Ecotoxicology and Environmental Safety 139 (2017) 481–487

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Antibiotics do not affect the degradation of fungicides and enhance the mineralization of chlorpyrifos in biomixtures

Humberto Castillo-González, Marta Pérez-Villanueva, Mario Masís-Mora, Víctor Castro-Gutiérrez, Carlos E. Rodríguez-Rodríguez*

Centro de Investigación en Contaminación Ambiental (CICA), Universidad de Costa Rica, 2060 San José, Costa Rica

ARTICLE INFO

Keywords:
Pesticides
Antibiotics
Degradation
Biopurification systems
Biomixture

ABSTRACT

The use of antibiotics in agriculture produces residues in wastewaters. The disposal of such wastewaters in biopurification systems (BPS) employed for the treatment of pesticides could result in the inhibition of the degrading capacity of the biomixtures used in the BPS. We assayed the effect of two commercial formulations of antibiotics used in agriculture, one containing kasugamycin (KSG) and the other oxytetracycline plus gentamicin (OTC + GTM), on the biomixture performance. Doses from 0.1 mg kg^{-1} to 1000 mg kg^{-1} of KSG increased the respiration of the biomixture, and low doses enhanced the mineralization rate of the insecticide ^{14}C-chlorpyrifos. On the contrary, OTC + GTM depressed the respiration of the biomixture and the initial mineralization rate of ^{14}C-chlorpyrifos; nonetheless, the antibiotics did not decrease overall mineralization values. The application of both formulations in the biomixture at a relevant concentration did not harm the removal of the fungicides carbendazim and metalaxyl, or their enhanced degradation; on the other hand, the biomixture was unable to dissipate tebuconazol or triadimenol, a result that was unchanged during the addition of the antibiotic formulations. These findings reveal that wastewater containing these antibiotics do not affect the performance of BPS. However, such a response may vary depending on the type of pesticide and microbial consortium in the biomixture.

1. Introduction

Biopurification systems (BPS) were designed as a biotechnological strategy to reduce the contamination by pesticide-containing wastewater produced during agricultural activities. Point source contamination by pesticides is linked to improper handling and spillages or leaks during mixing and cleaning of pesticide application equipment; this process is considered as one of the main causes of pesticide contamination in aquatic environments (Karanasios et al., 2012).

Within BPS, the removal process takes place in the biomixture, a biologically active matrix where pesticide degradation occurs due to the microbial activity provided by the biomixture components. These components include a humic substance-rich material (usually peat or compost) to increase pesticide retention, a lignocellulosic substrate to potentiate the activity of ligninolytic fungi (Borràs et al., 2011; Rodríguez-Rodríguez et al., 2013), and soil, ideally pre-exposed to the target pesticides, which provides adapted microbial populations with degrading capacity (Karanasios et al., 2012).

Besides pesticides, pest control in agriculture employs antibiotics for the prevention of bacteria-related diseases in crops. This activity translates in the production of antibiotic-rich wastewaters, analogous to those generated by pesticide application. The correct disposal of such wastewater is essential to avoid the inadequate release of antibiotics in environmental compartments. Given the mode of action of antibiotics, the presence of these compounds in the environment may cause deleterious effects on non-target microorganisms, related to the affectation of bioprocesses such as nitrification, iron reduction (Toth et al., 2011), biogas production (Mitchell et al., 2013), sulfate reduction, degradation of organic matter in sewage sludge (Kümmerer, 2009a), and the Anammox process (Jin et al., 2012). In addition, concern has arisen on the role of environmental exposure in the development of antibiotic resistance by human and veterinary pathogens (Kümmerer, 2009b; McManus et al., 2002).

