

## Bioreactors to Organize the Disposal of Phytosanitary Effluents of Brazilian Apple Production

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Safe disposal of pesticide waste is a concern that has been studied in recent years. Disposal through traditional means, such as incineration, it's too expensive to farmers. Inexpensive and safe methods have been developed in European countries, and being spread around the world. In Brazil, experiments with the use of bioreactors for the management of pesticide waste started four years ago, seeking to evaluate efficiency of systems for safety disposal of pesticide effluents, its recommendation for official use by the Brazilian government with international recognition, and besides its efficiency as a safety system to the environment, rural workers and food products from Brazil. Results obtained in this period indicate that, under Brazilian environmental conditions, some structural changes in relation to biobed are required, if we consider European standard, for maintaining similar efficiency in the system. In addition, it is necessary to continue the project to increase the number of pesticides evaluated by the system and substrate options, especially different types of straw and new sources of organic matter, replacing peat. The conclusions of these experiments are that the bioreactors in Brazil must be deeper (at least 1.0 m deep), compared to similar in Europe (0.5 m), ensuring moisture in the sample profile, but without excess of injury heat and lack aeration. In addition, they must have coverage, preferably translucent, to prevent rain water accumulation inside, but allowing the planting of grasses to keep evapotranspiration, and finally, use the European standard substrate, with composition of two wheat straw parts, one part of agricultural land and one part of peat, until new tests.

### 1. The problem of pesticides in Brazil

Pesticides are the greatest environmental contaminants involved in the agricultural process and, in some regions of the world, one of the most important concerns. Currently, due to its large agricultural area and the tropical and subtropical conditions, which greatly favors the occurrence of pests and diseases, Brazil is one of the world's largest consumers of pesticides. Despite the negative aspects of these products, it is not possible to maintain current standards of quality and quantity of food without giving up its use.

Some studies indicate that over 80% of products applied to cropping systems is lost in the diffuse form, ends up in the environment, resulting in source of contamination to air, soil and water. At this stage of work, diluted pesticide is applied in water and the active ingredient is located in the order of grams or kilograms per hectare (Castillo et al., 2008). This pattern receives safety approval from Brazilian ministries responsible for their authorization.

By the other hand, point sources of contamination, arising from the repetitive handling of pesticide, at sprayers handling pads, and the residue coming from the washing of the equipment after use, have increased the risk of contamination, and, in many cases, have turned into an equally point or more damaging than the diffuse contamination of the area. Point sources of contamination can be achieved polluting load values in the order of pounds per square decimeter (or tons per hectare), failing to provide safe handling (Wenneker et al. 2008).

Since much of the water used in urban water's supply comes from basins located in rural areas, vulnerable to contact with the issue of this waste, the concern under the risk of contamination grows every day. Moreover, the cost of the treatment process to ensure consumption of water to consumers, originated from sources contaminated by pesticides, can be very high, especially when there are legal requirements involved (Fogg et al., 2004).

### **1.1 Disposal of pesticide effluents**

Brazilian law provides that the residue of pesticides, not being mixed with other chemicals, must have proper disposal according to the manufacturer's labelling. In this case, it is observed that, both in legislation and in the label, the proper disposal indicates incineration under the responsibility of manufacturers. In Brazil, this disposal practice is acceptable in urban and industrial areas, where the use and disposal are close, but in agricultural areas, it's technically impossible to medium and small farmers, because distance between pesticides use sites and urban centres that exceed several hundred km.

In addition, incineration is only applied to the product that is pure commercial form, with no recommendations about the way the remains pesticide mixed with water found in the spray tank, the result of washing the application equipment or spills in refill point. Therefore, except for the pure commercial products (seized by justice or period of validity expired), Brazilian law allows specific interpretations which lack scientific basis.

Seeking solutions to similar problems of pesticide residue disposal, many countries preferred to make use of good environmental practices or agricultural processes, with ultimate goal of maximizing the mitigation actions based on natural attenuation (Grenni et al., 2012) or bioremediation (Monaci, et al., 2009). This line of action was taken to be technically and economically feasible within the farm, reducing the potential costs and reducing or eliminating the need to resort to industrial processes to address these problems (Coppola et al., 2011). Working with bioreactors in the form of biobeds, fulfil these premises have shown over time in countries where the system has been tested and has been adopted by a large number of them (Castillo et al., 2008).

Based on these experiences, we come running sequential research projects in Brazil in order to evaluate the validity of using different bioreactors substrates, comparing relative to the direct disposal on agricultural land as a final repository, assessing their capacity for retention and disposal of pesticides and residues. The aim of these projects is obtain an official recommendation of the Brazilian governmental bureaus with international recognition as a safe disposal system for the environment, rural workers and food products from Brazil.

