The Handbook of Environmental Chemistry 113
Series Editors: Damià Barceló · Andrey G. Kostianoy

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Pesticides in Soils

Occurrence, Fate, Control and Remediation

Springer

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ISSN 1867-979X ISSN 1616-864X (electronic)
The Handbook of Environmental Chemistry
ISBN 978-3-030-90545-3 ISBN 978-3-030-90546-0 (eBook)
https://doi.org/10.1007/978-3-030-90546-0

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The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Biopurification Systems: Current Advances and Future Prospects of On-Farm Biodegradation of Pesticides

Carlos E. Rodríguez-Rodríguez, Juan Carlos Cambronero-Heinrichs, Víctor Castro-Gutiérrez, and Gonzalo R. Tortella

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Abstract The mishandling of agrochemical residues in agriculture exerts an important risk for the environment due to point source contamination. To minimize environmental exposure to pesticides, biopurification systems (BPS) have been developed as a biotechnological tool for the on-farm treatment of pesticide-containing wastewater of agricultural origin. Although efficient in the removal of diverse pesticides, highly recalcitrant compounds have shown poor elimination in

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Pesticides in Soils: Occurrence, Fate, Control and Remediation,
Hdb Env Chem (2022) 113: 287–316, DOI 10.1007/698_2021_798,

Published online: 14 December 2021

BPS; moreover, the performance of BPS still needs to be evaluated for many agrochemicals and their sustainability should be assessed in real pesticide application cycles for specific crops. Recent studies describe the use of BPS for the removal of antibiotics of agricultural use; this approach required a previous assessment on the impact of antibiotics on BPS performance, which in most cases has revealed the absence of significant adverse effects on pesticide removal. Similarly, novel applications propose the potential use of BPS for the removal of pharmaceuticals from polluted matrices such as water or sludge. The degradation processes taking place within BPS and their link with the resident microbial communities have been scarcely studied to date; they are critical to achieve proper design and optimization of these systems. This chapter covers general aspects of BPS and their application scope to pesticides; special attention is given to novel topics such as the treatment and effect of antibiotics from agricultural wastewater and pharmaceutical-containing matrices, as well as the description of microbial communities within BPS.

Graphical Abstract

Keywords Antibiotics, Biobeds, Biopurification systems, Pesticides, Wastewater

1 Biopurification Systems: Tools for the Treatment of Pesticide Containing Wastewater of Agricultural Origin

Biopurification systems (BPS) are low-cost, simple, and effective tools that play an important role in the removal of pesticide-contaminated wastewater and the detoxification of point sources of pesticide residues, which represent potential risks as agents of water pollution [1, 2]. In general, a BPS is an ex situ bioremediation treatment technology, consisting of a solid-phase bioreactor or a composting facility, whose prophylactic purpose is to treat water contaminated with agrochemicals used in farming, aiming at their accelerated removal [3–6].

The inadequate management of pesticides and other farming chemicals may result in the exposure of soil, surface water, and groundwater to these residues [3, 7]. Hence, the main objective of a BPS is to minimize the contamination risk produced by point sources of pesticide pollution. These tools are devices intended to retain and degrade pesticide spills occurring during the filling of spraying equipment or tanks, resulting from leakages, caused by incorrect disposal of machinery-washing residues during and after the application process, and those comprised of wastewater from the rinsing of pesticide containers [1, 8–11].

BPS originated during the 90s in Sweden under the name of "biobeds" and in subsequent decades, this simple and effective strategy attracted the attention of many countries, mainly located in Europe [12, 13]. The biobed system has been adapted multiple times and even renamed as biomassbed in Italy, biofilter in Belgium, and Phytobac and biobac in France, for instance [1, 9]. The simplicity of BPS configurations has made them suitable for emulation in developing countries in Latin America, for example, Guatemala, Costa Rica, Chile, Peru, Ecuador, Brazil, and Argentina [1, 5, 10, 11].

These BPS or "biobeds" consist of a highly active biological biomixture confined in a container or excavation. The original European "biobed" consisted of three components: a clay layer at the bottom (to avoid contaminant breakthrough); topped by a mixture of peat, straw, and soil (1:2:1), known as biomixture; and a grass layer covering the surface, which helps to maintain the correct humidity, while also working as a useful indicator of pesticide spills [1, 12, 13]. Costa Rican adaptations of BPS, for example, are limited to a plastic container (without the clay layer) that functions as an impermeable material, and usually do not include the grass surface layer (Fig. 1a) [5, 14], while on the contrary, other container-scale biobeds in Brazil employ the grass cover (Fig. 1b); an excavated biobed is also shown in Fig. 1c. The biomixture represents the biologically active core of the BPS; this matrix can be prepared employing locally available materials, and adjustments to the original composition respond to material availability. For instance, alternative lignocellulosic substrates, other than straw, such as coconut fiber, barley husk, rice husk, oak husk, vine-branches, pine sawdust, and even newspaper, have been used; similarly, the substitution of peat by compost has resulted in the production of efficient biomixtures [5, 15–20]. Once spilled in the BPS, the vast majority of pesticides are retained in the upper parts of the biomixture and removed within 1 year [1, 9]. However, many pollutants have demonstrated to be highly recalcitrant (see Sect. 2).

The biomixture promotes the adsorption of organic compounds and their further removal by degrading microbial communities [1, 4]. The three main elements that compose the biomixture are: (1) a lignocellulosic substrate that promotes the growth of microbes, especially white-rot fungi; during the transformation of lignin-rich substrates, these organisms produce promiscuous enzymes (such as laccases and lignin peroxidases) characterized by their high and broad oxidative capacity that may enhance the removal of pesticides and many other organic pollutants [4, 21]; (2) a humic component such as peat or compost that regulates pollutant retention and maintains the BPS moisture, while simultaneously potentially providing degrading

Fig. 1 Different configurations of biopurification systems: container-scale biobed in Costa Rica (without grass cover; **a**) and Brazil (with grass cover; **b**); excavated biobed in Brazil (**c**)

microbial communities; and (3) soil, preferably pre-exposed to pesticides, from a farming site. Soil is the main source of the active degrading microbial populations which contain a plethora of gene products for the removal of organic contaminants. As these communities have been previously exposed to the pollutants, the use of primed soil usually results in an accelerated pesticide removal within the BPS, analogous to environments exposed to antibiotics, where higher frequencies of antibiotic resistant genes are developed [1, 2, 4, 9, 11].

