Influence of novel lignocellulosic residues in a biobed biopurification system on the degradation of pesticides applied in repeatedly high doses

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Abstract

Background: The biobed is a simple biopurification system used to prevent the point-source pesticide contamination that occurs at farm level. The typical composition of the biomixture used in this system is soil, peat and straw in volumetric proportions of 1:1:2. The principal component is straw due to its positive effects on biological activity and thus pesticide degradation. However, access to straw can be limited in some regions, so it must be replaced by other more readily available lignocellulosic residues.
Results: Therefore, two alternate lignocellulosic materials (barley husks and pine sawdust) were evaluated as partial substitutes for straw. The degradation of a repeatedly applied mixture of six pesticides by these alternates was assessed. The microbial respiration and fluorescein diacetate (FDA) hydrolysis activity were also assessed. The results showed that the highest degradation efficiency was found in mixtures containing straw and barley husks. Each biomixtures tested achieved a high degradation (50 to 90%) of all the pesticides used except iprodione. Repeated applications of pesticides resulted in a slowing of the degradation rate of all pesticide types in all biomixtures. FDA activity and microbial respiration were higher in the biomixtures containing barley husks and straw compared to the mixture with pine sawdust, a result consistent with the pesticide degradations observed.
Conclusions: This paper demonstrates that the straw in the traditional biomixture can be partially replaced by other lignocellulosic materials to efficiently degrade a mixture of pesticides, even when the pesticides are added in successive applications and high concentrations.

Keywords: biopurification system; biomixture; lignocellulosic materials; pesticide degradation.

INTRODUCTION

Pesticides play an important role in the success of modern farming and food production; however, inadequate pesticide management can cause surfaces as well as groundwater to be contaminated. Such contamination is extremely harmful to the environment and is a topic of international concern. Point sources, such as the accidental spillages that occur while filling a tank or cleaning spraying equipment, have been identified as major contamination risks (Müller et al. 2002).

Biobeds are simple and cheap biopurification systems that have been used successfully to reduce point source contamination by pesticides in Europe (Torstensson and Castillo, 1997; Castillo et al. 2008; Karanasios et al. 2012a; Marinozzi et al. 2012). A recent review of pesticide contamination

prevention using biobeds shows that the two factors controlling the depuration performance of biopurification systems, either individually or through their interaction, are the biomixture composition and water management (Karanasios et al. 2012b). The principal component in the biobed biopurification system is the biomixture, which is the principal element controlling degradation efficacy; the maturity of the biomixture affects its overall performance (Tortella et al. 2012). The biomixture is primarily composed of soil, peat and straw in volumetric proportions of 1:1:2 (Castillo et al. 2008). The biomixture efficiency is based on its ability to retain and degrade pesticides, and therefore a good biomixture must have a high biological catalytic activity for pesticide degradation (Castillo et al. 2008). In this sense, straw is the primary substrate in the biomixture. Straw allows the development of white rot fungi, which produce extracellular ligninolytic enzymes that promote pesticide degradation (Castillo et al. 2008; Tortella et al. 2012).

The traditional biomixture composition (straw, peat and soil) has proven efficient in the degradation of several pesticides (Castillo and Torstensson, 2007; Castillo et al. 2008). However, the adaptation of this biomixture in other countries has required the use of more available lignocellulosic residues than straw. Karanasios et al. (2010a) evaluated the use of corncobs, sunflower residues, grape stalks, orange peels and olive leaves as replacements for straw. They reported that grape stalks were the most efficient at degrading pesticides in the biomixture. Sunflower crop residues and corncobs showed a comparable degrading efficacy to the traditional composition. Coppola et al. (2007) reported that the use of urban compost and citrus peels in the biomixture caused a high chlorpyrifos degradation, but an accumulation of 3,5,6-trichloropyridinol (TCP) was also observed. However, the same authors reported that the TCP level decreased in a biomixture containing straw made from vine branches. Castillo et al. (2008) reported that residues with low lignin content may not support sufficiently high microbial activity to degrade pesticides. Recently, olive and winery by-products were used as a biomixture to treat high pre- and post-harvest volumes of wastewater from citrus production showing a high dissipation (Omirou et al. 2012).

Though several different residues have been evaluated to degrade pesticides in the biomixture of biobeds, studies have generally only modified the traditional composition of the biomixture by adding compost or other substrates. The use of wood residues such as pine sawdust, or readily available agricultural residues such as barley husks, has not been well evaluated. Moreover, degradation studies of modified biomixtures have been performed with only a single pesticide application. Therefore, the aim of this work was to evaluate the effects of pine sawdust and barley husks as a replacement for a fraction of straw in the biomixture of the biobed biopurification system. Their ability to degrade a mixture of pesticides added in successive applications at high concentrations was examined.

