Ecotoxicology and Environmental Safety 156 (2018) 97–105

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Lindane dissipation in a biomixture: Effect of soil properties and bioaugmentation

Juliana M. Saez[a], Ana L. Bigliardo[a], Enzo E. Raimondo[a], Gabriela E. Briceño[b,c], Marta A. Polti[a,d], Claudia S. Benimeli[a,e,*]

[a] Planta Piloto de Procesos Industriales Microbiológicos (PROIMI-CONICET), Avenida Belgrano y Pasaje Caseros, Tucumán 4000, Argentina
[b] Scientific and Technological Bioresource Nucleous (BIOREN-UFRO), Universidad de La Frontera, Av. Francisco Salazar 01145, Temuco 4780000, Chile
[c] Centro de Excelencia en Investigación Biotecnológica Aplicada al Medio Ambiente (CIBAMA-UFRO), Facultad de Ingeniería y Ciencias, Universidad de La Frontera, Av. Francisco Salazar 01145, Temuco 4780000, Chile
[d] Facultad de Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán (UNT), Miguel Lillo 205, Tucumán 4000, Argentina
[e] Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Catamarca, Av. Belgrano 300, Catamarca 4700, Argentina

ARTICLE INFO

Keywords:
Biomixture
Pesticides
Bioaugmentation
Biopurification system
Actinobacteria
Fungi

ABSTRACT

The biomixture is the major constituent of a biopurification system and one of the most important factors in its efficiency; hence the selection of the components is crucial to ensure the efficient pesticides removal. Besides, bioaugmentation is an interesting approach for the optimization of these systems.

A mixed culture of the fungus *Trametes versicolor* SGNG1 and the actinobacteria *Streptomyces* sp. A2, A5, A11, and M7, was designed to inoculate the biomixtures, based on previously demonstrated ligninolytic and pesticide-degrading activities and the absence of antagonism among the strains. The presence of lindane and/or the inoculum in the biomixtures had no significant effect on the development of culturable microorganisms regardless the soil type. The consortium improved lindane dissipation achieving 81–87% of removal at 66 d of incubation in the different biomixtures, decreasing lindane half-life to an average of 24 d, i.e. 6-fold less than $t_{1/2}$ of lindane in soils. However, after recontamination, only the bioaugmented biomixture of silty loam soil enhanced lindane dissipation and decreased the $t_{1/2}$ compared to non-bioaugmented. The biomixture formulated with silty loam soil, sugarcane bagasse, and peat, inoculated with a fungal-actinobacterial consortium, could be appropriate for the treatment of agroindustrial effluents contaminated with organochlorine pesticides in biopurification systems.

1. Introduction

Pesticides are among the most employed organic compounds worldwide and play an important role in modern agriculture and food production. However, their inadequate management can lead to contamination of soil, surface, and groundwater (Castillo and Torstensson, 2007; Chin-Pampillo et al., 2015).

Environmental pesticide contamination can occur through diffuse sources or point sources. Point source contamination by pesticides derives from improper handling or leaks of the spraying liquid, remnants, and washes of the spraying equipment, and is considered as one of the main causes of pesticide contamination in water and soils (Campos et al., 2017).

In the last decades, point source pesticides pollution has been rigorously addressed, through the evaluation and implementation bioprophylaxis protocols, whose objectives are to reduce or avoid point source pollution. For this purpose, biopurification systems or biobeds are among the most promising technologies. These systems initially developed in Sweden in the '90s, consist of a simple, ecological and cost-effective technology construction designed to retain and degrade pesticides. It consists of three main components: a clay layer at the bottom; a biomixture; and a grass layer that covers the surface, all arranged in an excavation in the soil at 60 cm-depth (Castillo et al., 2008). The biomixture represents the biologically active part of a biopurification system, where the processes of adsorption and degradation of pesticides take place. It is composed of a lignocellulosic substrate (originally straw), a humic rich component (peat or compost) and soil (Castro-Gutiérrez et al., 2017). Each component plays an important role in the dissipation of pesticides. The lignocellulosic substrate promotes the growth of ligninolytic microorganisms and the production of extracellular ligninolytic enzymes as peroxidases and phenol-oxidases; peat or compost provides high water retention and sorption capacity of

