Environmental Engineering and Management Journal August 2014, Vol.13, No. 8, 1939-1944
http://omicron.ch.tuiasi.ro/EEMJ/

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BIODEGRADATION OF THE HERBICIDE LINURON IN A PLUG-FLOW PACKED-BED BIOFILM CHANNEL EQUIPPED WITH TOP AERATION MODULES

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Abstract

A lab-scale packed-bed biofilm channel reactor that operates as a plug-flow system equipped with top aeration modules was constructed and evaluated. To assess the reactor performance a microbial community able to degrade linuron was used. Changing the fluid flow rates and the linuron concentrations in the inflowing medium the volumetric loading rates of linuron ($B_{V,L}$) were gradually increased from 0.29 to 14.93 mg/Lh. During the operation of the reactor, an airflow rate of 0.15 ± 0.05 L/min was maintained in each aeration module. In these working conditions, dissolved oxygen concentrations (OD) of about 5 mg/L were obtained in the aeration modules. For all $B_{V,L}$ values tested, the microbial community colonized the support and removed the linuron with efficiencies of about 100%. The highest removal efficiency measuring the chemical oxygen demand (COD) was about 84%, and no accumulation of aromatic intermediates was detected by HPLC analysis.
The results showed that the oxygen consumption rate was proportional to $B_{V,L}$. The dissolved oxygen was not entirely consumed in the bioreactor indicating that, even at the highest loading rates, the microbial community was not limited by oxygen. The use of top aeration modules was successful, and their place in a packed bed biofilm channel allowed enough dissolved oxygen at the beginning of each stage, and along the whole reactor. It is appropriate to mention that no reports of linuron removal in bioreactors could be found in the literature.

Keywords: biodegradation, biofilm, linuron, plug-flow

Received: February, 2014; Revised final: August, 2014; Accepted: August, 2014

1. Introduction

Plug-flow reactors are used in traditional suspended growth systems for wastewater treatment such as oxidation ditches and activated sludge processes (Alaya et al., 2010; Guo et al., 2013; Zhi et al., 2012). Although these systems are highly efficient for the treatment of municipal wastewaters (Chan et al., 2009; Kassab et al., 2010; Pitas et al., 2012), when they are mixed with some industrial or agricultural discharges the resulting inflowing can negatively affects the quality of the effluent, or can generate shock loading of the wastewater treatment plant (Boon et al., 2000; Kulkarni, 2012; Marrón-Montiel et al., 2006; Muhamad et al., 2012; Zhou et al., 2008). To avoid this, different treatment systems have been proposed; among them, biofilm systems are of considerable interest because of their higher removal rates and efficiencies compared with the suspended cells systems (Sharma et al., 2009; Singh et al., 2006). Comparatively, biofilm reactors produce considerably few biological solids and are more stable and resistant to shock loadings caused by the input of inhibitory or toxic compounds (Gómez-

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De Jesús et al., 2009; Mendoza-Espinosa and Stephenson, 1999; Sahoo et al., 2013).

The efficiency of a packed-bed biofilm reactor to remove pollutants depends on the microbial community used, the support material selected and the environmental conditions existing in the reactor (Biswas et al., 2013; Stephenson et al., 2013). In aerobic bioprocesses, the oxygen mass transfer is a key factor in bioreactor design. Moreover, the way in which oxygen is supplied to an aerobic biofilm reactor is essential because O_2 limitation could result in an energetically unaffordable and inefficient bioprocess (Daugulis et al., 2011; Garcia-Ochoa and Gomez, 2009; Gómez-De Jesús et al., 2009). Depending on the type of PBR, the oxygen supply could be carried out in different ways. In trickling filters, the liquid is aerated by aspersion or dripping on the biofilm support, or by injection of air in the bottom of the packed-bed (Gopinath et al., 2013; Mendoza-Espinosa and Stephenson, 1999). In submerged aerated filters, the oxygen supply to the packed bed could be done by the recirculation of liquid aerated in an airlift device (Gómez-De Jesús et al., 2009; Kermanshahi et al., 2005). In aerobic biobarriers, O_2 releasing compound (ORCs) into the reactor could also be used (Chevalier and McCann, 2008; Kao et al., 2001).