MATERIALS AND METHODS

Chemicals

Analytical standards of atrazine (ATZ, 99% purity), isoproturon (ISP, 99.3%), iprodione (IPR, 99.1%), chlorpyrifos (CHL, 99%), diazinon (DZN, 99%) and carbendazim (CARB, 99%) were purchased from Chem Service (West Chester, USA). Stock solutions (1000 mg L^{-1}) of the above analytical standards were prepared in acetone. Formulated pesticides containing the active ingredients ATZ (Atrazina 500SC), ISP (Fuego 50SC), IPR (Rovral 50SC), CHL (Chlorpyrifos S480), DZN (Sinpullkill), and CARB (Itabarb 50%) were used in the degradation studies.

Components and preparation of biomixtures

To prepare the biomixtures, we used Andisol top soil belonging to the Temuco Series (Typic Hapludands) was used, which has a loamy texture (37.1% sand, 34.2% silt and 28.7% clay) (CIREN, 2002). The soil was collected in southern Chile (38º 42'S, 73º 35'W) at a depth of 0-20 cm, then air dried at room temperature and sieved through a 2 mm mesh. The soil had a pH of 5.4, 18.6 mg kg^{-1} of available nitrogen, 17.1 mg kg^{-1} of available phosphorous and 11.7% organic matter.The lignocellulosic materials used as components in the biomixtures were wheat straw (WS), barley husks (BH) and pine sawdust (PS). BH and PS were tested as alternatives to partially replace the wheat straw in the biomixture. Wheat straw was collected from crop residues, barley husks were supplied by a brewing company and pine sawdust was collected from sawmill waste. The main physicochemical characteristics of the biomixture components are reported in Table 1.

To obtain a homogeneous biomixture, the straw, barley husks and pine sawdust were cut into small fragments (approximately 2-3 mm) using a food processor and mixed with top soil and peat in the volumetric proportions described in Table 2. Before the degradation studies, all biomixtures were pre-incubated in polypropylene bags. The moisture content was adjusted with distilled water to be 60% of the water holding capacity (WHC), and the biomixtures were stored in the dark at 20 ± 2ºC for 30 days.

Table 1. The physical and chemical properties of the soil, peat and lignocellulosic materials (WS, PS and BH) used in the biomixtures. The data are the average of three replicates.

Parameters	Components of the biomixtures				
	Soil	Peat	W S	P S	B H
Dry matter (%)	-	-	91.1 ± 1.8	87.8 ± 1.2	88.8 ± 1.0
Acid detergent fiber (%)	-	-	54.3 ± 1.2	76.6 ± 1.3	12.9 ± 0.5
Lignin (%)	-	-	9.9 ± 0.4	20.6 ± 0.6	2.4 ± 0.2
Cellulose (%)	-	-	41.8 ± 0.7	54.3 ± 0.4	9.6 ± 0.1
pH	5.4 ± 0.1	6.0 ± 0.2	5.9 ± 0.1	4.82 ± 0.1	5.8 ± 0.0
Organic carbon (%)	6.8 ± 0.9	43.7 ± 1.1	36.7 ± 1.2	34.3 ± 0.6	33.2 ± 0.9
TKN (%)	0.47 ± 0.3	0.57 ± 0.2	0.56 ± 0.3	0.28 ± 0.1	0.68 ± 0.6
C/N	14.4	76	65.5	122.5	48.8

TKN=total Kjeldahl nitrogen.

Table 2. The parameters of pH, organic carbon (OC) content, nitrogen (N) content and C/N ratio of the different biomixtures at the start (day 0) and the end of the biodegradation assay (day 90). The data are the averages of three replicates (n = 3).

Biomixture	Parameters at day 0				Parameters at day 90			
	pH	OC (%)	N (%)	C/N	pH	OC (%)	N (%)	C/N
C1	5.5	23.5 ± 1.2	0.77 ± 0.04	30.5	4.86	22.4 ± 0.9	0.84 ± 0.02	26.5
C2	5.8	22.8 ± 0.9	0.44 ± 0.04	51.8	4.5	24.5 ± 0.4	0.46 ± 0.01	53.3
C3	5.4	24.8 ± 1.1	0.85 ± 0.02	29.1	4.48	22.1 ± 0.6	1.44 ± 0.02	15.3

C1: soil (25%), peat (25%), straw (50%); C2: soil (25%), peat (25%), straw (25%) and pine sawdust (25%); C3: soil (25%), peat (25%), straw (25%) and barley husks (25%).

