows the current evidence and results obtained in the last decades that support the use of microorganisms belonging to the *Streptomyces* genus as a promising biotechnological tool for bioremediation of pesticides contaminated matrices. This review covers the degradation of pesticides in both liquid and soil systems, soil slurries and biobed biomixtures, by single, consortia, free and/or immobilized *Streptomyces* strains.

KEYWORDS

Biodegradation; bioremediation; immobilization; pesticides; mixed cultures; streptomyces

1. Introduction

Due to the actual demand for food, more than 2 million tons of pesticides are released into the environment each year (Storck, Karpouzas, & Martin-Laurent, 2017). The use of pesticides in agriculture has helped to improve yields and prevent crop losses. Despite the benefits of pesticides for the economic activities, the use of these compounds often induces possible negative effects for the environment and human health (Finger, Möhring, Dalhaus, & Böcker, 2017; Storck et al., 2017). There are three main groups of pesticides that have attracted a lot of attention because of the risk

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involved in their use. The first of them is the group of the organochlorine pesticides (OCP), which are compounds with a long half-life and high persistence in the environment (Verma, Jaiswal, & Sagar, 2014), so they were gradually replaced by the more biodegradable organophosphorus pesticides (OPP). These are characterized to be highly toxic, causing acute and chronic effects on the central nervous system of mammals including human beings (Singh, 2009). Today, the pyrethroid pesticides are viewed as a replacement of more toxic or recalcitrant compounds. However, there is evidence that they may have reproductive and neurological toxicity, act as endocrine disruptors and possible human carcinogenic (Chen, Hu et al., 2011).

The inappropriate pesticide management has resulted in the release of these compounds into the environment such that contamination of soil, water, air and food by pesticides has been reported worldwide (Fosu-Mensah, Okoffo, Darko, & Gordon, 2016; Pozo et al., 2016; Yu, Liu, Liu, Wang, & Wang, 2016). Pesticide contamination may result mainly from agricultural processes, but also from manufacturing, handling, improper storage, use in urban areas and inadequate disposal of pesticides and wastes (Köck-Schulmeyer et al., 2013; Morillo & Villaverde, 2017). Whatever the origin of pesticide wastes, they are characterized by high compound loads, high toxicity and serious problems of disposal and treatment (Karas et al., 2016). Another problem related to pesticides pollution is the production of a large amount of waste material and obsolete compounds deposits. These materials are, generally, chemically stable and very difficult either to reuse or to dispose of.

The extensive use and the lack of appropriate management of pesticides coupled to the contamination potential of these compounds have become the major concerns in the field of science and technology. Therefore, efforts have been made to develop technologies that guarantee that pesticide residues are eliminated in a harmless, effective and cost-effective manner. The currently proposed treatments for pesticide waste are physical, chemical and biological treatments. Today, incineration is the only method approved by the Environmental Protection Agency for pesticide destruction. However, this treatment presents serious public opposition because of their potentially toxic emissions; moreover, it is cost-restrictive and therefore not usually available in developing countries (Ghaly & Dave, 2012). Currently, promising methods include biological treatments, where microbial remediation strategies are developed to encourage the metabolism of environmental contaminants into inoffensive or less toxic compounds (Dzionek, Wojcieszy, & Guzik, 2016; Gupta, Pathak, & Fulecar, 2015). The toxicity of pesticides makes the removal of them from water and wastewater of great importance, especially for the production of drinking water and also for the maintenance of aquatic ecosystems. Bioremediation is among the most effective approaches for the removal of organic contaminants from a wide

range of industrial wastewaters (Ahmadi et al., 2017). The use of bioreactors for pesticide degradation has been studied in order to avoid and reduce wastewater containing pesticides before implementing a final disposal. Bioreactors are commonly employed as biotechnologies for the treatment of pesticides in wastewater by either free or immobilized microorganisms, which are evaluated principally in batch and continuous mode (Pradeep & Malavalli, 2016; Yadav, Srivastva, Sharan, Nath, & Kumar, 2014). Otherwise, soil bioremediation can be achieved by natural attenuation, biostimulation, and bioaugmentation. Bioaugmentation is considered a green technology defined as the improvement of the degradative capacity of contaminated areas by introducing specific microorganisms characterized by the desired catalytic capabilities (Cycón, Mrozik, & Piotrowska-Seget, 2017). Such microorganisms should be able to degrade specific pollutant compounds, survive in a foreign and unfriendly habitat and be genetically stable and viable (Dzionek et al., 2016). For this purpose, an extensive list of microorganisms has been isolated, characterized, and described as pesticide degraders (Cycón et al., 2017).

The biotechnological potential of actinobacteria for environmental purposes has been demonstrated through their ability to remove organic and inorganic pollutants, making them as good tools for the remediation process. For this reason, actinobacteria have received special attention as candidates for bioremediation (Álvarez et al., 2017). Among them, microorganisms belonging to the *Streptomyces* genus are one of the most representative pesticide-degrading actinobacteria due to their capacity to remove compounds from different chemical class. Pogell (1996) and De Schrijver and De Mot (1999) provided a comprehensive review of studies performed on pesticide degradation by *Streptomyces* and actinomycetes (currently actinobacteria), respectively. However, several advances in the use of these microorganisms in pesticide decontamination have occurred in the subsequent decades, where techniques such as bioaugmentation, the use of microbial consortia and immobilization have been incorporated for enhancement of the capabilities in pesticide degradation and bioremediation strategies in different environmental matrices. This review is focused on about different strategies using streptomycetes to improve the bioremediation of several matrices polluted with pesticides. We present evidence about biotransformation of these pollutants by employing *Streptomyces* for the bioaugmentation of liquid, soil and slurries systems. These matrices are representative of those that occur in actual environments, such as liquid effluents, soils and aquatic sediments contaminated with these xenobiotics. Also, this review compiles and updates information available on different strategies currently used for restoring these systems, as an approximation to *in situ* or *ex situ* bioremediation treatments.

2. *Streptomyces*: A promising genus for environmental applications

The phylum Actinobacteria is one of the major phyla in the domain Bacteria, as inferred from its branching pattern in the 16S rRNA gene tree. This phylum encompasses five classes, 19 orders, 50 families and 221 genera; however, many new taxa are being discovered (Ludwig & Klenk, 2005). Members of this taxon are widely distributed in aquatic and terrestrial habitats, including extreme habitats, such as deep-sea sediments (Pathom-Aree et al., 2006) and hyper-arid desert soils (Okoro et al., 2009). Thus, these microorganisms represent one of the most diverse groups and they are capable of surviving in a number of ecological niches due to their bioactive potential.

Within the phylum Actinobacteria, microorganisms belonging to the genus *Streptomyces* stand out because of its morphological and physiological versatilities. These microorganisms, generally named as Streptomycetes, are aerobic, Gram-positive, non-acid-fast and form extensively branched substrate and aerial mycelia. They are chemoorganotrophic, with oxidative metabolism. Colonies are discrete and present lichenoid, leathery or butyrous texture. Streptomycetes can produce a wide variety of pigments, responsible for the color of the vegetative and aerial mycelia. Also, colored diffusible pigments may be produced (Figure 1) (Kämpfer, 2012).

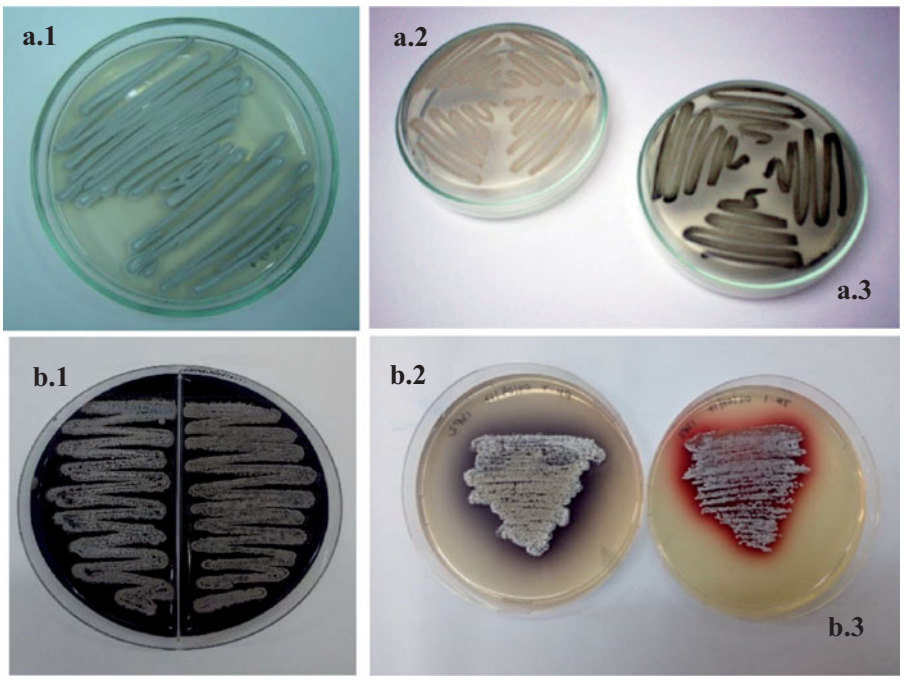


Figure 1. Cultures of *Streptomyces* strains isolated from pesticide-contaminated matrices in the Northwest of Argentine (a) and South of Chile (b). Strains *Streptomyces* sp. M7 (a.1), *Streptomyces* sp. A5 (a.2 and a.3) cultured in Starch Casein Agar medium, and strains *Streptomyces* sp. PM7 (b.1 and b.2), and *Streptomyces* sp. TM1 (b.3) cultured in ISP2 agar medium.