Some abiotic factors have to be taken into account to maintain the proper functioning of the biobeds. First of all, due to degradation of the lignocellulosic material, the carbon content in the biomixture decreases with time; consequently, the biomixture in the BPS should be regularly replaced. Even so, in the case of European biobeds, it is estimated they can last for up to 8 years under functional conditions [1]. Furthermore, the material removed from the biobed may contain residues of pesticides, and a subsequent composting process of 1 year is recommended to reduce the levels of pollutants under detectable limits in this waste [9]. Temperature is also a very important parameter, quicker pesticide removal can be recorded in warmer environments, while removal might substantially decrease in temperate regions during winter [1, 18]. Finally, water balance is a very delicate variable in BPS, as biobeds saturated with water will maintain primarily anaerobic conditions that are not suitable for the removal of pesticides; in this respect, some authors recommend using caps or roofing on biobeds to avoid rainfall saturation, which may also result in high risk of spilling pesticides [1, 9, 11, 18].

2 Application Scope: Removal of Pesticides from Diverse Chemical Families

A plethora of active ingredients are currently used in agriculture for the control of several plant pests such as insects, bacteria and fungi, nematodes or viruses. These compounds are grouped according to their mode of action, target pest, or by chemical group. Compounds grouped in the same family usually have a similar chemical core structure, and therefore similar chemical properties, including toxicological features, and their level of resistance to biodegradation.

As previously mentioned, BPS were designed to treat pesticide residues to avoid point source contamination. Therefore, given that under field conditions diverse pesticides from different chemical families are applied, the degradation capacity of BPS must be wide enough to be able to degrade different active ingredients (herbicides, insecticides, fungicides), sequentially applied alone or in mixture. Since BPS were first developed, degradation studies in these systems have included individual pesticides at different concentrations [22–24], but also mixtures of pesticides from the same chemical family [25] or from different chemical classes [26–28], including studies with real wastewater containing more than 50 active ingredients belonging to many chemical families [28].

Early studies report that soil degradation of pesticides such as atrazine [29] is fast, and the removal rate can increase rapidly when applied repeatedly due to adaptation of the microbiota [30] in a process known as accelerated pesticide degradation [31]. Similar results have been reported for chlorpyrifos, metolachlor, and terbuthylazine in soil, among many others [32, 33]. In contrast, other pesticides such as glyphosate show fast initial degradation, which decreases over time due to strong adsorption to the soil matrix [34]. Additionally, it has been reported that the presence of pesticide mixtures in soil can affect their degradation rate [35], either favoring or decreasing the removal. Moreover, it is well known that degradation rate, movement, distribution, and adsorption/desorption processes of pesticides in soil are governed by physicochemical properties of both soil and each specific pesticide [36]. Taking into consideration all these factors in soil, what happens with the degradation of different kinds of pesticides in BPS?

Undoubtedly, the factors that govern the movement and degradation process in soil are likely to be similar to those taking place within BPS. For instance, the accelerated degradation observed in soil for some pesticides has also been demonstrated in BPS for compounds such as carbofuran, carbendazim, or metalaxyl [37–39]. Likewise, its composition, as well as the type of substrates present in the organic biomixture, makes BPS a crucial and efficient system for retention and degradation of chemicals [12], compared to soil. Moreover, the presence of lignocellulosic substrates should promote the development and establishment of a robust and active microbiota responsible for the degradation of pesticides. In this regard, a great variety of substrates have been evaluated in BPS biomixtures, for the removal of numerous pesticides. Table 1 summarizes several examples of pesticide degradation from different chemical groups in BPS.

Table 1 Removal of pesticides in selected biomixtures and biopurification systems

Chemical group	Active ingredient	Biomixture evaluated	Concentration evaluated	Main removal results	Reference
Strobilurin	Azoxystrobin	Rice straw – Compost (RS + C) and corn cob – Compost (CC + C)	Two applications of 100 mg L^{-1}	81.5% in RS + C and 68.1% in CC + C	[40]
Neonicotinoid	Imidacloprid	Rice straw – Compost and corn cob – Compost	Two applications of 100 mg L^{-1}	100% in RS + C and CC + C	[40]
Dithiocarbamate	Ethylenebis (dithiocarbamate)	Sugarcane top, banana stem, or eucalyptus chip (at 30%, 50%, or 70% v/v) plus equal parts of sugarcane cachasse and Fluvisol soil	Several applications of 878 mg L^{-1}	The mixtures with 30% sugarcane top (30%) and eucalyptus chip (50%) yielded the best results	[41]
Organophosphate	Chlorpyrifos	Cereal bran, soil and peat (50: 25:25 v/v)	60 mg kg^{-1}	60–70%	[42]
Organophosphate Phenylurea Phenylamide Morpholine	Chlorpyrifos Malathion Linuron Metalaxyl Dimethomorph	Coconut fiber, compost, and soil pre-exposed to pesticides (50:25:25 v/v)	Five applications simulating field conditions between 64–240 mg kg^{-1}	100% metalaxyl and malathion; 20% chlorpyrifos; 10% dimethomorph	[6]
Phosphonoglycine	Glyphosate	Agricultural soil, alfalfa straw/wheat stubble, and river waste; biomixtures were evaluated with and without earthworms addition	1,000 mg kg^{-1}	Between 80% and 90% after 90 days, with or without earthworms, respectively	[43]
Organophosphate	Glyphosate and its metabolite (aminomethyl)-phosphonic acid (AMPA)	Biomixtures containing a mixture of an agricultural soil with alfalfa straw/wheat stubble, as lignocellulosic substrates, and river waste	1,000 mg kg^{-1}	85–99%	[44]
Phenylamide Neonicotinoid	Diuron Imidacloprid	Soil, peat, straw and soil, vermicompost of wet olive	Between 0 and 70 mg kg^{-1}	58–100% diuron; 19–61% imidacloprid; 12–49%	[45]