### **1.2 Environmental certification for Brazilian fruit trees and bioreactors**

Since Europe has been one of the main markets of fresh produce from Brazil and many other countries use standards based on European directives, validation and appropriation of environmental control systems recognized in the European community could be the way to avoid any non-tariff barriers, to ensure food safety to consumers and to control ecological risks.

With the approval of the new environmental law (Brazilian Forestall Code), the disposition of pesticide's remains and residues has returned to the agenda of debates in Brazilian society, linked to discussions involving the management area of chemical risks to water resources (urban supply), foods (consumers) and rural worker.

The need to prove that the production cycle of fresh food in Brazil presents are ecotoxicologically safe led the implementation of research proposal in bioreactors, capable of performing the treatment of pesticide effluents still under rural property, ensuring full respect for the environment, especially water resources, and rural worker. Biobed is one example of these bioreactors, composed by a mix of straw, agricultural soil and peat, inside a ditch, that receives all contaminants to biodegrade.

## **2. Evolution of research bioreactors for using in fruit growing**

Based on the need of the product's certification to the final consumer, it was necessary to establish a new line of researching for bioreactors substrates tests, because the investigation of degradation and environmental fate of pesticides in Brazil was linked to the residue found on agricultural land and in water.

Since work began with apple crop, was necessary to establish the products to be considered as models, allowing exploratory study of ecotoxicological dynamics of pesticides at the point of supply sprayers, places considered high environmental risk.

Pesticides selected for the initial phase were chlorpyrifos, insecticide of high toxicity also used in the production of apples, and glufosinate-ammonium, herbicide used in plant sprouting control phase. The objective was to assess, under Brazilian conditions, expected behavior differences from the literature, based primarily on the correlation ever recorded between chemical degradation of chlorpyrifos and the behavior of bio-indicators (Roffignac et al., 2008).

After the completion of the initial tests, we started working with other products, seeking to cover the maximum possible conditions in order to meet the government demand that the technology will be safe to the environment. Thus, the research has chosen mancozeb fungicide and insecticide phosmet, as representative pesticides applied on apple crop, but in its still very initial results.

Forecasting of the research line is to cover pesticides that fit at least the following conditions:

- a) Provide high resilience rating in the environment or as residues in food;
- b) Used in large volumes in apple orchards;
- c) Residual with high environmental toxicity;
- d) Provide low  $K_{ow}$  values and high water solubility.

If the pesticides fulfill at least two of these parameters, it will become a candidate for environmental degradation and dissipation tests in bioreactors.

## 2.1. Applied methodology

The methodology used in the studies varied according to the need of results, with the initial stage as exploratory and the following, like to management recommendation of the technology in the field.

### 2.1.1. Exploratory stage

At this stage, was executed the first research project (CNPq project number 471758/2011-6) and is finalizing the second (FAPERGS project number 081612-1/ARD 03/2012), formatted with complementary experiments with each other, which formed the basis for the default setting of the experiments of the following research phases.

In the first approach (CNPq), we tried to get answers about the following: a) the necessary depth to the bioreactors in Brazil, compared to the Swedish model (Castillo et al., 2008), taking into account some key differentiators such as the obligation of the reactors bottom waterproofing and here present a rainfall almost double in relation to original condition; b) The best substrates for use in the bioreactor in relation to a predominant agricultural soil in local apple orchards; c) The ecotoxicological potential behavior of different pesticides and their mixtures in different substrates and depths.

In the second study approach (FAPERGS), the proposed objective was to compare the substrate chosen previously with two different agricultural soils present in different areas of production apple orchards. Representative soils were collected of the two national centres of production, Vacaria (Rio Grande do Sul State) and São Joaquim (Santa Catarina State), far between about 200 km.

The field activity was based on a structure mounted outdoors to evaluate the effects of the local environment (temperature and humidity), made up of microcosm bioreactors, comprising two crates 3x9 space (27 cells), each with a polyvinyl chloride tube (150 mm diameter) filled with a substrate for analysis, without free drainage and 1.5 m height, ready about 30 d before beginning the experiment.

These 27 cells were arranged in blocks of 3x3 (9 cells each), with the following settings: S (orchard of soil of up to 10 cm deep); SS (soil mix – 50 %, and peach shredded pruning's composted for 3 months – 50 %); STP (standard Swedish system with wheat straw – 50 %, peat – 25 % and orchard soil – 25 %), each tested in height from 0 to 50 cm (top); 50 to 100 cm (middle) and 100 to 150 cm (reactor bottom).

