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Biodegradation of the Ametryne in Soil with Addition of Biofertilizer

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Abstract

The intensified use of pesticides and chemical fertilizers has resulted in threats to the environment. Thus it is crucial to the implementation of sustainable alternatives in order to mitigate the negative effects. The biofertilizer brings this proposal, as it improves soil fertility and minimize environmental risks. The ametryne herbicide is used for weed control, very soluble in water, bringing risks to terrestrial and aquatic ecosystem. Thus, the objective of this work was to study the degradation of ametryne in soil with the addition of liquid biofertilizers. After application of biofertilizer in soil with ametryne, the microbial activity increased in 39.149%, in CO_2 generation. In the assay for quantification of ametryne by HPLC-MS/MS there was reduction of concentration of the molecule in the presence soil

sample contaminated with the herbicide. Thus, the biofertilizer was able to assist in soil fertility, increasing metabolic activity, resulting in degradation of the herbicide.

Keywords: Biodegradation, Ametryne, Biofertilizer

1. Introduction

The ametryne belongs to the s-triazines group, is a selective herbicide for weed control in the pre and post emergency period (Duarte et al., 2009). It operates in the inhibition of electron transport in chloroplasts, resulting in phytotoxicological effects even at low concentrations (Sandoval-Carrasco et al., 2013).

This herbicide has low affinity for soil colloids, since it has a low coefficient of sorption and high solubility in water, become a threat to the aquatic ecosystem. The ametryne is soluble in water, a persistent compound and bioaccumulative in the environment, harming terrestrial and aquatic ecosystem. The triazine herbicides are considered one of the main pollutants due to its widespread use and toxicity to the environment. (Farré et al., 2002; Kasozi et al., 2012; Navaratna et al., 2012).

The main environmental pathways of contamination by pesticides (Banderi et al., 2012), are intensive application rates in cultures or accidental spillage (Lima et al., 2009). In order to bring changes in the crop, makes necessary the use of products with maximum yield from natural crops, contributing to pest control, stimulation of plant growth (Pešaković et al., 2013) and mineral enrichment in poor soil phosphorus and potassium (Andrade et al., 2013).

The biofertilizer has properties fermentation of organic compounds, or latent living cells of microorganisms and inorganic nutrients. These products have an intense microbial activity capable of protecting crops against pests and diseases, as well as increase the degree of bioavailability of nutrients to the plants through biological processes (Alfa et al., 2014).

Thus, the biofertilizer helps to increase microbiota in enhancing the photosynthesis process, inhibiting plant pathogens such as plant growth stimulators and detoxification of heavy metals. In studies with strawberry cultivation the biofertilizer, significantly improved fruit length of recommending the replacement of chemical fertilizer for use of biofertilizer, as it improves the financial cost and also the consumption of safe products to health and the environment (Pešaković et al., 2013).

Thus, the biofertilizer rich compound is beneficial microorganisms into the environment and nutrients, which can be used in organic cultures (Wu et al., 2005), through biological processes, resulting in improved soil quality (Jilani et al., 2007).

This study aimed to evaluate the biodegradation of ametryne in soil with addition of biofertilizer by analyzing CO_2 generation during the incubation period, but also to evaluate the presence of decreased ametryne by quantifying the molecule by chromatography HPLC-MS/MS.

2. Materials and Method

The biofertilizer MICROGEO® (patent # PI0207342 A2-0) was donated by Microbiol Biotechnology, in the Brazil. This biofertilizer consists of organic compounds, active and dormant cells of bacteria, fungi, algae and yeasts ((D'andrea, 2002).

The soil sample was collected in sugarcane growing area with application history, 20 cm deep, according to standard CETESB (1984). Below (Table 1) described the physicochemical characteristics of the soil.

2.1 Respirometry Test of Bartha & Pramer

The respirometry test was used to evaluate the generation of CO_2 in soil ametryne increase and addition of biofertilizer, 31 days incubation. We used the technical standard L6.350 (CETESB, 1990). Table 1 describes the compositions contained in each system respirometers.

Table 1. Respirometric assays composition

Systems	Composition
S1	50.0 g soil
S2	50.0 g soil+ ametryne (45.00 mg/kg) + biofertilizer (1% m/m)
S3	50.0 g soil + ametryne (45.00 mg/kg)
S4	50.0 g soil + biofertilizer (1% m/m)

The respirometric method Bartha & Pramer (1965), was analysed by generation of CO_2 from microbial respiration. The assay was described Régo et al. (2014). Readings of CO_2 values produced were measuring using standard solution of hydrochloric acid (HCl), solution of potassium hydroxide (KOH), solution barium chloride ($BaCl_2$) and phenolphthalein indicator.