Triazole Diphenyl ether	Tebuconazole Oxyfluorfen	cake, olive tree pruning (25:25:50 v/v, each)		tebuconazole; 47–74% oxyfluorfen.	
Triazine Triazole Organophosphate Carbamate Benzimidazole	Ametryn Atrazine Cyanazine Cyromazine Prometon Prometryn Simazine Terbuthylazine Bitertanol Cyproconazole Difenoconazole Coumaphos Dichlorvos Dimethoate Malathion Triazophos Carbaril Methiocarb Carbendazim (among others)	Coconut fiber, compost, soil (45:13:42 v/v)	Pesticide mixture containing 10 triazines, 13 triazoles and 20 organophosphates between 4–8 mg kg^{-1} each. -Treatment of real wastewater containing 38 pesticides at varied concentrations	Removal of triazines (59%), organophosphates (68%) and triazoles (failed). For real wastewater, removal of triazines (54%), organophosphates (90%), triazoles (73%), carbamates (71%), other groups (<90%)	[28]
Oxathiin Phenylamide Pyrazole carboxamide Phenylpyrrole Chloronitrile Benzimidazole	Carboxin Metalaxyl-M Fluxapyroxad Fludioxonil Chlorothalonil Thiabendazole	Straw, spent mushroom substrate and soil (25:50:25 v/v)	10, 20, and 150 mg kg^{-1}	Carboxin was the least persistent, followed by metalaxyl-M, while fludioxonil and fluxapyroxad were the most persistent compounds. DT_{50} varied from 2 days to 142 days	[46]
Phenoxyacetic acid Triazine	2,4-dichlorophenoxyacetic acid (2,4-D)	Agricultural soil, sisal pulp, and vegetable compost, corn	1.08, 2.50, 0.23, 0.34, and 0.36 mg mL^{-1} of	>98% for all pesticides after 20 days	[47]

(continued)

Table 1 (continued)

Chemical group	Active ingredient	Biomixture evaluated	Concentration evaluated	Main removal results	Reference
Carbamate Organophosphate Phosphonoglycine	Atrazine Carbofuran Diazinon Glyphosate	stover and seaweed in different proportions (soil accounted for 50% in all biobeds)	biomixture (total pesticide concentration)		
Phosphonoglycines	Glyphosate	Alfalfa straw, wheat stubble, river waste and two different soils	1,000 mg kg^{-1}	88–100%	[48]
Neonicotinoid Organophosphate Triazole Phenylamide Diphenyl ether	Imidacloprid Dimethoate Tebuconazole Diuron Oxyfluorfen	Two biomixtures with soil mixed with pruning and raw wet olive cake or vermicomposted (25:50:25 v/v); one biomixture with soil, straw and peat (25:50:25 v/v).	50 mg kg^{-1} each pesticide	100% dimethoate; 80% imidacloprid; 73% tebuconazole; 75% diuron; 50% oxyfluorfen.	[49]
Phenoxyacetic acid Hydroxybenzonitrile Sulfonylurea Pyrazolone Triazolone Pyrazolium Thiophenes	2,4-D Bromoxynil Thifensulfuron-methyl Tribenuron-methyl Pyrasulfotole Thiencarbazone-methyl Metsulfuron-methyl	Topsoil, composted manure and chopped wheat straw	0.6 mg g^{-1} for all pesticides except for bromoxynil that was 3.36 mg g^{-1}	100% 2,4-D, bromoxynil, and thifensulfuron-methyl, after 35 days; 93% tribenuron-methyl; 70% pyrasulfotole; 64% thiencarbazone-methyl; 34% metsulfuron-methyl	[50]
Phenoxyacetic acid Triazine Carbamate Organophosphate Phosphonoglycines	2,4-D Atrazine Carbofuran Diazinon Glyphosate	11 Biomixtures made of vegetable compost, sisal pulp, corn stover and seaweed	1.08, 2.50, 0.23, 0.34, and 0.36 mg cm^{-3} of biomixture, respectively	>99% for all pesticides	[51]
Carbamate	Carbofuran	Coconut fiber, garden compost and soil pre-exposed to carbofuran (50:25:25 v/v)	20 mg kg^{-1}	9.9% after first application; >88.5% in subsequent applications for 6 months	[52]

Dicarboximide	Iprodione	Soil, wheat straw and peat (25:50:25 v/v)	90.9 mmol kg^{-1} (dw)	DT_{50} between 3.4 days and 4.6 days	[53]
Triazine Organophosphate Dicarboximide	Atrazine Chlorpyrifos Iprodione	Soil, wheat straw and peat (25:50:25 v/v)	35 mg kg^{-1} each	>95% for all pesticides after 5 days	[54]
Triazine	Atrazine	Soil, wheat straw, and peat (25:50:25 v/v) inoculated with immobilized white-rot fungus	80 mg kg^{-1}	78–93%	[55]
Biphenyl Imidazole	Ortho-phenylphenol Imazalil	Spent mushroom substrate of *Pleurotus ostreatus,* either alone or in mixture with straw and soil at (50:25:25 v/v)	4062.5 g m^{-3} and 235.5 g m^{-3} of substrate, respectively	Reduced leaching of pesticides which corresponded to 0.014–1.1% (ortho-phenylphenol) and 0.120–0.420% (imazalil) of their initial concentration	[56]
Triazine Triazole Carboxamide Dinitroaniline	Terbuthylazine Difenoconazole Diflufenican Pendimethalin	Several biomixtures containing substrates such as cork, straw, coat pine and LECA (Light Expanded Clay Aggregates), with and without bioaugmentation with the fungus *Lentinula edodes*	20 mg kg^{-1} difenoconazole and diflufenican; 65 mg kg^{-1} pendimethalin and terbuthylazine	13–76% after 120 days in non-bioaugmented biomixtures; 45–99% in bioaugmented biomixtures	[57]
Carbamate	Carbofuran	Rice husk, compost, and soil biomixture (several compositions) bioaugmented with *Trametes versicolor*	~15 mg kg^{-1}	22–46% after 3 days	[58]

(continued)

Table 1 (continued)