Only a few antibiotics are currently used in the agricultural industry. Streptomycin is an aminoglycoside of relatively wide use commercialized in the USA since the 1950's for plant agriculture. Oxytetracycline (OTC) is a tetracycline of bacteriostatic action, used in the USA, Mexico and Central America mostly in fruit crops. Similarly, gentamicin (GTM) is widely used in Mexico and Central America in bacterial disease control in vegetable crops, and as streptomycin, it acts

* Corresponding author.
E-mail address: carlos.rodriguezrodriguez@ucr.ac.cr (C.E. Rodríguez-Rodríguez).

http://dx.doi.org/10.1016/j.ecoenv.2017.02.008
Received 9 July 2016; Received in revised form 30 January 2017; Accepted 7 February 2017
Available online 23 February 2017
0147-6513/

as a bactericidal compound by inhibiting protein synthesis (Stockwell and Duffy, 2012; Vidaver, 2002). Kasugamicin (KSG) is an aminoglycoside, originally used as a fungicide, but then as a bactericide for fire blight control (McGhee and Sundin, 2011). Other antibiotics used in agriculture include oxolinic acid and validamycin. Some formulations contain a mixture of antibiotics (such as OTC plus streptomycin or OTC plus GTM), therefore, the effects on non-target populations also depend on the antagonistic or potentiation interactions between the therapeutic agents applied.

A priori, BPS could be potentially used for the disposal of antibiotic-containing wastewater. However, for the reasons stated above, depending on the alteration on microbial communities, antibiotics could harm the capability of biomixtures to remove pesticides. In this context, this work aimed to determine the effects of two commercial formulations of antibiotics (one containing KSG and one containing OTC and GTM) on the performance of a biomixture used for pesticide removal. The respiration of the biomixture and its ability to mineralize the ^{14}C-radiolabeled chlorpyrifos (insecticide/nematicide of wide application) were assayed at different antibiotic doses; then a relevant dose was used to determine the effect on the removal of four fungicides of worldwide use (carbendazim, metalaxyl, tebuconazole and triadimenol). This work provides useful insights on the application range of BPS.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards metalaxyl (methyl N-(methoxyacetyl)-N-(2,6-xylyl)-DL-alaninate), carbendazim (methyl benzimidazol-2-ylcarbamate), tebuconazole ((RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol) and triadimenol ((1RS,2RS;1RS,2SR)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)butan-2-ol) were obtained from Chem Service Inc. (West Chester, Pennsylvania, USA). Commercial formulations of chlorpyrifos (Solver®, 48% w/v), carbendazim (Agromart®, 50% w/v), metalaxyl (Abak®, 24% w/v), tebuconazole/triadimenol 3:1 (Silvacur® Combi 30 EC, 22.5% and 7.5% w/v, respectively), KSG (Kasumin 2 SL®, 2.12% w/v) and oxytetracycline/gentamicin (OTC + GTM) (Agry-Gent Plus ® 8WP, 6% w/w and 2% w/w, respectively) were acquired from a local store. Radio-labeled chlorpyrifos (^{14}C-chlorpyrifos; [ring-2,6-$^{14}C_2$]-chlorpyrifos; 4.38×10^9 Bq g^{-1}; radiochemical purity 98.99%; chemical purity 98.34%) was obtained from Izotop (Institute of Isotopes Co., Budapest, Hungary). Carbofuran-d_3 (surrogate standard, 99.5%) and linuron-d_6 (internal standard, 98.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Potassium hydroxide (analytical grade) and glucose were purchased from Merck (Darmstadt, Germany). Ultima Gold cocktail for liquid scintillation counting was purchased from Perkin Elmer (Waltham, Massachusetts, USA). Solvents and extraction chemicals are listed elsewhere (Ruiz-Hidalgo et al., 2014).

2.2. Biomixture

A biomixture containing 45% (v/v) coconut fiber, 13% compost and 42% soil pre-exposed to carbofuran was employed in the assays (pH 6.4; C 4.83%; N 0.32%; C/N 15.2; P 0.22%; Ca 0.48%; Mg 0.71%; K 0.19%; S 0.07%; Fe 31 192 mg kg^{-1}; Cu 94 mg kg^{-1}; Zn 91 mg kg^{-1}; Mn 521 mg kg^{-1}; B 66 mg kg^{-1}; EC 0.6 mS cm^{-1}). The composition of this biomixture was previously optimized by Chin-Pampillo et al. (2015) in order to maximize the removal of carbofuran and the decrease of residual toxicity in the matrix.