2.2 Statistical Analyzes

For testing respirometry Bartha & Pramer was held analyses Friedman statistics, ORIGIN software 8.0 considered $p < 0.05$.

2.3 Kinetic Models

To evaluate the kinetics of biodegradation of ametryne was presented models adapted by Schmidt et al. (1985), to describe the maximum amount of accumulated CO_2 respirometry test expected in equation (1):

$$B = Bmax/(1 + \left[\frac{Bmax-Bo}{Bo}\right] e^{-rt}) \quad (1)$$

where B is the CO_2 produced, Bmax is the maximum amount of CO_2 produced, Bo is the initial CO_2 produced, r is the specific maximum production rate for each ametryne, t is the period in which the biodegradation occurs.

2.4 Quantitative Analyses

The assay was based on the methods in Analytical Methods for Pesticides Residues in Foodstuffs (2006) and Guidance Document the Pesticide Manual Residue on Analytical

methods (2010). The limits of detection were 0.15 mg/kg and the quantification limit was 0.3 mg/kg. The equipment used was HPLC-MS/MS API 2000.

3. Results

3.1 Assessment of Microbial Activity in Soil Contaminated by Ametryne and with Added Biofertilizer

The Figure 1 shows the amount of CO_2 accumulated in soil sample application ametryne addition of biofertilizer and analysed for 31 days and incubated at 28 C.

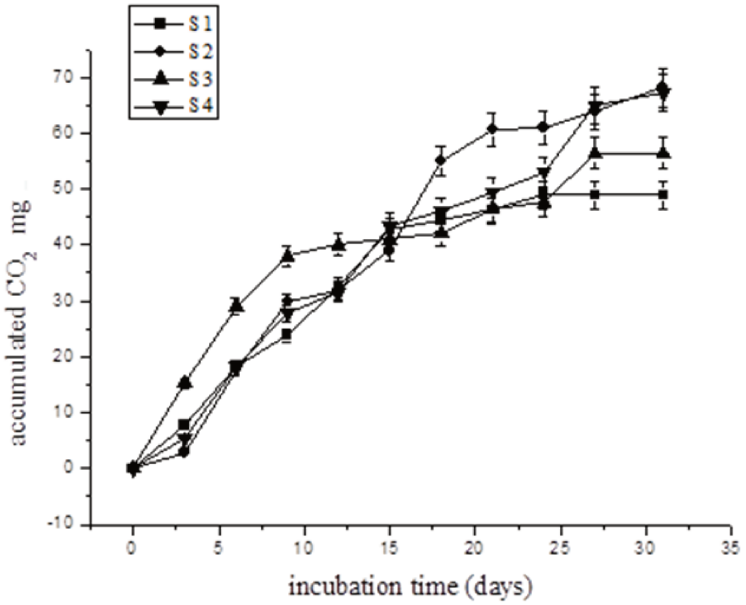

Figure 1. Generation of CO_2 accumulated for 31 test days and incubated at 28 ° C

The system (S4) with soil and biofertilizer, was the most CO_2 accumulated during the test, since it has a carbon source easily assimilated to soil microorganisms.

Contaminating the soil with the herbicide, CO_2 accumulation was lower compared to systems with addition of biofertilizer. After 15 days of testing, the system (S2) resulted in increased microbial activity even in the presence of ametryne. This was probably due to addition of biofertilizer. The ametryne probably have toxicity to soil microorganisms, presenting lower microbial activity (S3).

The system (S4) with soil and biofertilizer was obtained more accumulation of CO_2, with 43.571 mg. This occurred until the middle of the experiment, due to the nutritional character that has biofertilizer. Thus, it constitutes as a way of biostimulation of indigenous microbiota.

From the fifteenth day of incubation, the system (S2) with soil and biofertilizer ametryne generated more than 50.000 mg, exceeding the system (S4) with soil and fertilizer. This is due to the consumption of carbon source present in biofertilizers, ending with constant accumulation of CO_2.

The soil control (S1), which had ametryne application history accumulated less CO_2, with 48.981 mg over time relative to other systems.

