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New analytical method for chlorpyrifos determination in biobeds constructed in Brazil: Development and validation

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ABSTRACT

A quick and efficient method was optimized and validated to determine chlorpyrifos in biobeds using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Chlorpyrifos was extracted from the matrix with 30 mL of a mixture of acetone, phosphoric acid and water 98:1:1 (v/v/v). After homogenization, centrifugation and filtration, 125 μL of the extract was evaporated and reconstituted in 5 mL of methanol acidified with 0.1% acetic acid. Validation was performed by studying analytical curve linearity (r^2), estimated instrument and method limits of detection and limits of quantification (LOD_i, LOD_m, LOQ_i and LOQ_m, respectively), accuracy, precision (expressed as relative standard deviation, RSD), and matrix effect. Accuracy and precision were determined from the amount of pesticide recovered from biobed blank samples (i.e. without pesticide residue) spiked with chlorpyrifos at three different concentrations (2, 10 and 50 mg kg^{-1}), with seven replicates at each concentration. For all three concentrations studied, the average recovery values obtained were between 96 and 115% with RSD values lower than 20%. The validated LOQ obtained was 2 mg kg^{-1} (from recovery studies) and the matrix effect observed was lower than ± 20%, which demonstrated that there was neither considerable suppression nor enhancement of the analyte signal. The biobed system efficiently degraded chlorpyrifos in both 1) simulation of accidental spillage and 2) application of diluted pesticide solution. In the latter case, all the values obtained at the final sampling time (14 months) were below the validated LOQ_m.

1. Introduction

Pesticides play an important role in modern agriculture and food production [1]. However, the extensive use of pesticides is a serious public health problem in developing countries, especially those with economies based on agribusiness, as Brazilian case. Since 2008, Brazil has been considered the world's largest consumer of pesticides [2], due to the country's vast agricultural area in addition to its tropical and subtropical conditions, which favor the occurrence of pests and diseases during all over the year, due the lack of insect diapause period or stopping the growth of pathogens.

During repetitive application of concentrated pesticide at a given location, contamination spots can readily occur. This increases the risk that pesticide residue concentrations exceed the limit that it is considered safe in the environment [3-5]. For example, when filling and rinsing sprayer equipment at the same place, year after year, high pesticide residue concentrations have been detected [6,7].

A biobed is a *in situ* biological reactor system, developed in Sweden during the 1990's and nowadays implemented in several European countries, that adsorbs and degrades pesticides in order to minimize environmental contamination [8-10]. Biobed is a simple and low cost construction, designed to retain and degrade pesticides from spills on farms [6]. Due to its easy installation and use, it has also become an important environmental safety tool for Latin American countries [11].

The original Swedish biobed, whose substrate was replicated for this experiment, is an unlined system consisting of three layers: clay, biomixture and grass. Clay is used as an impermeable layer to decrease the water flow downward and to increase the pesticide retention time. Biomixture should have a good absorption capacity and a high microbial activity and it comprises straw, soil and peat in the ratio of 50:25:25 (w/w/w) [8]. Grass layer increases the efficiency of the reactor, retaining part of the pesticides, controlling the leaching of those with high mobility and regulating the system humidity [8,12]. The purpose of straw is to stimulate growth of white rot fungi

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(Phanerochaete chrysosporium) that degrade lignin through the production of ligninolytic enzymes, mainly phenoloxidase enzymes that have a high specificity and are thereby able to degrade a wide range of pesticides residues [10,13,14]. Soil provides sorption and should contain an appropriate amount of humus and clay to promote microbial activity [8,15]. Peat contributes to the sorption capacity, moisture control and abiotic pesticide degradation. It also decreases the biomixture's pH, favoring microbial activity [8,16].

Since the biomixture matrix used in the reactor is highly complex and it is usually based on materials found locally, it is necessary to have analysis methods suitable for each environment. In addition, the effectiveness of the pesticide degradation process in biobeds requires highly sensitive and validated analytical methods [11].