2.3. Experimental procedures

2.3.1. Microbial respiration in a biomixture in the presence of antibiotics

Microbial respiration was determined using the OxiTop Control OC 110® system (WTW Merck) and an adapted manometric procedure by Platen and Wirtz (1999). Briefly, biomixture samples (32.5 g dry weight, sieved < 2 mm) were placed in amber bottles and then amended with glucose (~1 g kg^{-1} biomixture). Moisture was adjusted to 55% WHC, before the application of the commercial formulation of either KSG or OTC + GTM to obtain final antibiotic concentrations of 0.1, 1, 10, 100, 500 and 1000 mg kg^{-1}. The treatments were performed in triplicates; control treatments (triplicates) lacking antibiotic were also prepared. The systems were completed with the addition of two NaOH pellets in the CO_2 trap chamber, and the OxiTop measuring heads, which were set to collect data continuously in pressure mode during 10 d (25 °C in the dark).

Respiration (milligrams of O_2 per kilogram of dry biomixture) was calculated using the pressure data, based on an adaptation of the ideal gas law, as described in Eq. (1):

$$MR = \frac{MM\ O_2}{R \times T} \times \frac{V}{m} \times \Delta p \quad (1)$$

where MR = microbial respiration; $MM\ O_2$ = molar mass of oxygen (32,000 mg mol^{-1}); R = general gas constant (83.14 $\frac{L \cdot mbar}{mol \cdot K}$); T = temperature (K); V = free gas volume in the system (L); m = mass of the dry biomixture (kg); and Δp = reduction in pressure (mbar).

O_2 consumption was modeled according to a first order model to determine O_2 consumption rate constants, which were analyzed by means of ANOVA tests to compare regression lines; analyses were performed in the STATGRAPHICS Centurion software (version XVII, Statpoint Technologies, Inc., VA, USA).

2.3.2. Mineralization of chlorpyrifos during co-application of antibiotics

The mineralization of ^{14}C-chlorpyrifos was determined through $^{14}CO_2$ production in biometer flasks containing $^{14}CO_2$ traps with 10 mL KOH (0.1 M). The biomixture (50 g), was weighed into each biometer flask and spiked with commercial chlorpyrifos (50 mg kg^{-1}) and ^{14}C-chlorpyrifos (5000 dpm g^{-1}), and either the commercial formulation of KSG or OTC + GTM to obtain each of the following concentrations of antibiotic: 0.1, 1, 10, 100, 500 and 1000 mg kg^{-1}. Every condition was evaluated in triplicate systems, which were incubated in the dark at 25 °C during 62 d; control treatments (triplicates) lacking antibiotic were also prepared. The KOH in the flasks was withdrawn at selected times and replaced with the same amount of fresh KOH. Activity of ^{14}C in the $^{14}CO_2$ produced from the mineralized pesticide was analyzed in the KOH samples as described in Section 2.4.1.

2.3.3. Removal of fungicides in a biomixture in the presence of antibiotics

Removal assays were performed in 50 mL polypropylene tubes containing 5 g of the biomixture. Each tube was spiked with commercial formulations of carbendazim, metalaxyl and tebuconazole/triadimenol to achieve nominal initial concentrations from 25 mg kg^{-1} to 60 mg kg^{-1}. A commercial formulation of KSG was applied to one set of tubes at a final concentration of 10 mg kg^{-1}; a second set of tubes was spiked with the commercial formulation Agry-Gent Plus ® (10 mg kg^{-1} antibiotic, equivalent to 7.5 mg kg^{-1} OTC and 2.5 mg kg^{-1} GTM). After manual homogenization, the systems were incubated in static conditions in the dark at (25 + 1) °C during a period of 68 d; water was added when necessary in order to keep a constant water content in the matrix. Triplicate tubes from each antibiotic treatment were periodically withdrawn to determine the remaining concentration of the fungicides.

2.4. Analytical procedures

2.4.1. Determination of $^{14}CO_2$ from mineralization assays

Scintillant liquid (8 mL) was added to 2 mL aliquots from the removed KOH solution samples and the ^{14}C activity from the trapped $^{14}CO_2$ was measured using a liquid scintillation counter (LS6000SC, Beckman Instruments Inc., USA). The total cumulative $^{14}CO_2$ activity