Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 2177–2181
BIORESOURCE TECHNOLOGY

Degradation of oxadiazon in a bioreactor integrated in the water closed circuit of a plant nursery

Paloma Pinilla, Juan Ruiz, María Carmen Lobo, María José Martínez-Iñigo *

Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), Finca El Encín, A-II Km 38,2, 28800 Madrid, Spain

Received 7 July 2006; received in revised form 17 May 2007; accepted 18 May 2007
Available online 12 July 2007

Abstract

Hardy ornamental nursery stock (HONS) use fertigation as a rational supply of nutrients all along the growth cycle of plants. Nevertheless, that frequency of irrigation increases the risks of nutrient and herbicide leaching and subsequent contamination of the waste water. Therefore, systems of water treatment are required in plant nurseries. *Pseudomonas fluorescens* strain CG5 cells were immobilized on a ceramic support (sepiolite) contained in a 150 l-bioreactor for the biodegradation of the herbicide oxadiazon in the re-circulated leachates. Percolation and inundation operating processes were assayed in the bioreactor. The levels of oxadiazon in water samples were determined by solid phase extraction on C_{18} columns and gas chromatography with electron capture detection system. Fifty eight percolation cycles resulted in a significant reduction of oxadiazon up to just 5 μg l^{-1} at the outlet. Similar herbicide elimination was achieved after two consecutive 68-h inundation periods. In addition, it was found that the nutrient content in the waste water at the bioreactor outlet was sufficient to support an adequate plant growth.

Keywords: Bioreactor; Plant nursery; Waste water; Oxadiazon; *Pseudomonas*

1. Introduction

Lately, a great development of the ornamental plant nursery industry has been observed in our country. Due to the intensive use of water, plant nutrients and pesticides, the benefits of this type of cultivation including the lack of dependence on arable soil, faster plant growth and higher plant quality, could be limited in terms of the environmental cost of the production. Alternative cultural practices like trickle irrigation can be incorporated in plant nurseries in order to ameliorate the management of water and fertilizers, which are directly applied to the substrate beneath plants. Nevertheless, the significant losses of N and P in leachates from containerized nursery crops are still a real menace of ground water pollution and eutrophication of surface waters. Furthermore, the contents of nitrate N (USEPA, 2004) and nitrates (European Community, 2000) have been limited to 10 mg l^{-1} and 50 mg l^{-1} in drinking water, respectively. The high consume of a scarce natural resource as water and the hazards of chemical diffuse contamination have led to investigate different systems of waste water treatment and recirculation for plant nurseries (Runes et al., 2003; Lubello et al., 2004).

Ronstar (25% w/v oxadiazon (5-*ter*-butyl 3-(2,4-dichloro 5-isopropoxyphenyl) 1,3,4-oxadiazol-2(3*H*)-one)) is commonly used in ornamental plant nursery industry for pre- and post- emergent weed control. Once applied to plant beds the mobility of pesticides depends on a combination of factors that includes the substrate and pesticide physicochemical properties, the crop cover, the timing of irrigation and the method of the chemical application. Previous studies on the leaching of oxadiazon in soil indicated that the strong adsorption of the herbicide to soil reduces the displacement towards the sub-surface layers (Wehtje et al., 1993; Ying and Williams, 2000). However, oxadiazon was found in the drainage water from an

* Corresponding author. Tel.: +34 91 8879471; fax: +34 91 8879474.
E-mail address: mjose.inigo@madrid.org (M.J. Martínez-Iñigo).

0960-8524/$ - see front matter
doi:10.1016/j.biortech.2007.05.039

ornamental nursery at levels above the limit allowed for drinking water (Harris et al., 1997).

Biological treatment of waste water from plant nursery industries could be a promising system that benefits from the natural ability of soil microorganisms to degrade herbicides (Chakraborty et al., 1995; Garbi et al., 2006).

