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Use of okara in the bioremediation of chlorpyrifos in soil: Effects on soil biochemical properties

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ABSTRACT

The main objective of this manuscript was to study the bioremediation capacity of okara (a byproduct of soy milk production) in soils contaminated by organic xenobiotics. To this aim, and under controlled laboratory conditions, a soil was contaminated with chlorpyrifos insecticide at a dose of 5 l ha^{-1}. Okara was added to this contaminated soil in two different manners: (1) pure okara (Op); and (2) a biostimulant/biofertilizer made from pure okara using the pH-stat technique. Several enzymatic activities (dehydrogenase, urease, β-glucosidase and phosphatase) and the evolution of the insecticide in soil were studied over an 80-day period. The results suggested that both forms of okara stimulated soil microorganisms and accelerated the degradation of chlorpyrifos in soil. It was, however, the biostimulant/biofertilizer that showed the greatest acceleration in insecticide degradation, possibly due to its higher content in low molecular weight (< 300 Da) peptides, easily available to soil microorganisms.

1. Introduction

Applying different sources of organic matter to soils contaminated by persistent pesticides is a widely-used environmental technique among scientists and environmental engineers (Tejada et al., 2011a, 2011b, 2014; Gómez et al., 2014). On the one hand, the mineralization of this organic matter releases nutrients that stimulate the growth of xenobiotic-tolerant microbes, causing an acceleration in the degradation of the said chemical compound by the microorganisms. On the other hand, the material is capable of adsorbing the said pesticide, reducing its concentration in the soil and consequently reducing its toxicity (Delgado-Moreno and Peña, 2009; Kadian et al., 2012; Gómez et al., 2014).

Okara is a byproduct obtained from the manufacture of soy milk, tofu, and their derivatives. At present, and according to Choi et al. (2015), worldwide an estimated 14 million tons of okara are generated annually. Its high fiber (56%) and protein (29%) content makes this byproduct an exceptional source for the food industry (Redondo-Cuenca et al., 2008; Villanueva-Suárez et al., 2013; Park et al., 2015). Several biostimulants/biofertilizers (BS) have, in recent years, been obtained from various organic wastes such as sewage sludge, chicken feathers, soluble wheat condensed distillers, rice bran extract, carob germ enzymatic extract – all with the purpose of being used in the bioremediation of soils polluted with various organic xenobiotics (pesticides and hydrocarbons) (Gómez et al., 2014; Tejada et al., 2010, 2011a, 2011b, 2014; Rodríguez-Morgado et al., 2015a, 2015b). Producing a new okara-based BS could, therefore, be another new alternative for recovering contaminated soils. This would be a completely new approach to the problem since, to our knowledge, there is nothing in the literature describing the use of okara either as an unaltered byproduct or as a BS in the bioremediation of soils polluted by organic xenobiotics.

In recent years, there is a strong concern regarding chlorpyrifos contamination in both soil and groundwater, due to its high persistence in soil (Korade and Fulekar, 2009; Tejada et al., 2011b, 2014). Chlorpyrifos [$C_9H_{11}Cl_3NO_3PS$ or *O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] is a non-systemic organophosphorus insecticide used to control insect pests in the soil and foliar treatments.

Most soil scientists, furthermore, consider biochemical properties (especially the study of enzymatic activities) as excellent soil quality indicators, because they are more sensitive than physical and chemical properties (Paz-Ferreiro et al., 2010; Bera et al., 2016). Therefore,

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Table 1
Chemical composition and protein molecular weight distribution (mean ± standard error, n = 3) of the two okara forms. Columns followed by the same letter(s) are not significantly different ($p > 0.05$).

	Op	Oh
Organic matter (g kg^{-1})	537a ± 14	448a ± 18
N (g kg^{-1})	60.7a ± 4.2	107.2b ± 11.4
P (g kg^{-1})	6.5a ± 1.7	11.8b ± 2.2
K (g kg^{-1})	9.1a ± 1.2	20.8b ± 3.1
S (g kg^{-1})	3.8a ± 1.1	11.8b ± 1.4
Ca (g kg^{-1})	1.8a ± 0.3	2.7a ± 0.8
Mg (g kg^{-1})	2.1a ± 0.8	3.3a ± 1.0
Fe (mg kg^{-1})	63.9a ± 4.9	63.4a ± 5.7
Cu (g kg^{-1})	10.9a ± 1.0	12.5a ± 1.6
Mn (g kg^{-1})	30.6a ± 2.2	27.2a ± 1.9
Zn (g kg^{-1})	28.0a ± 2.6	47.7b ± 3.8
Protein molecular weight distribution (Da)		
> 10,000	1.7b ± 0.5	0.7a ± 0.1
10,000–5000	64.0b ± 10.5	23.5a ± 2.8
5000–1000	1.2 ± 0.2	0
1000–300	0	0
< 300	33.2a ± 6.4	75.7b ± 10.6

measuring enzymatic activities can provide information on the behavior of chlorpyrifos in soil, as well as the effects that this insecticide can cause on soil metabolic activity (Tejada et al., 2011b, 2014; Franco-Andreu et al., 2016).

