BIODEGRADATION OF PESTICIDE: BROMUCONAZOL BY MICROBIAL CONSORTIUM IN BIPHASIC SYSTEM

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Abstract: The bromuconazol is a fungicide which is toxic for the environment. This present study was led with the aim of testing, in vitro, its biodegradation.
The bromuconazol was thus used in the presence of a microbial consortium resulting from a treated soil. A biphasic system MBS / oil of silicone was used during all the period of acclimatization. The fermentation was followed by measure of the optical density, the determination of the dry weight, the emulsifying power as well as the hydrophobicity of the selected consortium.
The results obtained after one year revealed a strong adaptation of this one to the bromuconazol. The use of the biphasic system allowed a better assimilation of the pesticide. The microbiological study allowed identifying a single bacterial strain capable of using the fungicide as unique source of carbon. It is about *Aeromonas hydrophila*. This strain shows a strong hydrophobicity as well as an emulsifying power lived in saw some pesticide.

Keywords: fungicide, Biodegradation, Bromuconazol, biphasic system, Batchs, microbial Consortium. *Aeromonas hydrophila*

INTRODUCTION

The intensification of agriculture has been accompanied by an extensive use of pesticides that generated a contamination of soil and water, major environmental problem of current. This situation is particularly worrying that the use of pesticides should be repeated periodically. This repetition in the long term, necessarily leads to an accumulation of pesticides and their residues in our natural environment, endangering the entire population by their multifaceted toxicity (Bouziani, 2007). The most frequent studies concern the presence of these molecules in the soil (Senesi, 1993), water contamination, biotic and abiotic degradation of these products (Muller et al. 1978; Bollag, 1982), and finally the identification of residues that apparently seem to have a relationship with them (Calderbank, 1989).

The pesticide to which we are interested in this study is bromuconazol, a fungicide widely used around the world. It is a systemic fungicide activity. It belongs to the family of triazoles which are inhibitors of sterol biosynthesis. It is intended to protect cereal crops against fungal diseases. Its target is a cytochrome P450 enzyme encoded by the gene CYP51 (Robbertsee and *al*., 2001). Bromuconazol is an environmental contaminant. Moreover, it is toxic to humans. Repeated exposure can cause health problems, adverse effects on the liver resulting in tissue lesions and impaired functions (anonymous 4, 2008)

The main objective of this study is in this context and aims to study the ability of a microbial consortium obtained from different biotopes, to degrade this fungicide

1. MATERIAL AND METHODS

1.1. Origin of the microbial consortium:

The first sample was taken from a ground agricultural nature located in the region of North- eastern Algeria. This soil is intented for crops of vineyards and has been extensively treated by bromuconazol. The second sample was collected from basins of biological treatment of wastewater also situated in North -eastern Algeria.

1.2. Bromuconazol:

This fungicide comes from Bayer (Rhône-Poulenc Agro- France). It is imported and widely marketed in Algeria as the Vectra. His brute formula is: $C_{13}H_{12}BrCl_2N_3O$ and chemical structure is presented in the following figure.

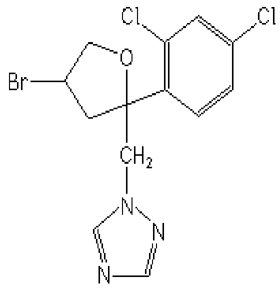

Fig.1. Chemical structure of the herbicide used: bromuconazol

1.3. Selection and acclimatization of microbial consortium:

The acclimatization and selection of the microbial consortium was conducted in biphasic system MBS / silicone oil (batch culture). Thus, at 40 ml of medium basic salin MBS (aqueous phase) are added 10 ml of silicone oil (organic phase). bromuconazol is added in the organic phase at 50µg/ml. The aqueous phase was inoculated with 10 ml of microbial inoculum (biomass). After incubation at 30°C and stirring at 190 rpm, cultures were centrifuged for 20 min at 5000 rpm. The culot obtained were washed with phosphate buffer and again centrifuged. Biomass collected will be used as inoculum for the next fermentation.

1.4. Biodegradation tests of bromuconazol in batch culture:

1.4.1. Biodegradation of bromuconazol in biphasic batch:

After several months of acclimatization of microbial consortium in presence of bromuconazol, biodegradation is followed in biphasic system (medium MBS / silicone oil). At flasks containing a culture medium composed of 40 ml MBS and 10 ml of silicone oil are added 10 ml of the consortium previously acclimated to bromuconazol. The latter is added at a concentration of 50µg/ml. After incubation for one week at 30°C with stirring of 200 rpm, fermentation was monitored by measuring the optical density, the determination of dry weight and changes in the pH of the medium. The emulsifying power and the hydrophobicity of the consortium were also studied.

