Degradation of the bendiocarb insecticide, using an adsorption and bio-desorption continuous process

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Abstract. In this work, the behavior of a multi-channel biofilm reactor (MCBR) was studied to attain an adsorption and bio-desorption system for the removal of pesticides. The MCBR operates with three airlift channels, and two channels packed with granular activated carbon (GAC) and fragments of volcanic stone (tezontle).

The adsorption coefficient *Qo* of bendiocarb, contained in the commercial insecticide "Ficam W" was determined on GAC. The high value of *Qo* favors high adsorption rates of bendiocarb, which was efficiently degraded by the immobilized community in the bioreactor, together with other Ficam W ingredients. It was evidenced that both processes, adsorption, and biodegradation, operated in the reactor since the bendiocarb removal rates and efficiencies were significantly higher in the MCBR than in batch cultures where the activated carbon was not present. The selected community efficiently degraded 92% of bendiocarb in batch cultivation and was able to remove most of the benzodioxol, which is an intermediary of the degradation of bendiocarb.

From the microbial community grown in MCBR, thirteen cultivable microorganisms were isolated and identified. They belong to the genera: *Pseudoxanthomonas, Ochrobactrum, Bosea, Pseudomonas, Agromyces, Bacillus, Ralstonia, Brevundimonas, Aminobacter, Paracoccus, Brevibacterium, Kakuria, and Gordonia.*

Keywords: Bendiocarb, Insecticide, Adsorption, Bio-desorption, Biodegradation.

1. Introduction

The use of pesticides in agriculture improves agricultural yields; however, the uncontrolled use of this agrochemical products has caused serious environmental problems in the areas of application since most pesticides are not a natural part of ecosystems. Therefore, they can lead to persistence problems in the environment. (Zacharia, 2011).

Bendiocarb is an insecticide used to control pests mainly in maize and sugar beet crops (Worthing, 1983). Through runoffs and soil leaching, these compounds can reach water bodies, resulting in contamination of both surface and underground waters (Hodgkin & Hamilton, 1993). With the increase of sickness and death by dengue, chikungunya, and zika, especially in tropical countries, the use of bendiocarb has been recently increased, being one of the most effective insecticides against mosquito vectors of these diseases (Dhimal, et. al., 2015).

Therefore, the development of simple, low-cost and efficient technologies for the elimination of pesticides is crucial. There are several technologies for the removal of pesticides, physical treatments such as adsorption, advanced oxidation processes (AOP) and biological treatments. Advanced operations, currently implemented in the treatment plants, such as reverse osmosis or adsorption with activated carbon, have been cited by the Environmental Protection Agency (EPA) of the United States of America as the best available environmental control technologies. However, with the use of these processes, the mineralization of xenobiotics is not achieved (Baggiani *et al*., 2004; USEPA, 1987). The biotechnological alternative has been used to degrade these contaminants. Pure strains or microbial consortia in suspended or immobilized cell systems could removal a wide variety of substrates. The use of biofilm reactors has been increased because of their resistance to compound toxicity and high loading rates. In this work, we evaluated the use of a combined system for the removal of bendiocarb from wastewater, where the insecticide is adsorbed in a bed of activated carbon and tezontle, eliminating it from the residual water. Subsequently or simultaneously the bendiocarb is degraded by the immobilized microbial consortium.

2. Materials and Methods

2.1. Chemicals

All components used in culture media were obtained from Merck (Darmstadt, Germany). Bendiocarb standard was acquired from Chem Service Inc, Pennsylvania. The solvents used for HPLC were purchased from J.T. Baker. For biodegradation experiments, a commercial formulation of the insecticide Ficam W, (Bayer, México), containing 80% of bendiocarb, was used.

2.2. Culture Media

Mineral salts medium (MSM) was used for all experiments. The medium contains, in g L^{-1}, KH_2PO_4, 0.2; K_2HPO_4, 0.40; $MgSO_4$, 0.20 and $CaCl_2$, 002. It was complemented with trace elements, obtaining a final concentration (in mg L^{-1}) of $FeSO_4.7H_2O$, 0.55; $MnSO_4.7H_2O$, 0.34; $ZnSO_4.7H_2O$, 0.23; $CoCl_2.6H_2O$, 0.065; $Na_2MoO_4.2H_2O$, 0.34. The insecticide Ficam W was added to MS medium to reach a bendiocarb concentration of 30 mg L^{-1} (MS-Ficam W medium).

2.3 Packed Bed Support

A mixture of granular activated carbon (GAC) and fragments of porous volcanic stone (tezontle) was used as biofilm support. GAC was used as the adsorbent material, and fragments of volcanic rock (tezontle) were included to avoid the bed compression and allow the formation of channels for air and liquid circulation, circumventing zones of low oxygenation that would affect the growth of the microorganisms. Tezontle is a low cost, porous and resistant material adequate for the immobilization of the cells.

Fragments of volcanic stone and fragments of activated carbon were considered ellipsoidal bodies with three characteristic radii: a, b, c. The calculated particle volume was used to determine the equivalent diameter of the porous fragments (Galíndez-Nájera *et al*., 2009). The average value was 6.18±1.57 mm for volcanic stone fragments and 2.74±0.86 mm for activated carbon particles.