Degradation studies

The pesticide degradation efficiency of the biomixtures prepared with two lignocellulosic materials instead of only wheat straw was evaluated. Additionally, the biomixture with only wheat straw was prepared for the purposes of comparison. One bulk sample of 2000 g dry weight (dw) of each biomixture (described in Table 2) was placed in a glass laboratory biobed (40 x 30 x 15 cm). The glass biobed was artificially contaminated with six pesticides (ATZ, ISP, IPR, CHL, DZN and CARB) taken from a stock solution. The first pesticide application was sprayed at a final concentration of 100 mg a.i. of each pesticide per kg of biomixture (dose 1). A second dose of 200 mg a.i. of each pesticide per kg of biomixture (dose 2) was applied on day 30 and a third dose of 300 mg a.i. of each pesticide per kg of biomixture (dose 3) was applied on day 60. The moisture content of the biomixtures was adjusted to and maintained at 60% of the WHC through the addition of distilled water. The laboratory biobeds were incubated in the dark at 20 ± 2ºC for 90 days. Before and after each pesticide application as well as at the end of the experiment (0, 30, 60 and 90 days), samples of 5 g of biomixture from surface to the bottom of glass biobed were removed for residual pesticide analysis. Each sample was tested in triplicate. To evaluate the changes in the composition of the biomixtures, samples were extracted to analyze the nitrogen (Kjeldahl) and organic carbon content at the beginning and end of the incubation period. Additionally, a sample was extracted to analyze the fungal colonization of the biomixtures by scanning electron microscopy (SEM).

Biological activities in the biomixtures

Samples were periodically taken from the laboratory biobeds used in the degradation study during the period of incubation (90 days) to analyze the total hydrolytic capacity. The total hydrolytic activity of the biomixtures was measured by fluorescein diacetate (FDA) hydrolysis, according to Schnurer and Rosswall (1982) with slight modifications. Briefly, 1 g dw of the biomixture was incubated in a 30 mL

conical flask with 9.9 mL of sterile 60 mM sodium phosphate buffer, pH 7.8. The reaction was started by adding 0.1 mL of the FDA solution (2.0 mg mL^{-1}). After 1 hr of incubation at 25 ± 1ºC, 10 mL of acetone was added to stop the reaction. The A$_{490}$ was measured spectrophotometrically after the biomixture was removed by centrifugation and filtration. The concentration of the released fluorescein was calculated by a calibration curve of standard FDA quantities, and the results were expressed as μg FDA g^{-1} h^{-1}.

Parallel to the degradation study, the microbial respiration was evaluated by measuring the CO_2 produced in the biomixtures according to the methodology of Iannotti et al. (1994). Each biomixture was weighed, and 50 g dw was placed into 500 mL glass flasks then contaminated with the same mixture of pesticides as described in the degradation study. A plastic cup containing 15 mL of 0.1 M NaOH, which was replaced at each sample point. Additionally, a vial containing 10 mL distilled water for maintaining a humid atmosphere was placed inside the glass flasks. The flasks were closed tightly and incubated at 20 ± 2ºC. The amount of CO_2 absorbed into the NaOH solution was determined by titration with a 0.1 mol L^{-1} HCl solution after the carbonate was precipitated out by 0.1 mol L^{-1} BaCl$_2$. The respiration was expressed as accumulated mg CO_2 per 100 g of biomixture.

Pesticide analysis

For the pesticide analyses, biomixture samples (5 g dw) were extracted by shaking (1 hr at 250 rpm) with 20 mL of acetone and by ultrasonication (30 min). After centrifugation (10,000 rpm), 5 mL of the supernatant was collected and evaporated to dryness under an N$_2$ flux, after which the residue was dissolved with 1 mL of acetonitrile and subsequently analyzed as described below. Recoveries of ATZ, ISP, IPR, CHL, DZN and CARB were all > 85%. The concentrations of ATZ, ISP, IPR, CHL, DZN and CARB were determined by HPLC using a Merck Hitachi L-2130 pump, a Rheodyne 7725 injector with a 20 μL loop and a Merck Hitachi L-2455 diode array detector. Separation of the different pesticides was achieved using a C18 column (Chromolit RP-8e, 5 μm 4.6 x 100 mm). Eluent A was 1 mM ammonium acetate, and eluent B was acetonitrile. The gradient conditions used for the pesticide separation were as follows: 95% A for 0-2 min, 95-70% A for 2-4 min, 70% A for 4-7 min, 70-30% A for 7-12 min, 30% A for 12-16 min, 30-95% A for 16-17 min, and 95% A for 17-20 min. The flow rate was set to the following conditions: 1.0 mL min^{-1} for0-12 min, increasing from 1.0 to 2.0 mL min^{-1} for 12-16 min and constant at 2.0 mL min^{-1} for 16-20 min. The column temperature was maintained at 30ºC. The detector was set at 220 nm (ATZ and IPD), 245 nm (ISP and DZN) and 290 nm (CARB) for data acquisition. Instrument calibrations and quantifications were performed against pure reference standards (0.1-10 mg L^{-1}) for each compound.