1.4. 2. Biodegradation of bromuconazol in monophasic batch:

After acclimatization of the microbial consortium in presence of xenobiotic, biodegradation is also followed in the same way in monophasic system, in the absence of the organic phase (MBS without silicone oil).

1.4.3. Biodegradation of bromuconazol in pure culture:

Biodegradation tests of bromuconazol in pure culture were performed in monophasic system. bromuconazol is added at 50µg/ml. The samples are incubated for one week at 30°C, with stirring at 190 rpm. The disappearance of the herbicide in the culture medium was performed using the technique of LC- MS -MS.

2. RESULTS AND DISCUSSION

2.1. Acclimatization of microbial consortium in batch culture:

After 4 weeks of acclimatization of the consortium in the presence of bromuconazol, the results obtained from different batch in biphasic system are shown in the following figure.

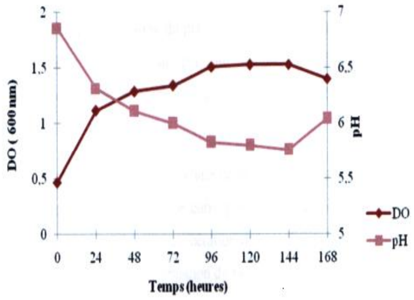

Fig.2. Growth of the microbial consortium in biphasic system (MBS/silicone oil) in the presence of bromuconazol at 50µg/ml

2.2. Study of the performance of biphasic system (MBS / silicone oil):

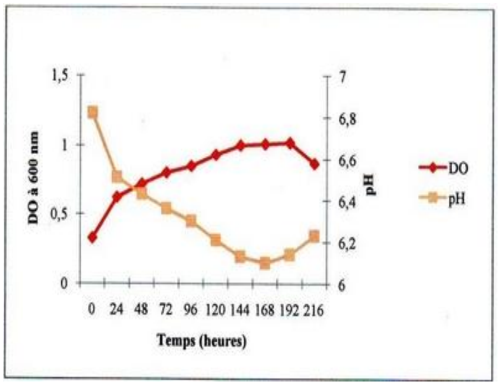

Fig. 3. Growth of the microbial consortium acclimated in batch monophasic

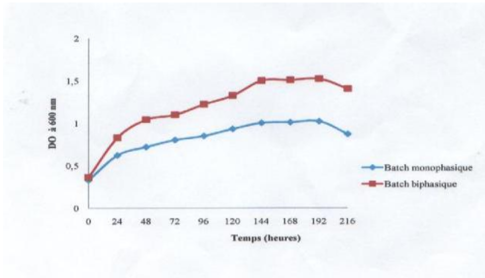

Fig.4. Evolution of the optical density of biomass in batch culture

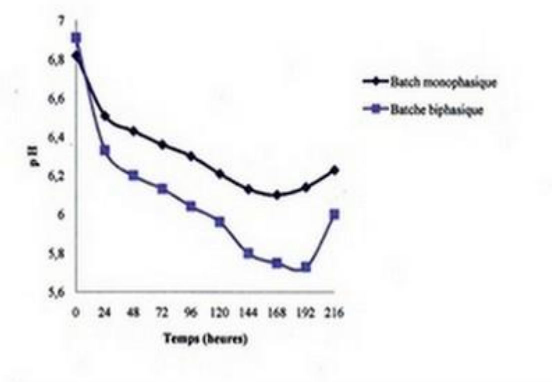

Fig.5. Evolution of pH in batch culture in the presence of bromuconazol

After about five months for the selection and acclimatization of the consortium implicated in the biodegradation of bromuconazol, the study of the biodegradation in batch monophasic (MBS only) was also studied. The aim of this comparative study between the two systems is to determine the usefulness and effectiveness of the organic phase (silicone oil) not only in the degradation process but also with regard to the viability of the consortium involved in the process. The results of test in monophasic systemc show clearly the complete absence of

inhibitory effect of bromuconazol on the growth of microbial consortium previously acclimated. In fact, a typically growth is thus obtained with an increase in the D.O of the culture and acidification of the medium. The maximum growth is obtained after 192 h of incubation at 30°C. The microbial consortium previously acclimated seems able to grow in the presence of bromuconazol as unique source of carbon and energy in monophasic system.
Comparing the curves obtained in monophasic and biphasic system show that the viability of the consortium is maintained in the two types of batch in the presence of bromuconazol. Although the same concentration of substrate (50µg/ml) was added, it does not appear to have the same effect on microbial activity. In the absence of silicone oil, the optical density of the medium reached an average maximum estimated at 1.02, whereas in the presence of the organic phase, the D.O recorded is 1.52. These observations were also found for pH.