Because water contamination by pesticides is an environmental concern, in this work a novel plug-flow packed-bed biofilm channel (PBBC) with top aeration modules, which promote the oxygen supply with low-energy consumption, is proposed for the treatment of water polluted with the phenylurea herbicide linuron. This herbicide has been frequently detected in soil, groundwater and surface water due to its extensive use in agriculture (Bers et al., 2012; Breugelmans et al., 2010).

2. Experimental

2.1. Herbicide

For biodegradation experiments, the culture medium was supplemented with a commercial presentation of linuron (3-[3,4-dichlorophenyl]-1-methoxy-1-methylurea), named Afalon 50WP, purchased from Proficol, Mexico, with a content of 50% of linuron and 50% of adjuvants.

For HPLC analysis, a standard of linuron (99.5% purity), was purchased from Chem Service Inc., USA.

2.2. Culture media

The liquid medium fed to the reactor was a minimal salts medium (MSM) containing different concentrations of linuron. The composition of MSM was (mg/L): $MgSO_4 \cdot 7H_2O$, 246; $NaH_2PO_4 \cdot H_2O$, 103; KH_2PO_4, 435; $FeSO_4 \cdot 7H_2O$, 2; $ZnSO_4 \cdot 7H_2O$, 0.08; $MnSO_4 \cdot H_2O$, 0.2; $Na_2MoO_4 \cdot 2H_2O$, 0.1; $Ca(H_2PO_4)_2 \cdot H_2O$, 0.4; H_3BO_3, 0.02. When a solid medium was needed, agar (20 g/L) was added.

2.3. Isolation of a microbial consortium able to degrade linuron

The microbial community used along this work was isolated from soil samples obtained from agricultural lands frequently treated with phenyl urea herbicides. The samples were collected from Tenango del Valle, Mexico. 10 g of mixed soil samples were inoculated in MSM medium containing 100 mg/L of Afalon 50WP as carbon and nitrogen sources (MSA medium). The flasks were incubated at 25 °C on a rotating shaker. During the incubation period, the concentration of linuron was spectrophotometrically monitored; when a clear diminution in linuron concentration was observed, an aliquot was transferred to fresh MSA medium. Blanks of non-inoculated MSA medium, or medium containing sterile soil, did not show abiotic losses of linuron. Six transferences were carried out.

To promote the prevalence of biofilm-forming microorganisms able to colonize the support material the flasks showing the highest substrate consumption rates were transferred to flasks containing fresh MSA medium and fragments of porous stone.

2.4. Packed-bed biofilm channel (PBBC)

A horizontal channel was constructed using methyl methacrylate plates. The channel body has five flow-directing panels that create a three-stage sinuous channel. Each stage starts with an aeration module where the inflowing liquid is aerated before entering to the U-channel that contains the microbial consortium attached to the porous support. In each stage, initially, the liquid moves downwards; then it moves upward. When the liquid medium reaches the end of the U-channel, it enters into a new aeration module, then flows to the next stage.

A scheme of the PBBC is shown in Fig. 1. The total volume of the channel was 5.66 L, the fraction of porous support (ε_S) in the packed-bed channel was 0.65 and the liquid fraction (ε_L) of 0.35; therefore, the operating volume in the channel was 2.0 L, and the total volume of liquid contained in the three small aeration modules was 0.25 L.

The support material used for the PBBC was a porous volcanic rock, named tezontle in Mexico. The characteristics of the porous stone, average diameter ($\boldsymbol{dp}$) of 7.44 mm ± 0.96, average particle volume ($\boldsymbol{Vp}$) of 0.227 cm^3 ± 0.091 and bulk density of the packed bed ($\boldsymbol{\delta}_s$) of 2.70 ± 0.34 g cm^{-3}, were determined according to Gómez-De Jesús et al. (2009).