The *Streptomyces* genus has gained special importance as the most potent source of antibiotics (Kandasamy, Muthusamy, Thangaswamy, & Senthilkumar, 2012) and other bioactive secondary metabolites (Solecka, Zajko, Postek, & Rajnisz, 2012). Their metabolic potential offers a strong area of research. Accordingly, the role of streptomycetes in biotechnology and medicine is well known and these industries are always looking for novelty bioactive compounds.

Streptomycetes are widely distributed in soil and play an important role in the recycling of organic matter. It is not surprising that the genomes of these microorganisms encode high numbers of predicted secreted proteins such as hydrolases, glucosyltransferases, xylanases, laccases, and proteases, among others (Shivlata & Satyanarayana, 2015; Ventura et al., 2007). These bacteria, therefore, are considered as a promising source of a wide range of enzymes, so they perform microbial transformations of organic compounds, degrading a wide range of hydrocarbons, pesticides, aliphatic and aromatic compounds (Sambasiva Rao, Tripathy, Mahalaxmi, & Prakasham, 2012).

Among the pesticide remediation strategies, biological treatments are an attractive approach for removing diverse kind of compounds. In this context, the use of indigenous streptomycetes for bioremediation of pesticide-contaminated environments is an attractive approach, since these microorganisms are already adapted to the soil and sediment habitats. In addition to their potential metabolic diversity, strains of *Streptomyces* may be well suited for soil inoculation as a consequence of their mycelial growth habit, relatively rapid growth rates, colonization of semi-selective substrates and their ability to be genetically manipulated (Shelton, Khader, Karns, & Pogell, 1996). Another additional advantage is that the vegetative hyphal mass of the *Streptomyces* can differentiate into spores that contribute to its spread and persistence; these spores are a semi-dormant stage in the cycle life that can survive in soil for long periods and impart resistance to low nutrient concentrations and water availability (Ensign, 1978; Karagouni, Vionis, Baker, & Wellington, 1993; Mayfield, Williams, Ruddick, & Hatfield, 1972).

Based on the above, different *Streptomyces* strains have received attention as candidates for bioremediation of environments polluted with recalcitrant organic compounds. The microorganisms belonging to this important genus exhibit a wide range of activities and have the ability to grow and degrade several chemical families of pesticides (Figure 2), including organochlorines, organophosphates, pyrethroids, ureas and chloroacetanilides (Álvarez et al., 2017; Briceño, Pizzul, & Diez, 2013). The following sections provide a review of the main results and trends related to the degradation of pesticides by *Streptomyces* strains and its environmental biotechnological applications.

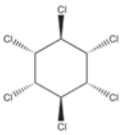
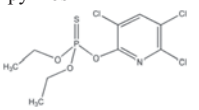
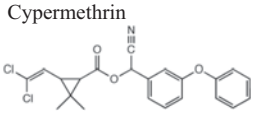
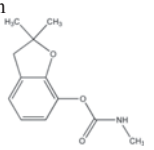
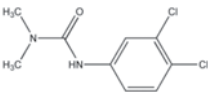
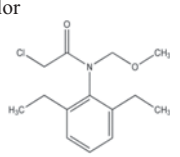
Chemical class	Molecular estructure of pesticide
Organochlorine	Lindane 
Organophosphate	Chlorpyrifos 
Pyrethroids	Cypermethrin 
Carbamate	Carbofuran 
Ureas	Diuron 
Chloroacetanilides	Alachlor 

Figure 2. Chemical structures of representative pesticides degraded by *Streptomyces* strains.

3. *Streptomyces* requirements and strategies to degrade pesticides in liquid media

For the effective use of microorganisms in bioremediation processes, it is extremely important to determine their potential for removal of pesticides in liquid media under optimal conditions (Cycón et al., 2017). In this sense, *Streptomyces* from diverse matrices contaminated or not with pesticides, have been isolated. In order to obtain alachlor resistant and degrading *Streptomyces* strains, Sette, Mendonça Alves da Costa, Marsaioli, and Manfio (2004); Sette, de Oliveira, & Manfio (2005) isolated fifty-three strains from alachlor contaminated soils, being sixteen of these able to grow at a high pesticide concentration (720 mg L^{-1}) and six strains were able to remove over 50% of alachlor from a mineral salt medium. Castillo,

Felis, Aragón, Cuesta, and Sabater (2006) studied the biodegradation of diuron by seventeen strains of *Streptomyces* spp. isolated from agricultural and nonagricultural soils. The pesticide was removed over 50% from ISP2 medium at five days of incubation by the strains isolated from the soil with a history of diuron application, while at the same time a lower degradation of diuron was obtained by the strains isolated from nonagricultural soils, thus confirming the adaptation response to the contaminant.

In general, pesticides are rapidly degraded by *Streptomyces* in liquid media, requiring only days or in some cases only a few hours. However, depending on the strain, the kind of pesticide, and its concentration, a longer duration may be required to obtain high or complete removal. Lin et al. (2011) showed that *Streptomyces* sp. HU-S-01 completely degraded cypermethrin within 24 and 30 h, at concentrations lower than 50 mg L^{-1}; however, at higher concentrations, between 150 to 250 mg L^{-1}, degradation was incomplete even after 48 h of incubation. An important variable in the pesticide-degrading ability is the supplementation of culture media with additional nutrient sources. In most cases, pesticide degradation by *Streptomyces* is evaluated by using the compound as the only source of nutrients in the form of C, N and P. However, when the pesticides are co-metabolized, an additional source of nutrients may increase its degradation. For instance, a study performed by Jayabarath, Musfira, Giridhar, Shyam sundar, and Arulmurugan (2010) showed that carbofuran removal increased about 30% in a glycerol asparagine broth contaminated with 20 mg L^{-1} pesticide and inoculated separately with *Streptomyces alanosinicus* and *Streptomyces atratus*, compared to the removal observed when the pesticide was used as only carbon source. Similarly, *Streptomyces* spp. strains showed a better performance in the presence of starch as co-substrate for the propoxur removal, which favored also its growth in the liquid medium (Rahmansyah, Agustiyani, Heddy, & Dewi, 2012).

There is a growing need to develop remediation strategies to remove toxic and persistent compounds. For instance, in the case of OCP, Benimeli, Amoroso, Chaile, and Castro (2003) isolated four *Streptomyces* strains able to grow in the presence of aldrin, chlordane, DDD, DDE, DDT, dieldrin, heptachlor, lindane, and methoxychlor. The authors found that the strain *Streptomyces* sp. M7 presented high ability to grow in the presence of these compounds. Then, focusing their studies on lindane removal, which was induced by glucose addition (Benimeli, Castro, Chaile, & Amoroso, 2006), they evidenced the release of chloride ions which could be associated to the presence of enzymes with dechlorinase activity in the cell-free extract after 48 and 96 h of incubation (Cuozzo, Rollán, Abate, & Amoroso, 2009). In the same way, Cuozzo, Fuentes, Bourguignon, Benimeli, and Amoroso (2012) reported chlordane removal from a

minimum liquid medium by the strain *Streptomyces* sp. A5, when this pesticide was added as the only carbon source. Regarding the removal of OPP, Briceño et al. (2012) isolated several strains of *Streptomyces* spp. from agricultural soils and studied the ability of these microorganisms to tolerate and remove chlorpyrifos from a liquid medium. They showed that 25 and 50 mg L^{-1} chlorpyrifos were efficiently removed through different patterns of removal and metabolite production. Moreover, the strain *Streptomyces* sp. AC5 contributed to a depletion of 3,5,6-trichloro-2-pyridinol over time, while using the strain *Streptomyces* sp. AC7 the metabolite concentration was increased. The better performance observed for *Streptomyces* sp. AC5 was related to the presence of the enzyme organophosphorus hydrolase involved in chlorpyrifos degradation (Briceño, Schalchli, Mutis, et al., 2016). The organophosphorus hydrolase enzyme has been also described for *Streptomyces venezuelae* ACT1, a marine isolate with a high potential to remove the insecticide parathion (Naveena, Annalakshmi, & Partha, 2013), which has been banned in all its formulation due to high toxicity for wildlife, environment, and humans.