2.4 Packed Bed Biofilm Reactor

The biobarrier consisted of a 40 cm channel subdivided into five sections, each section including a packed zone (triphasic) and an aerated zone (biphasic) where the liquid was oxygenated by a porous diffuser. The reactor was made of polymethylmethacrylate with cover and ports for sampling of liquid and fragments of colonized support material. The dimensions of the biobarrier were 40 cm length, 16 cm width, and 10 cm height. The operational volume of the biofilm reactor was 5.980 L, with a volume of packed support of 1.940 L and a liquid volume of 4.040 L.

2.5 Microorganisms

From soil samples from a composting plant located in the northeast of Mexico City, and using the method of successive transferences, an enriched microbial culture was selected. For the identification of the different microorganisms isolated, 16S rDNA fragments were amplified by the polymerase chain reaction (PCR), using the universal primers fd1 (Miyajima *et al*., 2002) and 1492r (Lane, 1991). The purified amplicons were sequenced (Macrogen Inc., Seoul, Korea). Using the BLAST program, the sequences were aligned with those deposited in the NCBI-GeneBank.

2.6 Determination of adsorption isotherm of bendiocarb in granular activated carbon

In 500 mL Erlenmeyer flasks containing 200 mL of minimal mineral medium added with different concentrations of insecticide (30, 40, 60, 70, 90 y 100 mgL^{-1}), 0.1 g of granular activated carbon was placed and incubated for 7 days under constant agitation and temperature (23±2 ° C). Samples were taken every 24 hours to determine the concentration of the insecticide in the liquid by HPLC. The amount of solute adsorbed was determined by the following formula (Hossein *et al*., 2013):

$$qe = \frac{\mathrm{V(Co - Ce)}}{\mathrm{m}}$$

qe = milligrams of solute adsorbed per gram of adsorbent in equilibrium

V = volume of the liquid

m = milligrams and granular activated carbon

Co = initial concentration

Ce = equilibrium concentration of adsorbate

Knowing the amount of solute adsorbed per gram of adsorbent in equilibrium (qe), the adsorption isotherm was obtained by plotting qe as a function of the equilibrium concentration of the adsorbate (Ce).

2.7 Determination of Bendiocarb

Samples were analyzed by HPLC using a Shimadzu SPD-10A HPLC System equipped with a Zorbax SB-Phenyl C18 Agilent Technologies column and a UV detector (λ=226 nm). The flow rate of the mobile phase was one mL min^{-1}. The mobile phase was a mixture of acetonitrile/water (60-40%) (Pérez-Ruiz *et al*., 2007).

2.8 Determination of Chemical Oxygen Demand (COD)

Samples were adequately diluted and analyzed using Hach Method 8000, 2012.

2. Results and Discussion

3.1| Adsorption isotherm of granular activated carbon

Initially the bioreactor was operated at a dilution rate D = 0.0167 h-1 in abiotic conditions, feeding it with MSM medium added with 20 mg L-1 of bendiocarb, in order to saturate the support material with the insecticide; however, after 43 days of operation, saturation of the system was not

reached. Therefore, it was necessary to obtain the adsorption isotherm to determine the maximum adsorption capacity of the commercial insecticide by GAC.

The adsorption isotherm obtained for the commercial insecticide Ficam W is shown in Figure 1, a logarithmic trend was observed.

When correlating the experimental data, its behavior was similar to the Langmuir isotherm (Figure 2).

This model is based on two assumptions: the interaction forces between the adsorbed molecules are insignificant, and once a molecule occupies a site no additional adsorption occurs, so the Langmuir isotherm refers to a homogeneous adsorption (Aharoni C, 1977; Foo K, 2013).

The maximum adsorption concentration (Qo) was determined from the mathematical model described for this type of isotherms.

$$qe = \frac{QobCe}{1 + bCe}$$

Figure 2. Mathematical model of Langmuir.

qe=solute adsorbed per gram of adsorbent in equilibrium (mg g^{-1}), Ce =concentration adsorbed in equilibrium (mg g^{-1}), Qo=Maximum adsorption capacity, b= Constant related to free energy adsorption (L mg^{-1}).

As a result, the maximum adsorption capacity Qo of the activated carbon used for Ficam insecticide was 133.82 mg / g GAC. Thus, the time required to achieve the abiotic saturation of the reactor by operating at different volumetric loads of the insecticide can be determined.

3.2 Removal of bendiocarb insecticide in the Packed Bed Biofilm Reactor operating in continuous regime

The biofilm reactor was inoculated with the selected microbial community and maintained with a loading rate B_V = 0.49 mg L^{-1} h^{-1}. Samples were taken every 24 hours, and a progressive decrease in insecticide concentration was observed. After 5 days, the insecticide was completely removed, pointing to a microbial degradation. Without microbial activity in the reactor, the concentration of the insecticide would have remained constant or with an upward trend.

Table 1 shows the results obtained at two different volumetric loading rates of the insecticide.