Analytical methods

The physicochemical analyses were carried out using the methodology described by Sadzawka et al. (2004). The soil pH was measured using a mixture of air-dried substrate and deionized water (1:5 w/v). Available nitrogen (the sum of ammonia N and nitrate N) was extracted by 2 mol L^{-1} KCl and quantified by titration with HCl and specific electrodes; available phosphorous was extracted by sodium bicarbonate (0.5 mol L^{-1}, pH 8.5) and determined colorimetrically with the molybdate-ascorbic acid method. The organic carbon content was determined by the dichromate oxidation method, using colorimetric determination of the reduced chromate (Cr^{+3}). The total Kjeldahl nitrogen (TKN) analysis determined both the organic and the inorganic form (NH_4^+) of nitrogen. The lignin content and the amount of acid detergent fibers (ADF) were determined using AOAC standard method 973.18. The cellulose percentage was calculated indirectly as the difference between the percentages of ADF and lignin. The WHC was measured gravimetrically following saturation of the biomixtures (30 g), with distilled water and Whatman Nº 1 filter paper in a funnel allowed to drain for 24 hrs.

Photomicrographs of the biomixtures were taken using SEM (JEOL JSM-6380 LV model). Three pieces of photomicrograph material (0.5 x 0.5 cm) with a thickness of either 0.3 or 0.5 cm were transferred to a Petri dish and dried for 12 hrs at 50ºC. The pieces of material were then submerged in glutaraldehyde (2.5%) for 15 min and washed with 0.1 mol L^{-1} phosphate buffer, pH 7.0. Then, the samples were placed in osmium tetroxide for 2 hrs and dehydrated by immersion in ethanol (at concentrations of 70, 80, 90 or 100%). The samples were deposited over the sample holder and coated with gold to make observations using SEM.

Statistical analysis of data

All experiments were conducted using three independent replicates. Data were subjected to a one-way analysis of variance (ANOVA), and the averages were compared using Duncan's multiple range test with a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Components of the biomixtures

To retain and degrade pesticides, a biomixture should have a good adsorption capacity and a high microbial activity. Therefore, the composition, homogeneity, age, moisture, and temperature of the biomixture should be taken into account (Castillo et al. 2008, Fernández-Alberti et al. 2012; Tortella et al. 2012). The compositions of the soil, peat and lignocellulosic materials used to prepare the biomixtures in this study are presented in Table 1. The organic content of the soil (6.8%) is high compared with soil used in other studies to prepare biomixtures for pesticide degradation (Fogg and Boxall, 2004; Karanasios et al. 2010a; Karanasios et al. 2010b). Soils with high organic carbon content should be rich in humus and consequently have a high level of microorganisms, which act as inoculum in the biomixture. A study developed by Fogg and Boxall (2004) showed that when different topsoils with organic carbon contents ranging from 0.9 to 3.6% were used in the biomixture, the leaching losses and degradation rates were similar and did not affect the level of treatment achieved.

The organic contents of the lignocellulosic materials used in this study were similar to other lignocellulosic components reported in the literature, including but not limited to cottonseed compost, olive leaf compost, pruning residues, coco chips (Castillo and Torstensson, 2007; De Wilde et al. 2009; Karanasios et al. 2010b; Coppola et al. 2011). The total Kjeldahl nitrogen was lowest in the pine sawdust and highest in the barley husks; therefore, the C/N ratio is very high in pine sawdust (122). The lignin content was highest in the pine sawdust (20.6%) and lowest in the barley husks (2.4%). The structure, levels of cellulose and hemicelluloses, monomeric content and composition of the lignin are some of the factors that can affect the biomixture's ability to degrade pesticides because they affect the level of lignin-degrading enzymes (Coppola et al. 2010).