2.5. Start-up and operation of the packed-bed channel

To verify that no abiotic losses of linuron occur the aerated channel was fed with MSA medium containing a linuron concentration of 19.5 mg/L. No significant difference between the linuron

concentrations in the inflowing and outflowing liquid was observed after operating the channel for 30 days.

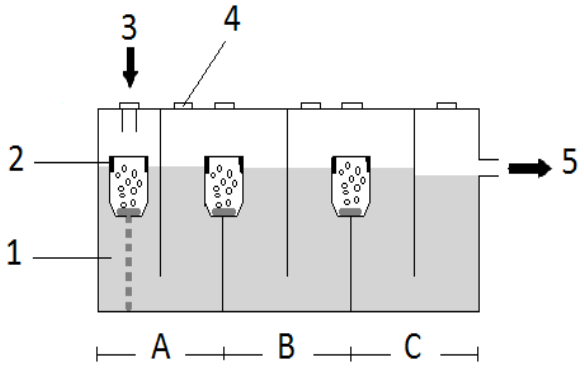

Fig. 1. Packed-bed biofilm channel used for the degradation of linuron.Packed-bed U-channel (1), aeration module (2), liquid input (3), ports for sampling or insertion of OD electrodes (4), liquid and air output (5), stages (A, B, C)

Once the support material was saturated with linuron, each stage was inoculated with the isolated community. The continuous culture was started with an MSA medium flow rate of 30 mL/h.

The linuron concentration was 19.5 mg/L and the aeration rate, maintained in each aeration module during the operation of the reactor, was 0.15 ± 0.05 L/min. Five flow rates (F) were used to evaluate the reactor performance. The F values varied from 30 to 480 mL/h and the linuron concentrations were gradually increased from 19.5 to 62.5 mg/L. At each operative condition, linuron concentration was monitored until it remained unchanged, meaning that a steady state was reached. Then, the total organic carbon (TOC), COD, DO and linuron concentrations in the different stages of the channel were determined.

2.6. Determination of linuron

Two methods were used for linuron determination. A spectrophotometric method (λ = 246 nm in a Beckman DU 650 spectrophotometer) was routinely used for linuron monitoring (Tunçeli et al., 2001). The quantitative analysis of the linuron was made by HPLC using a high performance liquid chromatography system, model 1260 Infinity of Agilent Technologies, equipped with a diode array detector and a Supercosil LC-18 column (dimensions 250 mm x 4.6 mm) with particle size of 5µm. The mobile phase used was water/acetonitrile (55:45) with a flow of 1.6 mL/min.

The linuron was quantified by UV absorbance at 250 nm. The method can also detect and quantify 3,4-dichloroaniline (3,4-DCA), a linuron metabolite.

2.7. Determination of COD, TOC and DO

The methods 8000 and 10129 from Hach Co. (2002) were used for COD and TOC determination, respectively, using a DRB 200 Hach reactor. For DO determinations, a dissolved oxygen meter model HI 9142 of Hanna instruments, USA was used.

3. Results and discussion

By measuring the difference of COD, TOC and linuron concentrations between the inflow and the outflow of the channel, the kinetic and stoichiometric behavior of the system were evaluated. The removal efficiencies (η) were expressed as the ratio of volumetric removal rates, (R_V) and volumetric loading rates (B_V) of COD, TOC, and linuron $\eta = \frac{R_V}{B_V}$ is calculated as $R_V = \frac{F(C_R - c)}{V_L}$ and $B_V = \frac{FC_R}{V_L}$, where F it is the flow of inflowing medium, C_R, and c are the linuron, COD or TOC concentrations in the inflowing and outflowing medium, respectively. COD and TOC determinations were used as indirect measurements of the chemically oxidizable adjuvants present in the commercial formulation of the herbicide.