On each block was applied in a single dose, a contaminant load of the following pure commercial pesticides without dilution, as an example of an accidental spill: A) 10 mL of insecticide chlorpyrifos 480 g L<sup>-1</sup> of a.i.; B) 50 mL of herbicide glufosinate-ammonium 200 g L<sup>-1</sup> of a.i.; C) 50 mL of pesticide mixture (40 mL of glufosinate-ammonium and 10 mL of chlorpyrifos).

Initial sampling was performed 48 hours after the contamination of the bioreactor with the pesticide. Other evaluations were carried out at intervals of 90 days between each sample, always performing the analysis for each of the three test depths.

Samples were taken from each third of each reactor cell, analyzing the estimation of microbial activity through fluorescein diacetate hydrolysis method - FDA (Tortella et al., 2010), the safety of the substrate was assessed using ecotoxicological tests, to acute and repellence behaviour, with earthworms (*Eisenia fetida*) and phytotoxic tests (*Lactuca sativa*) (Roffignac et al., 2008).

In the second project, the activity was also mounted outdoors, to assess the effects of the local environment (temperature and humidity) in the action of microorganisms, consisting of 310 L bioreactors, with drain at the bottom, forming two rows of reactors filled with soil or substrate (Monaci et al, 2009).

Treatments were arranged in blocks with two units with four collection points within each bioreactor, designated as "STP", standard Swedish system, with wheat straw (50 %), peat (25 %) and orchard soil (25 %), and bioreactors with soil (100 %) for comparison, being "L" for Oxisol and "N" for Nitosol, predominant soils in apple production areas of Southern Brazil.

In the first two blocks filled with substrate, the contaminant were applied in a single dose, a mixture of the commercial pesticides, insecticides chlorpyrifos one liter of 480 g L<sup>-1</sup> and 5 pounds fungicide mancozeb 800 g L<sup>-1</sup>. The first block received a loading of 50 L of water so that there was the initial moving the contaminant

profile in the bioreactor; while the second block received half of the hydraulic load 25 L of water. The purpose of these bioreactors was to establish an accidental spillage, the worst environmental situation, and compare it with the other blocks with substrate, which only received continued contamination. It was not planned to compare these two blocks with the behavior of pesticides in the agricultural soil block.

The remaining six bioreactors received effluent continuously made every orchard pesticide treatment, using agricultural machinery and implements washings, applying 50 L of effluent into each bioreactor of blocks 4, 6 and 8, and applied 25 L effluent within blocks 5, 7 and 9.

Laboratory tests involving the estimation of microbial activity by fluorescein diacetate hydrolysis, safety substrate with standardized ecotoxicological tests to chronic and reproduction (ISO protocols) of two types of worms (*Eisenia fetida* and *Enchytraeus crypticus*) and collembola (*Folsomia candida*) for residues of contaminant were carried out. Also, being made chemical degradation testing of pesticides in soil and substrate in analytical laboratory.

### **2.1.2. Definition of effluents and waste management recommendation step**

Currently, a stage was initiated to establish the technical recommendations for the construction and operation of bioreactors, particularly directed to the method for the evaluation of chemical degradation of waste, since there is already basic definition of substrate and sizing to be recommended to Brazil. Initially, it was proposed to adapt the extraction methodology and chemical analysis of the liquid phase effluent (Effluent input and output) and solid phase (Substrate) system, derived from the analysis method used in Sweden.

For analysis of the liquid phase of Phosmet, the initial methodology will follow the proposition Portolés et al. (2014), being adapted in the following experiment, whereas for the solid phase, the initial approach will be based on Fernandez-Alberti et al. (2012). Samples will be analyzed in UPLC-MS / MS system (Ultra Liquid Chromatography coupled to Mass Spectrometry).

Tests for Mancozeb methodology have two options for subsequent adoption which have better technical and economic results. Methodology adaptations for effluent analysis Mancozeb were obtained from scientific papers studying the surface residues of washing fruits and pesticide presence in the soil, different from the occurrence of direct effluent (high contaminating load) and substrate with high levels of organic matter.

Therefore, we intend to test variations of the method for analysis of atomic absorption spectrometry (Alves et al., 2013) and UV-VIS spectrometry (Rosa and Marques, 2011).

In both cases, the sensitive step involves the extraction of the analyzed element method. In atomic absorption spectroscopy the analysis target is manganese (Mn), whereas in the UV-VIS spectroscopy is carbon disulfide (CS<sub>2</sub>).

Also, the basic monitoring will be maintained by estimating microbial activity through the fluorescein diacetate hydrolysis method, justified by the behavior of economy and biomarker correlation with the existing bibliography.

