MINERALIZATION OF THE HERBICIDE ^{14}C-PROPANIL IN SOIL BY *Pseudomonas putida* AF7

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ABSTRACT

Many pesticides are persistent in soil environmental and the persistence depends on the dissipation processes. The herbicide propanil has been used extensively for the selective control of broad-leaved weeds in rice fields. Its widespread use has caused environmental concern on the basis of frequent detection in surface water and groundwater. In this work, it is described the isolation of rhizobacteria from rice roots and the investigation of the mineralization of ^{14}C-propanil in soil. Extensive biodegradation of propanil by a strain of *Pseudomonas putida* AF7 was demonstrated in nutrient cultures. The organism was capable of using propanil as the sole source of C, presenting up to 60% of degradation. Ring cleavage was confirmed in $^{14}CO_2$ evolution experiments. Between 17 and 19% of ring – ^{14}C was mineralized to $^{14}CO_2$ in natural soil and in soil inoculated with the bacteria, respectively. The formation of extractable metabolites of propanil were detected in natural soil (18%) and in soil supplemented with *P. putida* this value was of 20%. On the other band, bound residues were formed in higher amount in natural soil, while in soil with *P. putida* was a little smaller. These results suggest that it may be possible to develop practical systems based on the use of this bacteria to detoxify propanil-contaminated soil.

Keywords: Biodegradation, Propanil, *Pseudomonas*, Mineralization, Bound Residues.

1. INTRODUCTION

Propanil (3', 4' – dicloropropionanilide) is a herbicide extensively used around the world for control of weed in rice productions. Propanil and its metabolites are transported within characteristic ditch ecosystems in the production landscape of southeast Brazil. In soil, biodegradation of propanil liberates 3,4-dicloroaniline, which is converted by microbial peroxidades to 3'3,4,4'-tetrachloroazobenzene (TCAB) and other azo products. The TCAB may accumulate in rice-growing soils, and leach into groundwater. Studies have shown continued degradation into TCAB and TCAOB. There is great concem regarding TCAB and TCAOB because of embriolethal, teratogenic, and possible genotoxic effects shown in toxicity tests [1].
Repeated applications of herbicides are sometimes required during crop rotation and these may promote an increase in soil microorganisms able to attack a particular herbicide, a process known as enhanced degradation. Few studies on propanil degradation, its transport in soils and plants have been performed in tropical soils. The objectives of this study were to: i) quantify ^{14}C labeled propanil mineralization, and; ii) determine bound and extractable residues in soil samples

2. MATERIAL AND METHODS

2.1 Mineralization of ^{14}C Propamil

The biodegradation of the herbicide propanil was evaluated in soil, supplemented with ^{14}C–propanil diluted in technical grade propanil and inoculated with a strain of *Pseudomonas putida* AF7. This strain was isolated from the rhizosphere of rice grown in contamination soil with this herbicide, in southeast of Brazil.
Technical grade ^{14}C-ring labeled propanil, tested and confirmed to have a radiochemical purity of 98% and specific activity of 32.4 KBq.mg was obtained from Sigma Chemical Company. The supplementation solution was prepared using both labeled and unlabeled chemical.
Fifty grams (dry weight) of soil (four replicates) was placed into biometric flasks [2]. ^{14}C-propanil was added at final concentration of 7.43 KBq and 0.525mg.mL^{-1}. Vials containing solution of 0.2 KOH were placed in each flask to trap CO_2 evolved during the incubation period. The solution of KOH was collected after 1, 3, 5, 7, 14, 21 and 28 days.

2.2 Extractable and Bound Residues

Soluble residues were extracted from dried milled soil (50g) using methanol. The supernatants of each sample were combined and the radioactivity was measured in a liquid scintillation counter.
The initial radioactivity and remaining after the extracted residues was determined by combustion of 500 mg (dry weight) soil samples in a Biological Oxidizer – OX 600 and the total CO_2 evolved was trapped in a monoethanolamine solution.