Chlorpyrifos is an organophosphate insecticide [17]. It was introduced in the United States in 1965 by The Dow Chemical Company and it is known by several trade names, including Lorsban®. Lorsban® is one of the most used products for insect control worldwide [18,19]. Since its introduction into Brazil in 1972, Lorsban® has shown to be an important pest control agent, used for 36 plagues in 13 different crops [20,21]. This pesticide acts as an inhibitor of enzymes (e.g., cholinesterase) causing cholinergic syndrome and thus neurotoxic effect [22].

It is known that due to biological degradation occurring in the substrate of the biobed, chlorpyrifos will present some by-products (3,5,6-trichloro-2-pyridinol (TCP), 3,5,6-trichloro-2-methoxypyridine (TMP), O-ethyl O-(3,5,6-trichloro-2-pyridinol) (CYPO), which may have an inhibitory effect on the microbial activity inside the reactor [23,24]. From these, 3,5,6-trichloro-2-pyridinol (TCP) is considered one of the most important due to its antimicrobial characteristics, given the risk of affecting microorganisms populations that act on the biodegradation process in the system. However, there are no evidences that such metabolites were able to impede the efficiency of the system, even when applying chlorpyrifos alone or in combination to other pesticides [25-28].

The analytical methods for chlorpyrifos determination in biobeds are based on extraction with organic solvents. Most methods require > 30 mL of solvent, which generates large amounts of residue [29-32]. Other methods use less volume of organic solvents but are time-consuming [25,33].

The aim of this study was to optimize and validate an efficient and rapid analytical method to determine chlorpyrifos in a biobed system developed in southern Brazil [1,14]. The degradation of chlorpyrifos was studied in biobeds involving two situations: 1) simulating contamination by accidental pesticide spillage and 2) contamination with a diluted solution coming from the washing of agricultural machines used to apply pesticides. Those biobeds systems used in this study were assembled at the Embrapa Grape & Wine. Method optimization and validation, as well as biobeds sample analysis were performed at the Center of Research and Analysis of Residues and Contaminants (CEPARC) at the Federal University of Santa Maria (UFSM) - Brazil.

2. Materials and methods

In Brazil, studies concerning biobeds/bioreactor usage for pesticide residue management are relatively new and just very few analysis methodologies for monitoring the reactors developed for Brazilian conditions had been based on gas chromatography [7,14].

2.1. Biobed system

Field studies were conducted at the Embrapa Grape & Wine (southern Brazil), from December 2013 to February 2015 [1,34].

The reactors were constructed according to the Swedish model with a biomixture of straw, soil and peat at 50:25:25 (w/w/w) [6].

In order to evaluate chlorpyrifos degradation, nine reactors were constructed: five contained biomixture, usually made of wheat straw, peat and soil at 50:25:25 (w/w/w) (reactors 1 to 5), and four contained two subtropical soils, Typic Haploperox and Ultisol, representative of apple orchard regions were sampled at Campo Belo do Sul city (Santa Catarina State) and Vacaria city (Rio Grande do Sul State) in Brazil (reactors 6 to 9). Reactor 1 was not contaminated (blank reactor). Reactors 2 and 3 were contaminated with 1.0 and 0.5 L, respectively, of the commercial product Lorsban® (480 g L^{-1} chlorpyrifos) in order to simulate an accidental spillage. Reactors 4 to 9 received a diluted solution of Lorsban® (250 mL/100 L) originated from washing cleaning of agricultural machinery, according to Table 1.

The experiment was performed in duplicate. Sampling was done according to Table 2.

Composite samples of each individual reactor (~3 kg) were collected in 8–10 different points, extracted as a material column from zero to 20 cm depth in a grid pattern and then homogenized. These homogenized samples were stored in plastic bags and frozen (-20 °C) to avoid pesticide degradation. An amount (30–50 g) of biomixture and soils were separated for Chlorpyrifos chemical analysis.

Table 2
Sampling schedule applied for chlorpyrifos determination from biobeds samples after contamination with Lorsban®.