## **3. Obtained and expected results**

### **3.1 Results obtained in the exploratory phase**

Results obtained in the projects that made up the exploratory phase were aimed at getting answers on the microbial reaction capacity of substrates in relation to agricultural soil and analyze the influence of depth in relation to the climate of the place. Basically these results have been achieved in the first project, which is confirmed in the second, aggregating constructive suggestions derived from adverse weather events.

In the first project (CNPq), the analysis of orchard soil data in relation to both composts, showed no significant difference between them in relation to pH, but there were differences in relation to the retained moisture. Bioreactors filled with the standard mixture (straw wheat, peat and agricultural soil), were those that retained more moisture throughout the experiment, followed by agricultural soil and alternative substrate, which did not differ from each other.

In addition, microbial activity in SPT substrate always had a higher value, usually associated with efficiency, with positive statistical difference compared to other tested conditions, especially when there was the case of tests with pesticide mixtures, a situation that emulates the bioreactor in real activity.

As the depth, since the moisture had greater constancy in the bottom of the microcosm, it showed positive microbial activity statistical difference in the lower third of the tubes, suggesting that at least one meter would be more efficient than shallow reactors, as suggested by Sniegowski et al. (2011), recommending that in Brazil, due to intense evaporation, the effect of reduced air in depth would be offset by the maintenance of substrate moisture.

Tests with *Eisenia fetida* demonstrated that the worms strongly reacted to initially contaminants, with high repellency or mortality, but over time they arrived to give preference to contaminant material instead of the material-free.

*Lactuca sativa* phytotoxicity tests showed no significant differences for the tested pesticides and were discarded until the existence of tests with new herbicides.

In the second experiment (Fapergs), the tests were expanded to larger bioreactors, in meso scale, emulating the field conditions. In this case, the behavior has not been tested in depth but comparison the substrate chosen and two representative agricultural soils. The ecotoxicological tests, which are being finalized, have already pointed different behavior trend between meso and microorganisms in relation to the type of substrate. In the case of microorganisms, in the bioreactors containing the substrate, there was an average of 1 scale reactivity of magnitude related more with reactors containing the two types of soil. In this case, there was no significant difference between the types of soil. Although in tests with meso organisms, using populations of *Enchytraeus crypticus* and *Folsomia candida*, was possible to observe chronic effect in *F. candida* two months after the last contamination of bioreactors. Indeed, it's possible consider this population like extreme sensitivity to chlorpyrifos, demonstrating the need for joint chemical analysis.

In bioreactors with substrate that receiving continued contamination, the population of *Enchytraeus crypticus* showed a common behavior of degradation pesticide curve, with very low rate of reproduction in that time with highest application (ten weeks, once per week, without intervals) and larger reproduction after two months lacking application, while in the bioreactors with soils (L and N) the results are different.

In "L", the recovery after two months lacking application isn't enough for increase the rate of reproduction, since there was some mortality of populations in both times (highest and lower application). On the other hand, in "N", with lower application, the rate of reproduction was bigger than in the first moment.

However, population reproduction in both soils remains far below the values obtained in the blank tests. It's reinforces the assumption that to handle the effluent of pesticides on the substrate is more security than make this direct in the soil.

Nevertheless, into the bioreactors with substrate where was used single contamination that simulated an accidental spill, meso organisms behavior has confirmed that this is a critical situation, with total mortality and no reproduction in all samples, different from the estimate of microbial activity. This can be possible due the fact that recovery of microbiota is faster than meso organisms.

The tests with *Eisenia andrei* on this stage of work are starting and showed no further results.

Structurally, during the test period there were times that the bioreactors eventually flooded by heavy rains, demonstrating the necessity of a coverage system in tropical and subtropical conditions, even the bioreactors were built with soil insulation, no free drainage, as similar rainy weather situations in England, described by Fogg et al. (2004), regarding the requirement of environmental safety. Once the reactors with 0.5 m depth showed that there were impacts on microbiota in its substrate when flooded, there was no negative effect on reactors of 1.5 meters, which kept the moisture well distributed throughout the reactor profile, without prejudice to the microorganisms.

Even so, there has been progress in constructive recommendation of bioreactors, suggesting that they should follow the English model, with the separation of the pesticides management pads of the sites of bioreactors, separating rainwater to prevent flooding and system shutdown.

Regarding chemical analysis of pesticides, laboratory tests are still in the preparation phase and sample extraction, as the substrate which composes the biobeds is not commonly utilized in Brazil. The first evaluations involving chemical extraction tests using the standard method (Portolés et al., 2014) showed 61.3 % recovery in two replicates. By changing the method, replacing two hours of agitation in the table, for 1 minute at ultraturrax, the average recovery of analytes increased to 99.75 %. There are steps to be tested on this extraction stage prior to the completion of the process of residues' quantification.