Enzyme-based pesticide degradation is an innovative treatment method for the removal of pesticides from polluted environments (Verma et al., 2014). The advantage of enzymatic remediation over microbial treatment include high reaction activity towards recalcitrant pesticides, lower sensitivity to the pesticide concentration, coverage of a wide range of physicochemical gradients in the environment and easy control of field application (Gupta et al., 2015). Microorganisms belonging to *Streptomyces* genus represent an efficient source of oxidoreductases and hydrolytic enzymes. Amylase, protease, cellulase, xylanase, esterase, nitrile hydratase, laccase, dehydrogenase, and dehalogenase are some of the enzymes that could be involved in pesticides degradation (Karigar & Rao, 2011; Shivlata & Satyanarayana, 2015).

In the case of pyrethroid-degrading enzymes, the most reported correspond to the hydrolase family. However, a novel monooxygenase was described for *Streptomyces* sp. HU-S-01, isolated from a pyrethroid-contaminated sludge and able to degrade cypermethrin (Chen et al., 2013). The monooxygenase was able to catalyze the degradation of the insecticide beta-cypermethrin to form five simple aromatic by-products via hydroxylation and diaryl cleavage. *Streptomyces aureus* HP-S-01 also presents the capacity to biodegrade pyrethroid pesticides according to the reported by Chen, Lai et al. (2011). These authors reported that 50–300 mg L^{-1} deltamethrin were completely removed within seven days at the optimum conditions (27 °C, pH 7.8 and biomass amount of 0.55 g dry wt L^{-1}). Moreover, *S. aureus* HP-S-01was able to remove the main deltamethrin metabolite, 3-phenoxybenzaldehyde, and therefore no persistent

accumulative by-products were detected in the medium (Chen, Geng, Xiao, & Hu, 2012).

In general, biotechnological processes have been conducted using pure cultures or single microbial strains obtaining successful performances. Nonetheless, in many cases, microbial consortia have the potential to be more productive and robust than monocultures for their application in detoxification processes. Microbial consortia isolated from natural environments have been used for pesticide removal from wastewater showing promising results. However, naturally occurring microbial consortia are not free of difficulties associated with the culture conditions, long operation cycles, low efficiency and poor stability and controllability (Ding, Song, Wang, Liu, & Yuan, 2016). This is the reason why the design and formulation of microbial consortia have received considerable attention. In this context, the selection of highly effective strains is essential in order to obtain microbial consortia by combining the microorganisms with positive interaction for increasing their pesticides-degrading abilities (Hu et al., 2017; Zafra, Absalón, Anducho-Reyes, Fernandez, & Cortés-Espinosa, 2017). Microbial consortia have the capability to catalyze many processes through metabolic pathways and enzymatic systems of different organisms and to be more stable toward environmental perturbations; therefore, in bioremediation, the division of labor is crucial because complex toxic compounds often require several steps for degradation (Hays, Patrick, Ziesack, Oxman, & Silver, 2015).

Strategies for enhancing the pesticide biodegradation by using multiple strains have been widely studied during the last decade. In this context, significant researches have been conducted to evaluate the degradation of pesticides in liquid media by using microbial consortia constituted by *Streptomyces* spp. The removal of cypermethrin by a co-culture formed by *Bacillus cereus* ZH-3 and *Streptomyces aureus* HP-S-01 was described as the first mixed microbial consortium capable of metabolizing cypermethrin as the only carbon source in liquid media. It was also able to co-metabolize the pesticide in the presence of glucose, particularly with high cypermethrin concentrations reaching 500 mg L^{-1} (Chen, Luo, et al., 2012). The authors found that cypermethrin degradation was enhanced in the co-culture with a half-life of 13 h, whereas in the pure cultures a half-life of approximately 33–43 h was observed. In addition, the consortium converted cypermethrin into various metabolites by hydrolysis of ester linkages and oxidation of 3-phenoxybenzyl. However, the intermediate compounds were completely metabolized and no accumulation in the liquid medium was therefore observed (Chen, Luo, et al., 2012). Fuentes, Sáez, Benimeli, and Amoroso (2011) evaluated lindane degradation by pure and mixed cultures of streptomycetes previously isolated from a pesticide-contaminated environment.

The authors showed that both dechlorinase activity and lindane removal were improved by certain mixed cultures by up to 12-fold compared to the pure cultures, being the microbial consortia of two, three and four *Streptomyces* the most efficient ones. The efficiency of a microbial consortium can be increased throughout an acclimation period, as reported by Saez, Aparicio, Amoroso, and Benimeli (2015). In their study, a consortium formed by the strains *Streptomyces* sp. A2, A5, A11 and M7 showed a high stability of the members which was corroborated by the increased growth and capacity to remove between 20 to 50 mg L^{-1} lindane. When the consortium was acclimated using sequential and increasing concentrations of lindane, 40–97% of lindane was removed from liquid medium and the removal was decreased to 33–87% in absence of an acclimation period. Also, the use of *Streptomyces* mixed cultures has favored the diazinon removal which is influenced by the addition of glucose. The mixed culture metabolism throughout acid organic exudation caused a decrease in the pH of the liquid medium favoring that diazinon was rapidly hydrolyzed to its main metabolite 2-isopropyl-6-methyl-4-pyrimidinol (Briceño, Schalchli, Rubilar, et al., 2016). Moreover, the presence of an organophosphorus hydrolase enzyme in members of a *Streptomyces* mixed culture has favored the removal of chlorpyrifos and its main metabolite, 3,5,6-trichloro-2-pyridinol, which has antimicrobial properties, as well as a pesticide mixture composed by the insecticides chlorpyrifos, diazinon, azinphos-methyl and methidathion (Briceño, Schalchli, Mutis, et al., 2016).

Immobilization of microbial cells has received considerable attention in the field of bioremediation and specifically in the treatment of pesticide wastewater (Martins, Martins, Guedes, & Santaella, 2013; Pradeep & Malavalli, 2016; Yadav et al., 2014). Therefore, natural and synthetic organic and inorganic carriers have been used as support for cell attachment or entrapment (Dzionek et al., 2016). In this context, a carrier is suitable for cell immobilization when: is nontoxic, non-biodegradable and accessible, have a big surface for cell mass loading capacity and high mechanical, biological and chemical stability, is very durable, cost effective, amenable to scale-up and easily separable from the cells and the media. Immobilization has been widely proposed in bioremediation to achieve enhanced cellular viability, greater tolerance to high concentrations of pollutants and pesticide mixtures and better protection of cells from harsh environments. In relation to the immobilization of *Streptomyces* spp. there are some studies that have shown promising results when compared the pesticide removal by free and immobilized strains. The diazinon removal using calcium alginate for the entrapment of *Streptomyces* sp. AC1-6 was studied by Briceño et al. (2015). The authors demonstrated that the immobilized cells exhibited 60% higher diazinon removal than free cells and an

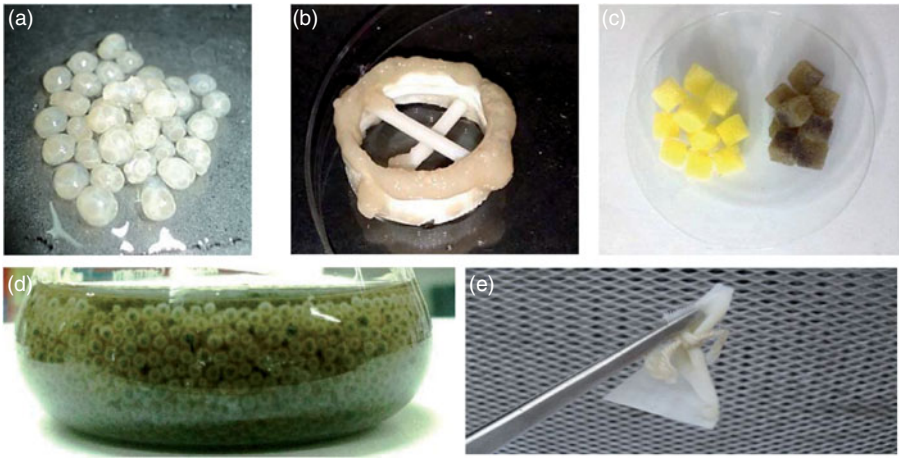


Figure 3. *Streptomyces* sp. strains immobilized in alginate beads (a), polypropylene caps (b), polyurethane sponge (c), biochar (d) and cloth sachets (e).