Considering the aforementioned working conditions, the corresponding volumetric loading rates for COD ($B_{V,COD}$) varied from 0.58 to 29.61 mg COD/Lh; for TOC ($B_{V,COD}$) from 0.23 to 11.84 mg TOC/Lh, and for linuron ($B_{V,L}$), from 0.29 to 14.93 mg linuron/Lh (Table 1.)

Table 1. Operational conditions and the corresponding volumetric loading rates used in the packed-bed biofilm channel

Operational condition	*Flow rate (mL/h)*	*Hydraulic retention time (h)*	*Afalon 50WP concentration (mg/L)*	B_V		
				Linuron (mg/Lh)	*COD (mg/Lh)*	*TOC (mg/Lh)*
1	30	67.0	50	0.29	0.58	0.23
2	60	33.5	50	0.58	1.16	0.46
3	120	16.8	50	1.16	2.33	0.93
4	240	8.4	50	2.33	4.66	1.85
5	480	4.2	50	4.66	9.31	3.70
6			75	7.00	13.85	5.56
7			100	9.34	18.63	7.40
8			125	11.65	23.16	9.27
9			160	14.93	29.61	11.84

3.1. Linuron removal

Fig. 2A shows that under all working conditions tested the volumetric removal rates of linuron ($R_{V,L}$) were equal to the corresponding volumetric loading rates ($B_{V,L}$) used; consequently, the removal efficiency (η) was 100 %. The metabolite 3,4-dichloroaniline was not detected.

3.2. COD Removal

The COD test indirectly measure the amount of the chemically oxidizable organic and inorganic

fractions present in a liquid sample. The commercial formulation used has 50% of linuron and 50% of adjuvants; thus, the COD values determined correspond to the quantity of oxygen required to oxidize the linuron and the organic adjuvants to CO_2, ammonia and water. According to the linuron oxidation reaction, the oxygen required to oxidize a milligram of linuron is 1.16 mg O_2.

$$C_9H_{10}Cl_2N_2O_2 + 9O_2 \rightarrow 9CO_2 + 2NH_3 + \\ + 2H_2O + 2Cl^-$$

Fig. 2B shows that in any of the working conditions tested the removal rate of COD ($R_{V,COD}$) reached the values of $B_{V,COD}$. Because linuron was entirely removed, and no aromatic catabolites were detected by HPLC, the COD values obtained could indicate that some herbicide adjuvants and possible, other catabolic intermediaries were not degraded by the microbial community. In general, it was found that working with flow rates between 30 and 240 mL/h and input COD values of 39 mg/L, the η_{COD} values were relatively low; about 50-60%.

Along the operation of the bioreactor, the η_{COD} values were gradually increased. Operating at a flow rate of 480 mL/h and COD concentrations between 39-124 mg/L, the η_{COD} reaches values rounding the 80%, and the highest efficiency achieved at the highest $B_{V,COD}$ tested (29.61 mg COD/Lh) was 84%. The improvement in η_{COD} values, could reflect the acclimation of the microbial consortium; phenomenon observed in the long-term operation of biodegradation processes (Nava-Arenas et al., 2012).

3.3. TOC removal

The carbon content of the linuron molecule is 0.434 mg C/[mg linuron]. According to the results obtained, 53% of the total organic carbon (TOC) present in the commercial formulation of the herbicide corresponds to linuron, and the remaining 47% to other organic molecules. Fig 2C shows than in any case the $R_{V,\,TOC}$ values reach the values of $B_{V,\,TOC}$. The TOC removal behavior is similar to that obtained for the removal of COD. These results indicate that the microbial community could not mineralize some organic compounds. The highest removal efficiency achieved was 85%, and as occurs with the COD removal, this efficiency was achieved at the highest $B_{V,\,TOC}$ (11.84 mg TOC/Lh).