However, the system with addition of soil and ametryne (S3) produced more CO_2 compared to control soil (S1). This soil had already ametryne load over time, making the indigenous microbiota adapted to the presence of the herbicide. By adding more ametryne, was the carbon source in the system increase, stimulating the metabolic activity of soil microorganisms.

By adding biofertilizer to the soil by applying ametryne, it is observed that there was a greater accumulation of CO_2 in the soil control and soil with added ametryne. This is due to higher source of bioavailable carbon for microbial metabolism. As the biofertilizer has nutrients and microorganisms in their composition, probably he was able to assist in the metabolism of organic compounds, with ametryne.

After 25 test days, CO_2 accumulation stabilizes, because the carbon source has been consumed, leaving the microbial biomass.

The Figure 2 shows the mathematical modelling of the test systems used respirometry Bartha & Pramer in order to simulate real systems predicting their behaviour over time.

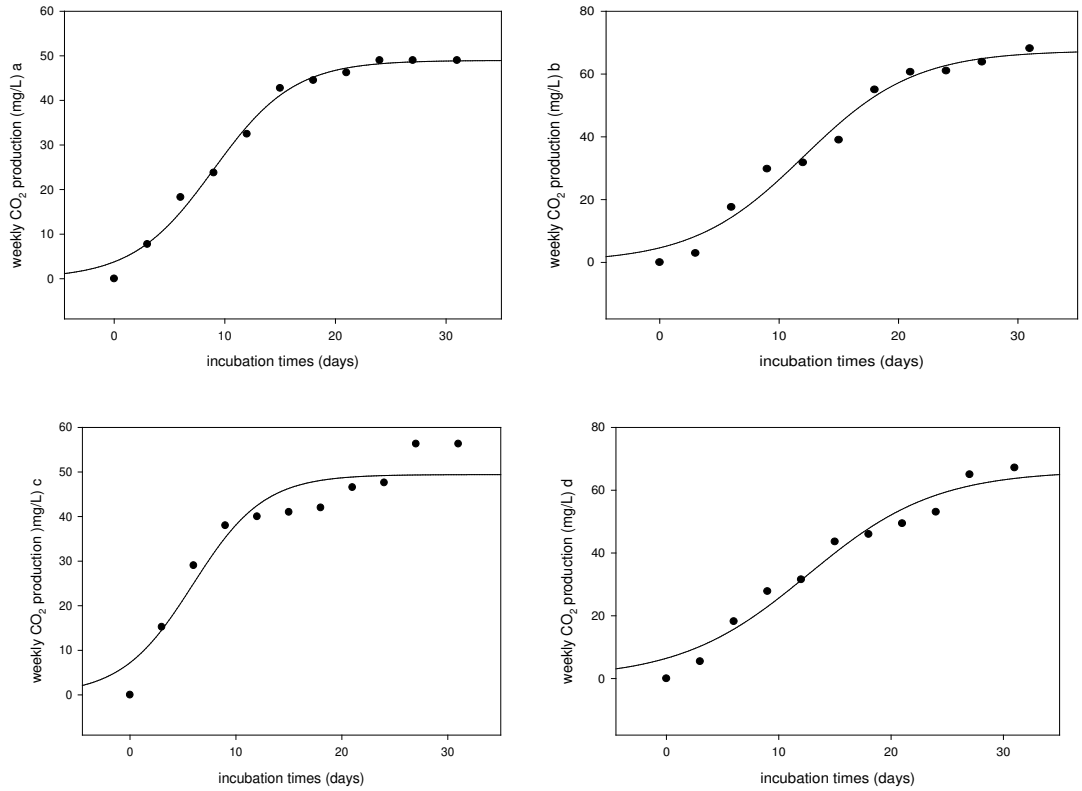

Figure 2. Chart of accumulated CO_2 generation, plotted second model of Schmidt et al. (1985) and adapted by Montagnolli et al. (2009), in systems containing control soil (a) soil with ametryne increase and fertilizer (b) soil with ametryne increase (c) and soil addition of biofertilizer (d)

It can be seen that the time of 31 day incubation period was sufficient to accumulate the CO_2 from the consumption of organic matter during the metabolic activity of the microorganisms.

In systems with soil and adding ametryne (c) and control soil (a) the CO_2 accumulation of settling time was lower compared to systems with biofertilizers increments. However, in systems with added ametryne and biofertilizer (S2) and soil biofertilizer (S4) the amount of CO_2 accumulated was greater, because the biofertilizer has easily absorbed organic substrates for microbial metabolism.

