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Identification of microbial species present in a pesticide dissipation process in biobed systems using typical substrates from southeastern Mexico as a biomixture at a laboratory scale

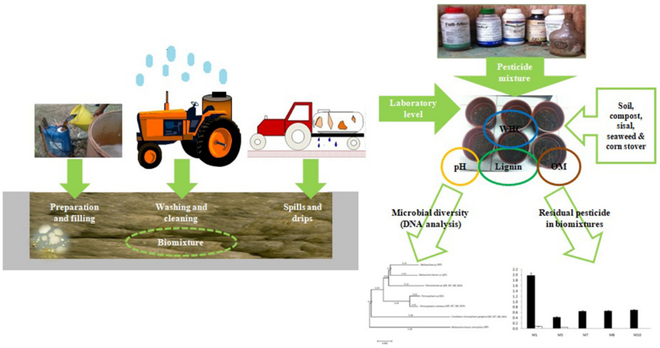
Virgilio R. Góngora-Echeverría [a],*, Carlos Quintal-Franco [a], María Leticia Arena-Ortiz [b], Germán Giácoman-Vallejos [a], Carmen Ponce-Caballero [a]

[a] Facultad de Ingeniería, Universidad Autónoma de Yucatán; Av. Industrias no Contaminantes por Anillo Periférico Norte s/n, Apdo, Postal 150 Cordemex, Cd., Mérida, Yucatán, Mexico
[b] Laboratorio de Estudios Ecogenómicos, Unidad de Ciencias y Tecnología de la UNAM en Yucatán, Parque Científico y Tecnológico de Yucatán, Carretera Sierra Papacal-Chuburná Puerto Km 5.1, 97302 Mérida, Yucatán, Mexico

HIGHLIGHTS

- Microbial diversity in biobed systems after pesticide dissipation was studied.
- >99% of the initial concentration of pesticides was dissipated at 41 days.
- Species of archaea (23), bacteria (598) & fungi (64) were identified in biomixtures.
- Biomixture type was significant on residual pesticides and microbial diversity.
- Microbial diversity and richness were significant on residual pesticides detected.

GRAPHICAL ABSTRACT



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ABSTRACT

Biobed systems are an important option to control point pollution in agricultural areas. Substrates used and microbial diversity present in a biomixture perform an essential function in pesticide dissipation. In this study, the effects of soil (50% of volume/volume [V/V] proportion for all biomixtures) and four soil-based biomixtures (miniaturized biobeds; addition of novel substrates from southeastern Mexico) on dissipation of high concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, carbofuran, diazinon, and glyphosate and on microbial diversity in biomixtures were evaluated. Small residual amounts of all pesticides at 20 (<2%) and 41 (<1%) days were observed; however, the lowest efficiency rates were observed in soil. Glyphosate was the only pesticide that completely dissipated in soil and biomixtures. Archaea, bacteria, and fungi were identified in biobeds, with bacteria being the most diverse microorganisms according to the identified species. The presence of white-rot fungi (normally related to pesticide degradation in biomixtures) was observed. Effects of the pesticide type and of biomixtures on pesticide dissipation were significant ($P < 0.05$); however, only the effect of biomixtures on microbial diversity was significant ($P < 0.05$); microbial diversity and richness had a significant effect on the residual amount of pesticides ($P < 0.05$). Microbial diversity in terms of phyla was directly related to physicochemical parameters such as organic matter, lignin, water-holding capacity, and pH of soil and biomixtures.

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* Corresponding author.
E-mail address: cponce@correo.uady.mx (V.R. Góngora-Echeverría).

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1. Introduction

Biological beds or biobeds are a technology whose purpose is to contain the drip and effluents contaminated with pesticides, thus providing maximum adsorption and optimal conditions for the degradation of pesticides by the microbial activity present in the biomixture (Torstensson and Castillo, 1997). In biobeds, a biomixture is the most important component, and its correct composition is a prerequisite for the successful degradation of pesticides in contaminated water flows. The efficacy of biobeds is based on their ability to retain and degrade pesticides via the microbial activity (Karanasios et al., 2010a; 2010b).

For example, the use of bacteria for degradation and detoxification of numerous toxic chemicals is an important approach to consider for decontamination of polluted sites (Mervat, 2009). Nevertheless, it is known that degradation processes in biobed systems respond to the microbial activity present, as is the case for often-mentioned white-rot fungi during degradation of pesticides such as atrazine (Castillo et al., 2008). Some studies on biobeds or related research on microbial diversity normally are conducted using a straw-based biomixture as in the original biobed. Coppola et al. (2011) reported no significant changes in the composition of the microbial community at the end of a pesticide degradation process. Tortella et al. (2014) established that the microbial community structure (bacteria and fungi) remained relatively stable over time when high diazinon doses were applied to the biomixture being tested. These findings were taken into account to conduct the present study; additionally, it is not known for certain what other types of degrading microorganisms are present in the systems in operation. Several microorganisms able to utilize pesticides as a source of energy have been isolated. Some fungi such as *Trametes hirsutus*, *Phanerochaete chrysosporium*, *Phanerochaete sordida*, and *Cyathus bulleri* can degrade lindane and other pesticides (Singh and Kuhad, 1999, 2000; Singh et al., 1999); white-rote fungi belonging to the genus *Phlebia* (*P. acanthocystis*, *P. brevispora*, and *P. aurea*) participating in aldrin and dieldrin degradation have been reported (Xiao et al., 2011). Nonetheless, most evidence suggests that soil bacteria are the principal components responsible for enhanced biodegradation (Walker and Roberts, 1993). Bacteria such as *Enterobacter cloacae*, *Bacillus cereus*, *Bacillus anthracis*, *Pseudomonas aeruginosa*, *Pseudomonas balearica*, *Pseudomonas indica*, *Pseudomonas otitidis*, *Ochrobactrum intermedium*, and *Providencia vermicola* have proven to be capable of degrading atrazine in soil (El-Bestawy et al., 2013). Other bacteria such as *Achromobacter xylosoxidans* and *Ochrobactrum* sp. have been shown to degrade chlorpyrifos (Akbar and Sultan, 2016), and *Streptomyces* sp. was reported to degrade diazinon (Briceño et al., 2016).