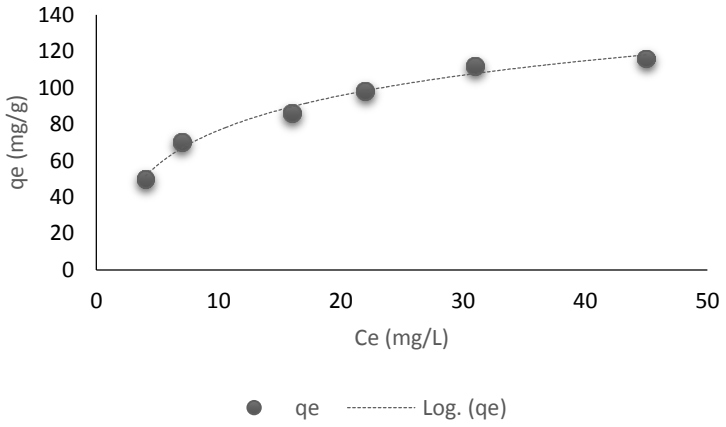

Figure 1. Adsorption isotherm of Ficam W insecticide on granular activated carbon (23±2°C). qe=solute adsorbed per gram of adsorbent in equilibrium; Ce = concentration adsorbed in equilibrium.

Table 1. Volumetric removal rates and removal efficiencies of bendiocarb in the bioreactor

Flow rate ($L\ h^{-1}$)	**Dilution rate** (h^{-1})	**Sr** ($mg\ L^{-1}$)	**s** ($mg\ L^{-1}$)	**B_V** ($mgL^{-1}\ h^{-1}$)	**$\eta_{\%}$**	**R_V** ($mgL^{-1}\ h^{-1}$)
0.1	0.024	100	5	2.5	96.26	2.35
0.2	0.049	100	9	5.0	90.5	4.5

Because the time to reach a steady state condition was greater than 200 h for the tested volumetric loads (B_V), it was decided to modify the system to perform a bendiocarb gradient feeding.

The gradient feed was achieved using two interconnected bottles; Rb (bendiocarb reservoir bottle) and Rg (bendiocarb gradient bottle), filled with MSM at the same height, but with different concentration of the insecticide. The Rb concentration, which remains constant along the procedure, was 172 mg L^{-1} , and the initial concentration in the Rg was 100 mg L^{-1}. A constant flow rate from Rg was pumped to the bioreactor. In this way, it was possible to carry out a fast evaluation of the system performance, determining the maintenance or loss of the bendiocarb removal efficiency.

The volumetric removal rate was maintained close to the volumetric loading during the gradient feed system; So that the removal efficiency was about 90%.

To verify that the removal of bendiocarb was due to a combined process of adsorption and biodegradation, and not only to an adsorption phenomenon, samples of the microbial community attached to fragments of tezontle were batch cultivated. The removal efficiencies rounded 60%. Similarly, a fedbatch cultivation was carried out in small columns packed with tezontle fragments colonized by the microbial community. In this system, efficiencies near 90% were obtained.

3.3 Bacterial strains identification

From the biofilm obtained at different operational conditions in the bioreactor, fourteen predominant microorganisms were isolated. Results are shown in Table 2.

It is documented that the genus *Pseudomonas* has the ability to degrade different carbamates such as carbofuran, carbaryl and aldicarb (Chapalamadugu *et al*., 1991; Ramanand *et al*., 1991; Topp *et al*., 1993); however, total mineralization of these compounds has not been achieved and intermediary compounds were formed. In the present work, according to the results of HPLC, COD and TOC, only the formation of the benzodioxol intermediary compound was observed, but its concentration in the effluents was minimal, so it can be assumed that the microbial consortium was also able to remove this compound. It is important to note that

Table 2. Predominant strains in the microbial biofilm.

Bacteria	Percentage of similarity	NCBI accession numbers
Pseudoxanthomonas spadix	98%	NC_016147.2
Ochrobactrum anthropi	97%	NC_009668.1
Bosea thiooxidans	99%	NZ_LMAR01000067.1
Pseudomonas denitrificans	99%	NC_020829.1
Agromyces sp.	98%	NZ_LMKQ01000001.1
Bacillus thuringiensis	97%	NC_022873.1
Pseudomonas alkylphenolia	98%	NZ_CP009048.1
Ralstonia sp.	92%	FJ774001.1
Brevundimonas sp.	97%	KU851032.1
Aminobacter aminovorans	96%	LN995690.1
Paracoccus sp.	97%	AM084012.1
Brevibacterium sp.	97%	KM507608.2
Kokuria sp.	97%	KU199722.1
Gordonia sputi	97%	FJ536318.1

Reported species showing the highest similarity were regarded as the isolated strains.

except for the genus *Pseudomonas*, none of the genera detected in the community has been documented as degrading bendiocarb insecticide.

3. Conclusions

The high bendiocarb Qo coefficient on GAC, allowed an efficient adsorption of the insecticide. A combined system of adsorption and biodegradation operated in the MCB reactor, since the removal rates were higher than in batch or fedbatch cultures where the activated carbon was not present.

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