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Review

Destination of pesticide residues on biobeds: State of the art and future perspectives in Latin America

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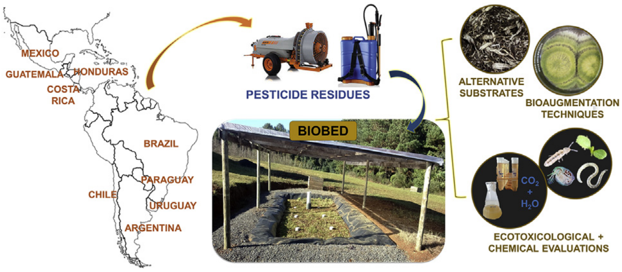
HIGHLIGHTS

- Biobed is a new technology in LA which requires adaptation/evaluation studies.
- This review brings the main results obtained in LA studies until June 2019.
- Advances include the search for alternative biomixture components.
- The role of bioaugmentation processes and abiotic factors are discussed.
- The use of ecotoxicological monitoring and chemical evaluations are presented.

GRAPHICAL ABSTRACT



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ABSTRACT

Land-use intensification with a high demand for pesticides is a consequence of human population increase. Feasible alternatives for correct concentrated residues discharge are necessary to avoid soil and water resources contamination. Biobeds are *in situ* bioreactors for treating pesticide residues, used by several European and American countries due to its low cost and simple construction, whose efficiency has been scientifically proved for over 20 years. This review presents the state of the art of biobeds in Latin America (LA), identifying advances and future research needs. Factors affecting the efficiency of biobeds are discussed, like ideal temperature, moisture, and microbial communities, followed by methods for evaluating the bioreactor's efficiency. It was necessary to adapt this technology to the climatic and economic conditions of Latin-American countries, due to its European origins. Guatemala is the LA country that uses biobeds as official technology. Brazil, Argentina, Costa Rica and Chile are examples of countries that are actively investigating new substrates and pursuing legal aspects for the establishment of the biobeds. Robust scientific evidences may enable farmers start using this technology, which is an environmentally safe system to protect water resources.

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Contents

1. Introduction

All processes involving pesticide use can offer some risks of environmental contamination. The contamination process that takes course during the pesticide application is called diffuse. However, when this happens while filling the spraying equipment or handling pesticide residues, it is called point-source contamination. Inadequate practices generate residues that may cause contamination of surface water, groundwater, soil and biota (Briceño et al., 2014; Rodríguez-Rodríguez et al., 2018). Through good agricultural practices, the contamination can be reduced or even avoided, and the biobed comprises one of those practices.

Biobeds are bioreactors developed for treating pesticide residues generated during agricultural activity, such as water from the washing of spraying equipment or any residue from the preparation of pesticide sprays (Castillo et al., 2008; Diez, 2010). The original structure and biomixture of the biobed have being adapted according to characteristics of soil, climate and organic materials available in Latin America (LA), in order to ensure that it will be affordable for all regions, as the efficiency of the biological treatment is preserved. Some studies have been focused on structural changes, e.g., to find an ideal depth, impermeabilization options and to evaluate the need of a roof, due to the high rainfall in some regions (Gebler et al., 2015; Lescano et al., 2018). The main approach in LA has been to test alternative component materials of the biomixture and evaluate bioaugmentation techniques with fungal or bacterial strains, testing the ability of different species to degrade pesticides. The maturation time and ideal substrate moisture conditions are also important aspects studied (Rodriguez-Rodriguez et al., 2018).

In this context, the objective of this paper was to analyse the biobeds studies in Latin America, bringing the state of the art and future perspectives for research and implementation of this system. Most of the data was obtained from databases like "Scopus" and "Google Scholar". The keywords included: "biobed", "*lecho biologico*" and "biopurification system", limiting the search results to Latin American authors. This research includes all papers published until June 2019. Other data sources were the official sites of biobeds and research groups in this theme (Biobeds.ORG, 2019; Lechos Biologicos, 2019). In addition, some questions were sent to leaders of Latin American research groups on biobeds, asking about their last scientific results and the actual state of the technology in their respective countries (whether it is official, legal aspects involved and acceptance by farmers).

2. Environmental contamination by pesticides

According to Fogg et al. (2003) point source contamination by pesticides is environmentally more aggressive than non-punctual contamination, although the second one results in major visual impacts, since it occurs during crop spraying and generates a pesticide fog beyond the crop area (Fogg et al., 2003). While point source contamination by pesticides is concentrated, reaching levels of grams or kilograms of products spilled over a few squared meters (Carter, 2000), in non-punctual contamination this equivalent volume is spread over several hectares of land.

Incorrect disposal of pesticides effluents can affect water resources, compromising environmental and human health (Gebler and Fialho, 2011). According to Fernandes Neto and Sarcinelli (2009), drinking water can be an important source of human exposure to pesticides, besides being toxic to living organisms and causing bioaccumulation in the trophic web. Apparently, the risk is common to all LA countries, as agriculture is one of the main socio-economic activities. Thus, one of their biggest challenges is the definition of appropriate legislation instruments, including alternatives for waste disposal.