Before and after the test respirometry Bartha & Pramer the quantification of ametryne the soil matrix was performed. In the system with soil and ametryne was 41.03 mg/kg and the system with soil contaminated with ametryne and addition of biofertilizer was 34.74 mg/kg. Thus, after 31 days of incubation of the soil, the herbicide has suffered biodegradation. The application of biofertilizers, enhanced the biodegradation of ametryne. Therefore, the addition of biofertilizer helped ametryne degradation in the soil, decreasing its persistence in the environment, favouring the aquatic and terrestrial ecosystem, ametryne since the molecule is very soluble in water.

4. Discussion

In the system with control soil (S1), the CO_2 accumulation was lower compared to other systems, resulting in 48.981 mg, since it had low amount of carbon source available to the indigenous microbiota. It is known that the presence of persistent compounds in the environment may cause changes in the amount of nutrients available to the terrestrial microorganisms. This can worsen when organic matter is scarce and the nutritional carbon source is unavailable will microbial metabolism (Saha, 2012).

However, the control soil sample (S1) presented ametryne application history for the control of weeds in sugar cane crop, and during the test there was no addition of the herbicide. Thus, the molecule of ametryne is persistent in the environment and slow degradation (Lovecka et al., 2015), and may remain in the soil for many years (Shegunova et al., 2007).

By adding ametryne the soil (S3), the CO_2 accumulation was higher in the control soil (S1). This is due to the soil with application ametryne history, and this probably has a specific microorganisms able to consume the molecule ametryne. Thus the herbicide served as carbon source for the local microflora (Zabaloy et al., 2008).

Probably there was a recovery of the indigenous microbial population in response to increasing the supply of nutrients arising from bacterial decomposition, resulting in the development of species resistant to the herbicide treatments (Gómez et al., 2014; Moreno et al., 2007).

It can be seen that when added to the soil biofertilizer with the addition of ametryne (S2), the CO_2 concentration increased, totalling 68.157 mg after 31 days of incubation, by passing the system with addition of soil and biofertilizer (S4) with 67.149 mg, in a total of 39.149 % increase compared to control soil. This is due to the biostimulation and bioaugmentation produced by chemical and biological composition of biofertilizer (Pešaković et al., 2013).

The biodegradation of ametryne can be assessed by soil microbial respiration, as the quantification of CO_2 production. Thus, can to determine the optimal application rate of an herbicide. And evaluated application rates and nutrient concentration (Fiúza & Vila, 2004; Miles & Doucette, 2001; Montagnolli et al., 2009; Wu et al., 2004).

Adding biofertilizer, the amount of ametryne present in the soil decreased significantly, from 41.03 mg/kg in soil with ametryne to 34.74 mg/kg in soil with ametryne and biofertilizer. Thus, the organic substrate reduced the toxic effect of ametryne to the biological properties of the soil (Gomez et al., 2014).

The system S2 with soil and fertilizer ametryne, it can be seen that with the nutritional composition rich biofertilizer has meant that the enzymatic activity of the indigenous microflora to be stimulated and passed to metabolize possible degrading the soil, resulting in increased bacteria and fungi (Gomez et al., 2014), checked in increased generation of CO_2.

Therefore, the introduction of alien species of microbes in conjunction with indigenous species to the contaminated soil, causes the environmental impacts caused by the presence of ametryne is reduced (Adesemoye & Kloepper, 2009).

Thus, biofertilizer as organic compounds are rich in nutrients and microorganisms, are promising to be used integrated with other agricultural products, in order to alleviate environmental problems, since they have low financial cost (Pešaković et al., 2013; Hole et al., 2005).

5. Conclusion

The application of ametryne on the soil impaired microbiota metabolic activity, resulting in a low accumulation of CO_2 throughout the incubation period. The addition of biofertilizer to the soil contaminated with ametryne favoured the accumulation of CO_2, increasing microbial activity.

During the incubation period, the addition of biofertilizer assisted in biodegradation ametryne metabolizing molecule in the soil. Therefore, the addition of biofertilizer in soil with ametryne of application history, was essential to mitigate the degrading effect by caused the application of herbicides in the environment.

It is suggested the application of biofertilizer along with ametryne in soil contaminated by ametryne, in order to soften the aggressive and negative effect, due to the presence of molecule, promoting of the quality to terrestrial ecosystem.

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Conflicts of interest

There are no conflicts of interest in this research.

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