3. RESULTS AND DISCUSSION

In laboratory tests *P. putida* AF7 was able to degrade over 50% of propanil when evaluated by HPLC. At the same time, this bacteria produced high amount of indole acetic acid. When inoculated In seeds of rice, the strain promoted the plant growth and colonized the root system. The process of colonization of root was monitoring using a field emission scanning electron microscopy (Zeis + Leica) (Figure 1).
The $^{14}CO_2$ data presented enable comparison of the rates of propanil mineralization in non-sterilized soil. Mineralization of propanil was high within 14 days. After 14 days of the incubation the mineralization was reduced (Figure 2), suggesting an accelerated degradation in the early period.
The $^{14}CO_2$ production from ^{14}C-propanil supplemented into the soil was 18.67% in natural soil infested with the bacteria and 17.02% in soil without the bacteria (Figure 3).

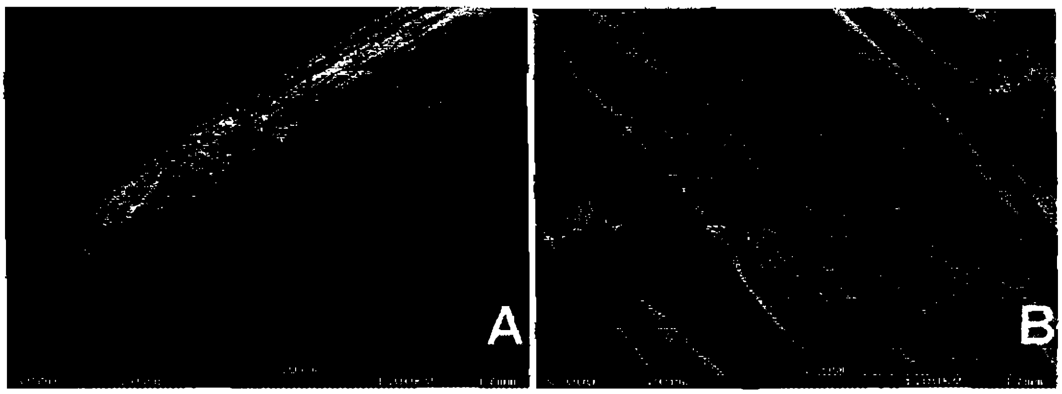

Figure 1. Colonization of rice roots by *Pseudomonas putida* AF7, viewed by a field emission electron microscopy. The images show the bacteria colonizing the root hair of rice.

a) General of the root tip, where the bacteria was observed
b) Colonization of Pseudomonas cells in the root hair. Image shows the attachment of the bacteria and biofilm formation.

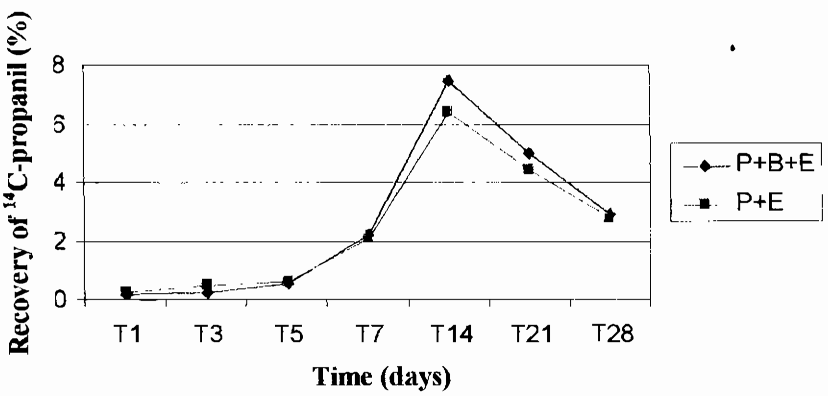

Figure 2. Evolution of ^{14}C from ^{14}C-propanil in soil inoculated with *Pseudomonas putida.*