3. The biobed system

Biobed is a technology developed in Sweden by Torstensson and Castillo (1997). It was originally described as a trench in the soil filled with the biomixture, which is a mixture of peat (25%), wheat straw (50%) and agricultural soil (25%), and covered by grass. It was designed to dispose pesticide residues, derived from contaminated machinery washings and accidental spillages, in order to be degraded by the microbiota developed in its substrate (Cooper et al., 2016). Simultaneously, the adsorption of the pesticide residues takes course into the biobed's substrate (or biomixture) particles, decreasing their chemical availability (Castillo et al., 2008). Fig. 1 illustrates the field biobed systems that are currently under study in Brazil.

Diez et al. (2013c) state that in Chile each biobed can be used for up to 4–5 years. That period will be lower depending on the climate features of each country, and it can be tracked by the changes in the

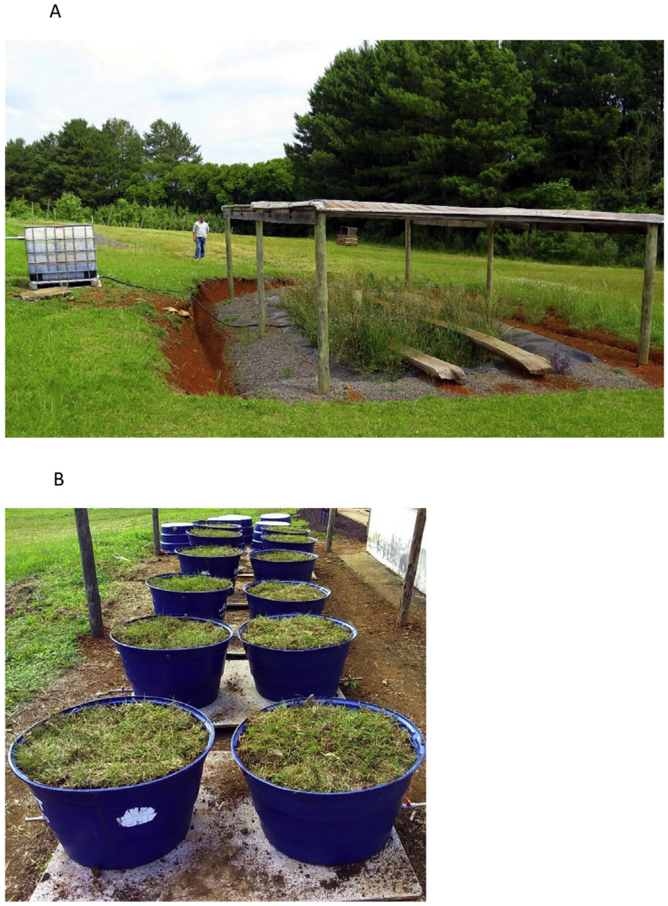

Fig. 1. Biobeds installed in Brazil. A) A full size bioreactor, designed to pass the farm sprayer over it. B) Small reactors used for experimental purposes or disposing small amounts of pesticide waste.

substrate's depth. A biobed system that received carbofuran applications continuously during a year, in Costa Rica, revealed a decreased efficiency after six months of use, suggesting that climate conditions in the tropics might result in biobeds of shorter shelf-lives if compared to their operation in temperate regions (Castro-Gutiérrez et al., 2017). These results indicate the importance of evaluating the limiting shelf life in each country, before applying the biobed technology as an environmentally safe system on the field.

The right moment to replace the entire biobed's substrate is when it reaches 1/3 of the initial depth (Diez et al., 2013c). At this moment, the aged biomixture must be submitted to a composting process (Torstensson, 2000), which has to be carried out according to legal standards defined by each country or region. The required composting time depends on the country weather. Diez et al. (2013c) state that in Chile, six months would be necessary for composting. This period could be shorter for the LA countries where weather is warmer. After composting, the biomixture can be discarded on the land through landfarming techniques. Masin et al. (2018) state that another option is to submit the aged biomixture to vermicomposting, which consists in using earthworms to convert organic matter into a humus-like matter.

4. Overview of the system and background in Latin America

Several studies from the United States and European Union (EU) countries in biobeds, in testing phase or under official use, reported an effective degradation of several pesticide residues. The system has being used to prevent point-source environmental contamination, specially of water bodies (Torstensson and Castillo, 1997; Cooper et al., 2016; Diez et al., 2017). The United Kingdom, Sweden (country of origin of the biobed technology, with more than 1500 units installed), France, Belgium, Guatemala, Spain and Poland are among the countries that officially use this technology (Biobeds.ORG, 2019).

The EU countries that officially adopted the biobed system must attend the legislative acts that limit the presence of pesticides in drinking water since 1980 (Carter, 2000), such as the first Drinking Water Directive no. 80/778/EEC, revised by Directives 98/83/EC and 2015/1787. In 2008, the Environmental Quality Standards Directive (Directive nº 2008/105/EC) established limiting concentrations for 33 priority substances and eight other pollutants in surface water in the EU (European Commission, 2016).

In LA, scientific studies on biobeds are focused on Chile (Diez et al., 2013c; Briceño et al., 2017), Costa Rica (Castro-Gutierrez et al., 2018), Brazil (Gebler, 2015), Argentina (Lescano et al., 2018), Mexico (Gongora-Echeverría et al., 2018) and Uruguay (Rivero et al., 2016). These studies report substantial levels of pesticide degradation for many compounds. In order to share information and the latest results from their studies, LA groups periodically organize workshops, which take place every 3 years in a different country.