3.4. Oxygen concentration throughout the channel

In aerobic processes, the maintenance of an adequate DO concentration during the culture is essential. For this reason, the channel design considered aeration modules between each stage of the channel to replenish the oxygen consumed along the PBBC. Fig. 3 shows the oxygen concentration at the end of each channel's stage. The horizontal dashed lines show the average DO concentrations achieved in each one of the aeration modules located at the beginning of each stage. Each column indicates one loading rate, the values of the loading rates increase from left to right.

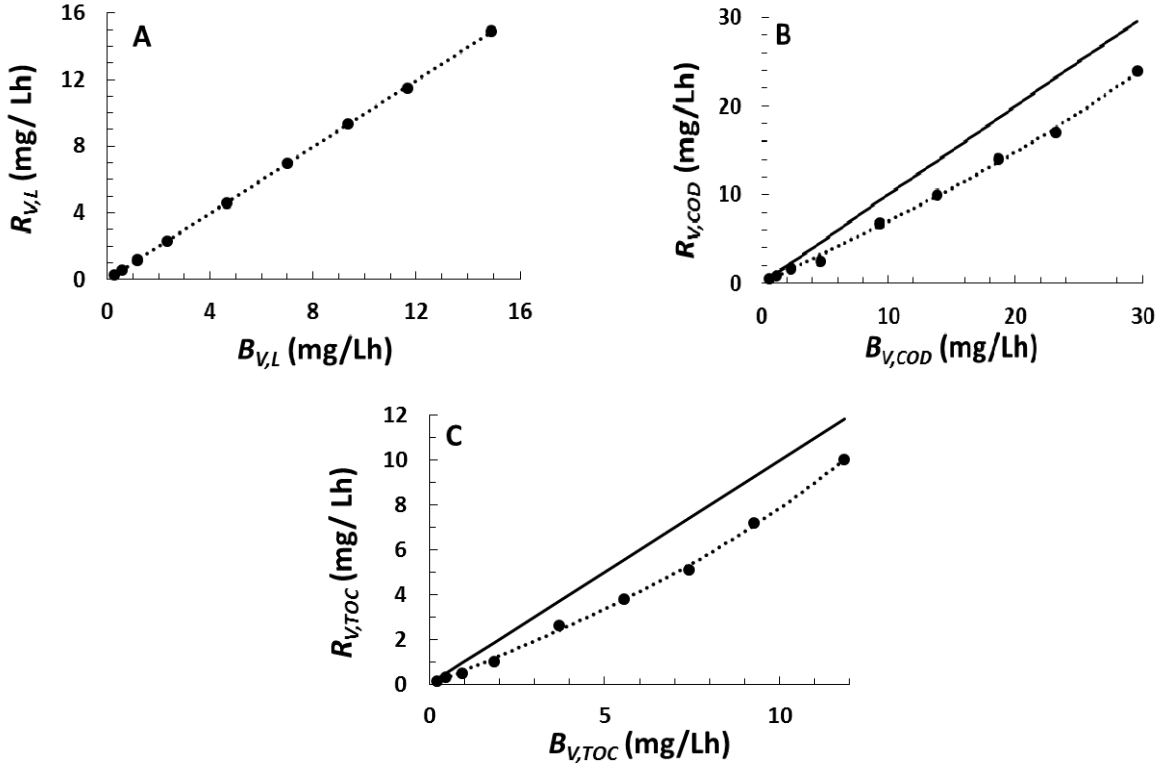

Fig. 2. The volumetric removal rates R_V of linuron **(A)**, COD (**B)** and TOC **(C)**, respectively, obtained at different loading rates B_V (the solid line represents the 100% of removal efficiency, and the markers are the experimental values resulted)