The main objectives of this manuscript are to study the method of obtaining a new BS from okara, as well as its effectiveness in bioremedying a chlorpyrifos-contaminated soil by observing its impact on the biochemical properties of the said soil.

2. Material and methods

2.1. Characteristics of okara and enzymatic hydrolysis process

Pure Okara (Op) was supplied by the Spanish company Soria Natural S.A. The chemical characteristics of this by-product are shown in Table 1. The determinations of organic matter, macro- and micronutrients, as well as protein molecular mass distribution were performed following the methodology described in Rodríguez-Morgado et al. (2015c).

Hydrolysis process took place in a bioreactor following the pH-stat methodology (Adler-Nissen, 1977), using an endoprotease obtained by liquid fermentation of *Bacillus lichiniformis* ATCC 21,415 as the hydrolytic agent. Fig. 1 shows this process of enzymatic hydrolysis schematically. Table 1 shows the chemical properties of the new BS (Oh) obtained from pure okara. The methodology used in determining the chemical properties of this new fertilizer was the same as previously described.

2.2. Soil and chlorpyrifos characteristics

The soil used in the study was classified as Vertic Argiudoll (USDA, 1999), with 173 ± 23 g kg^{-1} sand, 585 ± 31 g kg^{-1} silt, and 242 ± 29 g kg^{-1} clay. Soil pH was 6.5 ± 0.1, 30 g kg^{-1}C, and 1.9 ± 0.1 g kg^{-1} N. The methodology used in the determination of the soil parameters is described in Tejada et al. (2014).

The insecticide used was chlorpyrifos. The Senator® 48 (48% chlorpyrifos) commercial formulation was purchased from Bayer CropScience (Madrid, Spain). According to Giménez et al. (2004), the recommended dose of insecticide to cause toxic effect on soil biochemical properties is 5 l ha^{-1}.

2.3. Experimental design

Two hundred and fifty grams of dried and sieved (< 2 mm) soil was mixed with chlorpyrifos insecticide and the forms of okara in 1-L glass bottles at a rate of 1.5%. Distilled water was added to each soil to bring it to 60% of its water-holding capacity. An unamended polluted and amended non-polluted soil were used as control. The incubation treatments are detailed as follows:

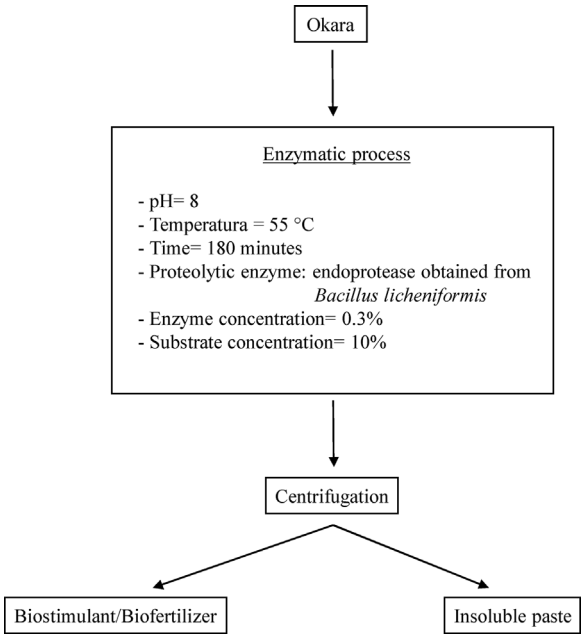

Fig. 1. Enzymatic hydrolysis process used for obtaining biostimulants/biofertilizers from okara.

C, Control soil, unamended and non insecticide polluted soil. (1)

C + Op, soil amended with Op and non-insecticide polluted (2)

C + Oh, soil amended with Oh and non-insecticide polluted (3)

C + I, soil non-organic amended and insecticide polluted. (4)

C + Op + I, soil amended with Op and insecticide polluted (5)

C + Oh + I, soil amended with Oh and non-insecticide polluted (6)

For each treatment, triplicate glass bottles were kept at 25 ± 1 °C. The moisture content was controlled gravimetrically and moisture loss was replaced by distilled water as necessary.

2.4. Soil analysis

On days 3, 7, 15, 30, 50 and 80, dehydrogenase, urease, β-glucosidase and phosphatase activities were determined using the methods described by García et al. (1993), Kandeler and Gerber (1988), Eivazi and Zakaria (1993) and Tabatabai and Bremner (1969), respectively.

Chlorpyrifos in soil was also extracted and determined on days 3, 7, 15, 30, 50 and 80 after the start of incubation. Chlorpyrifos extraction from soil followed the Menon et al. (2004) criteria and its concentration was determined by high-performance liquid chromatography (HPLC) using a UV detector set at 240 nm using a C18 reverse phase column (4.6 × 250 mm^2) at 25 °C. Hexane/dichloromethane/methanol/2-propanol (80:15:0.2:4.8) was used as the mobile phase.

2.5. Statistical analysis

With the data obtained, a two-way analysis of variance (ANOVA) was performed with treatment and sampling time as factors followed by Tukey's significant difference as a post hoc test, considering a significance level of $p < 0.05$ throughout the study and using