

# Monitoring the biodegradation of benzonitrile herbicide - ioxynil octanoate - by HPLC-MS/MS

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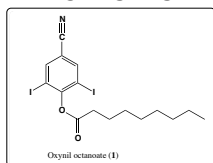
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## Introduction

Totril® (ioxynil octanoate, **1**) is an herbicide used to control weeds that compromise onion and garlic crops. This herbicide is toxic, harmful to health and can pollute the environment.<sup>1</sup> The aim of this work is monitoring the biodegradation of Totril® by microorganisms isolated from onion farms soil samples that are routinely sprayed with this herbicide through HPLC-MS/MS.



## Results and Discussion

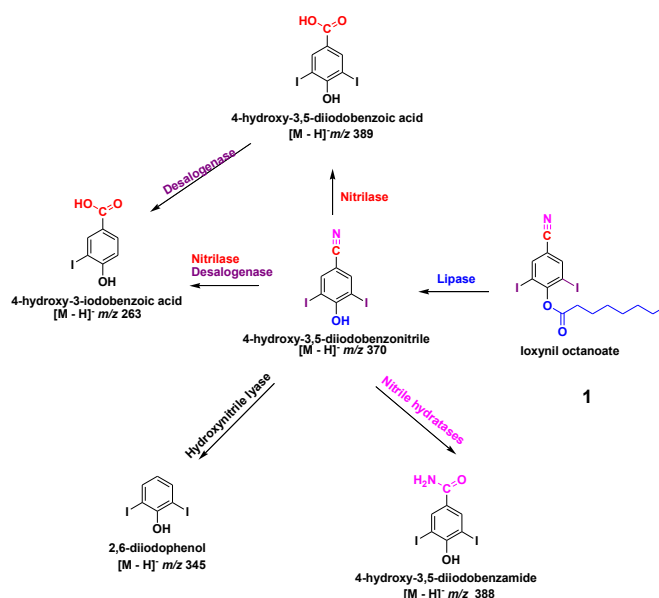
The main route of benzonitrile biodegradation are the enzymatic systems involved in the conversion of nitriles such as nitrilase, nitrile-hydratase and amidases.<sup>2</sup> The first step of the current work involved a colorimetric enzymatic screening assay to prospect these enzymes. Four positive hits were obtained from the 27 microorganisms evaluated. These microorganisms were isolated from soil samples of four onion farms located in São José do Rio Pardo city, one of the main producers of onion in São Paulo State.

The study of ioxynil octanoate biodegradation was performed using the microorganisms encoded as: MLH-31, MLH-54, MLH-61, and MLH-60. These microorganisms are currently under taxonomic identification. The reaction was carried out in erlenmeyer flasks containing 90 mL of Sorensen's buffer (100 mM) pH 7.5, biomass (OD<sub>610</sub> = 2) and 0.1 mM of Totril® herbicide solubilized in 4.5 mL of ethanol. Five biodegradation products were detected during the seven days of monitoring, and were identified and characterized by HPLC coupled to Mass Spectrometer with an electrospray ionization source and ion trap analyzer operating in negative mode (Figure 1).

Ioxynil octanoate biodegradation rates of microorganisms MLH-60, MLH-31, MLH-54, MLH-61 were 58.71%, 96.61%, 46.51% and 75.04%, respectively. In addition to the enzymes (nitrilase, nitrile hydratase and amidase) previously assessed

during the enzymatic screening assays, dehalogenase and lipase enzymes, responsible for production of some observed degradation compounds, were also detected.

**Figure 1.** Suggested enzymes involved in ioxynil biodegradation and their respective observed products.



## Conclusion

Four microorganisms were selected for the biodegradation of the Totril® herbicide. Among them MLH-31 was the best, achieving almost complete biodegradation (96.61%) in 7 days with biodegradation products detected and characterized by HPLC-MS/MS. The results described herein are superior to those reported in the literature in terms of percentage of biodegradation and period (days).

## Acknowledgments

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<sup>1</sup> MAPA - Ministério da Agricultura, Pecuária e Abastecimento. **AGROFIT: Sistema de Agrotóxicos Fitossanitários**. Brasília, DF, Brasil, accessed in 20<sup>th</sup> Jan 2015: <[http://extranet.agricultura.gov.br/agrofit\\_cons/principal\\_agrofit\\_cons](http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons)>.

<sup>2</sup> RANTEK, P. W.; MAURICE, N. G.; JOSEPH, B.; WADHER, B. J. Nitrile converting enzymes: an eco-friendly tool for industrial biocatalysis. **Biotechnol. Appl. Biochem.**, v. 60, n. 5. p. 459 - 481, 2013.