Times of sampling	Month and year of sampling	Time of contamination (months)
T0	December 2013	No contamination
T1	February 2014	2
T2	April 2014	4
T3	July 2014	7
T4	August 2014	8
T5	February 2015	14

Table 1
Reactors contaminated with Lorsban® (250 mL/100 L) including date of application and pesticide amount applied.

Application Date	Reactors/ Duplicate/ Volume Lorsban® (L)											
	4 Biomixture		5 Biomixture		6 Latosol		7 Latosol		8 Nitosol		9 Nitosol	
	A	B	A	B	A	B	A	B	A	B	A	B
16/12/13	50	50	25	25	50	50	25	25	50	50	25	25
07/03/14	20	20	10	10	20	20	10	10	20	20	10	10
10/03/14	20	20	10	10	20	20	10	10	20	20	10	10
17/03/14	20	20	10	10	20	20	10	10	20	20	10	10

2.2. Chemicals and reagents

Acetone, methanol, and toluene, all pesticide grade, and phosphoric acid 85.0% were obtained from Mallinckrodt (Phillipsburg, NL, USA); ammonium formate (99.0%) was from Fluka Analytical (Seelze, Germany) and acetic acid 99.9% from J. T. Baker (Center Valley, PA, USA). Reference standards of chlorpyrifos (98.5%), propoxur (99.5%) and quinalphos (99.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

2.3. Analytical solutions

Analytical stock solutions of chlorpyrifos as well as the internal standards (Quinalphos and Propoxur) were prepared at 1000 mg L^{-1} in toluene. Each compound was individually weighed directly into 20 mL glass flask and immediately dissolved in the appropriate solvent considering its purity and stability. Finally, the solutions were placed in an ultrasonic bath for 5 min to obtain complete dissolution.

Analytical stock solution of chlorpyrifos was diluted to 100 and 10 mg L^{-1}. These solutions were used to obtain analytical curves (0.1, 0.5, 1.0, 5.0, 20.0, 100.0 and 250.0 ng mL^{-1}) and for each spike concentration studied. Analytical solutions were stored in freezer at −20 °C. Before use, all solutions were removed from the freezer and allowed to stand until room temperature was reached. Subsequently, the solutions were placed in an ultrasonic bath for 5 min to obtain complete dissolution.

In this study, two internal standards were used. Quinalphos, as procedure internal standard (P.I.S), was added to the extraction solvent (acetone/water/phosphoric acid, 98:1:1, v/v/v) at 500 ng mL^{-1}. A solution of the instrument internal standard (I.I.S.) (Propoxur at 40 ng mL^{-1}) was prepared in methanol and used to redissolve the evaporated residue extract before UPLC-MS/MS analysis.

2.4. UPLC-MS/MS experimental conditions

The chromatographic system consisted of an ACQUITY UPLC coupled to a XEVO TQ-S tandem MS from Waters (Milford, MA, USA).

Chromatographic separations were carried out using a BEH C18 column (2.1 mm i.d. × 100 mm, 1.7 μm particle size), maintained at 40 °C. The UPLC mobile phases consisted of 0.3 g L^{-1} ammonium formate solution (solvent A) and methanol (solvent B). The following gradient elution (0.45 mL min^{-1} flow rate) was performed: 90% A at the injection time, decreasing linearly to 0% A over 7.75 min. This eluent composition was maintained for 0.75 min and then increased to 90% A. This condition was maintained until the end of the chromatographic run.

The MS was operated in the positive electrospray ionization mode (ESI^+). Two ion transitions were monitored in the multiple reaction monitoring (MRM) mode: for chlorpyrifos (350.1 > 97.0 for quantification; 350.1 > 197.9 for confirmation), propoxur (210.1 > 110.9 for quantification; 210.1 > 92.9 for confirmation) and quinalphos (299.0 > 243.0 for quantification; 299.0 > 271.0 for confirmation).

Preliminary tuning experiments were carried out via direct infusion of diluted standard solution (chlorpyrifos at 200 ng mL^{-1}) into the MS in order to establish the optimal determination conditions. The infusion flow rate was maintained at 5 μL min^{-1}.

