SELECTION AND ACCLIMATION OF A DICAMBA AND 2,4-D DEGRADING MICROBIAL CONSORTIUM

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Introduction. Dicamba and 2,4-D (2,4-dichlorophenoxyacetic acid) are two widely used herbicides. Both agrochemicals are toxic to aquatic organisms, and due to their solubility they have been found as pollutants in groundwater, riverine and lacustrine systems [1, 2]. Because of its environmental importance, the selection and acclimation of a microbial consortium capable of degrading both compounds was carried out.

Methods. The sources of isolation were agricultural soil samples, where maize and sorghum were cultivated. The herbicide studied was Banvel (Syngenta, which contains 2,4-D and Dicamba (25.5 and 12.65 % respectively). The soil was inoculated into a glass column packed with basaltic rock fragments with a porous glass diffuser at the bottom. To allow the bacterial colonization of the support material and the biofilm formation, initially the selector was batch operated. When a significant change in the absorption spectrum of the herbicidal mixture was observed, the operational mode was changed. A minimal salts medium containing the herbicide was continuously supplied. The reactor was operated at room temperature with an aeration rate of 0.1 LPM. The minimum salts medium (MS) fed into the reactor was supplemented with 50 ppm of the herbicide formulation under study (33.5 and 16.5 ppm for 2,4-D and Dicamba respectively). The absorption spectrum of the selector's output was determined in a UV-VIS spectrophotometer at λ = 283 nm. Further analyzes of chemical oxygen demand COD and liquid chromatography HPLC were realized. The isolation of cultivable microorganisms comprising the microbial consortium was performed by plate counting. The microbial growth of the microbial isolates was made in MS medium plates supplemented with Banvel, Dicamba or 2,4-D.

Results. After 150 days of operation, high removal efficiencies of both herbicides were achieved in the continuous selector. Values of OD_{283} values and COD in the reactor's effluent remained stable; so, it was inferred that the support material of the reactor was colonized by the microbial consortium (Table 1). The results obtained by spectrophotometry and COD showed that some herbicidal components were not entirely degraded; thus, the reactor effluent was analyzed by liquid chromatography. By HPLC, the complete disappearance of 2,4-D and Dicamba was observed; however, a catabolic byproduct of either of the herbicides was detected. The accumulation of this intermediate and the incomplete degradation of the additives contained in the herbicide could explain the relatively low values of the COD removal efficiency.

Table 1. Removal efficiency determined by various methods.

Method	Removal effiiciency
UV photometry	86.4%
DQO	79.5%
HPLC	100%

From this packed bed column, six colonies with different colonial morphology were isolated. All of them were able to grow in MS medium plates containing Dicamba or 2,4-D as the sole carbon sources (Table 2). This precludes the possibility that some of these isolates grow only on the adjuvants of the commercial formulation of the herbicides.

Table 2. Microbial growth over different substrates as a sole carbon source.

Isolated culture	Banvel®	2,4-D	Dicamba
SSC1	+	+	+
SSC2	+	+	+
SSC3	+	+	+
SSC4	+	+	+
SSC5	+	+	+
SSC6	+	+	+

Conclusions. The traditional enrichment method of successive transferences in batch culture was substituted by a packed bed chemostat (PBC) acting as a continuous selector. With this method, a microbial consortium capable of degrading the herbicides 2,4-D and Dicamba, were selected in the PBC. Complete biodegradation of both herbicides was achieved after 150 days of continuous operation. However, accumulation of a catabolic byproduct was detected. Despite of this, six bacterial isolates were able to use Banvel, Dicamba and 2,4-D herbicides as carbon sources..

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References.

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