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Enhancing pesticide degradation using indigenous microorganisms isolated under high pesticide load in bioremediation systems with vermicomposts

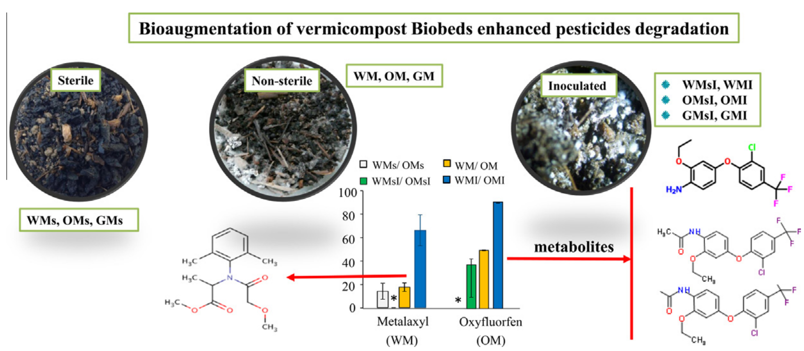
Jean Manuel Castillo Diaz [a], Laura Delgado-Moreno [a], Rafael Núñez [b], Rogelio Nogales [a], Esperanza Romero [a,*]

[a] Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (EEZ-CSIC), Department of Environmental Protection, C/Profesor Albareda, 1, 18008 Granada, Spain
[b] Estación Experimental del Zaidín, Consejo Superior de Investigaciones Científicas (EEZ-CSIC), Scientific Instrumentation Service, C/Profesor Albareda, 1, 18008 Granada, Spain

HIGHLIGHTS

- Vermicomposts as an alternative to peat in biobed bioremediation systems (BBSs).
- Vermicompost BBSs as source of bacteria and fungi pesticide degraders.
- The best fungus growth was in the BBSs with vermicompost from olive mill wastes.
- Enhanced biodegradation of tebuconazole, oxyfluorfen and metalaxyl by bioaugmentation.
- Four metabolites of oxyfluorfen and one of metalaxyl determined by GC/MS.

GRAPHICAL ABSTRACT



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ABSTRACT

In biobed bioremediation systems (BBSs) with vermicomposts exposed to a high load of pesticides, 6 bacteria and 4 fungus strains were isolated, identified, and investigated to enhance the removal of pesticides. Three different mixtures of BBSs composed of vermicomposts made from greenhouse (GM), olive-mill (OM) and winery (WM) wastes were contaminated, inoculated, and incubated for one month (GMI, OMI and WMI). The inoculums maintenance was evaluated by DGGE and Q-PCR. Pesticides were monitored by HPLC-DAD. The highest bacterial and fungal abundance was observed in WMI and OMI respectively. In WMI, the consortia improved the removal of tebuconazole, metalaxyl, and oxyfluorfen by 1.6-, 3.8-, and 7.7-fold, respectively. The dissipation of oxyfluorfen was also accelerated in OMI, with less than 30% remaining after 30 d. One metabolite for metalaxyl and 4 for oxyfluorfen were identified by GC–MS. The isolates could be suitable to improve the efficiency of bioremediation systems.

1. Introduction

Climate change requires urgent attention to water conservation worldwide and the development of affordable technologies for wastewater depuration. The European Union has established more stringent requirements concerning pollution of surface and groundwater (Directives 2000/60/EC, 2006/118/EC). Among pollutants, pesticides are organic compounds frequently detected in water due to their diverse properties, activities, and applications, which contribute to their widespread presence in the environment, threatening ecosystems and human health (Campo et al., 2013).

* Corresponding author.
E-mail address: esperanza.romero@eez.csic.es (E. Romero).

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Disposal of pesticide wastewater must be undertaken properly to prevent the deterioration of water quality (Castillo et al., 2008). However, most current methods used for pesticide removal from water have containment problems, high costs, and ineffectiveness. Biobed bioremediation systems (BBSs) constitute a viable option for on-farm use and have also been used successfully to treat polluted complex agricultural and industrial wastewater (Castillo et al., 2008; Sniegowski et al., 2011; Sniegowski and Springael, 2015; Omirou et al., 2012; Karanasios et al., 2012).

The BBSs composed of biomixtures of topsoil and organic materials (most often peat and straw) have been widely investigated in Europe for the removal of pesticides from wastewater (Castillo et al., 2008). However, when the peat or straw are unavailable, as in Spain, this technique requires the use of other organic substrates. The use of composts or vermicomposts from agricultural organic wastes can be an optimum alternative since these substrates affect the sorption and degradation of the pesticides and stimulate microbial activity, reducing the adverse effect in the environment (Delgado-Moreno and Peña, 2009; Castillo et al., 2016). Vermicomposts possess greater microbial biodiversity than do composts and boost the metabolic capacities of soil microorganisms to degrade organic pollutants (Vivas et al., 2009; Castillo et al., 2016). Previous vermicomposting studies reveal that vermicomposts have a higher microbial functional diversity which can tolerate pesticide and thus could possibly be useful for managing pesticide pollution in agriculture (Castillo et al., 2013). Furthermore, some indigenous microorganisms such as *Aspergillus niger* and *Fusarium* species from winery vermicomposting can metabolise 3,4-dichloroaniline (Castillo et al., 2014). The use of vermicomposts in biobed systems can also promote a sustainable agriculture development.