2.5. Optimization of extraction parameters

Based on the method proposed by Racke et al. [33], the optimization experiments were performed in triplicate at 10 mg kg^{-1}.

In order to perform the experiments including the validation study, blank samples from reactor 1 were used.

The following three experiments were performed to assess whether the efficiency of the extraction process could be improved. The goal of the first experiment (Experiment 1) was to evaluate the efficiency of mechanical shaking for 2 h followed by ultrasonication for 30 min, as proposed by Racke et al [33]. Experiment 2 evaluated the efficiency of homogenization. For this, mechanical shaking for 2 h was exchanged by 1 min of homogenization using ultra-turrax (Polytron PT/MR 3100 Model, Kinematica - Switzerland) and keeping the use of ultrasonication. Finally, experiment 3 evaluated the efficiency of ultrasonication (frequency of 48 KHz and intensity of 55 W) after 10 and 20 min, as well as samples not subjected to ultrasonication step.

2.5.1. Optimized extraction method

A homogenized biobed sample (5 g) of an analytical test portion was transferred to a 250 mL polypropylene centrifuge tube. Subsequently, 30 mL of the extraction solvent acetone/water/phosphoric acid (98:1:1, v/v/v) containing the P.I.S. (Quinalphos at 500 ng mL^{-1}) was added and the mixture was vigorously dispersed by using an ultra-turrax (15000 rpm) for 1 min. The tubes were centrifuged at 7800 g for 10 min. After centrifugation, an aliquot of 2 mL was taken and filtered using a 0.2 μm PTFE membrane. Finally, 125 μL of the filtered extract was evaporated at 50 °C and 450 mBar until dryness (7 min) and redissolved in 5 mL of methanol acidified with 0.1% acetic acid containing the I.I.S. (Propoxur at 40 ng mL^{-1}). The final extract was injected into the UPLC-MS/MS system.

2.6. Validation study

The optimized method was evaluated in order to prove its fit for purpose. The validation procedure was carried out by assessing accuracy (recovery), precision (RSD), LOD and LOQ, dynamic linear range (r^2) and matrix effect according to SANTE [35].

2.6.1. Linearity

Linearity of analytical curves was evaluated by comparing the solutions prepared in neat solvent with those prepared in the blank biomixture extract at seven concentrations: 0.1, 0.5, 1.0, 5.0, 20.0, 100.0 and 250.0 ng mL^{-1}.

2.6.2. Detection and quantification limits

The LOQ_m is defined as the lowest concentration at which the tested analyte (in this case chlorpyrifos) can be reliably detected, with recovery and RSD values in accordance with acceptable accuracy (i.e. recovery results between 70 and 120%) and precision (i.e. RSD less than 20%).

Data obtained from the linearity study were employed to calculate LOD_i, LOD_m, LOQ_i and LOQ_m. Whilst the LOQ calculation is based on data obtained from analytical curve results, the validated LOQ_m was defined as the lowest spiked concentration of Lorsban® that showed acceptable accuracy (i.e. recovery results between 70 and 120%) and precision (i.e. RSD ≤ 20%).

2.6.3. Matrix effect

The matrix effect was evaluated by comparing the analytical curve slopes obtained from solutions prepared in organic solvent with those prepared in blank matrix extract, as can be seen in equation (1).

$$Matrixeffect\,(\%) = \left[\left(\frac{Slopeanalyticalcurvestdinmatrix}{Slopeanalyticalcurvestdinsolvent}\right) - 1\right] x100 \quad (1)$$

2.6.4. Accuracy and precision

In order to evaluate accuracy and precision seven blank biomixture samples were spiked at 2, 10 and 50 mg kg^{-1} (n = 7) and submitted to the extraction procedure for posterior UPLC-MS/MS determination.