Pesticide degradation in different biomixtures

The degradation percentages of ATZ, ISP, IPR, CHL, CARB and DZN in biomixtures containing different lignocellulosic materials and contaminated with three successive pesticide doses (100, 200 and 300 mg a.i. per kg of biomixture) are summarized in Figure 1. A high degradation (50% to 90%) of all pesticides was achieved in the different biomixtures, except for IPR. The difference in pesticide degradation among the different biomixtures was significant ($p < 0.05$). A relatively low degradation of all pesticides was obtained in the biomixtures containing PS (C2) when compared with the traditional biomixture (C1), after three pesticide applications (Figure 1). Only the degradation of DZN in C2 (> 60%) was similar to that observed in C1. On the other hand, a significantly larger (P < 0.05) degradation of CARB, ATZ and DZN was achieved in C3 than was seen in C1 (Figure 1a, 1b, 1f) after three pesticide applications. For ISP and CHL, the degradation levels of C3 were similar to those of C1, and only the degradation of IPR was lower in C3 than in C1. The biomixture composition in C3 had a fundamental role in pesticide removal. Straw is an effective component, providing carbon and nutrients primarily for white-rot fungi, which are actively involved in the degradation of many different pesticides (Bending et al. 2002). However, some studies (Coppola et al. 2010; Karanasios et al. 2010a; Karanasios et al. 2010b) have shown that straw can be partially or completely replaced in the biomixture by other lignocellulosic substrates that are more easily available in a particular region. The results reported here demonstrate that a portion of WS in the biomixture can be replaced by BH, increasing pesticide degradation.

On the other hand, when PS was used as partial substitute for wheat straw, lower degradation rates were obtained than in C1 (only WS) and C3 (WS and BH). Lower degradation rates in biomixturescontaining PS could be attributed to the high lignin content of PS (Table 1), which may allow a greater adsorption of pesticides but decrease their bioavailability to be degraded by microorganisms. Rodríguez-Cruz et al. (2009) reported that lignin has a greater adsorption capacity of

both non-ionic and ionic pesticides than does cellulose, and a high correlation with the lignin content in the wood residues was found. Alternatively, the lower degradation in biomixtures with PS could be due to their high C/N ratio (51.8) (Table 2), causing a low availability of nutrients for the microorganisms, so that they establish themselves more slowly. According to Castillo and Torstensson (2007), a C/N ratio of approximately 30, obtained with a biomixture composed of a straw:peat:soil ratio of 50:25:25% v/v, is the recommended composition for field biobeds. Their recommended composition best promotes ligninolytic activity and consequently pesticide degradation. In this context, the addition of substrates such as pine sawdust, with a high C/N ratio (122.5) (Table 1), into the biomixture can affect the structure and activity of the microorganisms, decreasing pesticide degradation. Similar results were reported for a biomixture containing aged manure, in which bromuconazole and isoproturon were slowly degraded due to the absence of easily metabolized organic matter (Torstensson and Castillo, 1997).

In this study, the short time periods between each application were used to simulate high agricultural activity, where the possibility of accidental spillage or leakage during pesticide handling can be high. As shown in Figure 1, in most cases repeated pesticide application affected negatively the degradation efficiency in biomixtures C1 and C3 more than C2 biomixture, where only DZN and IPR were affected negatively by repeated applications of pesticides. However, considering the high and increasing doses added (100, 200 and 300 mg a.i. per kg of biomixture) and the short time (30 days) between applications, a large amount of degradation of all the pesticides in the mixture was achieved (Figure 1). For example, in C1 the residual CARB concentration after the addition of 611 mg a.i. kg^{-1} over 90 days was 199 ± 20 mg a.i. kg^{-1}, corresponding to a remaining 33% of the total concentration added. Similar results were obtained for C2 and C3, where the residual concentrations of CARB were 43% and 17%, respectively (Figure 1). An unusual situation occurred with IPR in the three biomixtures evaluated; its degradation was less than 30% of the total applied amount (Figure 1d). In this context, this response could be explained by the low pH in the biomixtures (Table 2), because IPR transformation could be favoured at high pH values in the biomixtures. Walker (1987) demonstrated that the transformation of iprodione in soils with pH < 5.5 tends to be slower than in soils with a higher pH. Also, it is possible that this particular pesticide had a negative effect on the microbial communities, so that it was not degraded as efficiently as the other pesticides. However, molecular studies with this pesticide and its interaction with other pesticides are needed to corroborate this result. Wang et al. (2004) reported that iprodione at concentrations of 5 and 50 µg g^{-1} of soil caused a change in soil bacterial communities in the first days of incubation, as evaluated by denaturing gradient gel electrophoresis (DGGE). In another study on the topic, Coppola et al. (2011) reported that DGGE analysis showed an obvious modification of microbial diversity after the addition of the fungicides usually applied in vineyards. However, at the end of the degradation process (112 days), no significant changes in the composition of the microbial community were observed.