* Corresponding author at: PROIMI-CONICET, Avenida Belgrano y Pasaje Caseros, Tucumán 4000, Argentina.
E-mail address: cbenimeli@yahoo.com.ar (C.S. Benimeli).

https://doi.org/10.1016/j.ecoenv.2018.03.011
Received 1 November 2017; Received in revised form 15 February 2018; Accepted 5 March 2018
Available online 20 March 2018

pesticides, while the soil is an important source of bacteria and fungi which can degrade the pesticides (Castro-Gutiérrez et al., 2017; Chin-Pampillo et al., 2015).

Agricultural and forestry residues generated as lignocellulosic wastes increase every year the environmental pollution. However, these natural compounds can be converted to several value-added products (Elgueta et al., 2016). In this sense, the design of a biopurification system should be adapted to every region; hence the composition of the biomixtures will depend on the availability of agricultural by-products of low commercial value, and the physicochemical parameters of local soils, such as pH, organic matter content, moisture, temperature, among others (Góngora-Echeverría et al., 2017; Ruiz-Hidalgo et al., 2015). Intensive researches have been carried out in order to adapt the systems to the local conditions, availabilities, and needs, obtaining good performances even when the pesticides were added in successive applications and high concentrations (Chin-Pampillo et al., 2015; Góngora-Echeverría et al., 2017; Ruiz-Hidalgo et al., 2015).

The bioaugmentation of biopurification systems with pesticide-degrading microorganisms represents a very interesting approach in the design and optimization of these systems (Ruiz-Hidalgo et al., 2016). Among a variety of microorganisms known to degrade or mineralize pesticides, actinobacteria, especially those belonging to the *Streptomyces* genus, stand out as they have a great capacity to degrade various pesticides such as lindane, chlordane, methoxychlor, chlorpyrifos, diuron, diazinon and pentachlorophenol (Alvarez et al., 2017; Benimeli et al., 2008; Briceño et al., 2016, 2012; Fuentes et al., 2016, 2014). On the other hand, given that a half of the biomixture consists of lignocellulosic substrates, the bioaugmentation with ligninolytic fungi is of particular interest to potentially increase the degrading capacity of the biomixture. Also, ligninolytic fungi such, as white rot fungi, are recognized for their ability to transform a wide range of organic pollutants, including pesticides (Camacho-Morales et al., 2017; Purnomo et al., 2017; Ruiz-Hidalgo et al., 2016; Tortella et al., 2015).

In this regard, several studies have evaluated the bioaugmentation of biopurification systems with ligninolytic fungi obtaining satisfactory results (Elgueta et al., 2016; Madrigal-Zúñiga et al., 2016; Ruiz-Hidalgo et al., 2016). Moreover, Verhagen et al. (2013) demonstrated that bioaugmentation of a biopurification system with a pesticide-degrading enriched mixed culture showed an improvement in the removal of chloropropham compared to the systems inoculated with a single pesticide-degrading strain. Only a few studies have evaluated the bioaugmentation of biomixtures with either single or mixed bacterial cultures (Briceño et al., 2017; Campos et al., 2017; Karas et al., 2016), but none, to our knowledge, have explored the use of a fungal-actinobacterial mixed culture for the bioaugmentation of biopurification biomixtures.

