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ACCLIMATION OF A COMPLEX MICROBIAL COMMUNITY TO DEGRADE A COMBINATION OF ORGANOCHLORINE HERBICIDES IN A BIOFILM REACTOR

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Abstract

The most widely organochlorine herbicides used in North America are 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, simazine and diuron, in minor grade. These herbicides can be simultaneously or sequentially applied in agricultural fields; thus, the contamination of water bodies usually occurs by a combination of two or more herbicides. For this reason, the acclimation of an integrated microbial community able to degrade a variety of organochlorine herbicides in a packed-bed biofilm reactor (PBR) is proposed.

The community was composed by the combination of two microbial consortia, one that degrades triazine herbicides, and another able to degrade diuron. A Burkholderia strain, capable of degrading 2,4-D, was added to the integrated community.

The reactor used was an aerated packed bed biofilm reactor (PBR). The salts medium used a combination of the following herbicides, in mg L^{-1}, diuron, 20; atrazine, 20; simazine, 5 and 2.4-D, 50. After inoculation, the reactor was fed continuously, increasing the herbicide loading rates ($B_{V,H}$) from 3 to15 mg $L^{-1}h^{-1}$.

The attached cell mass was analyzed for their viable cell counting, and the content of herbicides, chlorides, COD and TOC was determined in the PBR influent and effluent.

After six months of PBR's operation, the results showed that colonization of the porous support had an initial period of slow growth followed by an accelerated growth at the highest loading rate, until the cell counting reaches a stable value.

In concordance with the community enrichment, a decrease in the concentration of herbicides and various metabolic by-products was observed. At the end of the continuous bioprocess, no herbicides or their metabolic intermediates, with the only exception of cyanuric acid, were detected. The efficiency of dehalogenation and the reduction of COD and TOC reached values of 100, 91 and 92% respectively.

Several genes involved in the degradation of different herbicides were detected throughout the process. The genes *tfdA*, *tfdC* and *tfdD*, involved in the catabolism of 2,4-D were amplified. The *puhB* gene involved in the degradation of diuron was also detected. Amplicons for the genes atz*A*, *atzB* and *atzC*, responsible for the degradation of triazine herbicides were obtained; however, the gene *trzD* that encodes for the cyanuric acid amidohydrolase was not always detected, indicating its low abundance; this could explain the accumulation of cyanuric acid in the reactor.

The acclimatization of the integrated microbial community was evidenced by the accelerated biofilm growth, consistent with the decrease in the concentrations of herbicides and their metabolic intermediaries. Finally, the integrated community resulted functionally efficient for the degradation of the combination of the halogenated herbicides 2,4-D, atrazine, simazine and diuron.