Chemical group	Active ingredient	Biomixture evaluated	Concentration evaluated	Main removal results	Reference
Carbamate	Carbofuran	Five different lignocellulosic materials (sugarcane bagasse, coconut fiber, rice husk, wood chips and newsprint paper) mixed with either compost or peat and soil (50:25:25 v/v)	30 mg kg^{-1}	98.5% after 16 days at best conditions	[59]
Triazine Organophosphate Dicarboximide	Atrazine Chlorpyrifos Iprodione	Soil, wheat straw and peat at (25:50:25 v/v)	35 mg L^{-1} each added at 0.6 L and 1.2 L of water per day	>95%	[60]
Chloronitrile Neonicotinoid	Chlorothalonil Imidacloprid	Soil, spent mushroom, wheat straw (25:25:50 v/v)	100 μg g^{-1} each	Dissipation rate constant: Chlorothalonil 0.031–0.105, imidacloprid 0.009–0.031	[61]
Triazine	Atrazine	Soil, wheat straw, peat (25:50:25 v/v) amended with rich terpene substrates (5% w/w)	10 and 20 mg kg^{-1}	95–98%	[62]

The diversity of chemical structures that can be efficiently treated in BPS is remarkable. As shown in Table 1, several neonicotinoids, dithiocarbamates, organophosphates, phenylureas, acylalanines, triazines, triazoles, nitriles, dicarboximides, carbamates, benzonitriles, and dinitroanilines, among others, have been degraded in BPS to some extent, even reaching a high elimination of over 80%. Despite the high versatility demonstrated by BPS in terms of their removal capacity of diverse compounds, some pesticides have shown little or no degradation in these systems. In this respect, various works have described a high recalcitrance of some insecticide neonicotinoids [63] and fungicide triazoles [64] in soil, even when the microbial degradation of these groups is considered to be the most efficient method for their elimination [64, 65]. Interestingly, the same resistance to degradation has also been observed for several neonicotinoids [27, 66] and triazoles [27, 38, 67] in BPS. The low removal observed for triazoles might be related to their harmful effect as fungicides on ligninolytic fungi developed in the biomixture, since this group of microorganisms is associated with the first steps in some degradation processes within BPS [1].

On the other hand, it is clear that removal of a specific pesticide may vary depending on the biomixture (i.e., materials selection) employed, as well as the operating conditions and even the combination of pesticides treated in the BPS. For example, the combined application of azoxystrobin (strobilurin) and imidacloprid (neonicotinoid) in a BPS composed of rice straw/compost or corn cob/compost biomixtures resulted in an efficient retention of both pesticides, the complete degradation of imidacloprid, and azoxystrobin degradation ranging from 68.1% to 81.5% depending on the biomixture [40]. Interestingly, another study carried out by Kumari et al. [68] revealed that imidacloprid degradation in the same biomixtures was reduced by five- to nine-fold when unconditioned biomixtures were employed (without a previous incubation period); a change that did not affect azoxystrobin removal. Similar studies carried out with the organophosphate chlorpyrifos showed that pre-conditioning of the biomixture (straw, peat, soil, 50:25:25 v/v) did not cause a delay in the degradation processes, reaching values >50% [24]; however, the degradation rate was affected by the concentration of chlorpyrifos applied. Vischetti et al. [33] described the composition of the biomixture as a more determinant factor in chlorpyrifos degradation, where low pH and low C/N ratio favored greater degradation of the compound. Moreover, different composition of biomixtures can lead to the accumulation of 3,5,6-trichloro-2-pyridinol (TCP), the main degradation product of chlorpyrifos; this metabolite exhibits antimicrobial properties that may cause alterations in microbial communities of BPS, thus hindering the degradation of pesticides [17]. Similarly, the production of metabolites with antimicrobial properties, reported for other pesticides in soil [69–71], may affect bacterial composition of BPS, also translating into delays in pesticide degradation.

Modification of biomixture composition results in removal variations. For instance, the degradation of a mixture of imidacloprid, oxyfluorfen, tebuconazole, and diuron in a biomixture composed of straw, peat, and soil (50:25:25 v/v) resulted in complete removal of diuron and partial removal of the remaining compounds (from 45.7% to 55.3%) [45]. When the biomixture was modified by the addition of

vermicompost a decrease in the degradation of all pesticides was observed, ranging from 11.8% to 58.8%, including diuron.

Removal of specific pesticides also seems to be dependent on the combination of pesticides simultaneously applied or coexisting in the biomixture. In this respect, higher degradation rates for individual pesticides have been described compared to those achieved when the active ingredients were applied as mixtures in the BPS [46]. Such behavior was observed in a work by Masís-Mora et al. [28], who evaluated the degradation of pesticide mixtures from single chemical families (10 triazines, 13 triazoles, and 20 organophosphates, at 4–8 mg kg^{-1} each), on a biomixture composed of coconut fiber (45%), compost (13%), and farm soil (42%) at laboratory scale. The results showed that organophosphates were degraded at the largest extent (68%), followed by triazines (59%), while the degradation of triazoles failed. The same work also evaluated the degradation of 38 pesticides contained in real wastewater of laboratory origin at low concentrations on a pilot-scale BPS; interestingly, in this case triazoles were degraded at 73%, while triazines, organophosphates, and carbamates were removed at 54%, 90%, and 71%, respectively, showing a delay or an increase in the removal of some pesticides depending on their initial concentrations applied in the BPS.

In this chapter, we made a brief summary of the diversity of pesticides whose removal has been evaluated in BPS. Although we cannot include all structures assayed so far, it is remarkable that BPS exhibit in most cases a higher capacity to remove pesticides than soil. As shown in Table 1, different removal patterns are observed even for the same pesticide depending on factors such as the co-application with other active ingredients or the composition of the biomixture employed, which severely affects degradation kinetics. Hence, it is difficult to draw a general recommendation or a specific biomixture composition for a particular pesticide or mixture of pesticides. In this respect, field conditions must be evaluated depending on each particular situation to select the biomixture that favors the highest pesticide degradation and detoxification in the BPS, and to assay the efficiency of a BPS to remove complex mixtures of pesticides; regarding the latter recommendation, the evaluation of BPS during complete pesticide application cycles of specific crops represents a critical gap in the current knowledge of these systems.