Intermediate precision was studied to evaluate the analytical method repeatability. Two different analysts performed recovery experiments on two different days. For each analyst, the average recovery of seven replicates (for each spike level), was calculated. Subsequently,

Table 3
Average recovery and RSD values obtained from experiments 1, 2 and 3 (n = 3) for spiking procedure with chlorpyrifos at 10 mg kg^{-1}.

	Experiment 1 (mechanical shaking)	Experiment 2 (ultra-turrax)	Experiment 3 (ultrasound)		
			0 min	10 min	20 min
Recovery (%)	42.9	99.2	97.9	103.1	107.6
RSD (%)	2.1	1.7	2.8	4.6	0.3

the averages of the results from two analysts, at each spike concentration and RSD, were calculated.

2.7. Sample analysis

Diluted solutions of Lorsban® were applied to biobed systems with different compositions: biomixture, latosol and nitosol.

Contaminated samples from reactors 2 to 9 were analyzed using the same analytical procedure described in section 2.5.1.

3. Results and discussion

3.1. Extraction procedure

Based on the proposed method of Racke et al. [33], biobed samples were spiked at 10 mg kg^{-1} and submitted to three separate experiments as described in section 2.5.

Recovery values obtained from extraction procedure using mechanical shaking for 2 h (experiment 1) were below 70% (42.9%). In the experiment 2, which used homogenization for 1 min, a considerable improvement in the recovery value (99.2%) was observed. Moreover, the total analysis time was considerably shortened. Recovery results from experiment 3 (0, 10 and 20 min ultrasound) showed no considerable difference compared with those from experiment 2. Thus, it was decided not to include this step in subsequent procedures. The RSD values were less than 20% for all experiments. Table 3 shows the recovery and RSD values obtained for those three experiments.

3.2. Method validation

3.2.1. Linearity

The method linearity was evaluated using analytical solutions (n = 7) prepared in organic solvent and blank matrix extract. The analytical curve equations obtained were y = 6759 × – 969 and y = 6767 × – 2255, respectively.

The determination coefficient (r^2) numerically expresses the percentage of total analytical signal variation due to the analyte concentration variation. The determination coefficient (r^2) values were satisfactory for standards solutions in solvent (0.990) and in the biobed extract (0.995), demonstrating that the method is linear in the range studied (0.1 to 250.0 ng mL^{-1}). Grubbs test applied to these results showed that there were no outlier values in the seven replicates [36].

3.2.2. Detection and quantification limits

There are limited literature studies that present LOQ_m values related to chlorpyrifos determination in biobeds although Fait et al. [30] and Vischetti et al. [31] reported satisfactory LOQ_m results; however, both procedures used large volume of solvent, 100 mL and 80 mL, respectively. Such excessive use of solvent generates high passive waste volumes and it becomes an expensive analysis.

The estimated LOD_i, LOD_m, LOQ_i and LOQ_m values were calculated using data obtained from the linearity study, for chlorpyrifos pesticide in organic solvent and matrix extract. The results were 0.1 ng mL^{-1}, 0.02 mg kg^{-1}, 0.3 ng mL^{-1} and 0.07 mg kg^{-1}, respectively for LOD_i, LOD_m, LOQ_i and LOQ_m, either in organic solvent as in matrix extract.

Table 4
Recovery and RSD values for samples spiked with chlorpyrifos at three different concentrations and intermediate precision (RSD) of chlorpyrifos recoveries obtained on different days by different analysts.

Spiked concentration (mg kg^{-1})	Recovery average % (n = 7) / RSD%		RSD (%) intermediate precision
	Analist 1	Analist 2	
2	115/8.5	118/6.5	6.6
10	100/3.2	106/4.6	3.7
50	96/5.1	101/4.7	3.8

The validated LOQ_m of 2 mg kg^{-1} for chlorpyrifos was determined by the proposed method (Table 4).