The aim of this study was to assess the feasibility of a bioreactor consisting of immobilized *Pseudomonas fluorescens* strain CG5 cells to clean-up oxadiazon in the waste water from an ornamental conifer nursery. The integration of the bioreactor in a water closed circuit that saves the excess of nutrients from the release to the environment and re-uses them in plant fertigation was also evaluated.

2. Methods

2.1. Microorganisms

The oxadiazon-degrading bacteria *Pseudomonas fluorescens* strain CG5 was used in the immobilized-cell system proposed for the waste water treatment (Garbi et al., 2006).

2.2. Chemicals

Ronstar (25% w/v oxadiazon) herbicide was purchased from Aventis. The chemical reference standards at the highest purity commercially available were obtained from Dr. Ehrenstorfer GmbH (Reference Materials ISO 9001 certified).

2.3. Experimental site and herbicide application

The study was conducted on a plot of 42 m^2 isolated from the underlying soil by an impermeable membrane. Four different species of conifers (*Cupressus sempervirens* L., *Cupressocyparis leylandii* Dallim & A.B. Jack, *Cupressus arizonica* Greene, *Cedrus deodara* (D. Don) G. Don) were grown in 65 containers placed on the plot. The substrate for bedding plants consisted of a commercial mixture of peat, vermicompost humates, puzzolane and sand (5:1:2:2, by volume). Three kilograms of the mineral fertilizer osmocote (18N–9P–10K) was supplied to one cubic meter plant bed. This 12–14 moths controlled-release fertilizer provides 9% of N as nitrate N, 8% as ammonium N and 1% as urea N. In addition, nutrient-enriched groundwater was delivered by trickle fertigation to each container (Eymar et al., 2005). Drainage water from containers was collected and sent to the bioreactor for treatment. Ronstar was manually spread over the top of the containers at the dose of 120 kg ha^{-1}.

2.4. Bioreactor experiments

A 150-l bioreactor was installed in an ornamental conifer nursery for the treatment of the water lixiviated from a 42 m^2-cultivated area. The industrial scale bioreactor was filled with 124 dm^3 of the ceramic support sepiolite. The method used for the immobilization of *P. fluorescens* strain CG5 cells on the granular sepiolite has been previously described (Garbi, 2002). Bacterial cells were grown aerobically at 30 °C in minimal MB medium (Gerhardt et al., 1995) with oxadiazon at the concentration of 3 mg l^{-1} supplied in 0.3% ethanol. The culture at the exponential phase of growth was added to 150 l of MB medium, representing an inoculum charge of 1% of the volume applied to the bioreactor. After 2-day inundation period, the initial immobilized inoculum (6×10^{-6} CFU g^{-1} sepiolite) was determined according to Hrenovic et al. (2005). The bioreactor operated with a working volume of 75 l either under flooding or re-circulating conditions.

2.5. Analytical methods

Herbicide was extracted from 5-g samples of the substrate from each container by adding 16 ml of acetone:water (1:1, v/v) acidified with phosphoric acid to pH 2. The soil suspension was introduced into an ultrasonic bath for 15 min. The extract was decanted and diluted with 72 ml of water.

Solid phase extraction of drainage water and substrate extract samples was carried out using C_{18} columns (Mega Bond Elut, 1 g, Varian, Harbor City, CA) that were consecutively conditioned with 2 ml of dichloromethane, 2 ml of methanol and 2 ml of purified water. Four millilitre aliquots of either waste water or substrate extracts were loaded on the column at reduced vacuum. The cartridge was then washed with 1 ml of purified water and allowed vacuum-dry for 30 min. Afterwards, the herbicide was eluted in 2 ml of dichloromethane. Solvent was then evaporated under vacuum and the herbicide re-dissolved in 2 ml of hexane.