3 Description of Microbial Communities During the Operation of BPS

As mentioned before, soil represents the main source of pesticide-degrading microorganisms and their catabolic gene pool in BPS. In consequence, it is generally accepted that the degradation and mineralization processes in BPS occur broadly in a similar way as they do in soil. For instance, bacteria and fungi represent the dominant organisms in soil regarding biomass and metabolic activity [72] and are considered the main groups with respect to pesticide degradation both in soils [73]

and in BPS. Oftentimes, fungi are in charge of transforming pesticides through minor structural changes in the molecule, rendering it non-toxic and susceptible to further degradation by bacteria [74]. It is important to point out that pesticide mineralization in complex natural matrices is often carried out by the combined action of microbial communities and abiotic factors, instead of isolated microbial species only [75]. Nonetheless, only a limited number of studies have described the structure and changes in the microbial communities of BPS upon contaminant influx. This information is important because the composition of the microbial community may strongly influence the efficiency of pesticide degradation.

Earlier works involved the use of phospholipid fatty acid analysis (PLFA) [76] and denaturing gradient gel electrophoresis (DGGE) [52, 77–83], while more recent works utilized quantitative PCR (qPCR) [53, 56] next-generation amplicon sequencing [47, 84–87] and DNA Stable Isotope Probing (DNA-SIP) [88].

Several studies have found little or no effect of pesticide applications on the microbial community when assessed by DGGE. For instance, elimination of atrazine, chlorpyrifos, and iprodione and microbial community changes in biomixture column systems with and without a plant cover were assessed and it was found that presence of rhizosphere enhanced pesticide dissipation, but microbial communities were not affected by the application of the pesticide mixture [80]. A similar study was undertaken at a larger scale with repeated applications of the pesticides reaching similar conclusions [79]. Likewise, it has been observed that repeated atrazine and carbendazim applications had only a minor and transient effect on the microbial community structure of biomixtures composed of straw, peat, and soil [81, 82]. A study investigating repeated applications of carbofuran to a biomixture composed of straw, compost, and soil revealed modest changes on microbial community patterns occurring through time [52]. Similarly, it has been observed that addition of the antibiotic oxytetracycline to a biomixture used to degrade a mix of pesticides (atrazine, carbendazim, carbofuran, and metalaxyl) did not affect the efficiency of pesticide removal or the bacterial and fungal community structure [77].

On the other hand, other studies using similar molecular fingerprinting techniques have revealed more evident changes in the microbial composition of these systems. Fungicide dissipation and microbial changes were assessed in a biomixture composed of pruning residues and straw. Both penconazole and fludioxonil generated a notorious change in the microbial community, mainly attributed to inhibition of the fungal fraction by these compounds, however these effects were transitory [76]. Another study also investigating the effect of fungicides in a similar matrix showed changes in the microbial community through DGGE analysis, but likewise, the modification was only temporary [78]. An additional study employed pilot-scale biofilters that were treated with chlorpropham and found that community diversity significantly increased over time [83].

In general, when assessed by molecular fingerprinting techniques such as DGGE, a moderate modification of the overall community structure with the addition of some pesticides can be observed, while for others, the effects appear to be very limited.

Techniques capable of targeting specific microbial groups, such as qPCR, have also been used to evaluate the abundance of microbial populations of interest in BPS systems. For instance, an increased abundance of total bacteria and fungi was observed in pilot-scale BPS exposed to high levels of pesticides in simulated effluents from fruit packaging plants. It was found that the community was dominated by Actinobacteria, Firmicutes, and α-Proteobacteria [56]. In another study, bioaugmentation and rhizosphere assisted biodegradation were successfully used to maximize the dissipation of iprodione in BPS packing material. qPCR showed that changes in the abundance of α-Proteobacteria and Firmicutes were driven by the presence of rhizosphere while bioaugmentation had no significant effect in this respect [53]. This technique is suitable when targeted evaluation of microbial populations is required.

Molecular techniques capable of simultaneously targeting a broad range of known and unknown organisms, such as next generation sequencing, have added to the body of information and have increased our understanding of the microbial changes during pesticide addition and dissipation in BPS [2]. Pyrosequencing, in this case combined with DGGE, was used to study an operational BPS exposed to a variety of pesticides. Significant community changes were detected, most notably, a decrease in community diversity. Also, certain groups such as Gammaproteobacteria showed an increased relative abundance, while a decrease of Firmicutes and Bacteroidetes was observed [87]. Illumina amplicon sequencing was used to study the variations in bacterial and fungal communities within BPS exposed to different pesticide rinsates. It was found that exposure to pesticides generated an increase in bacterial diversity, while the effect on fungal diversity was variable [85]. The same method was used to assess microbial diversity in miniature biobed systems with different kinds of biomixture exposed to atrazine, carbofuran, diazinon, glyphosate, and 2,4-D. The type of biomixture had a significant effect on diversity in all biomixtures. Additionally, diversity had an important effect on the residual amount of pesticides at the end of the assay [47]. Oxford Nanopore sequencing of amplicons has also been used to characterize the microbial population of a biomixture obtained from an operational BPS repeatedly exposed to a mixture of pesticides, including chlorpyrifos and iprodione. It was found that the microbial community composition was mainly formed by the phyla Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria [86].

Finally, DNA-SIP has been used as a culture-independent approach to identify microorganisms of the genera *Ramlibacter*, *Variovorax,* and an unknown genus of the family Comamonadaceae as the dominant linuron degraders in a biomixture. *Ramlibacter* could not be isolated by selective enrichment from the matrix. This study highlights the biases that cultivation techniques introduce when used to identify pesticide-degrading microorganisms [88].

These newer, more informative techniques will provide further knowledge on the dynamics of microbial communities within biomixtures. Gathering this information potentially represents a key step to improve the performance of BPS. Furthermore, the identification of the most important degrading communities would provide more targeted inputs for biostimulation/bioaugmentation of BPS.

4 Alternative Applications: Effects of Antibiotics of Agricultural Use During Co-Disposal in BPS

The application of agrochemicals in agricultural activities is not restricted to pesticides; plant growth regulators (promoters or retardants), fertilizers, and other crop protection chemicals such as antibiotics also make up the diversity of compounds employed in this industry [89]. Considering that most antibiotic application strategies employed in agriculture resemble those used for pesticide application, on-farm production of antibiotic-containing wastewater is also a consequence of pest management.

