

## Stability of a Biofilm Reactor Able to Degrade a Mixture of the Organochlorine Herbicides Atrazine, Simazine, Diuron and 2,4-Dichlorophenoxyacetic Acid to Changes in the Composition of the Supply Medium

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**Abstract :** Among the most important herbicides, the organochlorine compounds are of considerable interest due to their recalcitrance to the chemical, biological, and photolytic degradation, their persistence in the environment, their mobility, and their bioaccumulation. The most widely used herbicides in North America are primarily 2,4-dichlorophenoxyacetic acid (2,4-D), the triazines (atrazine and simazine), and to a lesser extent diuron. The contamination of soils and water bodies frequently occurs by mixtures of these xenobiotics. For this reason, in this work, the operational stability to changes in the composition of the medium supplied to an aerobic biofilm reactor was studied. The reactor was packed with fragments of volcanic rock that retained a complex microbial film, able to degrade a mixture of organochlorine herbicides atrazine, simazine, diuron and 2,4-D, and whose members have microbial genes encoding the main catabolic enzymes atzABCD, tfdACD and puhB. To acclimate the attached microbial community, the biofilm reactor was fed continuously with a mineral minimal medium containing the herbicides (in mg•L<sup>-1</sup>): diuron, 20.4; atrazine, 14.2, simazine, 11.4, and 2,4-D, 59.7, as carbon and nitrogen sources. Throughout the bioprocess, removal efficiencies of 92-100% for herbicides, 78-90% for COD, 92-96% for TOC and 61-83% for dehalogenation were reached. In the microbial community, the genes encoding catabolic enzymes of different herbicides tfdACD, puhB and, occasionally, the genes atzA and atzC were detected. After the acclimatization, the triazine herbicides were eliminated from the mixture formulation. Volumetric loading rates of the mixture 2,4-D and diuron were continuously supplied to the reactor (1.9-21.5 mg herbicides •L<sup>-1</sup> •h<sup>-1</sup>). Along the bioprocess, the removal efficiencies obtained were 86-100% for the mixture of herbicides, 63-94% for COD, 90-100% for COT, and dehalogenation values of 63-100%. It was also observed that the genes encoding the enzymes in the catabolism of both herbicides, tfdACD and puhB, were consistently detected; and, occasionally, the atzA and atzC. Subsequently, the triazine herbicide atrazine and simazine were restored to the medium supply. Different volumetric charges of this mixture were continuously fed to the reactor (2.9 to 12.6 mg herbicides •L<sup>-1</sup> •h<sup>-1</sup>). During this new treatment process, removal efficiencies of 65-95% for the mixture of herbicides, 63-92% for COD, 66-89% for TOC and 73-94% of dehalogenation were observed. In this last case, the genes tfdACD, puhB and atzABC encoding for the enzymes involved in the catabolism of the distinct herbicides were consistently detected. The atzD gene, encoding the cyanuric hydrolase enzyme, could not be detected, though it was determined that there was partial degradation of cyanuric acid. In general, the community in the biofilm reactor showed some catabolic stability, adapting to changes in loading rates and composition of the mixture of herbicides, and preserving their ability to degrade the four herbicides tested; although, there was a significant delay in the response time to recover to degradation of the herbicides.

**Keywords :** biodegradation, biofilm reactor, microbial community, organochlorine herbicides

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