Oxadiazon in hexane extracts was analyzed in a gas chromatograph with electron capture detector (Varian CP3800). A fused silica capillary (5% phenyl 95% dimethyl polysiloxane; CP Sil 8CB, Varian) column (30 m × 0.25 mm I.D., 0.25 μm film thickness) was used. Aliquots of 1 μl were injected in splitless mode and the chromatographic conditions were as follows: the initial temperature held at 80 °C for 1 min was raised to 250 °C at the rate of 15 °C min^{-1} and the final temperature held for 5 min. Gas carrier was helium. Injector and detector were heated at 300 °C. Alachlor was used as internal standard. The limit of detection for the herbicide was 4 ng ml^{-1}.

Nutrients Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} in waste water were determined by Atomic Absorption (Varian SpectraAA-600).

Nitrate and phosphate anions were analyzed by ionic chromatography (Dionex DX 500) using an AG9-HC precolumn and an Ion Pac AS9-HC column with a 9 mM Na_2CO_3 solution as mobile phase.

3. Results

In the ornamental conifer nursery the trickle fertigation system was adjusted to deliver either 500 l or 700 l of nutrient enriched water during the last days of spring under study. The 24-h drainage volume following each fertigation represented at between 11% and 20% of the water received (Fig. 1). In all cases, the concentration of nutrients in leachates was increased as compared to that in the fertigation water (Fig. 2). The losses of nitrate N and phosphate P from the containers for the whole period of 17 days were measured as the charge of potential contaminants towards ground and surface waters that would have received the soil beneath the containers (Fig. 3). Up to 49 kg ha^{-1} nitrate N and 17 kg ha^{-1} phosphate P were released.

The pH and conductivity of leachates were determined in order to complete the physicochemical characterization of the waste water that fed the bioreactor. The conductivity values were found at between 2.9 and 4.3 dS cm^{-1}. pH was close to neutrality in the first drainage and became consistently acid (pH 6). Regarding herbicide leaching, the highest peak of concentration was found shortly after application varying from 37 to 17 μg oxadiazon l^{-1}. In contrast, analysis of the herbicide in plant beds rendered high concentrations of oxadiazon, ranging from 0.25 to 5 μg g^{-1}. These values accounted for the strong adsorption of the herbicide (Goodwin and Beach, 2001) that resulted not evenly distributed over the different containers due to the application method used.

The efficiency of the cell-immobilized bioreactor was assayed under two distinct operating procedures (Fig. 4).

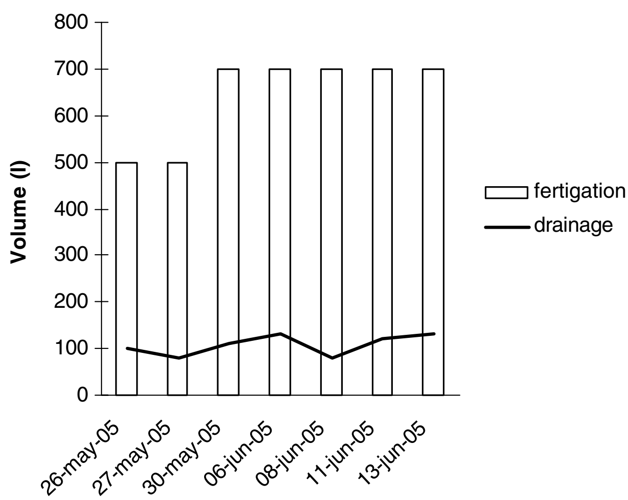

Fig. 1. Volume of water delivered in each fertigation and drained during the following 24-h.

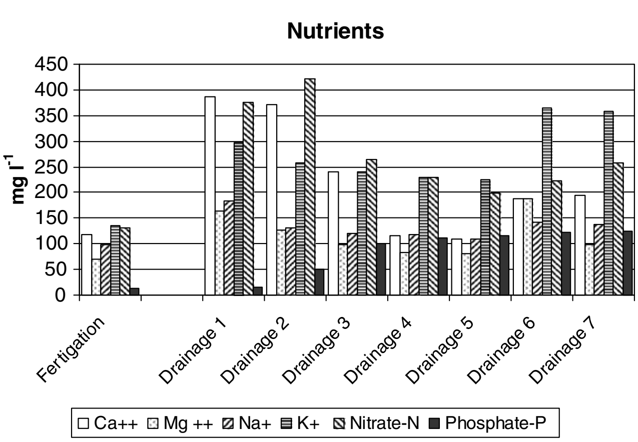

Fig. 2. Concentration of nutrients in the fertigation and drainage water from the ornamental conifer nursery. The standard deviations were less than 10% of the mean value presented.