Antibiotics used in agriculture are restricted to a small number of molecules, in contrast to what happens in human and veterinary medicine. Currently, only two drugs, oxytetracycline and streptomycin, are registered by the EPA for use in plant agriculture [90]. However, other antimicrobials including gentamicin, oxolinic acid [91], kasugamycin, and validamycin [92] are also used in several geographical regions for the same purpose.

The release of wastewater containing high loads of antibiotics represents an important environmental risk, which is why they should be treated to avoid antibiotic exposure to ecosystems. Due to their action over non-target organisms, the release of antibiotics in the environment may exert adverse effects on diverse natural and anthropogenic-related processes, such as nitrification and iron reduction in aquatic systems [93, 94], enzymatic activity in soils [95], sulfate reduction and decomposition of organic matter in wastewater treatment plants [96], anaerobic digestion and biogas production [97, 98], and the Anammox process [99]. In addition, the potential spread of antibiotic-resistance genes has also been linked to environmental exposure to antibiotics [100], a problem that is partially solved by the use of different antibiotics, at least in animal/human medicine.

For these reasons, the use of BPS for the disposal and treatment of antibiotic-containing wastewater has been considered as a novel application of such systems. However, the mechanism of action of antibiotics would apparently stand as the main disadvantage against this use, as their bactericidal or bacteriostatic action might result in the inhibition of microbial communities and subsequently in the removal capacity of BPS. It is true that some deleterious effects of antibiotics are reported only at concentrations usually higher than those detected in real environmental samples; however, considering the nature of agricultural wastewater and their corresponding disposal, these high concentrations could be reached within BPS.

Most of the studies conducted so far on the effect of antibiotics during co-disposal of pesticides contradict the hypothesis of inhibition in the efficiency of pesticide degradation in BPS. Such effects have been evaluated in the mineralization and removal of several pesticides, when different doses of oxytetracycline, kasugamycin, or the commercial formulation of oxytetracycline+gentamicin were simultaneously applied to functional biomixtures.

A pesticide mineralization process indicates that the agrochemical is completely oxidized to yield inorganic compounds (CO_2 and H_2O under aerobic conditions),

thus preventing the accumulation of organic transformation products of potential toxicity in the BPS. Pesticide mineralization analyses are usually based on the use of ^{14}C-radiolabeled pesticides, whose mineralization yields $^{14}CO_2$ that can be easily distinguished from the CO_2 produced in the biomixture through the metabolization of other sources (respiration of other substrates in the matrix) [101]. The effect of antibiotics on the mineralization of pesticides has been analyzed in biomixtures, mainly employing ^{14}C-chlorpyrifos and ^{14}C-carbofuran as model pesticides (Table 2).

The co-application of a commercial formulation of oxytetracycline resulted in the stimulation of ^{14}C-chlorpyrifos mineralization rates at low doses of 0.1 and 1 mg kg^{-1}; on the contrary, high antibiotic doses of 100, 500, and 1,000 mg kg^{-1} caused an important decrease in mineralization rates [102]; the dose of 10 mg kg^{-1}, the one more likely to occur within BPS biomixtures, seemed to affect this parameter the least. This pattern has been described as a hormetic-like response to the antibiotic, in which low, non-inhibitory concentrations enhance certain metabolic activities of microbial communities, while higher, inhibitory doses, evolve into their depression [105]. An analogous hormetic-like response was also observed during the co-application of the same oxytetracycline formulation during ^{14}C-carbofuran removal in biomixtures [39]; however, in that case, stimulation in the mineralization rate was observed at concentrations ranging from 0.1 mg kg^{-1} to as high as 500 mg kg^{-1}, and only the extreme dose of 1,000 mg kg^{-1} delayed mineralization.

The co-application of kasugamycin also produced stimulation in the mineralization of ^{14}C-chlorpyrifos; in particular, the mineralization rate also exhibited a hormetic-like response, with markedly increased rates at concentrations ranging from 0.1 to 100 mg kg^{-1}, and a deleterious effect only at the dose of 1,000 mg kg^{-1} [37]. The stimulation of the process was remarkably higher than that achieved with oxytetracycline, reaching a value of more than 250% in the parameter of mineralization rate at a dose of 0.1 mg kg^{-1}, with respect to the system without antibiotics. Curiously, the maximum mineralization estimated was higher for each of the tested antibiotic doses, even those that decreased the mineralization rate.

A different panorama has been observed when the commercial formulation of oxytetracycline+gentamicin was co-applied with ^{14}C-chlorpyrifos, resulting in an important decrease of mineralization rates, from 15 to 50%, at every dose tested [37]. Despite reducing the speed of mineralization, most doses, on the contrary, produced an increase in the maximum estimated mineralization, thus not discouraging at all the potential co-disposal of this formulation with pesticides in BPS.

The observations achieved with oxytetracycline and kasugamycin formulations revealed an enhancement in the removal of recalcitrant pesticides; moreover, they indicate that BPS might be used for the co-disposal of pesticides and antibiotics, without necessarily jeopardizing the pesticide removal capacity of the system. The fact that potential antibiotic concentrations in BPS (5–50 mg kg^{-1}) fall within those for which mineralization enhancement has been achieved supports the relevance of this practice. Nonetheless, the pattern observed for ^{14}C-chlorpyrifos and ^{14}C-carbofuran cannot be generalized, and the response of other pesticides should be evaluated, as well as the effect of other antibiotic formulations.