The first scientific paper on biobeds in LA was published by Diez (2010), from the University of La Frontera, Chile. It involved a literature review about environmental contamination by the use of pesticides, alternatives for its biological treatment and the microbiota involved in this process, presenting the biobed system and its purposes of use.

Diez and other authors are part of the research group named "Lechos Biológicos.CL" from University of La Frontera, which has records of studying biobed systems since 2008, being considered pioneers in LA. This group published the first guideline for the Construction and Operation of Biobeds in Spanish (Diez et al., 2013c), where detailed instructions are provided to farmers from La Araucanía region, in Chile, in order to manage pesticide residues using biobeds. Advances in treatment of pesticide residues in Chile are discussed by Briceño et al. (2014), confirming that this country was the pioneer of biobeds technology in LA, installing bioreactors in real scale. Their lines of work involve evaluating new organic materials for the biomixture (Diez et al., 2013a), including the co-application of terpenes for degradation enhancement (Tortella et al., 2013b), bioaugmentation strategies and the benefits of the rhizosphere environment (Campos et al., 2017; Diez et al., 2017).

In Costa Rica, there is a research group on biobeds as part of the Centre for Research in Environmental Contamination, at the University of Costa Rica. This group presented their first paper as a literature review on the role of white-rot fungi in the treatment of pesticides contaminated water, within biobed systems (Rodríguez-Rodríguez et al., 2013). In addition, the group has been working on defining new substrate composition for the biomixture, evaluating their efficiency through monitoring ecotoxicity along the process (Chin-Pampillo et al., 2016), and evaluating the effect of co-disposal of antibiotics from agricultural use, in association with a pesticide mixture (Jiménez-Gamboa et al., 2018). Additional studies have involved the removal of highly recalcitrant products (Rodríguez-Castillo et al., 2018) and complex pesticide mixtures (Masís-Mora et al., 2019). This group published a chapter in a Springer Protocols book that proposes methods to evaluate pesticide toxicity and biodegradation (Rodríguez-Rodríguez et al., 2018), including ecotoxicity tests as well as chemical and mineralization analysis for monitoring the biomixture efficiency throughout the maturation process.

Brazil was responsible for the first studies in LA proposing ecotoxicity tests associated to chemical analysis to evaluate the efficiency of the biobed system in comparison to soil disposal, performed by Gebler et al. (2015) and Carniel (2015). This team is composed by Brazilian Agricultural Research Company, Federal University of Santa Maria, and Santa Catarina State University. Soil invertebrates were used to monitoring the decrease of ecotoxicity of mancozeb and chlorpyriphos in biobeds, where the biomixture was more efficient in ecotoxicity reduction than Brazilian Oxisol and Utisol. Furthermore, the tests indicated risk to soil fauna when pesticide effluents were discarded in soil (Carniel, 2015). Most recently, the advances in using gas chromatography to determine dithiocarbamates in biobeds were presented by Vareli et al. (2018). Gebler (2015) also produced the first guideline in Portuguese, regarding the use of biobeds to provide correct disposal for pesticide residues from the culture management of temperate fruits in Brazil. Currently, new partnerships were included to expand Biobed research groups in Brazil, such as the inclusion of the Federal University of Santa Catarina, Campus of Curitibanos, resulting in the proposal of local materials for the biomixture.

In Uruguay, Rivero et al. (2016) developed analytical approaches to evaluate the use of biobed in treating a highly recalcitrant organochlorine pesticide. New substrates with local materials were proposed in Mexico by Góngora-Echeverría et al. (2018) and Argentina by Lescano et al. (2018). As reported by a researcher from the Nacional University of the Litoral (Argentina), the regulation for constructing and operating biobeds in Argentina is under development, as a partnership with the Soil Commission of the Argentine Institute for Standardization and Certification. Studies from Argentina also show results related to bioaugmentation techniques (Saez et al., 2018) and ecotoxicity tests (Masin et al., 2018).

Different from the other LA countries, in Guatemala the use of biobeds was not originated from academic research, but through AGREQUIMA, an associated group from pesticide industries in this country. In this case, AGREQUIMA acts on developing training courses and indicating new technologies for the farmers in Guatemala, in order to assure the responsible handling of pesticides to avoid environmental contamination. Due to that agroindustrial demand, Guatemala was the first LA country to adopt this system as an official technology for pesticide wastewater disposal. Nowadays, the technology adapted by AGREQUIMA has been applied to a large scale over the country, under the name of Biodep, having over 3000 units implemented (Agrequima, 2019).

5. Research issues on biobeds in LA countries

The biobed efficiency depends on the bioreactor's intrinsic components, such as the organic materials source, the role of microbiota and grasses in the substrate, besides abiotic factors like moisture and temperature. Recent studies in LA have focused on these aspects, and are presented below.

5.1. Organic materials for the substrate

One of the main issues on biobeds research is to propose alternative substrates for the biomixture, so that farmers can use readily-available materials from their own countries, to keep this technology as a cost-effective system. As the original technology was developed in Sweden, the biomixture was proposed as a mixture of two parts of wheat straw, one part of peat and one part of soil (Castillo et al., 2008). However, peat is an expensive material in LA, while wheat straw is not available on a large scale for some regions.