increase of greater than 50% in diazinon removal was observed when the immobilized cells were used in a second batch reactor. Similarly, reusability of immobilized *Streptomyces* strains during three 96-h periods for lindane removal by pure and mixed cultures was reported previously by Saez, Benimeli, and Amoroso (2012), demonstrating the continuity of the catalytic activity in sequential batch cultures. Immobilization by means of entrapment is simple, inexpensive, and nontoxic for the microorganisms, and protects the microorganisms against the toxicity of xenobiotic compounds while allowing the development of spatially organized microenvironments controlling the degree of protection, the rate of cell release and the association of cells with nutrients, agents or chemicals (Kurzbaum et al., 2017). Streptomycetes immobilized in alginate beads could be a promising biotechnological technique for the removal of pesticides from wastewater (Fuentes et al., 2013). However, low stability of this support due to a restricted pH range (Dzionek et al., 2016) motivate the search of other supports such as volcanic rocks, biocarbon, polyurethane sponge, polyvinyl alcohol-alginate, silicone tubes or cloth sachets (Saez et al., 2012; Yadav et al., 2014) (Figure 3). The immobilization of *Streptomyces* strains in silicone tubes has provided better performances than polyvinyl alcohol-alginate in relation to the higher microbial growth observed. Moreover, cloth sachets showed an improvement of around 25% in lindane removal for both pure and mixed cultures of *Streptomyces* spp. Also, a *Streptomyces* mixed culture formed by six strains characterized by degrading OPP and/or OCP exhibited an increase in the percentages of simultaneous removal of chlorpyrifos and pentachlorophenol by the immobilized cells (71% and 14%, respectively), respect to free cells (40% and 5%, respectively) (Fuentes et al., 2013). Table 1 shows a summary of the main results obtained in the

Table 1. Pesticide degradation in liquid medium by *Streptomyces* strains.

Chemical class/pesticide	Microorganisms	Conditions	Comments	Reference
Carbamate				
Carbofuran	*S. alanonosinicus* isolated from saline soil	Minimal broth and broth glycerol asparagine with 20 mg L^{-1} of carbofuran	- After 10 d, 65.5% was degraded as sole carbon source and 95.3% was degraded as co-metabolism	Jayabarath et al. (2010)
Propoxur	Pure *Streptomyces* spp., isolated from a mixture of rice field sprayed with pesticides and soil of banana plantation	Yeast extract starch broth with 0–1800 mg L^{-1} of propoxur	- *Streptomyces* spp. were able to grow and use propoxur efficiently	Rahmansyah et al. (2012)
Chloroacetanilide				
Alachlor	Pure *Streptomyces* spp. isolated from soil treated with alachlor	MSM with 144 mg L^{-1} of alachlor	- 60 to 75% of degradation after 14 d	Sette et al. (2004)
	Pure *Streptomyces* spp. isolated from soil treated with alachlor	MSM with 72 mg L^{-1} of alachlor	- ≥50% degradation after 7 d	Sette et al. (2005)
Organochlorine				
Aldrin	*Streptomyces* sp. M7 isolated from wastewater sediment of a copper filter plant	Nutrient source in LMM with 48 μg L^{-1} of aldrin	- 90% degradation after 72 h	Benimeli et al. (2003)
Lindane	*Streptomyces* sp. M7	Nutrient source in LMM with 100 μg L^{-1} lindane and addition of 0.6 and 6 g L^{-1} glucose	- 50 and 80% of lindane was removed in LMM with 0.6 and 6 g L^{-1} glucose, respectively, after 20 h - Cl^- release was observed	Benimeli et al. (2006)
	Streptomyces sp. M7	Nutrient source in LMM with 10 μg L^{-1} of lindane or co-metabolism with addition of 6 g L^{-1} glucose	- 44.3% of lindane was removed after 72 h under aerobic condition - Glucose improved lindane degradation and microbial biomass	Benimeli, Castro, Chaile, & Amoroso (2007)
Lindane-chlordane-methoxychlor	Pure *Streptomyces* spp. isolated from soil contaminated with OCP	Nutrient source in LMM with 1.66 mg L^{-1} of pesticide	- 53 to 82% removal of lindane after 7 d - Chlordane was not detected after 7 d - 27 to 100% removal of methoxychlor after 7 d	Fuentes, Benimeli, Cuozzo, & Amoroso(2010)
Lindane	Pure *Streptomyces* sp. strains A2, A5, A8 and A11 isolated from soil contaminated with OCP	Nutrient source in LMM with 1.66 mg L^{-1} of lindane	- Dechlorinase activity was observed - 22 to 30% of lindane degradation after 96 h	Fuentes et al. (2011)
	Streptomyces strains A2, A5, A11 and M7 immobilized in agar cubes, polyvinyl alcohol-alginate beads, silicone tubes and cloth sachets	Nutrient source in LMM with 1.66 mg L^{-1} of lindane	- Removal was higher in immobilized cells than in free cells - Best removal efficiency in silicone tubes and cloth sachets	Saez et al. (2012)

Lindane	Pure *Streptomyces* sp. MC1 and a quadruple consortium	Nutrient source in LMM supplemented with 250 μg L^{-1} of lindane and Cr(VI) 25 mg L^{-1}	- 52% of lindane removal after 5 d	Aparicio, Saez, et al. (2018)
Chlordane	*Streptomyces* sp. A5 isolated from contaminated soil with OCP	Nutrient source in LMM with 1.66–16.60 mg L^{-1} of chlordane	- Optimum degradation conditions were pH 7.0, 30 °C and agitation at 200 rpm - 99.8% degradation and high release of Cl^- occurred after 24 h	Cuozzo et al. (2012)
Methoxychlor	Pure *Streptomyces* sp. strains A3, A6, A12 and A14 isolated from pesticide-contaminated sediments	Nutrient source in LMM with 1.66 mg L^{-1} of methoxychlor	- 97 to 100% degradation after 7 d	Bourguignon, Fuentes, Benimeli, Cuozzo, & Amoroso(2014)
Pentachlorophenol	Pure and consortia of free and immobilized *Streptomyces* spp. isolated from OCP contaminated soils and sediments	Nutrient source in LMM with 1.66 mg L^{-1} of pentachlorophenol and chlorpyrifos in mixture	- 10.6% and 10.1% was removed by *Streptomyces* sp. A5 and the consortia formed by strains A2, A5, A11 and M7 respectively - 5.2 and 14.7% was removed by free and immobilized consortium formed by *Streptomyces* spp. strains A2, A5, A11, M7, AC5 and AC7	Fuentes et al. (2013)
Pyrethroid				
Cypermethrin	*Streptomyces* sp. HU-S-01 isolated from wastewater sludge	Nutrient source in Gause synthetic N° 1 media with 50 mg L^{-1} of cypermethrin	- 92 and 100% of degradation after 24 and 30 h, respectively - Optimum degradation condition at temperature of 26–28° and pH 7.5	Lin et al. (2011)
β-Cypermethrin	*S. aureus* HP-S-01 in co-culture with *Bacillus cereus* ZH-3 isolated from activated sludge contaminated with pyrethroids	Nutrient source in MSM with 50 mg L^{-1} of cypermethrin	- 23 to 37.5% was degraded by single strains - 73.1% was degraded by co-culture after 24 h - Strain HP-S-01 used the β- cypermethrin metabolite, 3-phenoxybenzaldehyde, as nutrient source	Chen, Luo et al. (2012)
Organophosphorus				
Chlorpyrifos	Pure *Streptomyces* sp. strains AC5 and AC7 isolated from agricultural soil sprayed with OPP	Co-metabolism in liquid medium with glucose and 25 and 50 mg L^{-1} of chlorpyrifos	- 90% degradation after 24 h	
			- The main chlorpyrifos metabolite, TCP was produced and metabolized	Briceño et al. (2012)
			- 99.2% removal by strain M7 isolated from organochlorine contaminated sediment	Fuentes et al. (2013)

(*continued*)

Table 1. Continued.