3.2.3. Matrix effect

The matrix effect is observed as an increase or decrease in the detector response for a particular analyte present in the matrix extract compared to the detector response for the same analyte in organic solvent [37]. Considering UPLC-MS/MS analysis, this effect is usually caused by interference of matrix components eluting near or at the same retention time of the analyte and competing with it during the ionization process. At low analyte concentrations, the matrix effect can become important, because there is a reduction on the analyte's ionization due to its low concentration in the matrix [38]. According to SANTE [35] if matrix effects exceeds ± 20%, a different quantitation approach should be applied in order to compensate for signal suppression/enhancement. The matrix effect values for chlorpyrifos at 0.1, 0.5, 1.0, 5.0, 20.0, 100.0 and 250.0 ng mL^{-1} concentration were 15, 14, 7, 4, −3, −1 and 0, respectively. Thus, it can be concluded that the proposed extraction method did not influence the chromatographic signal for chlorpyrifos.

3.2.4. Accuracy and precision

The recovery of chlorpyrifos was conducted on blank biobed samples spiked at 2, 10 and 50 mg kg^{-1} concentrations. The recovery values obtained were between 96 and 118% with RSD values lower than ± 20%, for all three concentrations studied, as shown in Table 4. The acceptance criterion for fortification tests requires recovery values within the range of 70 to 120% with RSD values below 20% [35]. The results of this study are therefore consistent with these requirements.

In Fig. 1, the MRM chromatograms for chlorpyrifos (in the ESI positive mode) are shown for standard solution prepared in blank matrix extract and in organic solvent at 250 ng mL^{-1} and blank biobed sample fortified at 2, 10 and 50 mg kg^{-1}. Besides, from the LOQ_m value reported, the good sensitivity of this method can be seen from the similar, high responses at 2 mg kg^{-1} spike level, compared with the chromatogram at 50 mg kg^{-1} spike level.

In the intermediate precision experiment, RSD values results were between 3.7 and 6.6% indicating no considerable difference between the experiments irrespective of the analyst and day of sample analysis, under otherwise identical conditions. Results are shown in Table 4.

A number of methods for chlorpyrifos determination have been proposed and satisfactory values of recovery (percentage) and RSD reported [15,25,29,32]. However, these methods require time-consuming extractions and are usually costly, making the processes unsuitable for routine analysis. Fogg et al. [29] proposed a method for chlorpyrifos determination using 1 h of shaking and the method developed by Omirou et al. [32] used a longer shaking time of 90 min. Coppola et al. [25] and Fernández-Alberti et al. [15] used a similar extraction solvent volume to that used in the current method (30 mL of acetone). However, Coppola et al. [25] applied overnight mechanical shaking to the samples and Fernández-Alberti et al. [15] exposed samples to 2 h mechanical shaking plus an additional 30 min of ultrasonication.