Table 2 Effect of co-disposal of antibiotics on the removal of pesticides in biopurification systems

Pesticide	Chemical group/ function	Process assayed (mineralization/removal)	Co-disposed antibiotic	Effect on pesticide elimination	Reference
Ametryn	Triazine/herbicide	Removal	Oxytetracycline	Ranged from no alteration to duplication of DT_{50}	[27, 102, 103]
Atrazine	Triazine/herbicide	Removal	Oxytetracycline	No effect regardless of the mixture of pesticides treated in the BPS	[27, 77, 102, 104]
Carbendazim	Benzimidazole/ fungicide	Removal	Oxytetracycline	Ranged from no alteration to increase (three-fold) of DT_{50} depending on the mixture of pesticides treated in the BPS	[27, 77, 104]
			Kasugamycin	No alteration	[37]
			Oxytetracycline +Gentamicin	No alteration	
Carbofuran	Carbamate/insecti-cide-nematicide	Mineralization	Oxytetracycline	Hormetic-like effect in mineralization rate: increase at low-high doses (0,1 to 500 mg kg^{-1}), decrease at very high doses (1,000 mg kg^{-1})	[39, 77, 104]
		Removal	Oxytetracycline	No alteration	
Chlorpyrifos	Organophosphate/ insecticide	Mineralization	Kasugamycin	Hormetic-like effect in mineralization rate: increase at low-medium doses (0.1, 1, 10, 100 mg kg^{-1}), decrease at high doses (1,000 mg kg^{-1}). Increase in maximum mineralization at every dose.	[37, 102]
			Oxytetracycline	Hormetic-like effect in mineralization rate and maximum mineralization: increase at low doses (0.1 and 1 mg kg^{-1}), no apparent effect at medium dose (10 mg kg^{-1}), decrease at high doses (100, 500, 1,000 mg kg^{-1})	
			Oxytetracycline +Gentamicin	Decrease in mineralization rate at every dose (0.1 to 1,000 mg kg^{-1}). Increase in maximum mineralization value at most doses	

(continued)

Table 2 (continued)

Pesticide	Chemical group/ function	Process assayed (mineralization/removal)	Co-disposed antibiotic	Effect on pesticide elimination	Reference
Metalaxyl	Phenylamide/ fungicide	Removal	Oxytetracycline	Ranged from no alteration to increase (1.5 to 2.7-fold) of DT_{50} depending on the mixture of pesticides treated in the BPS	[27, 77, 104]
			Kasugamycin	No alteration	[37]
			Oxytetracycline +Gentamicin	No alteration	
Linuron	Urea/herbicide	Removal	Oxytetracycline	Ranged from no alteration to slight increase (1.3-fold) of DT_{50} depending on the mixture of pesticides treated in the BPS	[27, 102]
Terbutryn	Triazine/herbicide	Removal	Oxytetracycline	Slight decrease (0.67-fold) of DT_{50}	[103]

A different scenario has been observed for pesticide removal (instead of mineralization) in BPS during co-application of antibiotics: the process mostly ranges from no significant alteration to delays in the removal, revealed as an increase in the half-life (DT_{50}) values for some pesticides, depending on specific conditions (Table 2). That said, contrary to mineralization, enhancement or stimulation has not been described as a common feature in the removal of pesticides in the presence of antibiotics (with some exceptions, as indicated below). The following discussion refers to observations obtained using potential antibiotic concentrations in BPS (from 10 to 40 mg kg^{-1}), according to real application practices.

The removal of several herbicides belonging to the group of triazines has revealed different patterns when co-applied with oxytetracycline. There seems to be no alteration on atrazine removal, as DT_{50} values have not significantly differed from control biomixtures without the antibiotic; this pattern has been the same regardless of the mixture of pesticides treated, ranging from just a few herbicides to complex mixtures of up to nine active ingredients [27, 77, 102, 104]. On the contrary, ametryn removal showed no significant alteration in a biomixture simultaneously treating nine pesticides (three herbicides, two fungicides, and two insecticides) [27], while its DT_{50} was doubled (from 43.9 days to 92.4 days) in a simpler mixture containing only the herbicides [102]. A marginal enhancement in the removal of triazines was also described by Cambronero-Heinrichs et al. [103] in the cases of ametryn (DT_{50} decreased from 34.8 days to 28.4 days) and terbutryn (DT_{50} decreased from 51.0 days to 34.0 days), during their simultaneous removal in biomixtures containing oxytetracycline. Some differences might also be ascribed to the heterogeneity in microbial communities from one biomixture to another, depending on the origin of the soil and other materials employed in their production.

Removal of linuron, a phenylurea herbicide, followed a pattern that resembled that from ametryn during co-application of oxytetracycline: no alteration during the elimination of a complex mixture of pesticides [27], and some delay (DT_{50} increased from 30.7 days to 40.1 days) only in the removal of three herbicides [102]. Interestingly, such delays observed in the case of herbicides do not affect final removal values.

The effect of oxytetracycline was also assayed during the elimination of the insecticide carbofuran, whose removal was not affected even by successive co-applications of the antibiotic (every 7 days; four applications) at relevant BPS concentrations [39]. Moreover, a second dose of carbofuran applied 14 days after the first one revealed accelerated degradation of this compound, a process that also was not inhibited by the antibiotic. Furthermore, no alteration was demonstrated during the elimination of carbofuran in biomixtures used for the simultaneous elimination of other pesticides [77, 104]; nor during the removal of its transformation products [39].

The effect on the removal of the fungicides carbendazim and metalaxyl was evaluated with several antibiotics of agricultural use. First, when oxytetracycline was co-applied in a biomixture for the elimination of six pesticides (four fungicides plus two insecticides), the removal of both fungicides was delayed: DT_{50} increased from 8.9 days to 26.8 days for carbendazim, and from 6.5 days to 17.5 days for

metalaxyl [27]. Then, during the elimination of nine pesticides (the latter plus three insecticides), no delay was observed for carbendazim due to oxytetracycline (although the DT_{50} was significantly higher in the absence of the antibiotic), while a lower delay was still observed for metalaxyl (DT_{50} increased from 8.8 days to 12.9 days). On the contrary, no alterations were recorded in the DT_{50} values for both fungicides when the effect was assayed with the commercial formulations of kasugamycin or oxytetracycline+gentamicin [37]. However, in this case the biomixture had been previously exposed to carbendazim and metalaxyl, and accelerated degradation was achieved for both fungicides (observed as much lower DT_{50} values); such effect could have masked potential effects due to antibiotics after first pesticide/antibiotic application, as most reports describe. Conversely, these findings further support the evidence for co-disposing antibiotics and pesticides in the same BPS, without significant depletion of its removal capacity.