Straw is the lignocellulosic substrate that supplies the initial energy for the fungi community, stimulating the production of extracellular ligninolytic enzymes. In addition, it acts as physical support for the establishment of these communities (Urrutia et al., 2013; Tortella et al., 2013c). After the maturation time, where the establishment of the microbial community takes place, biobed is ready to receive the pesticide residues.

The agricultural soil used in the biomixture provides the microbiota in charge of pesticide degradation. This natural microbiota is already adapted to pesticides exposure, when primed-soil is employed (Góngora-Echeverría et al., 2018; Rodríguez-Rodríguez et al., 2018). Regarding alternative materials to replace the use of wheat straw, Saez et al. (2018) tested sugarcane bagasse, with positive results for lindane degradation. Diez et al. (2013a) tested barley husk and pine wood sawdust at different proportions to promote the degradation of a six pesticides mixture. The highest degradation rate occurred in the biomixture composed of barley husk and wheat straw.

Peat is a porous humic compound which provides high sorption capacity, acting both in the physical treatment of the residues as well as retaining the humidity of the biomixture, due to the great number of micropores (Diez et al., 2013a; Urutia et al., 2015). The replacement of peat by other materials has been a complex process due to the specificity of its physical characteristics. Diez et al. (2013b) carried out several experiments using biochar produced from the pyrolysis of *Pinus radiata* wood mixed with peat in different proportions. Results showed that biochar could be a promising substitute for the Chilean region, being efficient to promote the degradation of atrazine, carbendazim, chlorpyrifos, and other pesticides after 40 days, however, degradation occurred in lower rates than in the peat-based system.

In Costa Rica, researchers are testing biobeds with a modified biomixture whose degradation efficiency was evaluated through chemical analysis and ecotoxicity tests, for chlorpyrifos, carbofuran, imidacloprid, thiamethoxam and other pesticides. That biomixture was composed of coconut fiber, composted vegetal residues and soil (Chin-Pampillo et al., 2016; Rodríguez-Castillo et al., 2018). In Mexico, Góngora-Echeverría et al. (2018) evaluated sisal pulp, composted vegetal residue, corn husk and seaweed. These organic materials were mixed with the agricultural soil in different proportions, replacing peat and wheat straw. Degradation for a pesticide mixture (atrazine, carbofuran, diazinon, glyphosate and 2,4-D) was over 90%. The most efficient substrates had corn husk in their composition.

Urrutia et al. (2013) evaluated the use of barley husk, wood sawdust and oat husk as total or partial substitutes for wheat straw (50% of the lignocellulose source). The best efficiency was observed for oat husk. In Argentina, Lescano et al. (2018) evaluated laboratory-scale biobeds for glyphosate degradation with a mixture of alfalfa straw, wheat stubble and river sludge, all mixed with a percentage of agricultural soil. The biobed composed by soil and wheat stubble achieved a degradation level of 99% after 63 days. Table 1 summarizes alternative biomixture compositions to be used in biobed systems, successfully evaluated in LA.

5.2. Influence of abiotic factors

Other factors that influence on the efficiency of biodegradation are the maturation time, moisture and temperature. Fernández-Alberti et al. (2012) evaluated chlorpyrifos degradation and its metabolite TCP (3,5,6-trichloro-2-pyrinidol) in a traditional biobed with different maturation periods (0, 15 and 30 days), and different moisture levels (40, 60 and 80% of the water holding capacity). The highest levels of degradation were achieved after 15 days and 30 days of maturation, using 60% of water holding capacity.

Maturation time before the first pesticide application may ensure the degradation efficiency, although this subject lacks enough studies (Tortella et al., 2012). Over this period, moisture

Table 1
Summary of alternative substrates, with different composition and material proportions, evaluated for biobeds in LA.

Biomixture Composition	Reference
Coconut fiber (45%), composted vegetal residue (13%), soil (42%)	Chin-Pampillo et al. (2016)
Rice husk (30%), composted vegetal residue (43%), soil (27%)	Ruiz-Hidalgo et al. (2016a)
Sugarcane bagasse (50%), peat (25%), soil (25%)	Saez et al. (2018)
Oat husk (50%), peat (25%), soil (25%)	Urrutia et al. (2013)
Barley husk (25%), wheat straw (25%), peat (25%), soil (25%)	Urrutia et al. (2013)
Pine sawdust (25%), wheat straw (25%), peat (25%), soil (25%)	Urrutia et al. (2013)
Wheat straw (50%), biochar (25%), soil (25%)	Diez et al. (2013b)
Wheat straw (45%), pine leaves (5%), peat (25%), soil (25%)	Tortella et al. (2013b)
Wheat straw (45%), eucalypt leaves (5%), peat (25%), soil (25%)	Tortella et al. (2013b)
Wheat straw (45%), orange peels (5%), peat (25%), soil (25%)	Tortella et al. (2013b)
Soil (50%), sisal pulp (12,5%), composted vegetal residue (12,5%), corn husk (12,5%), seaweed (12,5%)	Góngora-Echeverría et al. (2018)
Wheat stubble (50%); soil (50%)	Lescano et al. (2018)

conditions in the biomixture are adjusted to provide the microbial community establishment. Tortella et al. (2012) evaluated a traditional biobed at different maturity stages (0, 15 and 30 days) on the degradation of chlorpyrifos at increasing concentrations. Results showed similar and efficient degradation over the three evaluated periods, and its efficiency decrease only happened due to the increasing concentrations of the pesticide.