The results obtained in this studied showed that a larger amount of pesticide degradation was observed in C1 and C3 than in C2. However, the tested pesticides were degraded in different proportions. This could be attributed to three factors: (a) the lignocellulosic substrates used generated different microbial consortiums capable of using different pesticides as carbon sources or promoting metabolic or co-metabolic degradation processes; (b) differences in the adsorption capacity of the biomixtures caused differences in the pesticide bioavailability (Castillo et al. 2008); and (c) possible production of tannins or phenols, depending on the biomixture, could have acted as phenoloxidase inhibitors, as has been reported by Castillo et al. (1997). Repeated use of some pesticides in soils can result in enhanced pesticide degradation due to the adaptation and proliferation of specific microbial communities (Torstensson et al. 1975). However, our studies showed that the rate of pesticide degradation in the biomixtures decreased with each application of the pesticides, similar to the results found by Fogg et al. (2003) for isoproturon, chlorothalonil, chlorpyriphos, dimethoate and pendimethalin. Our decreasing degradation rates may be due to the high pesticide concentrations used, so the negative effects of these high concentrations masked any increase in individual microbial activity.

The changes in biomixture composition during the incubation period were due to the biological activity of the microorganisms, particularly the lignin-degrading fungi that colonize and degrade lignocellulosic materials. Figure 2 shows a white-rot fungus growing on wheat straw after 7 days and after 90 days of incubation in biomixture C1. At the beginning of the assay (day 7), the fungus completely colonized thestraw, and by the end (day 90) the straw was degraded and the level of fungal hyphal colonization had decreased. Rubilar et al. (2011) demonstrated that a white-rot fungus, *Anthracophyllum discolor*,

efficiently colonized not only wheat straw but also wheat grains and wood chips. White-rot fungi degrade lignocellulosic materials such as straw through their non-specific ligninolytic enzymatic system (Gianfreda and Rao, 2004).

A slight decrease in organic carbon content was observed in biomixtures C1 and C3, and a slight increase was observed in C2. A slight increase occurred in total nitrogen content in all biomixtures (Table 2), probably due to the nitrogen content from pesticide residues used in this study. These results modified the C/N ratio in the biomixtures; thus, the C/N ratio in C1 and C3 decreased after 90 days of incubation, and the C/N ratio of C2 increased (Table 2). The variation in the C/N ratio is an indicator of the durability of the biomixture in the biobed. A study by Castillo et al. (2008) indicated that when the C/N ratio decreases to values similar to those found in agricultural soils, the biomixture should be changed.

Biological activities in biomixtures

The FDA hydrolytic activity in the three biomixtures is shown in Figure 3. The FDA hydrolytic activity during the 90-day degradation trial varied significantly month to month ($p < 0.05$). In general, a high FDA hydrolytic activity was achieved at the start of the incubation (day 0) in the three biomixtures evaluated. However, the FDA activity decreased over time after the application of pesticides. This was most evident in biomixture C1, containing only WS (Figure 3). The opposite effect was observed in the biomixture containing PS (C2), in which the highest FDA activity was obtained at day 90 of incubation. Interestingly, in the biomixture containing BH (C3), a high FDA hydrolytic activity was achieved during the degradation study and only decreased to a level similar to that obtained with C1 at days 45 and 75 ($1.1 \ \mu g^{-1} \ h^{-1}$).

The microbial respiration in the different biomixtures is shown in Figure 4. A significantly higher microbial respiration was evident in biomixture C1 when compared with C2 and C3. The lowest microbial respiration was observed in the biomixture containing PS (C2), which can be attributed to the high lignin content of PS (20.6%) as compared to WS and BH (lignin contents of 9.9 and 2.4%, respectively). The recalcitrant characteristics of PS, as opposed to WS or BH, may affect the decomposing ability of the microorganisms in the biomixture. Studer et al. (2011) demonstrated that the sugars released from lignocellulosic material are negatively correlated with lignin content because sugars bound to the cell wall in substrates with high lignin content are less accessible to microorganisms.

Similar FDA values were found in all biomixtures over time. Low values of microbial respiration were found in C2, where PS replaced a fraction (25%) of WS as the lignocellulosic material. However, high pesticide degradation levels were not always found in this biomixture. High enzymatic activity does not always represent efficient pesticide degradation, as has been reported by Coppola et al. (2007). Castillo et al. (2008) reported that it is necessary to not only quantify microbial activity but also qualify whether that activity is appropriate.