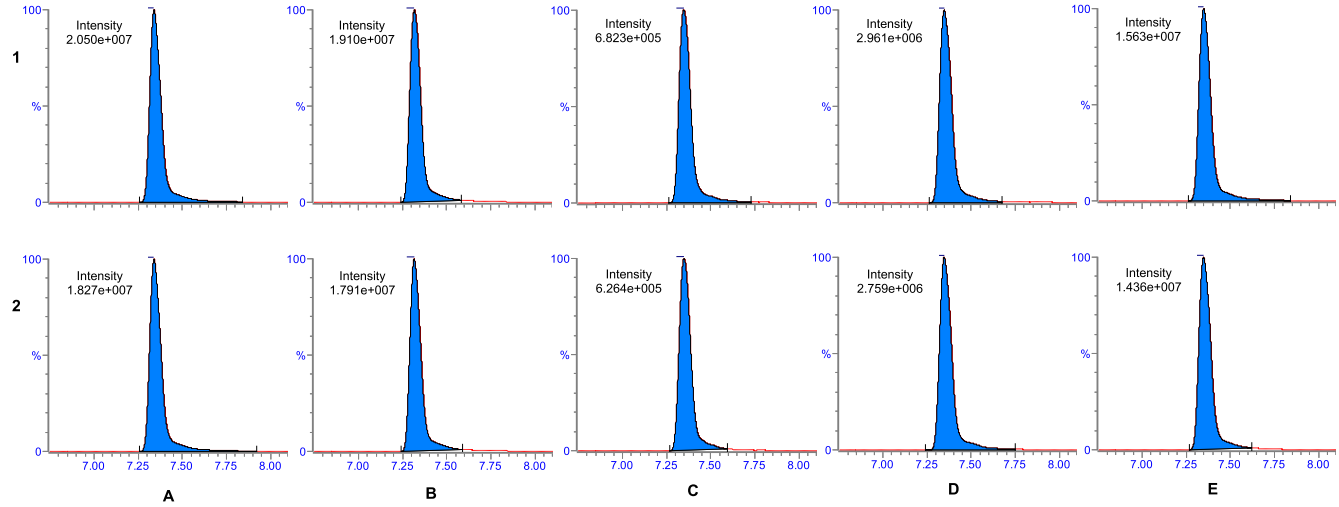

Fig. 1. MRM chromatograms for chlorpyrifos (retention time: 7.34 min): (1) quantification transition (350.1 > 97.0) and (2) confirmation transition (350.1 > 197.9) obtained by UPLC-MS/MS ESI positive ionization mode for (A) standard solution prepared in blank matrix extract and (B) in organic solvent at 250 ng mL^{-1}; blank biobed sample fortified at (C) 2 mg kg^{-1} (D)10 mg kg^{-1} and (E) 50 mg kg^{-1}.

3.3. Chlorpyrifos degradation in biobed systems

Results of sample analysis (n = 3 for each sample) are shown in Table 5.

For 2A, 2B and 3B reactors, at sampling times T1 and T5, chlorpyrifos concentrations decreased, demonstrating that degradation of the pesticide occurred at some stage between its application and the final sampling (February 2015). Considering all sampling times, outlier values (in italics) of chlorpyrifos concentration were detected: T2 to T4 for reactor 2A and 2B reactor. Given the samples were obtained in the field, the sampling procedure may have influenced chlorpyrifos concentrations found. Moreover, dry biobed reactors cannot guarantee a homogeneous mixture. It means that clods of soil (or peat) may be present in the biomixture (straw, soil and peat) of the biobed. Thus, at any given time of the sampling procedure, samples containing clods with low or zero chlorpyrifos concentration may have been collected, while at other times, samples may have contained high concentrations of chlorpyrifos.

Interestingly, chlorpyrifos concentration for samples from reactor 3A did not decrease over sampling time. It is believed that samples from this reactor may have contained a clod of peat (or soil) with a large amount of pesticide adsorbed during the accidental spillage simulation. Indeed, failure to completely homogenized peat or soil could produce clods that would retain the pesticide to various extents, influencing both sampling and pesticide degradation processes. This could explain the inconsistent results found among the sampling times and between duplicates. Regarding the RSD values (n = 7 for each sample analyzed), 90% of them were in accordance with the recommended limit. The use of biobeds in Brazil is relatively new [11]. Thus, it is important to consider that the biobed construction and sampling process may require further adjustments [1].

. As shown in Table 6 some outlier values of Lorsban® concentration for all three biobed compositions were observed and values between duplicates were inconsistent. Even so, degradation of chlorpyrifos was successful, since in T5 (final sampling time) all concentration's values obtained were below the LOQ_m (2 mg kg^{-1}).

Table 5
Chlorpyrifos concentration in the biobed at five different sampling times after simulation of accidental spillage with Lorsban®.

Reactor	Chrorpyrifos concentration (mg kg^{-1})/RSD%				
	T1	T2	T3	T4	T5
2A	370.0/6.5	*572.5/2.0*	234.6/6.8	143.4/7.8	47.6/19.7
2B	552.7/4.3	446.2/5.3	425.2/14.3	*498.7/3.9*	15.9/20.2
3A	403.3/2.8	163.6/5.7	274.4/8.0	< LOQm/16.2	347.7/9.0
3B	468.2/11.7	468.4/2.8	446.1/6.4	215.7/25.6	43.5/6.9

Validated LOQm: Method quantification limit (2 mg kg^{-1})

4. Conclusions

In Brazil, the use of bioreactors for pesticide waste management is relatively new and there are scarce published studies on the determination of pesticide residues developed for local conditions, and the existing ones were focused on the use of gas chromatography. This new analysis methodology, using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS), presents advantages in relation to the reduction of the analysis time and the volume of the reagents involved. The creation of new analytical technologies also meets the concept that the introduction of biobed technology in a new country requires some adaptation of its composition and structure to specific local environmental needs.