### **3.2 Expected results for the recommendation phase**

For the recommendation phase that begins, the establishment of minimum conformation to be recommended to the end user (the farmer) is expected, and the continuity of new pesticide molecules degradation tests, alone and mixed with other substances that may be part the effluent in order to strengthen confidence in the pesticide wastewater treatment system based on bioreactors.

It is also expected to obtain the approval of the Brazilian government to adoption or official recommendation of safe waste environment disposal system and rural workers, avoiding future problems of contamination in Brazilian agricultural areas, negative effects at water resources and trade problems arising from environmental issues related to pesticides.

## **4. Conclusions**

Brazilian's bioreactors must be deeper (at least 1.0 meter depth) compared to that proposed in Europe (0.5 meter), ensuring the moisture profile, without be damaged by the heating, flooding or lack of aeration.

Bioreactors should provide coverage, preferably translucent, to prevent rain water accumulation inside, and allowing the planting of grasses to keep the evapotranspiration.

Substrate recommended for current use should be the same of the Swedish standard, with composition of 50 % wheat straw, 25 % of agricultural soil and 25 % peat.

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### References

- Alves M.R.R., Torres M.C.L., Soares N.F.F., Melo N.R., Geraldine R.M., Mizubuti E.S.G., Silveira M.F.A., 2010, Efeito de soluções de enxágue na remoção de resíduos de mancozebe em tomates de mesa. *Pesquisa Agropecuária Tropical*, 40, 96-101.
- Castillo M.D.P., Torstensson L., Stenström J., 2008, Biobeds for environmental protection from pesticide use – a review. *Journal of Agriculture and Food Chemistry*, 56, 6206-6219.
- Coppola L., Castillo M.D.P., Vischetti C., 2011, Degradation of isopropuron and bentazone in peat- and compost-based biomixtures, *Pest Management Science*, 67, 107-113.
- Fernandez-Alberti S., Rubilar O., Tortella G.R., Diez M. C., 2012, Chlorpyrifos degradation in a Biomix: Effect of pre-incubation and water holding capacity, *Journal of Soil Sci. Plant Nutr.*, 12, 785-799.
- Fogg P., Boxall A.B.A., Walker A., Jukes A., 2004, Leaching pesticides from biobeds: effect of biobed depth and water loading, *Journal of Agricultural and Food chemistry*, 52, 6217-6227.
- Grenni P., Falconi P., Barra Caracciolo, A., Microcosm experiments for evaluating natural biorremediation of contaminated ecosystems, *Chemical Engineering Transactions*, 28, 7-12.
- Monaci E., Coppola L., Casucci C., Perucci P., Vischetti C., 2009, Retention capacity of an organic bio-mixture against different mixtures of fungicides used in vineyards, *Journal of environmental Science and Health Part B*, 44, 724-729.
- Portolés T., Mol J.G.J., Sancho J.V., Hernández F., 2014, Use of electron ionization and atmospheric pressure chemical ionization in gas chromatography coupled to time-of-flight mass spectrometry for screening and identification of organic pollutants in waters, *Journal of Chromatography A*, 1339, 145 – 153.
- Roffignac de L., Cattan P., Mailloux J., Herzog D., Bellec F.L., 2008, Efficiency of a bagasse substrate in a biological bed system for the degradation of glyphosate, malathion and lambda-cyhalotrin under tropical climate conditions, *Pest Management Science*, 64, 1303-1313.
- Rosa A.C.P., Marques M.R.C., 2011, Metodologia para preservação do fungicida mancozebe em amostras de solo, *Química Nova*, 34, 1639-1642.
- Sniegowski K., Bers K., Van Goetem K., Ryckeboer J., Jaeken P., Spanoghe P., Springael D., 2011, Improvement of pesticide mineralization in on-farm biopurification system by bioaugmentation with pesticide-primed soil, *FEMS Microbiology Ecology*, 76, 64-73.
- Tortella G.R., Rubilar O., Cea M., Wulff C., Martinez O., Diez M.C., 2010, Bioestimulation of agricultural biobeds with NPK fertilizer on chlorpyrifos degradation to avoid soil and water contamination, *Journal of soil science and plant nutrition*, 10, 464-475.
- Wenneker M., Beltman W.H., De Werd H.A.E., Van De Zande J.C., 2008, Identification and quantification of point sources of surface water contamination in fruit culture in the Netherlands, *Aspects of Applied Biology*, 84, 369-375.