Chemical class/pesticide	Microorganisms	Conditions	Comments	Reference
	Pure and consortia of free and immobilized *Streptomyces* spp. isolated from OCP and OPP contaminated soils and sediments	Nutrient source in LMM with 1.66 mg L^{-1} of chlorpyrifos and pentachlorophenol in mixture	- 91.5% removal by consortium formed by strains AC5 and AC7 isolated from organophosphorus contaminated soil - 40.2 and 71% removal by free and immobilized consortia (strains A2, A5, A11, M7, AC5, and AC7) respectively	
	Pure and consortia of *Streptomyces* spp. strain AC5, AC9, GA11, and ISP13 isolated from organophosphorus contaminated soil	Nutrient source in LMM with 50 mg L^{-1} of chlorpyrifos	- 32 to 72% removal by single strains - Presence of the enzyme organophosphorus hydrolase - 36 h of chlorpyrifos half-life in LMM and 20 h in liquid medium with glucose addition and *Streptomyces* consortium - *Streptomyces* consortium removed a mixture composed by other OPP	Briceño, Schalchli, Mutis et al. (2016)
	Consortium of *Streptomyces* spp. isolated from organophosphorus contaminated soil	Nutrient source in LMM with 100 mg L^{-1} of chlorpyrifos plus diazinon	- 90% of chlorpyrifos removal after 24 h - 85% of diazinon removal after 168 h	Briceño et al. (2017)
Diazinon	Free and immobilized *Streptomyces* sp. AC1-6 isolated from agricultural soil sprayed with OPP	Co-metabolism in liquid medium with 25 and 50 mg L^{-1} of pesticide.	- Immobilized cell showed 60% higher diazinon removal than free cells	Briceño et al. (2015)
	Pure cultures and consortia of *Streptomyces* spp. isolated from agricultural soil sprayed with OPP	Nutrient source in LMM with 50 mg L^{-1} of diazinon	- 10.8 to 31.7% removal by single strains - 13 to 61.7% removal by *Streptomyces* consortia - Diazinon hydrolysis to IMHP was increased in liquid medium with glucose addition	Briceño, Schalchli, Rubilar et al. (2016)
Parathion	*S. venezuelae* ACT1 isolated from marine water sample	Co-metabolism in starch casein broth with 100 mg L^{-1} of pesticide	- Maximum specific growth rate and the enzyme activity rate were 0.571 h^{-1} and 0.472 h^{-1}, respectively	Naveena et al. (2013)
Urea				
Diuron	*S. albidoflavus*	Co-metabolisms in ISP2 medium with 4 mg L^{-1} of diuron	- 95% of degradation after 5 d and no residues after 10 d	Castillo et al. (2006)

OCP: organochlorine pesticides; OPP: organophosphorus pesticide; LMM: Liquid minimal medium; MSM: Mineral salt medium; TCP: 3,5,6-trichloro-2-pyridinol; IMHP: 2-isopropyl-6-methyl-4-pyrimidinol.

last time related to the use of *Streptomyces* strains for the removal of pesticides from liquid medium.

4. *Streptomyces* requirement to degrade pesticides in soil

Soils are the basis of terrestrial ecosystems containing mineral components and organic matter, with microorganisms being involved in both soil formation and soil function (Schütze et al., 2014).

When pesticides reach the soil, it acts as an active filter, where they can be degraded by chemical, physical and biological means. Such degradation is a function of their availability and the ability of microorganisms to utilize the pesticides (Fuentes, Raimondo, Amoroso, & Benimeli, 2017). Clearly, the physicochemical properties of the pesticides, as well as soil properties and management will govern its behavior and movement in the soil compartments (Briceño, Palma, & Duran, 2007).

Adsorption is the first process that takes place when pesticides are in contact with soil, affecting other processes such as leaching, bioavailability or toxicity against non-target organisms (Morillo & Villaverde, 2017). There is substantial evidence demonstrating that the uptake of organic pesticides by soil is strongly dependent on the soil organic matter (SOM) content (Kah & Brown, 2006), because it provides a number of binding sites for organic pollutants, especially hydrophobic compounds (Álvarez et al., 2017). However, the role of the mineral composition in pesticide adsorption cannot be ignored mainly in soils poor in organic matter amount (Čadková, Komárek, Kaliszová, Vaněk, & Balíková, 2013; Kodešová et al., 2011).

The soil cover and specific pesticide adsorption in soils are factors that determine pesticides contamination of groundwater. Groundwater is an important source of drinking water in many countries in the world, so it is important to assess the risk of the selected pesticide leaching into these matrices (Kodešová et al., 2011). Thus, remediation of pesticides contaminated soils is a long-standing, high-priority goal in many countries and the subject of numerous research studies. Both physicochemical and biological approaches for removal of different kind of pesticides from soils have been investigated (Qu, Xu, Ai, Liu, & Liu, 2015). Bioremediation, using microorganisms and phytoremediation, has been applied to remove pesticides from contaminated soils; proving to be safe and effective techniques for pesticide removal (Zhang, Wang, & Yan, 2011).

Bioaugmentation, i.e. the inoculation of given matrices with microorganisms characterized with desired catalytic capabilities, belongs to the green technologies that are used to remove organic contaminants from several environments (Cycón et al., 2017). However, soil bioaugmentation has

sometimes unpredictable outcomes, because many abiotic (temperature, pH, moisture, SOM, initial pesticide concentration, additional carbon sources) and biotic factors affect its final result. Among the biotic factors, some of the most important seem to be the interactions between autochthonous and inoculated microorganisms, the ability of inoculants to survive in the contaminated environment, the inoculum size and the adaptation of introduced strains to soil conditions, among others (Cycón et al., 2017).

It is well known that streptomycetes possess many properties that make them good candidates for their use in bioremediation of soils. In this context, there are several studies concerning the use of microorganisms belonging to the *Streptomyces* genus for the removal and degradation of different kind of pesticides from soil, including triazines, pyrethroids, organophosphates, and organochlorines, among others. For instance, Shelton et al. (1996) and Fadullon, Karns, and Torrents (1998) reported 70 and 78% of atrazine biotransformation by *Streptomyces* PSI/5 in a sterile and non-sterile soil, respectively, both supplemented with chitin. Their results suggest that indigenous soil microbes contribute but not preponderantly to atrazine degradation. Moreover, they propose chitin as a semi-selective substrate for *Streptomyces*, which mixed with contaminated soils may stimulate their metabolism to degrade pollutants such as pesticides. Similarly, the ability of *S. aureus* HP-S01 to eliminate β-cypermethrin and its metabolite 3-phenoxybenzaldehyde in a sandy loam soil, under laboratory and field conditions was shown (Chen et al., 2012). In the laboratory conditions (sterilized soils), around 80% and 73% of an initial dose of 50 mg kg^{-1} or β-cypermethrin and 3-phenoxybenzaldehyde were removed, respectively. Nevertheless, in field conditions, the disappearance rate was around 7% higher for both compounds, thus confirming the great potential of this *Streptomyces* strain to be inoculated in real polluted soils. Also, as with the chitin, the addition of sucrose to soils, enhanced the pesticide-removal ability of the *Streptomyces* strain, suggesting that a biostimulation of the pesticide-polluted soils, could be useful joined to a bioaugmentation with *Streptomyces* strains. Zaborina, Baskunov, Baryshnikova, and Golovleva (1997) demonstrated that a sandy loam soil inoculated with *S. rochei* 303 reached almost 90% of removal of pentachlorophenol in three months, whereas in non-inoculated soils (control) only 25% of pentachlorophenol was removed. Moreover, two by-products of pentachlorophenol were detected after five-month treatment, being their concentration 10 and 100 times higher in the non-inoculated control.

In the last two decades, these studies were deepened and important advances were obtained. In this sense, Benimeli, Fuentes, Abate, and Amoroso (2008) reported 68% of lindane removal in non-sterile soil samples inoculated with *Streptomyces* sp. M7 after 14 days of incubation,

whereas no evident changes in the concentration of the pesticide were detected in the uninoculated soil, indicating that the native microorganisms were not involved in the pesticide removal. Besides, normal germination of maize seeds and an increased in the seedling vigor, compared to the control, were observed after soil bioremediation with the *Streptomyces* strain, indicating that no toxic metabolites would have been accumulated. In the same report, the influence of different *Streptomyces* inoculum size (0.5–4.0 g kg^{-1}) was studied, determining 2 g kg^{-1} as the inoculum to obtain an efficient lindane removal (56%). Thereafter, the degradation studies using OCP were performed using an inoculum of 2 g kg^{-1} with the results described below (Table 2).