Although bacterial activity is important for pesticide degradation, materials where microorganisms are deposited and growing are crucial too. Correct materials favor the immobilization process to carry out the biodegradation of pesticides. Materials such as chitosan, sawdust, straw, charcoal, plant fibers, corncob, bagasse, rice, husks of sunflower seeds, diatomite, and mycelium have proven to be good support materials in a process of biodegradation of pesticides (Dzionek et al., 2016).

Before we conducted the present research, Góngora-Echeverría et al. (2017) showed high pesticide dissipation rates and that a biomixture composed of soil–corn stover in the ratio 1:1 (V/V) is the most effective; additionally, it was proved that the biomixture and its physicochemical characteristics (lignin, pH, carbon nitrogen ratio [C/N], and water-holding capacity [WHC]) have significant effects on pesticide dissipation during testing of 11 soil-based biomixtures and soil; however, microbial diversity was not considered, and its relation with residual amounts of pesticides and with biomixtures was not studied.

Thus, the aim of this study was to identify the microbial diversity (archaea, bacteria, and fungi) during dissipation of five pesticides (2,4-D, atrazine, carbofuran, diazinon, and glyphosate) in miniaturized biobed systems at a laboratory scale involving local materials (agricultural soil, compost, sisal, corn stover, and seaweed) from southeastern Mexico as biomixture components. This is because they are available in different agricultural areas in Yucatán State. Accordingly, the relations of the microbial taxa with a biomixture and its physicochemical parameters were studied.

2. Materials and methods

2.1. Substrates and biobed implementation

Previously, for microorganism identification in biobeds after a pesticide degradation process, these systems were implemented at a laboratory scale. Substrates used in biobeds were agricultural soil, sisal pulp, and vegetable compost, corn stover and seaweed in different proportions (soil always constituted 50% in all biobeds). According to the study (Góngora-Echeverría et al., 2017) where these substrates were tested, the main physicochemical characteristics were as follows: organic matter (OM) = 35.51%, total organic carbon (OC) = 20.60%, water holding capacity (WHC) = 154%, nitrogen (N) = 1.71%, carbon/nitrogen ratio (C/N) = 12.05%, and pH = 7.25 for soil; OM = 44.00%, OC = 25.52%, lignin = 20.84%, WHC = 285%, N = 0.83%, C/N = 30.60%, and pH = 7.25 for compost; OM = 72.48%, OC = 42.04%, lignin = 11.75%, WHC = 715%, N = 0.83%, C/N = 53.86%, and pH = 8.34 for sisal; OM = 87.89%, OC = 50.98%, lignin = 6.46%, WHC = 1270%, N = 0.75%, C/N = 73.49% and pH = 7.85 for corn stover; finally, OM = 61.78%, OC = 35.83%, lignin = 7.13%, WHC = 530%, N = 2.17%, C/N = 18.15% and pH = 7.48 for seaweed. Substrates were selected because of their easy availability on Yucatan Peninsula, Mexico. All the substrates were dried at environmental temperature for 24 h. Vegetable substrates were chopped with an electric device and soil was sieved through 0.5 mm sieve.

All individual biomixtures were physicochemically characterized (Table 1). OM and OC were studied by wet digestion and colorimetry (Okalebo et al., 1993), total nitrogen (N) by the Kjeldahl method (Okalebo et al., 1993), lignin by Ankon's method (Van Soest and Wine, 1968), pH according to the Oficial Mexican Standard NOM-021-SEMARNAT-2000 (2002) (soil and compost) and the Oficial Mexican Standard NMX-F-317-S-1978 (1978) (other substrates), %WHC according to the method of biotechnology-IPN (Juárez et al., 2009), and texture by the method of Bouyocus (Estrada Medina and Álvarez Rivera, 2011) were determined.

A total of 11 miniaturized biobeds with mixture of substrates (biomixture) were implemented during 41 days, and the most effective biomixtures after pesticide degradation experiment were selected plus soil-like control (Table 1). The systems were incubated indoors at a temperature and humidity of 29.6 °C ± 2 °C and 58.5% ± 5.8%, respectively; illuminance indoors was 74.139 °C ± 162.821 lmm^{-2}.

Table 1
Composition, proportion (V/V) and physicochemical characteristics of selected biomixtures.

Biomixture	V/V[a] %	OM[b] %	OC[c] %	Lig[d] %	WHC[e] %	N[f] %	(C/N)[g]	pH[h]
M1[I]	1	35.51	20.60	ND	154	1.71	12.05	7.25
M5[II]	1:1	40.05	23.23	0.56	251	1.63	14.28	7.63
M7[III]	2:1:1	30.34	17.60	3.99	210	1.19	14.74	7.65
M8[IV]	2:1:1	31.02	18.00	4.00	223	1.16	15.48	7.67
M10[V]	2:1:1	36.24	21.02	1.59	275	1.32	15.95	7.63

[a] Volume-volume proportion.
[b] Organic matter.
[c] Organic carbon.
[d] Lignin.
[e] Water holding capacity.
[f] Total nitrogen.
[g] Carbon nitrogen ratio.
[h] Hydrogen potential.
[I] Soil.
[II] Soil-corn stover.
[III] Soil-compost-seaweed.
[IV] Soil-compost-corn stover.
[V] Soil-sisal-corn stover.