The analytical method proposed in this study was validated for chlorpyrifos determination in biobeds by UPLC-MS/MS. The validated LOQ_m was 2 mg kg^{-1} and the matrix effect was lower than ± 20%, which demonstrates that there was no considerable enhancement or suppression of chlorpyrifos chromatographic signal. Furthermore, this method uses a low solvent extraction volume compared to the majority of existing methods for biobeds or similar matrices. Homogenization of the samples prior to analysis produced excellent results on recovery experiments and clearly shortened the time required for sample analysis. Recovery values between 96 and 118% and RSD values lower than 20% were obtained for samples spiked with each of those three chlorpyrifos concentrations studied.

The biobed system was efficient in degrading chlorpyrifos in both of the two experimental conditions: 1) one that simulated an accidental spillage of the pesticide and 2) one in which diluted solutions of Lorsban® from agricultural machine washings were applied to the biobed.

There is not Maximum Residue Limit (MRL) established in Brazil for pesticide residues in biobeds as well as no monitoring data available. This reality shows the importance of the current study towards

Table 6
Chlorpyrifos concentration obtained at five different sampling times after application of a diluted solution of Lorsban® (250 mL/100 L), for three biobed compositions: biomixture (reactors 4 and 5), latosol (reactors 6 and 7) and nitosol (reactors 8 and 9).

Reactors	Chrorpyrifos Concentration (mg kg^{-1})/RSD%				
	T1	T2	T3	T4	T5
4A	4.9/16.0	2.4/16.7	< LOQm /48.6	< LOQm /8.0	< LOQm /9.4
4B	3.4/19.0	< LOQm /41.3	2.3/19.4	3.5/8.8	< LOQm /17.3
5A	2.8/6.0	< LOQm /5.8	< LOQm /46.7	< LOQm /12.1	< LOQm /52.3
5B	< LOQm/42.6	< LOQm /17.4	< LOQm /21.1	< LOQm /36.3	< LOQm /32.0
6A	2.5/12.4	< LOQm /8.3	< LOQm /16.6	< LOQm /7.9	< LOQm /7.5
6B	2.0/8.9	< LOQm /9.6	< LOQm /20.3	< LOQm 10.4	< LOQm /27.1
7A	4.7/9.6	2.8/5.3	2.4/17.0	< LOQm /0.8	< LOQm /19.9
7B	3.9/18.7	2.9/2.6	< LOQm/15.1	2.2/12.2	< LOQm/9.9
8A	< LOQm/13.7	2.1/9.6	< LOQm/48.3	44.2/1.0	< LOQm/0.0
8B	5.9/17.9	< LOQm/36.2	< LOQm/33.7	< LOQm/8.4	< LOQm/13.9
9A	2.3/13.6	3.5/24.8	< LOQm/17.1	< LOQm/17.3	< LOQm/31.5
9B	< LOQm/6.3	< LOQm/5.0	< LOQm/19.9	< LOQm/5.3	< LOQm/34.6

Validated LOQm: Method quantification limit (2 mg kg^{-1})

developing qualified analytical methods for pesticides determination and to contributing to biobed implementation in Brazil, at the farm level.

CRediT authorship contribution statement

Gustavo D. Quatrin: Conceptualization, Methodology, Writing - original draft. **Ionara R. Pizzutti:** Conceptualization, Funding acquisition, Methodology, Supervision. **Luciano Gebler:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - review & editing. **Jonatan V. Dias:** Methodology, Writing - original draft. **Carmem D. Cardoso:** Conceptualization, Writing - original draft, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2020.122285.

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