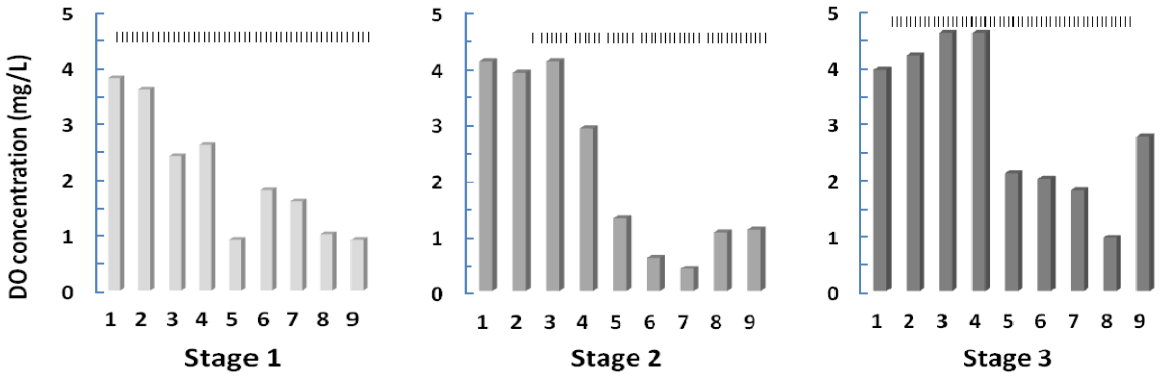

Fig. 3. Dissolved oxygen concentrations (DO) at the end of each channel stage along the nine working conditions evaluated

The aeration rate supplied to the modules was 0.15 ± 0.05 L/min. With this rate, a DO concentration near five mg/L, which corresponds to the value of ≈ 70% saturation, was maintained in each aeration module. As seen in Fig. 3, at the distinct operating conditions, the DO was not exhausted in the packed bed, indicating that even at the highest volumetric rates the microbial community was not limited by oxygen. From this figure, it could be noticed that the highest O_2 consumption occurs in the first stage of the channel and that the O_2 consumption tends to increase with the volumetric loading rate of the herbicide, proportionally with the increase in the $B_{V,L}$ values.

The overall evaluation of the PBBC behavior indicates that the bioreactor was highly efficient for linuron removal. Although the maximum removal efficiencies achieved for COD and TOC were slightly higher than 80%, these values were satisfactory, considering that the commercial presentation of the herbicide contains 50% of adjuvants. The highest removal efficiencies were achieved when the herbicide loading rates were increased. Evidently, these values occur after a long period of continuous operation (about 14 months), when the biofilm was acclimatized and established in a large area of the porous support (Nava-Arenas et al., 2012).

The channel works as a plug-flow reactor in which, each zone has differences in attached cell mass, and reactants concentrations. These local differences joined to the composition and feed rate of the inflowing medium should affect the local rates of O_2 consumption and contaminant biodegradation. When the channel was operated at the lowest concentration of linuron (19.5 mg/L), and at relatively low flow rates (30 to 240 mL/h), the linuron was exhausted in the first stage of the channel.

Finally, as can be observed, the design of the aeration system (aeration modules) was successful, and its location in the bioreactor allowed enough dissolved oxygen at the beginning of each stage, and along the whole channel.

4. Conclusions

Under the working conditions, the microbial community was able to remove linuron completely. The highest COD and TOC removal efficiency reached in the PBBC were higher than 80%. With the aeration rate used, DO concentrations of about 5 mg/L were maintained in the aeration modules. In any stage of the bioreactor, the DO was entirely consumed, indicating that, even at the highest loading rates, the microbial community was not limited by oxygen. Therefore, the design of the aeration system was adequate for this kind of packed-bed biofilm channel.

Acknowledgements

Marrón-Montiel E is holder of a research grant from Conacyt. González-Cuna S was holder of a research grant from Conacyt and BEIFI, IPN. Ruiz-Ordaz N., and Galíndez-Mayer J., are holders of grants from COFAA-IPN, SIP-IPN, and SNI-Compact. Poggi-Varaldo H is holder of a grant from SNI-Conact.

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