Atrazine, a highly toxic herbicide, had its degradation evaluated in association with artificial terpenes, which are volatile organic compounds, in order to improve degradation rates. Degradation reached values higher than 70% in the original biomixture (Tortella et al., 2013a). Once the efficiency increased by addition of alphapinene, eucalyptol, and limonene terpenes, Tortella et al. (2013b) evaluated common organic residues rich in terpenes, including pine leaves, orange peels, and eucalyptus leaves. Degradation rates were similar or higher than the control (80%) in all mixtures.

The effects of oxytetracycline, kasugamycin and gentamicin, antibiotics of agricultural use, were assayed on the biobeds performance during the removal of a range of insecticides, fungicides and herbicides mixtures. Overall, results suggested that the antibiotics application did not affect the biobeds performance substantially, although there was some increase in phytotoxicity responses due to the oxytetracycline effect (Huete-Soto et al., 2017). Castillo-González et al. (2017) highlight that the antibiotics influence on degradation rates depends on the type of pesticide and on the microbial consortium present in the substrate.

Gebler et al. (2015) adapted a biobed structure to southern Brazil, conducting tests in PVC column reactors to identify the ideal depth for biobeds in this region. Chlorpyrifos and glyphosinate were applied separately and mixed together as models. Results suggested an ideal depth between 0.80 m up to 1 m, to guarantee optimal temperature and moisture. The authors stated that Brazilian biobeds must have a translucent cover, to prevent flooding from the intense rainfalls in this region.

5.3. Rhizosphere influence

Grass cover in the biobed has an important role on contaminants dissipation and in promoting microbial activities in the plant's rhizosphere environment. Urrutia et al. (2015) set up containers with a biomixture of peat, soil and oat husk in the laboratory, with and without grass cover, for the treatment of a mixture of atrazine, chlorpyrifos and isoproturon. After the increase of fungal biomass activity, it was noticed that the most efficient dissipation occurred due to the grass rhizosphere. Diez et al. (2017) obtained the same result, assessing atrazine, chlorpyrifos and iprodione dissipation, with increased degradation over the initial 30 cm of the substrate, reaching levels higher than 95% of degradation.

Campos et al. (2017) verified the influence of two strategies for biobeds optimization, the presence or absence of the rhizosphere and a bioaugmentation technique with *Arthrobacter* bacteria. The association of the two factors (presence of the grass rhizosphere and bioaugmentation) optimized the treatment of iprodione.

5.4. Microbial communities and bioaugmentation

When the biobed structure and composition is already evaluated for a specific country, bioaugmentation by fungi or bacteria can be a way to increase the biodegradation process, through the inoculation of microorganisms whose capacity to degrade pesticides is already known. Also, the technique may represent a way to improve degradation levels of highly recalcitrant pesticides, such as triazoles and neonicotinoids (Lizano-Fallas et al., 2017; Masís-Mora et al., 2019).

Tortella et al. (2013a) suggest that the microbial community in a biobed is negatively affected immediately after the addition of the pesticide, and it recovers after a process of adaptation. Saez et al. (2018) evaluated lindane degradation in a biobed inoculated with the fungus *Trametes versicolor* and with the bacteria *Streptomyces* sp., which are not antagonists. Degradation was monitored along the contamination and recontamination processes, where the rate of degradation decreased, but still reached a good level at the end of 66 days, due to the bioaugmentation techniques. Briceño et al. (2017) carried out tests with the same bacteria and concluded that the inoculum promoted an increase in the degradation rate for a mixture of chlorpyrifos and diazinon.

In Costa Rica, Rodríguez-Rodríguez et al. (2017) studied bioaugmentation with the ligninolytic fungus *T. versicolor* in a biobed composed of rice husk, composted vegetal residue and soil. In general, results showed that bioaugmentation did not increase degradation rates, but reduced the transformation products on the first stages of three carbamates treatment. Castro-Gutiérrez et al. (2016) identified a bacterial consortium (genus *Cupriavidus, Achromobacter* and *Pseudomonas*) that was able to degrade carbofuran, stating that microbial consortia could be better than isolated strains for bioremediation of recalcitrant compounds. Such bacterial consortium was applied and evaluated in a bioaugmentation process (Castro-Gutiérrez et al., 2018). In this case, there were no differences arising from the technique and the most influencing factor over microbial population shifts was the biomixture aging.

T. versicolor grown on rice husk was also tested on carbofuran degradation. It was possible to evaluate the bioaugmentation technique in a biobed composed of rice husk (pre-colonized by the fungi), compost and soil, and other biobed composed of rice rusk, peat and soil. Bioaugmentation only improved the removal of carbofuran in the peat-based biomixture (Madrigal-Zúñiga et al., 2016). Diez et al. (2016) evaluated the effect of inoculating in a biobed three white-rot fungi species (*Inonotus* sp., *Stereum* *hirsutum* and *T. versicolor*) immobilized in a pelletized support. *Stereum hirsutum* showed higher atrazine degradation results, after 60 days of incubation.