CONCLUDING REMARKS

This study provides evidence that novel lignocellulosic materials such as BH and PS, which are more readily available in some countries, can partially substitute for WS as the lignocellulosic material in a biopurification system. Biomixtures containing alternative lignocellulosic materials showed a degradation higher than or similar to most pesticides compared to the traditional biomixture. FDA hydrolytic activity was similar in all biomixtures whereas microbial respiration decreased in the order C1>C3>C2. Therefore, our results indicated that the two lignocellulosic materials used for replacing a fraction of the straw in this study can be used in the biomixture. They successfully degraded a mixture of pesticides that were added in successive applications. However, lignocellulosic material with elevated lignin content, such as PS, should be analyzed carefully because it can lead to a restriction of the pesticide degradation.

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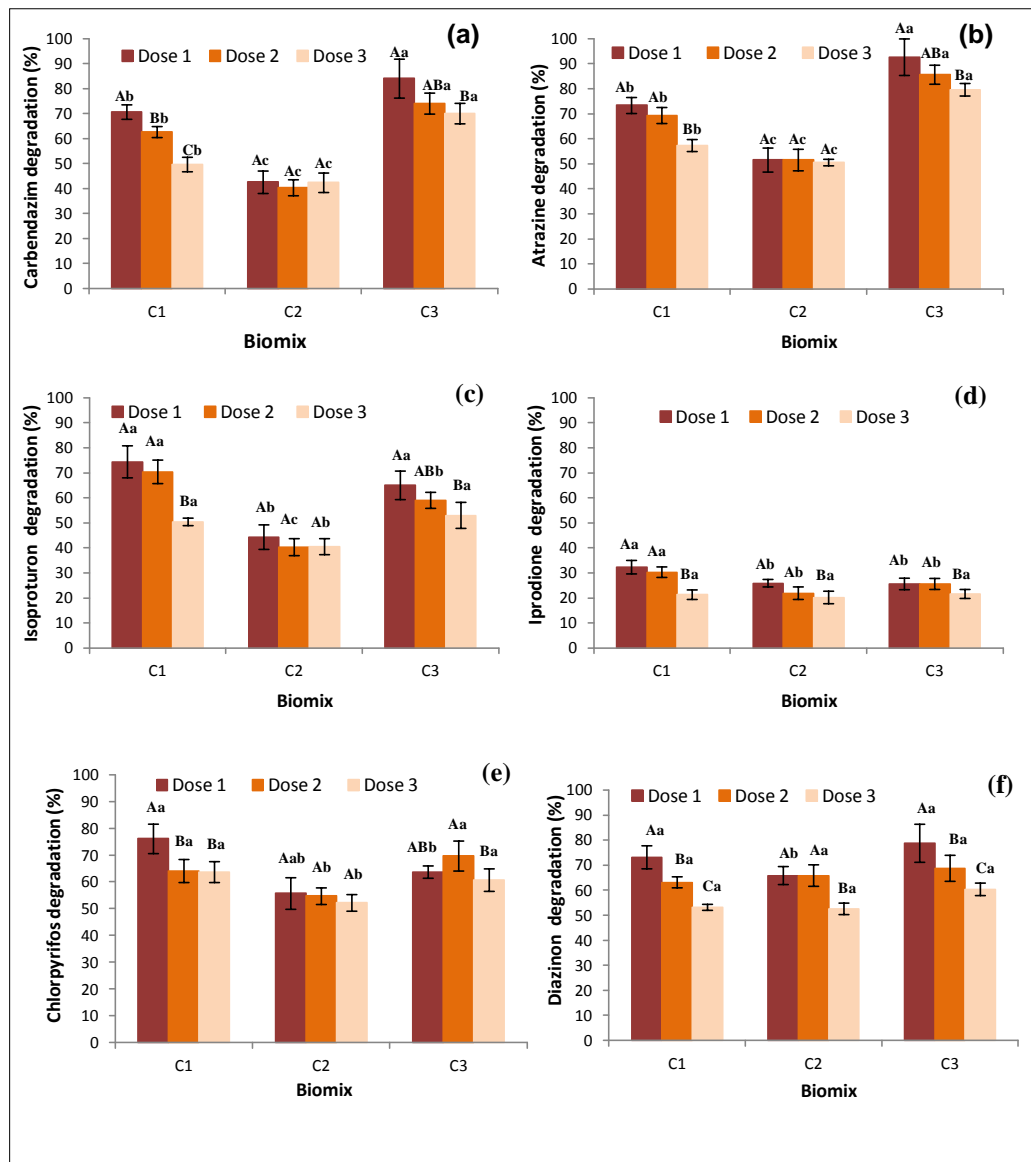
Figures