2.3. Determination of emulsifying activity of the consortium:

After acclimatization in the presence of the herbicide, the study of the emulsifying activity of the consortium shows that it evolves in a similar way to the cell growth for reaching a maximum production after 144 h of incubation. So, the shape of the curve (Fig.6) shows clearly, a strong emulsifying power exhibited by the microbial culture in the presence of bromuconazol.

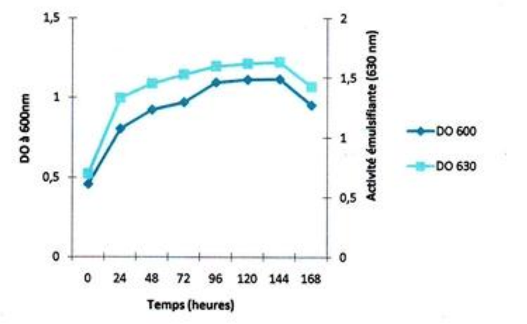

Fig.6. Emulsifying activity of the consortium acclimated to bromuconazol

Fig.7. The emulsifying power of the consortium in the presence of bromuconazol

2.4. Study of the hydrophobicity of the microbial consortium:

The study of the hydrophobicity performed according to the protocol BATH (bacterial adhesion to hydrocarbons) (Rosenberg, 1984) revealed that 55.67 % of the cells are hydrophobic. The hydrophobicity is a key factor in the selection of microorganisms for degradation of xenobiotics, demonstrating the strong adaptation of the culture of the consortium.

2.5. Identification of microorganisms in the consortium degrading bromuconazol:

After a period of acclimatization and adaptation after more than one year in the presence of bromuconazol used as the sole source of carbon and energy, the results obtained after tests of isolation and purification revealed the presence of a single type of colony. After the macroscopic and microscopic examination, the biochemical tests API 20 E and API 20 NE have identified the bacterium implicated in the biodegradation of bromuconazol. It is the species *Aeromonas hydrophila*

2.6. Results of the biodegradation of bromuconazol by *Aeromonas hydrophila*:

Different concentrations of bromuconazol shown in Fig.9 are those obtained after a series of dilutions performed on the initial sample for reasons of handling required by the technique used for the determination of the compound. The form of the curve indicating a gradual disappearance of the herbicide in the culture medium and an increase in the biomass of *Aeromonas hydrophila*, confirming the ability that has this species in the elimination of bromuconazol. The maximum growth, estimated by dry weight is reached after 144 h. The maximum degradation of bromuconazol seems occur during these first 72h.

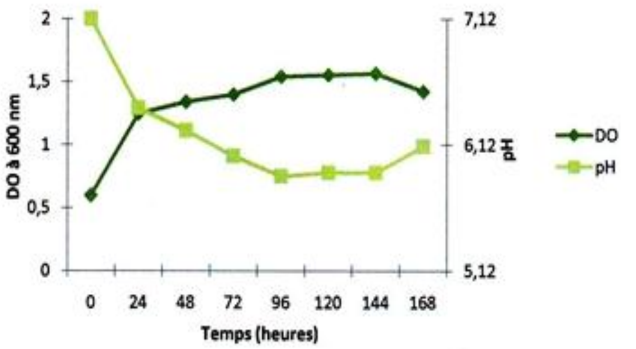

Fig.8. Growth of Aeromonas hydrophila in batch monophasic in the presence of bromuconazol

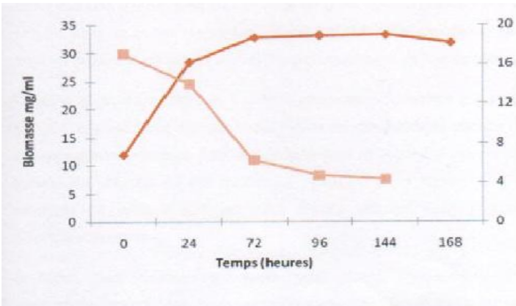

Fig.9. bromuconazol biodegradation by *Aeromonas hydrophila* in monophasic system

CONCLUSION

The use of system biphasic has not only lifted the inhibition due to the substrate, but also allowed better pesticide assimilation compared to that obtained in monophasic system (mineral medium).
After one year of adaptation and acclimatization, the bacterium *Aeromonas hydrophila* has been isolated from a consortium initially inoculated in the MBS culture medium in the presence of bromuconazol.
The disappearance of bromuconazol in the medium culture confirms a real use of herbicide as unique source of carbon and energy by *Aeromonas hydrophila* and the ability possessed by this bacterial species to acclimatize and metabolize the herbicide. This ability is largely due to the presence of the organic phase (silicone oil).

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