More recently, Fuentes et al. (2011); Fuentes, Alvarez, Saez, Benimeli, & Amoroso (2014), in an intention to provide closer insights before an *in situ* intervention for decontamination could be carried out, demonstrated that a *Streptomyces* defined consortia were able to grow and remove lindane and methoxychlor from sterile soil microcosms. Notably, in methoxychlor-polluted soils, the microbial biomass of the *Streptomyces* consortium was significantly higher than in the control without pesticide, thus reinforcing the hypothesis that these microorganisms are well adapted to proliferate in natural soils contaminated with xenobiotics. In fact, on the opposite to the occurred in the control, in the presence of the pesticide, the *Streptomyces* defined consortium did not reach the stationary growth phase at the end of the assay, suggesting that pesticide provides an additional source of carbon to support the cells growth, besides to own soil organic matter.

Considering that real polluted environments generally contain more than one type of contaminant, several researchers have focused their studies on the removal and/or conversion of two or more pollutants together (Aparicio, Saez, Raimondo, Benimeli, & Polti, 2018) . In this context, it has been demonstrated that microorganisms belonging to the *Streptomyces* genus have excellent properties for this purpose. For instance, Polti, Aparicio, Benimeli, and Amoroso (2014) revealed that *Streptomyces* strains, as pure and mixed cultures, removed efficiently both lindane and Cr(VI) from co-contaminated soils. Furthermore, they observed that in a sterile soil, the colonization of the actinobacteria prior to contamination improved their development, whereas in a non-sterile soil the addition of the pesticide at the beginning of the assay may have exerted a selective pressure in favor of lindane-resistant actinobacteria, allowing its growth to surpass that of the native microbiota. Later, Aparicio, Simón, Benimeli, Amoroso, and Polti (2015) demonstrated that the strain *Streptomyces* sp. M7 exhibits strong versatility, being able to bioremediate co-contaminated soil samples at several physicochemical conditions such as temperatures between 25 and 35 °C and humidity between 10 and 30%. Moreover, in accordance with

Table 2. Degradation of pesticides in polluted systems involving soil by *Streptomyces* strains.

Systems	Microorganism	Conditions	Comments	Reference
Soil	*Streptomyces* sp. M7	200 g sterilized soil spiked with 100 μg kg^{-1} lindane, microbial concentration of 2.0 g kg^{-1}, and incubation for two weeks	- 56% of lindane was removed within 14 d. Activity of the strain was not inhibited by the natural soil microorganisms	Benimeli et al. (2008)
	Streptomyces sp. A5	200 g sterilized soil spiked with 16.6 mg kg^{-1} lindane, microbial concentration of 2.0 g kg^{-1} soil, and incubation for 4 weeks	- 56% of chlordane removal was observed after 4 weeks. Aerobic degradation of the pesticide by the studied strain was observed	Cuozzo et al. (2012)
	Streptomyces sp. A14	200 g sterilized soil spiked with 8.33 and 16.60 mg kg^{-1} methoxychlor, microbial concentration of 2 g kg^{-1}, and 4 weeks of incubation	- 40 and 76% degradation after 4 weeks. The higher degradation in inoculated soil was obtained with the highest pesticide concentration	Bourguignon et al. (2014)
	Streptomyces sp. M7 and *Streptomyces* consortium	200 g sterilized and non-sterilized soil spiked with 25 μg kg^{-1} lindane and 50 mg kg^{-1} Cr(VI). Microbial concentration of 2.0 g kg^{-1} soil	- Between 22–46% of lindane was removed by single cultures and consortium in sterilized soil. In non-sterilized soil, lindane degradation was close to 50% and no differences in the use of pure culture and the consortium were found	Polti et al. (2014)
	Streptomyces sp. M7	200 g of soil contaminated with 25 μg kg^{-1} lindane and 50 mg kg^{1} Cr(VI), inoculated with 0.5, 1, 2, or 4 g kg^{-1} of biomass	- Lindane was removed in 6% with an inoculum of 4 g kg^{-1}. Maximum lindane removal (38%) was observed when an inoculum of 2.0 g kg^{-1} was used	Aparicio et al. (2015)
	Consortium formed by *Streptomyces* sp. M7, MC1, AC5 and *Amycolatopsis tucumanensis* DSM 45259T (strain AB0), isolated from soils and sediments contaminated with OCP and heavy metals	200 g of soil contaminated with 40 mg kg^{1} of lindane and 80 mg kg^{1} of Cr(VI). Microbial concentration of 2.0 g kg^{-1} soil. Biomass was obtained from vinasse as feedstock	- 50% lindane removal after 14 d	Aparicio, Benimeli, Almeida, Polti, and Colin (2017)
	Single and actinobacteria consortia, strains Streptomyces sp. M7, *Amycolatopsis tucumanensis* DSM 45259, *Streptomyces* sp. MC1, *Streptomyces* sp. A5	200 g of soil contaminated with 25 μg kg^{1} of lindane and 50 mg kg^{1} of Cr(VI). Microbial inoculum of 2 g kg^{-1} soil	- Lindane removal by single actinobacteria was between 50–60%, while lower than 35% removal was observed by mixed cultures of two or three strains	Aparicio, Saez, et al. (2018)
	Consortium formed by *Streptomyces* sp. M7, MC1, A5, and *Amycolatopsis tucumanensis* DSM 45259 (strain AB0)	Real polluted soil samples in Argentina. Lindane contamination over 10 μg kg^{-1}. Temperature between 25–30 °C and humidity of 30%. Lab scale	- Lindane concentration decreased in all samples with exception in one which was associated with the soil properties (low pH and organic matter content). Optimal condition were determined by factorial design	Aparicio, Raimondo, et al. (2018)

	Pure strains and consortia of *Streptomyces* spp.	200 g sterilized soil with 1.66 mg L^{-1} lindane, microbial biomass of 2 g kg^{-1} and 4 weeks of incubation. Microbial consortia of 2, 3, and 4 strains	- After 28 d, up to 32% lindane was removed	Fuentes et al. (2011)
	Pure strains and consortium of *Streptomyces* spp.	Interaction lindane-plant (maize)-consortium	- After 21 days up to 61% lindane removal was observed	Álvarez et al. (2015)
	Native *Streptomyces* consortium	Sterile and non-sterile soil microcosms contaminated with a mixture of lindane, chlordane and methoxychlor at 1.66 mg kg^{-1} each one	- Removal of pesticides in different types of soils was: clay silty loam > loam > sandy. In non-sterile clay silty loam soil removal was highest for lindane and methoxychlor	Fuentes et al. (2017)
	Streptomyces mixed culture strains AC5, AC9, GA11 and ISP13	Sterile soil contaminated with a mixture of chlorpyrifos and diazinon (100 mg kg^{-1}). Microbial biomass of 5%	- After 28 days, 14% and 50% of chlorpyrifos and diazinon was removed respectively	Briceño et al. (2017)
Slurry	*Streptomyces* consortium immobilized in cloth bags	Two slurry concentrations contaminated with 2–50 mg kg^{-1} of lindane	- After 7 days, 35.3 mg kg^{-1} of lindane was removed using 10^7 CFU g^{-1} of inoculum added to the slurry prepared with a proportion of 2:3 soil/water	Saez, Alvarez, Benimeli, & Amoroso (2014)
	Native *Streptomyces* consortium	Slurry prepared with 40 g of sterile soil and 60 mL of sterile distilled water and contaminated with lindane, chlordane, and methoxychlor at 1.66 mg kg^{-1} each one	- The order of pesticide degradation was methoxychlor (26%)>lindane (12.5%)>chlordane (10%)	Fuentes et al. (2017)
Biobed biomixture	*Streptomyces* mixed culture strains AC5, AC9, GA11 and ISP13	Sterile biomixture (straw, soil, and peat) artificially contaminated with a mixture of chlorpyrifos and diazinon (100 mg kg^{-1})	- The culture colonized the biomixture and high microbial activity was observed. After 28 days, 6% and 60% of chlorpyrifos and diazinon was removed respectively	Briceño et al. (2017)
	Mixed culture of the *Trametes versicolor* SGNG1 and *Streptomyces* sp. A2, A5, A11, and M7	Biomixture (sugarcane bagasse, different soil types, and peat) artificially contaminated with 100 mg kg^{-1} lindane	- Lindane half-life was between 20–24 d in bioaugmented biomixture and between 28–52 d in non-bioaugmented biomixture	Saez et al. (2018)

Benimeli et al. (2008), the maximum lindane removal (38%) was obtained when 2 g kg^{-1} of *Streptomyces* sp. M7 was inoculated in soil samples.