Fig. 1 The pesticide degradation (%) in biomixtures composed of wheat straw (C1), pine sawdust (C2) and barley husks (C3). The biomixtures were given three pesticide applications of 100 mg a.i. kg^{-1} (dose 1), 200 mg a.i. kg^{-1} (dose 2) and 300 mg a.i. kg^{-1} (dose 3) over 90 days, with incubation at 20 ± 2ºC. The different capital or lower case letters show the significant differences between samples of different concentrations in the same biomixture or between the same concentrations in different biomixtures. Differences between mean values (n=3) were determined with Duncan's test (p < 0.05).
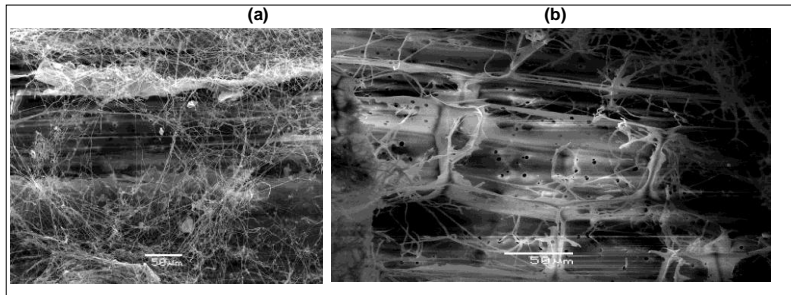
Fig. 2 Electronic micrographs of white-rot fungus grown on wheat straw after 7 days (a) and after 90 days (b) of incubation in biomixture C1.
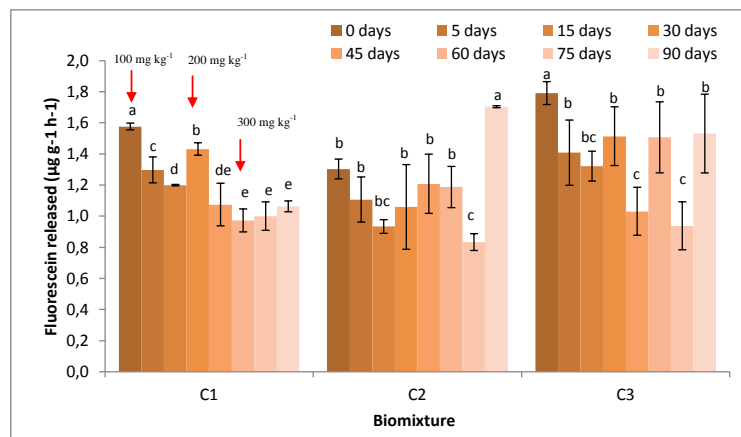

Fig. 3 The hydrolytic activity (FDA) in biomixtures containing wheat straw (C1), pine sawdust (C2) and barley husks (C3). The biomixtures were contaminated with a mixture of pesticides applied in 3 doses-100 mg a.i. kg^{-1}, 200 mg a.i. kg^{-1} and 300 mg a.i. kg^{-1}- one dose every 30 days.
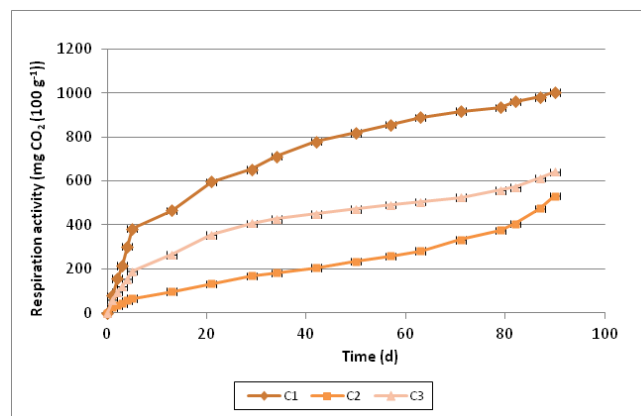

Fig. 4 Microbial respiration in biomixtures containing wheat straw (C1), pine sawdust (C2) and barley husks (C3). The biomixtures were contaminated with a mixture of pesticides applied in 3 doses-100 mg a.i. kg^{-1}, 200 mg a.i. kg^{-1} and 300 mg a.i. kg^{-1}- one dose every 30 days.