Lindane is an organochlorine pesticide, primarily used as an insecticide and fumigant against a wide range of soil-dwelling and phytophagous insects. Although the use of lindane has been banned or severely restricted in at least 52 countries (Madaj et al., 2017; Ministerio de Salud, 2016), some developing countries are still using it for economic reasons. Therefore, it was chosen as model pesticide because due to its widely utilization in the past and its persistence in the environment, it is still being found in different environmental compartments such as water courses, sediments, soils, animal and plant tissues (Li et al., 2015; Villaamil Lepori et al., 2013; Yadav et al., 2015); thus it is imperative to develop methods to minimize, or if possible avoid, its release to the environment.

In view of the above, the aim of this study was to evaluate the performance of biomixtures formulated with an agro-industrial by-product derived from a local industry and different soil textures, and the effect of the bioaugmentation with a consortium of actinobacteria and fungi, on their lindane removal capacity.

2. Materials and methods

2.1. Microorganisms

The actinobacteria *Streptomyces* sp. A2, A5, A11, and M7 were used in this study. These strains were isolated from soils and sediments contaminated with organochlorine pesticides (Benimeli et al., 2003; Fuentes et al., 2010), and selected based on its lindane removal capacity (Fuentes et al., 2011).

Filamentous fungi *Trametes versicolor* S5NG1, *Fusarium solani* S2EG3, and *Trichoderma atroviride* S1EG1, isolated from leaf litter collected from a mountain forest of Northwestern Argentina (Fernandez et al., 2017), were used due to its lignocellulolytic activities.

2.2. Culture media and chemicals

Tryptic Soy Broth (TSB) was used for the actinobacteria inoculum preparation. It consists of ($g\,L^{-1}$): tryptone, 15; soy peptone, 3; NaCl, 5; K_2HPO_4, 2.5; glucose, 2.5 (pH 7).

Starch Casein (SC) medium, containing in $g\,L^{-1}$: starch, 10; casein, 1; K_2HPO_4, 0.5; agar, 15 (pH 7), was used for the antagonism assay among the strains (Hopwood, 1985).

Yeast extract-Malt extract (YM) broth, containing in $g\,L^{-1}$: glucose, 10; peptone, 5; malt extract, 3; yeast extract, 3 (pH 6.4) (Pajot et al., 2011), was used for the fungus inoculum preparation.

Plate Count Agar (PCA) medium was used for counting the total heterotrophic microorganisms. It contains ($g\,L^{-1}$): tryptone, 5; glucose, 1; yeast extract, 2.5; agar, 15 (pH 7).

All the culture media were sterilized by autoclaving at 121 °C for 15 min.

Lindane (γ-HCH, 99% pure) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and all other chemicals used in this study were purchased from certified manufacturers. A stock solution of γ-HCH dissolved in acetone ($50\,mg\,mL^{-1}$) was employed to contaminate the biomixtures. Solvents were of pesticide grade and all other chemicals were of analytical grade.

2.3. Antagonism assay

Each of the filamentous fungi was seeded in the center of a Petri dish containing SC medium and faced transversally with the actinobacteria, making all possible combinations. Petri dishes were incubated at 28 °C for 7 days. At the end of the assay, the development of the microorganisms was macroscopically evaluated and the presence of antagonism between the four actinobacteria and the three fungi under study, evidenced as an inhibition halo, was determined. The strains presenting no antagonism among them were selected for the bioaugmentation assays.

2.4. Soils sampling and conditioning

Three types of soils with different textures were collected from various regions of Tucumán province, free of contamination with OPs. Soil samples were taken from the upper layer (5–15 cm deep) and were stored in dark at 15–20 °C. The main physicochemical properties of the soils are detailed in Table 1.

Soil samples were conditioned; the undesirable macroscopic particles were separated and soils were dried at 30 °C for three days for decreasing the humidity rate. Table 2

2.5. Preparation of the biomixtures

According to the methodology described by Castillo et al. (2008), the biomixtures were prepared using a lignocellulosic material, peat, and soil in a proportion of 50:25:25 vol%. Sugarcane bagasse, provided by the Sugar mill Cruz Alta (Tucuman, Argentina), was used for the