Tortella et al. (2010) performed the biostimulation technique by adding the NPK inorganic fertilizer (nitrogen, phosphorus, potassium) in different doses, in order to evaluate chlorpyrifos degradation. Results indicated that the dose that increased chlorpyrifos degradation rate was up to 0.5% of NPK for the first treatment days. Other doses modified the bacterial communities, showing less efficient results.

5.5. Degradation efficiency assessment

5.5.1. Chemical analysis

Chemical analysis is the approach that is commonly used to monitor pesticide degradation in biobeds. For this purpose, chemical methods of extraction and quantification of pesticides have been adapted, as biobeds substrates are complex matrices that cannot be analysed by the conventional methods developed for soil or water alone.

Rivero et al. (2016) evaluated a series of different analytical methods to determine chlorpyrifos, its main metabolites, and endosulfan in a laboratory scale biobed composed of bran, peat and soil, that was inoculated with the fungus *Abortiporus biennis* mycelium. The ultrasound-assisted extraction with ethyl acetate was considered as the best methodology, which achieved recoveries between 80% and 110% of the actual remaining pesticides in the sample, after 27 days of degradation. In Brazil, Vareli et al. (2018) reported an analytical method to determine mancozeb (dithiocarbamate) residues using gas chromatography-tandem mass spectrometry (GC-MS). This method reached recoveries between 89% and 96%.

Chilean researchers used High Performance Liquid Chromatography (HPLC) after extraction with acidified acetone, followed by a diode array detector, to evaluate residual concentrations of chlorpyrifos, atrazine, carbendanzim and diazinon in biobeds (Fernández-Alberti et al., 2012; Diez et al., 2013b; Briceño et al., 2017). In Costa Rica pesticide removal quantification has been done by ultra-high performance liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS), to evaluate carbofuran (Chin-Pampillo et al., 2015), carbofuran and oxytetracycline (Jiménez-Gamboa et al., 2018), chlorpyrifos co-applied with antibiotics (Castillo-González et al., 2017), oxytetracycline and herbicides (Cambronero-Heinrichs et al., 2018), among others.

Rodríguez-Castillo et al. (2018) used LC-MS/MS to measure the removal of imidacloprid and thiamethoxam in biobeds and soil. At the end of 228 days of treatment, removal rates identified by the LC-MS/MS were low. These results bring out the problem of high persistence of neonicotinoids in the substrate proposed by the authors, who suggested that additional methods must be evaluated in order to optimize neonicotinoids degradation.

Mineralization studies using radiolabeled compounds and liquid scintillation approaches, have being performed in Costa Rica to determine complete oxidation of pesticides, as described by Ruiz-Hidalgo et al. (2014) on evaluating carbofuran mineralization. Castillo-González et al. (2017) used this method for chlorpyrifos, Chin-Pampillo et al. (2016) during carbofuran and chlorpyrifos co-application, and Rodríguez-Castillo et al. (2018) used this method to evaluate imidacloprid mineralization, with mineralization half-lives for this highly recalcitrant product ranging from 3466 days in the biomixture to 8667 days in soil.

Gebler et al. (2015) used the fluorescein diacetate hydrolysis method (FDA) to evaluate the microbial activity of a traditional biobed contaminated by chlorpyrifos and ammonium - glufosinate, with some success in identifying contaminant decomposition trends. Tortella et al. (2013b), in Chile, also used the FDA method to monitor the hydrolytic activity of a traditional biomixture that was amended with the terpenes alpha-pinene, eucalyptol, and limonene, individually and as mixtures, to evaluate atrazine biodegradation. The FDA hydrolysis presented similar results to the obtained for the phenoloxidase activity, which was also evaluated in the experiment. Both activities suffered temporarily stimulation by the terpenes, indicating the enhancement effect on atrazine degradation. In Tortella et al. (2013a), enzymatic activity analysis was applied in order to evaluate atrazine impact on the microbial communities from a biobed. Phenoloxidase activity, acid and alkaline phosphatase activities, and dehydrogenase activity (DHA) showed that atrazine may inhibit or stimulate their patterns, but negative effects only happened initially, showing a recovery after some time of pesticide application.

5.5.2. Ecotoxicological assessment

Pesticides may exert negative effects on non-target organisms. Some sensitive species are used in ecotoxicity tests, according to well stablished methodologies, due to their high sensitivity to pollutants and changes in their environment. That sensitivity allows to estimate detoxification, from the response of these organisms to the contact with the substrate from a contaminated biobed. Therefore, ecotoxicity tests combined to chemical analysis results allow to evaluate the environmental safety of contaminated and aged substrates (Masin et al., 2018; Rodríguez-Rodríguez et al., 2018).