Besides organic and inorganic mixed contamination, it is known that pesticides are usually applied simultaneously or one after another for crop protection, leading to a combined contamination of these compound in the soil environment (Chu et al., 2008). In this context, Fuentes et al. (2017) revealed the ability of a defined consortium of four *Streptomyces* strains to remove a mixture of three OCP from soil microcosms with different textures, although the rate of removal differs for each soil texture and for each pesticide probably due to a difference in the bioavailability of pesticides. It was observed that the order of removal of OCP in soils was clay silty loam- > sandy soil > loam soil. Clay silty loam soil has a predominance of clay (30.9%) and silt (56.4%) particles, providing more sites for bacteria to attach and leading to a higher utilization of pesticides as a carbon and energy source. This higher specific surface area of silts and clays in the fine soil textures enhances the availability of the compounds adsorbed in the soil particles for the microorganisms. Also, it was demonstrated that sandy soils, with small amounts of organic matter (0.4%) and large particles (97% sand), retain pesticides less than soils with high clay and/or organic content, reducing the efficiency of microorganisms to remove contaminants (Rama Krishna & Philip, 2008; Fuentes et al., 2017).

As a form to increase the pesticide bioavailability on soil, Saez, Garcia, and Benimeli (2017) studied the effect of stable microemulsions formed with Tween 80, 1-pentanol and three vegetable oils on the removal of a high lindane concentration (100 mg kg^{-1}) by *Streptomyces* sp. M7. The main results of this work showed that in a loam soil, the microemulsion favored lindane solubilization, allowing an 87% of lindane removal by the *Streptomyces* strain. The authors postulated that microemulsions combined with streptomycetes could be used as potential tools in soil washing technologies or *ex situ* bioremediation.

Plants and pollutants-degrading microorganisms are also a suitable combination for the remediation of pesticides contaminated soils. In this regard, *Streptomyces* strains in combination with maize plants or its root exudates, have demonstrated to be a useful biological system for reducing pesticides in soils. The inoculation of maize planted soils with a *Streptomyces* consortium led to an increase in the vigor index of plants and protected them against the toxicity of lindane (Álvarez, Benimeli, Saez, Giuliano, & Amoroso, 2015). However, further research is still needed in order to develop a better understanding of plant-bacteria partnerships and, thereby, to enhance the bio-phytoremediation potential.

The presented data suggest that *Streptomyces* may have sufficient metabolic diversity to be useful as a soil inoculant to bioremediate pesticide-

polluted soils. Many microbes are capable of utilizing pesticides in optimized laboratory conditions where they show higher efficiency; however, under field conditions, a considerable reduction in degradation could be possible. Therefore, more and deeper studies are needed to address the limitations in the field in order to ensure the successful bioremediation of sites contaminated with pesticides by using streptomycetes (Odukkathil & Vasudevan, 2016). Table 2 shows a summary of the main results obtained in the last time related to the use of *Streptomyces* for the removal of pesticides from soil.

5. Bioremediation of pesticide-contaminated slurry systems by *streptomyces*

Among bioremediation techniques, the *ex situ* bioremediation, which involves the removal of the contaminants that will be treated elsewhere, is advantageous when a safe and effective intervention is required (Kensa, 2011). The treatment in slurry bioreactors (SBs) under controlled environmental conditions is one of the best *ex situ* techniques for the bioremediation of soils contaminated by recalcitrant pollutants (Christodoulatos & Koutsospyros, 1998; Mueller, Lantz, Blattman, & Chapman, 1991; Venkata Mohan, Shailaja, Rama Krishna, Reddy, & Sarma, 2006). The SBs may be used to create a three-phase (solid, liquid and gas) mixing condition, to increase the bioremediation rate of soil-bound and water-soluble pollutants, as a water slurry of the contaminated soil and biomass capable of degrading target contaminants (Kensa, 2011). The advantages of SBs include the feasibility of controlling the operating parameters such as mixing, pH, temperature, nutrients and amendments such as surfactants, and the possibility to reduce the processing time in comparison with the time required for the *in situ* bioremediation processes (Robles-González, Fava, & Poggi-Varaldo, 2008; Troy, 1994; Venkata Mohan, Ohkuma et al., 2006; Venkata Mohan, Shailaja et al., 2006; Venkata Mohan, Sirisha, Sarma, & Reddy, 2004). The SBs typically increase mass transfer rates such as desorption of pollutants from soil, which usually translates in faster and higher rates of contaminant biodegradation (Robles-González et al., 2008). This technology is applied for the bioremediation of poorly degradable contaminants such as pesticides and explosives (Pavlostathis, Prytula, & Yeh, 2003), and can be used with either native soil microbiota or by inoculating strains with specific capabilities to metabolize the target contaminants (Venkata Mohan, Shailaja et al., 2006).

As mentioned earlier in this review, there is much research on pesticide bioremediation by *Streptomyces* strains; however, the available data on bioremediation of pesticides in slurry systems by these microorganisms is still scarce. There are some reports which demonstrate the abilities of

Streptomyces strains composing microbial consortia to remove OCP in SBs (Fuentes et al., 2014, 2017; Saez et al., 2014). In this sense, Fuentes et al. (2014) evaluated the methoxychlor bioremediation by a defined consortium composed of four environmental *Streptomyces* strains inoculated with a microbial concentration of 2 g kg^{-1} on sterile soil and 2 g L^{-1} on sterile slurry systems. In this work, significant differences were not found between the methoxychlor removal percentages in both assayed systems incubated at 30 °C. Despite that, the time required to reach similar removal percentages were four weeks in soil systems while in the SBs was just one week. This finding reinforces the advantages of *ex situ* bioremediation methods which allow more efficient removal of pollutants by controlling the physicochemical parameters, increasing the mass transfer rate such as desorption, resulting in a shortening of the total time of reclamation (Dzionek et al., 2016; Robles-González et al., 2008). It has been shown that xenobiotic degradation by a microbial population can be enhanced by the addition of rate-limiting nutrients to the contaminated system (Delille, Coulon, & Pelletier, 2004). In this sense, Fuentes et al. (2014) evaluated a possible stimulating effect on the microbial consortium growth and pesticide removal, by using SBs formulated with the rich culture medium tryptic soy broth (SB-TSB) in comparison with the SBs formulated with distilled water (SB-water). In this case, the maximum growth of the *Streptomyces* consortium was detected on SB-TSB spiked with methoxychlor at the third day. Also, the duplication time of the streptomycetes was lower on the SB-TSB (3.13 h) than in SB-water (3.94 h). However, no statistically significant differences were found between biomass values reached with and without nutrient supply, and with and without methoxychlor, at the end of the experiments. This phenomenon is possible because the assayed *Streptomyces* strains were isolated from pesticides contaminated soil, so the microorganisms could be adapted and to not need additional carbon sources such as methoxychlor and/or TSB. Also, regarding the pesticide dissipation, methoxychlor removal was 10% higher on SB-TSB (56%) than on SB-water (46%) at the end of the assay. In contrast, Robles-González et al. (2012) detected lower lindane removal in aerobic SBs biostimulated with sucrose than in aerobic SBs without stimulation.

On the other hand, and in order to increase the efficiency of pesticides removal in the SBs, it is also possible to use the microbial biomass immobilized. For this reason, Saez et al. (2014) evaluated lindane removal from slurry systems by a four *Streptomyces* strains-consortium immobilized in cloth sachets, at different inoculum, pesticide and slurry concentrations. The higher pesticide removal, over 70%, was obtained with an inoculum of 10^7 CFU g^{-1} and at the highest pesticide concentration tested (50 mg kg^{-1}). However, when concentrated slurry (soil/water ratio 2:3) was used,

high percentages of lindane removal were also detected in the abiotic controls (slurries without microorganisms). This is probably due to the adsorption of the xenobiotic to soil particles. In a more diluted slurry (soil/water ratio 1:4), although lindane removal by the *Streptomyces* consortium was lower (almost 60%), the authors highlight that the pesticide removal registered in the abiotic controls was half of the obtained in the more concentrated slurry. Hence, the authors proposed the diluted slurry as the more efficient system for lindane dissipation at the assayed conditions. The mentioned work highlights the importance to study combined bioremediation technologies to achieve optimum performance in removal of pesticides from soil.