To facilitate the breakdown of more persistent pesticides or to minimize their impact on microorganisms, bioremediation techniques often introduce organic wastes and/or specialized strains with catabolic capabilities against targeted pollutants (Chowdhury et al., 2008; Pigeon et al., 2006). The use of genetically modified strains may constitute an efficient strategy to degrade pesticides (Nikel et al., 2014). However, legal restrictions make this solution unfeasible over the short term. By contrast, native organisms exposed to pesticides can acquire the capability of degrading these substances (Castillo et al., 2016; Sniegowski et al., 2011). In fact, this technique has been designed for the remediation of environmental pollution with pesticides (Barreiros et al., 2012; Lima et al., 2009). The use of mixed bacterial consortia or pesticide-primed materials has been shown to be more advantageous in comparison to pure cultures for improving the degradation and mineralization capacity of pesticides in BBSs (Sniegowski and Springael, 2015). In addition, BBSs can be an optimal microcosm to develop adapted microorganisms able to enhance the metabolisation of pesticide residues from wastewaters (Dunon et al., 2013). However, the bioaugmentation technique for a multiple biodegradation of pesticides in wastewaters at high concentrations, as occurs under real conditions, remains poorly understood (Sniegowski and Springael, 2015). In fact, only scant information is available on the potential of bacterial strains or mixed microbial consortia from biobeds to improve the performance of these systems (Verhagen et al., 2013).

The BBSs composed of vermicomposts from agroindustrial wastes represent a more sustainable and less expensive alternative to retain and degrade pesticides than do the traditional biobed systems based on soil, peat, and straw mixtures, especially in countries where peat is scarce, such as Spain. The present work investigates the reincorporation of native microorganisms in biobed bioremediation systems composed of vermicomposts of agro-industrial wastes to reduce the degradation time of pesticide residues with the aim of enhancing the functionality of these low-cost remediation systems for wastewater depuration. Three vermicomposts made from organic wastes of olive-oil and wine production as well as from greenhouse tomato crops were assayed.

The pesticides selected are widely used in olive, vineyard and greenhouse crops where these organic agroindustrial wastes are abundant, low cost and easily available for the development of these types of biobed bioremediation systems (BBSs). Pesticides were applied at high dosages to check the robustness of these systems. In the inoculated BBSs, molecular techniques were applied to evaluate: (l) the prevalence and abundance of the consortium inoculated, (2) the disappearance of the pesticides and the presence of metabolites. This study offers a new approach with special focus on promoting and improving low-cost bioremediation systems.

2. Materials and methods

2.1. Soils, vermicomposts, and biomixtures

The agricultural soil (0–25 cm) from a farm in Granada, south-eastern Spain (S2, 0436148-4211209, zone 30S) was sieved at 4 mm. This soil is a silty-clay loam containing 39.0% clay, 47.9% silt, 13.0% sand, and 39.1% carbonates. The organic wastes selected to make the vermicomposts came from the olive-oil and wine industries or refused tomatoes from greenhouses. The first vermicompost was made by vermicomposting a mixture of wet olive cake (O) or *alperujo* supplied by Romeroliva, S.L. (Deifontes, Spain), which is the main waste from two-phase olive-oil extraction, and cattle manure at a ratio of 4:1 (d.w:d.w). The winery vermicompost (W) was made by mixing grapevine shoots and biosolid vinasse at 4:1 (d.w:d.w) ratio. Mixed plants, tomato plants, and damaged tomato-fruit wastes, which are three of the most abundant greenhouse vegetable wastes in Andalusia (southern Spain), were collected from an organic waste-treatment plant located in El Egido, Almería (Spain), mixed with cow dung at at a ratio of 4:1 (d.w:d.w) to make the vermicomposts from greenhouse wastes (G). The vermicomposting was carried out with the earthworm *Eisenia fetida* for 6 months on a pilot scale and 2 months more for maturation and drying. Further information is described elsewhere (Castillo et al., 2013; Fernández-Gómez et al., 2010).

Three BBSs were performed by using three biomixtures. Olive-pruning, grapevine-pruning and tomato-plant debris were dried at 25 °C, ground to <4 mm size, and used as bulking materials in the biomixtures. The first biomixture (OM) contained the soil, vermicompost from the olive mill by-product, and the olive-pruning debris at a ratio of 1:2:1 (v:v:v). The second biomixture (WM) contained the soil, vermicompost from grapevine shoots, and vineyard-pruning debris at a ratio of 1:2:1 (v:v:v). The third biomixture (GM) contained the soil, vermicompost from greenhouse wastes and tomato-plants wastes at a ratio of 1:2:1 (v:v:v). The main physical–chemical properties of these BBSs are given in Table 1.

The biomixtures in flasks plugged with cotton were sterilized using an autoclave (OMs, WMs, and GMs). The process was repeated 3 consecutive times at 121 °C for 20 min. The complete sterilization was checked by plate counting using LB agar medium.

2.2. Chemicals and culture media

Imidacloprid (I) (99%), metalaxyl (M) (99.5%), tebuconazole (T) (99.6%), oxyfluorfen (O) (98%), dimethoate (Dt) (99.6%), and