Analysis of a bacterial community able to degrade a mixture of organochlorine herbicides (atrazine, simazine, 2,4-D and diuron)

Nava-Arenas Ixchel, Marron-Montiel Erick, Chávez-Rodríguez Connie Marisol, Cabrera-Orozco Alberto, Peña-Partida José Carlos, Ruiz-Ordaz Nora, Galíndez-Mayer Juvencio; Instituto Politécnico Nacional, Escuela Nacional de Ciencias Biológicas, Depto. Ingeniería Bioquímica, Prolongación de Carpio y Plan de Ayala S/N, Santo Tomas, Miguel Hidalgo, Ciudad de México, Distrito Federal, Cp. 11340, juvenciogm@yahoo.com.

Degradation, herbicides, microbial consortium

Introduction. The most widely organochlorine herbicides used in North America are 2,4-dichlorophenoxyacetic acid (2,4-D), atrazine, simazine, and, in minor grade diuron. These herbicides can be simultaneously or sequentially applied in agricultural fields; thus, the contamination of water bodies usually could occur by a combination of two or more herbicides. For this reason, the study of communities able to degrade a mixture of these organochlorine herbicides is proposed.

Methods.

In this study, the bacterial community was composed by the combination of three microbial consortia. Each of them can degrade a specific herbicide.

The community was immobilized in a 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 continuously fed, increasing the herbicide loading rates ($B_{V,H}$), which ranged between 3 and 15 mg $L^{-1}h^{-1}$.

The attached cell mass was analyzed for their viable cell counting. The concentration of herbicides, chlorides, chemical oxygen demand (COD), and total organic carbon (TOC) was determined in the PBR outflowing liquid. The concentration of herbicides was measured by high-performance liquid chromatography (HPLC).

At the end of PBR's operation, the bacterial community was detached from the support. Bacterial strains were isolated, identified and screened for the presence of genes involved in the degradation of the different herbicides.

Isolated genomic DNA from each strain of the bacterial community was used as PCR template for identification and screening purposes.

Results.

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.

The enriched culture obtained was composed of eight bacteria and several genes involved in the degradation of different herbicides were detected in each strain (Table 1). The genes *tfdA, tfdC* and *tfdD* (Hogan *et al.*, 1997; Chang y Ka, 1998; Morimoto y Fujii, 2009), involved in the catabolism of 2,4-D, were amplified. The *puhB* gene involved in the degradation of diuron was also detected (Khurana *et al.*, 2009). Amplicons for the genes *atzA, atzB*, *atzC* and *atzD*, (De Souza *et al.,* 1998; Martinez et al. 2001) responsible for the degradation of triazine herbicides were also amplified.

Table 1. Catabolic genes detected in the bacterial community attached to the PBR' porous support.

	Strain	Max Ident	Accession number	Catabolic genes detected								
				tfdA	*tfdC*	*tfdD*	*puhB*	*atzA*	*atzB*	*atzC*	*atzD*	*atzF*
C1	*Pseudomonas* sp.	99%	JN093012.1	♦	♦	♦		♦		♦	♦	♦
C2	*Bosea* sp.	99%	HQ260890.1		♦	♦		♦	♦	♦		
C3	*Gordonia* sp	99%	GU367115.1	♦	♦	♦						
C4	*Mycobacterium* sp	99%	JF772584.1	♦	♦						♦	
C5	*Bosea* sp.	99%	DQ440824.1	♦	♦	♦					♦	
C6	*Kaistia* sp.	98%	FJ006913.1	♦		♦				♦		
C7	*Mycobacterium smegmatis*	99%	NR_074726.1	♦	♦	♦	♦			♦	♦	
C8	*Nocardioides simplex*	99%	AF005013.1	♦	♦	♦	♦					

Conclusions.

The integrated community was functionally efficient for the degradation of the combination of the halogenated herbicides 2,4-D, atrazine, simazine and diuron.

All strains have a role in the degradation of the different herbicides.

Acknowledgements. N-A,I; C-R,CM; C-O,A, and P-P,JC receive a grant from PIFI-IPN. R-O,N; and G-M,J. receive a grant from SIP-IPN

References.

1. De Souza M. L., Sefernick J., Martínez B., Sadowsky M. J. y Wackett L. P. (1998). *J. Bacteriol*. 180:1951-1954.
2. Chang Min-Jae y Ka Jon-Ok (1998). *J. Microbiol*. 36 (4): 256-261.
3. Hogan D.A., Buckley D.H., Nakatsu C.H., Schmidt T.M. y Hausinger R.P. (1997). *Microb. Ecol.* 34:90–96.
4. Ibrahim M. A., Bond G. G., Burke T. A., Cole P., Dost F. N., Enterline P. E., Gough M., Greenberg R. S., Halperin W., E., McConnel E., Munro C., Swenberg J. A., Zahm, S. H. and Graham J. D. (1991). *Environ Health Persp* 96:213-222.
5. Khurana J. L., Jackson C. J., Scott C., Pandey G., Horne I., Russell R. J., Herlt A., Easton C. J. y Oakeshott J. G. (2009). *Biochem. J.* 418: 431–441.
6. Martinez B., Tomkins J., Wackett L. P., Wing R. y Sadowsky M. J. (2001). *J. Bacteriol.* 183:5684-5697.
7. Morimoto S. y Fujii T. (2009). *Appl Microbiol Biotechnol.* 83:389–396.
8. Parsons J. K., Hamel K. S., Madsen J. D. And Getsinger K. D. (2001). *J. Aquat. Plant Manage.* 39:117-125.
9. Tworkoski T. and Miller S. (2001). *HortScience*. 36(7):1211–1213.