Regarding pesticides mixed pollution, Fuentes et al. (2017) investigated the simultaneous removal of three OCP (lindane, chlordane, and methoxychlor) from slurry systems formulated with different textured soils by employing a quadruple native *Streptomyces* consortium. The soils textures and assay conditions (sterility, slurry formulation) were determining factors that influenced the removal of each pesticide from the mixture. However, the authors reported similar microbial growth levels in the presence or absence of the pesticides mixture. The favorable development of the consortium growing in the presence of the contaminants may be due to a selective pressure of the polluted environment from which these *Streptomyces* strains were isolated (Benimeli et al., 2003; Fuentes et al., 2010). Regarding the pesticides removal, when clay silty loam soil-SB was used, an increase in the percentage of methoxychlor removal (26%) was detected, in relation to the removal percentage obtained in soil microcosms with the same kind of soil (21%). In this sense, different efficiencies in the biodegradation of pesticides mixture were also reported in submerged soil systems, soil slurries and liquid systems (Fuentes et al., 2013; Rama Krishna & Philip, 2011; Monsalvo, Garcia-Mancha, Puyol, Mohedano, & Rodriguez, 2014).

Based on the above, SBs may be considered as a successful *ex situ* bioremediation technique, which allows under controlled conditions to exacerbate the capacities of microorganisms with versatile metabolisms, such as the belonging to the *Streptomyces* genus, capable to remediate sites contaminated with pesticides. Table 2 shows a summary of the main results obtained in the last time related to the use of *Streptomyces* for the removal of pesticides from SBs.

6. *Streptomyces* as bioremediating agents in biobed systems

Biobeds are biological systems originated in Sweden in response to the need for a simple and effective system to minimize environmental contamination from pesticide manipulation, especially when filling the spraying

equipment, a typical point source of contamination (Castillo, Torstensson, & Stenstrom, 2008; Castillo & Tortensson, 2007). In Sweden and other countries in Europe and Latin America (Chile, Costa Rica, Ecuador, Guatemala, Perú), there are numerous examples of biobeds being used on farms which have shown to be efficient at reducing pesticide water-body contamination (Castillo et al., 2008; Chin-Pampillo, Carazo-Rojas, Pérez-Rojas, Castro-Gutiérrez, & Rodríguez-Rodríguez, 2015; Vischetti, Coppola, Monaci, Cardinali, & Castillo, 2007). Biobeds are based on the adsorption and potential degradation of organic biomixtures composed commonly of top soil, peat, and straw (25: 25: 50 vol %) that fill a deep pit (60 cm) in the ground and a grass layer that covers the surface (Castillo et al., 2008; Castillo & Tortensson, 2007). Biobeds are a low-cost alternative for the treatment of pesticide waste and washings, providing a matrix to absorb the pesticides and facilitate their biodegradation by microorganisms that constitute the biomixture, i.e. the core of a biobed. In the biomixture, straw stimulates the growth of ligninolytic microorganisms and the production of extracellular ligninolytic enzymes, such as peroxidases and phenoloxidases. The peat contributes to sorption capacity, moisture control and abiotic degradation of pesticides and decreases the pH of the biomixture, which is favorable primarily for fungi and their pesticide-degrading enzymes (Castillo et al., 2008; Castillo & Tortensson, 2007). The soil enhances the sorption capacity in the biobed, and is also an important source of pesticide-degrading bacteria, including actinobacteria, which can act synergistically with fungi.

As was previously discussed, *Streptomyces* can effectively degrade a wide range of organic pollutants like pesticides. Due to the large amount of favorable results obtained in the field, the use of biobeds to prevent pesticide pollution is growing and many countries are adopting and adapting the system to local conditions and needs. Kipping in mind these facts, it is important to highlight the potential application of bacteria belonging to the *Streptomyces* genus as a strategy for the optimization of biobeds performance (Briceño et al., 2017; Campos et al., 2017).

Streptomycetes possess several characteristics that make them useful for the inoculation of biobeds: a) the ability to degrade lignin and to produce phenoloxidases; b) mycelial growth that allows efficient colonization of the biomixture, and c) the production of spores under adverse conditions that can be important for survival in case of, for example, high fluctuations in moisture levels. Moreover, compared to white-rot fungi, streptomycetes can degrade lignin at high N levels and are therefore less dependent on the C/N ratio of the materials used for the biomixture (Briceño et al., 2013).

The use of *Streptomyces* in biobeds is a not very explored area, and there is a need for new knowledge. Currently, research on bioaugmentation of a biobed with *Streptomyces* is being performed in Argentina and Chile. The

candidate microorganisms are OCP- and OPP-degrading *Streptomyces*, which has shown good degrading activity and viability when inoculated in different matrices (Álvarez et al., 2017; Briceño et al., 2014, 2017). In this sense, two actinobacteria strains, isolated from agricultural soils and identified as *Streptomyces* sp. AC5 and AC16, were used for the bioaugmentation of a biomixture contaminated with 25 mg kg^{-1} of chlorpyrifos and diazinon (Briceño et al., 2014). The inoculation of *Streptomyces* sp. AC5 improved the enzymatic activity and microbial respiration of the biomixture. Similarly, in a recent study performed by Briceño et al. (2017), after *Streptomyces* mixed culture inoculation in a biobed biomixture contaminated with diazinon plus chlorpyrifos (100 mg kg^{-1}), high dehydrogenase and acid phosphatase activities were observed in the course of time. Then, it was confirmed by q-PCR technique the ability of streptomycetes to colonize efficiently the biomixture and to favor the dissipation of the pesticides, mainly diazinon, reducing its half-life to 11 days. Recently, a study performed in Argentina by Saez et al. (2018) showed that biomixtures prepared with sugarcane bagasse used in replacement of wheat straw, soils of three different textures and peat, inoculated with a compatible consortium formed by the fungus *Trametes versicolor* SGNG1 and the actinobacteria *Streptomyces* sp. A2, A5, A11, and M7 improved lindane dissipation achieving 81–87% of removal at 66 d of incubation in the different biomixtures. However, when the pesticide was re-applied in the biomixtures, only the bioaugmented biomixture of silty loam soil enhanced lindane dissipation and decreased the $t_{1/2}$ from 100 d to 19 d. Table 2 shows the main results obtained in the last time related to the use of *Streptomyces* for the removal of pesticides from biobed biomixture used in biopurification systems.

7. Concluding remarks

The capability of actinobacteria, specifically from those belonging to *Streptomyces* genus, to degrade pesticides have been explored due to the need to develop biotechnological processes which allow the removal or decrease of pesticides concentration from diverse environmental matrices or polluted systems and/or prevent their entry into the environment and final human exposure. In this context, the review performed at studies reported during the last two decades indicates that:

- The isolation of *Streptomyces* strains from contaminated environments contributes to their adaptation to pesticides, so that those pesticides can be removed rapidly from liquid media by these strains. Diverse chemical class of pesticides as pyrethroids, carbamates, ureas, organochlorines, chloroacetanilides and organophosphorus can be degraded efficiently by

Streptomyces in liquid samples. These microorganisms can express enzymes such as monooxygenases, dechlorinases, and organophosphorus hydrolases, respectively, which favor the degradation of these compounds.

- Strategies to optimize the pesticide degradation by *Streptomyces* include the use of microbial consortia and immobilization. The use of microbial consortia of *Streptomyces* strains has shown promising results. In most cases, the pesticides degradation is increased and the by-products are metabolized efficiently compared with the use of single strains. On the other hand, immobilization of *Streptomyces* using alginate beads constitute the most used support even though alternatives as silicone tubes and cloth sachet have represented a good alternative for the immobilization and entrapment of *Streptomyces* cells.
- Studies performed in sterile and non-sterile soils have indicated that *Streptomyces* strains have the capacity to adapt and colonize natural and contaminated soils. Moreover, pure and mixed cultures can remove pesticides in combination with inorganic contaminants as metals. In general, over 32% of OCP can be removed using *Streptomyces* inocula of $2\,g\,kg^{-1}$ of soil. The soils texture constitutes an important factor to be considered in the bioaugmentation of *Streptomyces* for pesticides removal due to limited bioavailability of pesticides caused by adsorption to soil particles, which could be improved by incorporation of microemulsions.
- Streptomycetes can efficiently colonize complex matrices as biobed biomixtures, stimulating some enzymatic activities in the system and degradation of OPP and OCP. In the same way, despite that few studies have been performed in slurry bioreactors, the inoculation of free and immobilized *Streptomyces* in this system operated in batch allowed the degradation of organochlorine compounds as lindane and methoxychlor.

Streptomyces genus is a good alternative to remove pesticides from contaminated environmental matrices. However, the field-scale bioaugmentation strategy is still limited and environmental variables influencing the degradative activity of inoculated streptomycetes are unknown. Studies on pesticide degradation by streptomycetes must be deepened and the results optimized in order to obtain adequate and efficient treatment systems to give a response to the problem of contamination by pesticides.

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