The rationale behind disposing antibiotic-containing wastewater in BPS is aiming to achieve antibiotic removal, as in the case of pesticides. In this respect, current evidence reveals that oxytetracycline is actually removed in biomixtures. Jiménez-Gamboa et al. [39] and Cambronero-Heinrichs et al. [103] reported DT_{50} values of 34 days and 38 days, respectively, which are shorter than those described in soil [106] and in some anaerobic systems [97, 107], although they are similar or longer than values achieved in matrices containing manure [106, 108]. More research is necessary to enhance oxytetracycline removal in BPS; moreover, the dissipation of other agricultural antibiotics such as kasugamycin or gentamicin is yet to be evaluated in BPS.

Even though elimination delays have been described for some pesticides, the removal capacity of BPS is not significantly hindered in the presence of relevant antibiotic concentrations. On the contrary, the enhancement of processes such as mineralization supports the practice of co-disposal of antibiotics and pesticides in the same BPS, thus resulting in lower operational costs and smaller areas devoted for BPS within agricultural fields. As DT_{50} values for pesticide elimination depend not only on the co-application of antibiotics, but also on the mixture of pesticides disposed in a specific BPS, effects and removal performance (including antibiotic removal) should be assayed for each specific case of pesticide application cycle for a given crop.

5 Alternative Applications: Use of BPS for the Removal of Pharmaceuticals

Despite being typically employed for the removal of pesticides, or more recently for the elimination of antibiotics of agricultural use from wastewater of agricultural origin, the application of BPS for the removal of pharmaceutical-containing effluents has been recently under exploration. This incipient approach clearly depends on how feasible a BPS configuration can be adapted to treat the high volume of effluents

usually polluted with pharmaceuticals. Hence, the studies here discussed employed small biomixture systems, of up to a few kilograms.

Delgado-Moreno et al. [49] described the use of biomixtures containing soil and raw olive mill cake or its vermicompost for the removal of diclofenac, ibuprofen, and triclosan from wastewater. Removal values over 94% were achieved over a period of 84 days in the best scenario, and biodegradation of triclosan and diclofenac was determined to be controlled by their bioaccessible fraction in the system. One of the main advantages of the biomixtures evaluated was their higher adsorption efficiency (compared to soil), which might avoid pollutant transport to other compartments. A similar assay demonstrated slower dissipation of these pharmaceuticals when they were simultaneously applied in the biomixture, and differential alteration in the composition and relative abundance of bacterial taxa, depending on the pharmaceutical [84]. The bioaugmentation of a BPS with an ibuprofen-degrading bacterial strain of *Sphingopyxis granuli* successfully enhanced the degradation of this pharmaceutical even after successive weekly applications, increasing dissipation rates by up to three-fold in the biomixture, and reducing the accumulation of transformation products [109]. Bioaugmentation of the BPS with aqueous extracts from a previously acclimated biomixture (exposed to the same compounds) at least partially improved the dissipation of the pharmaceuticals [110].

Similarly, other biomixture-like matrices composed of wheat straw pellets (lignocellulosic substrate) mixed with sewage sludge naturally contaminated with pharmaceuticals were successfully employed for the removal of several compounds, including atorvastatin, clarithromycin, fenofibrate, hydrochlorothiazide, ibuprofen, and ranitidine [111]. Such mixtures have commonly been evaluated as bioaugmented matrices, particularly with the ligninolytic fungus *Trametes versicolor*; this approach has achieved efficient elimination of diverse pharmaceuticals (the previously mentioned plus atenolol, bezafibrate, cimetidine, diclofenac, mefenamic acid, naproxen, sulfamethazine, among others) but lower elimination of psychiatric drugs like diazepam and carbamazepine [111–113]. Analogous to traditional biomixtures aimed at pesticide removal, this approach (bioaugmentation aside) also relies on the presence of pharmaceutical microbial degrading communities within the contaminated matrix (i.e. sewage sludge), which is pre-exposed to these compounds.

The treatment of effluents, either wastewater or sludge, still requires further optimization, and in particular, proper approaches to scale up the process, considering that such effluents are of higher flow/volume than those containing pesticides produced at farm level.

6 Challenges and Future Prospects

Research on BPS has been mostly performed in Europe and Latin America, and despite a few exceptions in European countries (and Guatemala in Latin America), extensive application has not been reached yet. The lack of legislation regarding the

treatment of pesticide residues, as well as the explicit inclusion of BPS as a treatment option, represents one of the biggest challenges to achieve the generalized implementation of the BPS technology. Contrary to those few European countries where BPS are included in local legislation, gaps in their Latin American counterparts limit their extensive application. Another challenge for a more generalized utilization of this technology is the delivery of adequate training to the farmers, particularly in less developed countries, especially because they might not have been exposed to enough information regarding the use and advantages of BPS.

Further research on the evaluation of real pesticide application cycles to assay the potential accumulation of the most recalcitrant compounds is necessary to properly establish whether single or multiple BPS are required in farms where intensive agricultural activities are undertaken. Furthermore, it is important to assess the removal of different pesticide mixtures, as this represents the real scenario during agricultural production, and mixture complexity may modulate removal performance. In this respect, additional efforts should be targeted at demonstrating detoxification or mineralization of the compounds within the system, and not only their removal or transformation.

Given that only scarce reports describe the long-term efficiency of BPS [52], the useful life of these systems is yet to be determined. Similarly, since no formal studies regarding the disposal of aged biomixtures have been reported, more research should focus on the evaluation of composting, the widely recommended approach, or alternative strategies for BPS discarding; moreover, the disposal of BPS should follow a strict ecotoxicological monitoring of the biomixture waste, to minimize the potential environmental risk.

Experimental evidence suggests that a single BPS could be used for the simultaneous removal of antibiotics and pesticides, thus resulting in lower operational costs and smaller areas devoted for BPS within agricultural fields. Nonetheless, as there is no single golden recipe for biomixture composition, the removal performance should be evaluated for each desired combination of agrochemicals aimed to be degraded in the BPS. Newer, more informative microbial community analysis technologies have started to increase our understanding of the dynamics of degrading communities and will provide novel insights on their identification, selection of the most suitable specialized strains, and identification and monitoring of specific degrading genes. This information will prove vital for the enhancement of BPS performance in the near future.

Acknowledgements This work was supported by Vicerrectoría de Investigación at Universidad de Costa Rica (project 802-B8-510), and Ministerio de Ciencia, Tecnología y Telecomunicaciones de Costa Rica (MICITT, Project FI-197B-17).

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