In Costa Rica Huete-Soto et al. (2017) performed ecotoxicity tests with the microcrustacean *Daphnia magna* and *Lactuca sativa* seed germination tests in elutriates from a biobed composed of coconut fiber, composted plant material and soil. The 115-day degradation of two groups of pesticides (herbicides/insecticides/fungicides, or insecticides/fungicides) and oxytetracycline was evaluated. Chemical analyses showed that the herbicides removal levels were higher, and there was no meaningful removal towards neonicotinoid insecticides and triazole fungicides. The biomixtures remained toxic for *D. magna* in all cases, with a significant decay only in the phytotoxicity (*L. sativa*), which relates to a higher herbicide removal. Also, Lizano-Fallas et al. (2017) performed the same tests to evaluate the degradation of a mixture composed of chlorpyrifos and three herbicides from the triazine group. For this case, ecotoxicity to *D. magna* reduced rapidly over 60 days of treatment, and no significant decrease was observed for *L. sativa* phytotoxicity, despite consistent herbicide elimination levels according to chemical analysis.

In Brazil, Carniel (2015) conducted chronic (reproduction) tests with collembolans (*Folsomia candida*), earthworms (*Eisenia andrei*) and enchytraeid (*Enchytraeus crypticus*), in order to determine the efficiency of a field-scale biobed (360 L capacity in plastic boxes), comparing the conventional biomixture with two subtropical soils (Utisol and Oxisol) for pesticide degradation. For that, the substrates received a mixture of mancozeb and chlorpyrifos, through a sequence of applications. All treatments presented ecotoxicity at 90 and 270 d after residues disposal, and both the biomixture and the Utisol showed detoxification after 420 d. However, in Oxisol, 420 d were not sufficient to eliminate ecotoxicity, especially to collembolans.

The worst-case scenario of an accidental spillage was also evaluated by Carniel (2015), discarding a single dose (1 L) of a chlorpyrifos based insecticide in the biobed. The same test organisms were used in chronic tests. Significant negative effects on earthworms and enchytraeids reproduction were observed until 270 d, but no longer observed after 420 d of aging. Collembolans were the most sensitive organisms at this case too, as negative effects were observed 420 d after contamination. The author indicated the potential of reproduction tests with collembolans to be used for monitoring biobed's efficiency.

In Gebler et al. (2015), the use of earthworms to evaluate glufosinate-ammonium and chlorpyrifos detoxification during several applications in a traditional biomixture, showed high mortality and avoidance rates during the initial applications. Over time and after more pesticide applications, the behavioural response changed, indicating a preference for the contaminated biomixture. Detoxification rates of mancozeb and chlorpyrifos were also evaluated, using *Eisenia fetida, E. crypticus* and *F. candida* reproduction tests. Collembolans showed the most severe ecotoxicity effects, which decreased after 2 months of the last application.

As the sensitivity of the test organisms depends on the pesticides involved, data concerning pesticides and ecotoxicological parameters for the test species, can help to determine the most suitable species for biomonitoring purpose. Table 2 summarizes the main ecotoxicity studies involving biobeds in LA.

6. Challenges for biobeds implementation in LA

Considering that Guatemala is the only LA country where biobed is already applied as an official technology all over the country, there is a discussion about the moment that other countries in the region could reach this point. The main difference between those two cases was brought up earlier in this review. In Guatemala there was a private initiative focused on exporting products, associated with the pesticide industry, to train the farmers and apply their biobed system (Biodep) officially on the field (Agrequima, 2019). In other countries the main initiatives to study biobed come from public institutions such as universities and governmental research institutes. Those studies have considerable advances in biobed's topics that would allow this technology to be installed on the field. However, it is still necessary, in all cases, to make it an official technology.

Guatemala has defined not only the set of laws that state the use, management and disposal of pesticide residues, but also provided official guidance for the correct disposal of residues. Information was obtained from research teams or through agents from governmental and international institutions from Argentina, Brazil, Chile, Costa Rica, Honduras, Paraguay, and Uruguay. With the exception of Argentina, which does not have specific pesticide use legislation, and Chile, which does not have specific pesticide waste disposal legislation, all the other countries have pesticide-related legislation. However, one of the major findings is that none of them has official guidance, suggestion or clarification on how to properly dispose of pesticide residues, with the exception of pesticides that may be classified as Hazardous Organic Products (POPs) such as organochlorines.

Moreover, as in Guatemala, where the private initiative was responsible for the introduction and legal standardization of biobeds, only Argentina and Paraguay declared the interest from industry and other sectors in the subject, with advances towards methodological standardization or regularization by the government. The other countries consulted found no sign of explicit support from the private sector for introducing the system as a solution to the pesticide residues pollution problem.

Nevertheless, in all countries there are different levels of movement from their governments, demonstrating an interest in the use of pesticide waste disposal systems, including the Biobed system. The approach is mainly through Good Agricultural Practice (GAP) programs, or through technical manuals from government research companies, such as in Brazil (Gebler, 2015, 2017), Chile (Diez et al., 2013c), Costa Rica (MAG, 2019) and Uruguay (Digegra, 2014). These documents and all the studies carried out by the research groups aim to make the biobed technology cost-effective and, overall, applicable according to their countries' legislation. With that, the biobed application would support all the requirements of environmental inspection in complying with legal demands and, at the same time, guarantees technical and legal security to the rural producers.