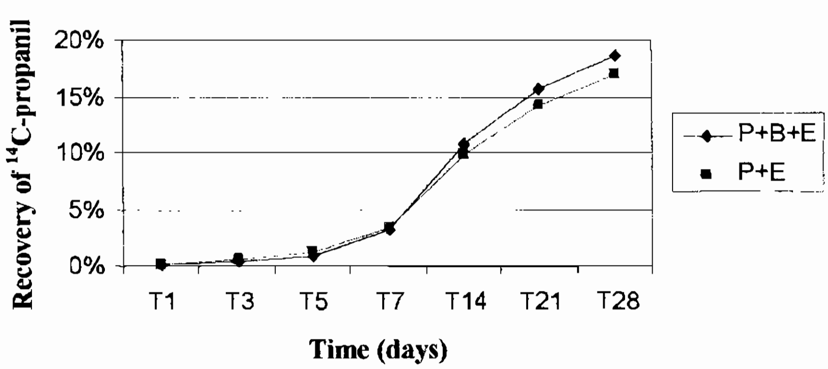

Figure 3. Evolution of $^{14}CO_2$ accumulated from ^{14}C-propanil in soil inoculated with *Pseudomonas putida.*

The values obtained from ^{14}C extractable and bound residues are presented in Figures 4 and 5. In 24 hours after incubation was possible observe the production of $^{14}CO_2$ (0.035%) and, immediately after 48 hours, the production of CO_2 presented an increase of about 10 times, suggesting the start of an accelerated degradation.
The production of $^{14}CO_2$ accumulated in soil as bound residues was of 2.77% after 28 days of incubation. Thus, the biodisponibility of residues was of 8.34%, fraction of the molecule that may be degraded by the microorganisms.

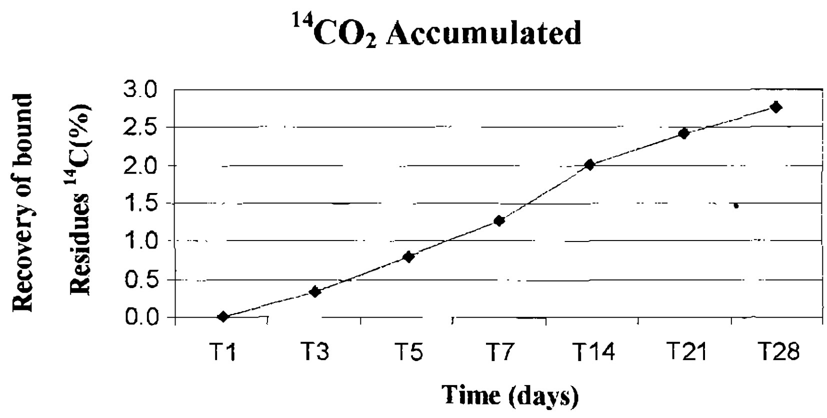

Figure 4. Evolution of $^{14}CO_2$ of bound residues using *Pseudomonas putida* AF7.

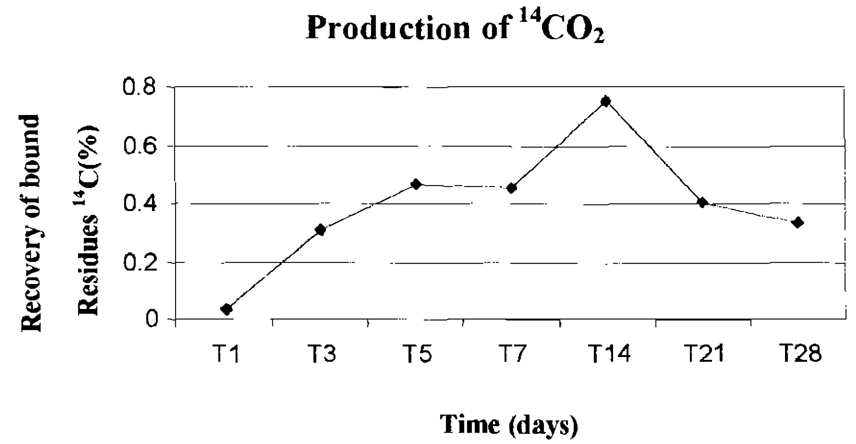

Figure 5. Evolution of $^{14}CO_2$ from ^{14}C of bound residues in soil inoculated with *Pseudomonas putida*.

4. CONCLUSION

The mineralization of propanil was higher in soil inoculated with *Pseudomonas putida* than in soil without the bacteria. The bound residues of this herbicide were of approximately 2%. This work shows the potential of *Pseudomonas putida* for bioremediation of contaminated soils with pesticides. This bacterial strain also promotes the plant growth.

REFERENCES

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