GAP programs are tools for risk management developed specifically for each farm, with its application being monitored by surveillance authorities (IICA, 2017). Their application is focused on environmental protection, besides worker, animal and food safety. It involves every production stages, the use of pesticides, harvesting, product transportation and residue management (Gebler, 2015; IICA, 2017). In this respect, biobeds have been included in GAP programs in Costa Rica, with a user manual aimed at farmers from a specific region (CICA, 2015). Similarly, the research groups in Chile and Brazil published user's guides for constructing and operating biobeds (Diez et al., 2013c; Gebler, 2017). Such input is necessary to spread the used of biobeds, not only in LA, but worldwide.

Table 2

Test species, endpoints and pesticides in ecotoxicity studies in LA involving biobeds.

Bioindicator	Endpoint	Pesticide(s)	Reference
Daphnia magna/ Lactuca sativa	Immobilization (acute test)/Seed germination	Several Mixtures:atrazine, amethrin, linuron, imidacloprid, thiamethoxam, carbendazim, metalaxyl, tebuconazole, triadimenol	Huete-Soto et al. (2017)
D. magna/L. sativa	Immobilization (acute test)/Seed germination	Mixture: atrazine, terbuthylazine, terbutryn, chlorpyrifos	Lizano-Fallas et al. (2017)
D. magna	Immobilization (acute test)	Mixture: aldicarb, carbofuran, methiocarb, methomyl	Rodríguez-Rodríguez et al. (2017)
D. magna	Immobilization (acute test)	Mixture: metalaxil, epoxiconazole, fenbuconazole, carbendanzin, tebuconazole, triadimenol, edifenphos	Murillo-Zamora et al. (2017)
D. magna	Immobilization (acute test)	Mixture: carbofuran and chlorpyrifos	Chin-Pampillo et al. (2016)
D.magna/Oreochromis aureus	Semi-static reproduction (chronic test)/Physiological alterations	Carbofuran	Ruiz-Hidalgo et al. (2016b)
Eisenia fetida	Survival (acute test) and Reproduction (chronic test)	Glyphosate	Masin et al. (2018)
Folsomiacandida/Enchytraeus crypticus/E. fetida/L. sativa	Reproduction (chronic test)/ Avoidance test/Seed germination	Mixtures: glufosinate-ammonium and chlorpyrifos/mancozeb and chlorpyrifos	Gebler et al. (2015)
F. candida/E. crypticus/Eisenia andrei	Reproduction (chronic test)	Mixture: mancozeb and chlorpyrifos	Carniel (2015)

7. Future perspectives

It is noticed that one of the main technical issues that guarantees an efficient application of the biobed technology in LA is the assessment of new substrates. Considering the positive results of some experiments presented in this paper, the original substrates (wheat straw and peat) can be substituted by equivalent materials that are different according to each country, but are considered to be as efficient as the original ones. It is important that, prior to the actual application of this technology by the farmer, these new local substrates have their efficiency evaluated in the laboratory, on a pilot scale, and also on the field, being exposed to natural variations from the weather.

While evaluating new organic compounds, it should also be considered the high persistence of some neonicotinoids, triazoles, and organochlorine compounds. The development of complexes biomixtures, pre or post-treatment of residues, and strategies that are able to improve degradation levels of these pesticides need more studies. This is an important gap pointed by authors, in regards to the efficiency of new-studied biomixtures (Masís-Mora et al., 2019; Huete-Soto et al., 2017; Rivero et al., 2016).

Structural design adaptations should be considered to ensure the bioreactor's efficiency in different climatic regions of each country, avoiding soil and groundwater contamination. Studies involving the biobed shelf life and necessary time for efficient degradation of every pesticide molecule, especially highly recalcitrant ones, are important considering each biobed model. Equally necessary are the assessments of the minimum degradation time that guarantees environmental protection when the old substrate is removed and disposed on a composting or vermicomposting system. Considering the current practices of pesticide wastes disposal in agricultural LA areas, the biobed system can be considered as an instrument for the water bodies protection (surface and groundwater) within each river basis, as used in England (Fogg et al., 2003; Fogg et al., 2004).

Taking into account the efficiency of this system on degrading several pesticide residues, it is recommended additional Latin-American studies involving the use of biobeds for the treatment of agroindustrial effluents, as it was proposed in Greece, where the biobed system was evaluated in optimizing the depuration of the pesticide-contaminated wastewaters from the fruit-packaging industry (Karas et al., 2015; Karas et al., 2016). First, the authors proved that modified biobeds generated higher pesticide dissipation capacities than the industrial treatment methods based on anaerobic and aerobic sewage sludge. After that, a biobed system associated to bioaugmentation strategies was proved to optimize the depuration of the pesticide-contaminated wastewaters. Further research should also focus on the evaluation of biobeds during pesticide application cycles for specific crops, to determine the pesticide volume they can receive during a crop cycle.

Research has advanced and made available technical information about the Biobed System. It showed that the system is safer than the solutions used for the final disposal of pesticides currently applied in LA, which are generally the simple disposal in soil of rural properties. It is necessary to involve the public and private institutions (industry, commerce, farmers and environmental agencies), in order to discuss and promote the biobed official implementation for legal and environmental safety purposes, such as in European countries where this technology is usually recognized and adopted. Because biobed is a simplified and low-cost system, it would represent a quick and concrete solution to the problems of inadequate handling and disposal of pesticide residues that are currently causing punctual pesticide contamination in LA.

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