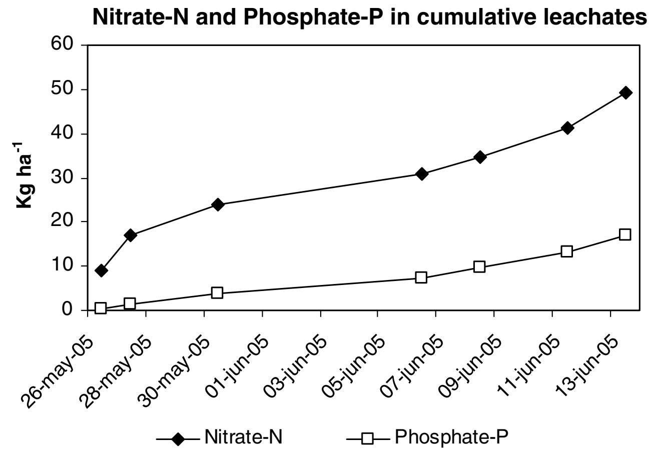

Fig. 3. Cumulative loss of nitrate N and phosphate P from conifer containeized crop. The standard deviations were less than 10% of the mean value presented.

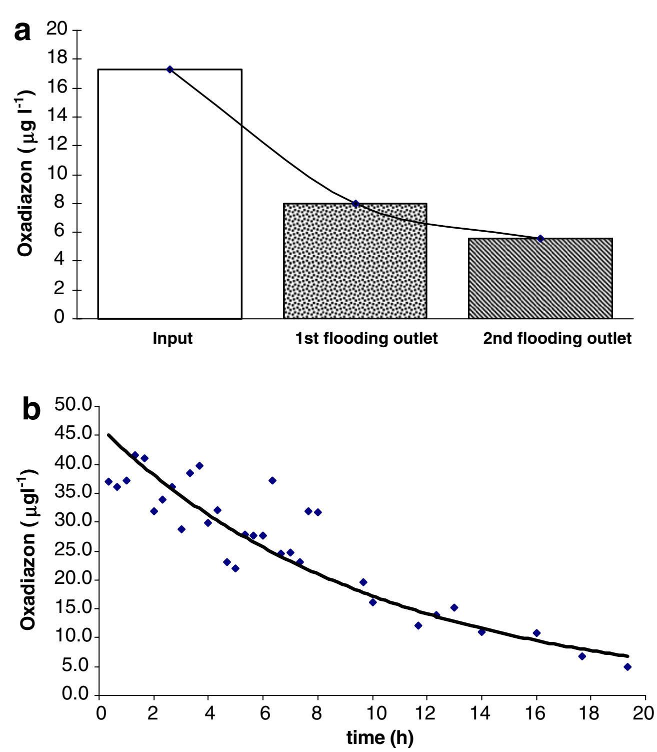

Fig. 4. Oxadiazon concentration during the treatment of the waste water from the ornamental conifer nursery in the bioreactor operating under (a) flooding and (b) percolation conditions. The standard deviations were less than 10% of the mean value presented.

Flooding conditions were essayed with the lowest charge of oxadiazon in the waste water (17 μg l^{-1}), in an attempt to eliminate the herbicide by operating for short periods without aeration (Fig. 4a). After two repeated 68-h inundations separated by a resting period of 4 h with the bioreactor emptied for aeration, the level of oxadiazon descended to the limit of detection. On the other hand, 58 percolation cycles of 20 min hydraulic retention time (HRT) each were necessary to clean-up the waste water contaminated with 37 μg oxadiazon l^{-1} (Fig. 4b). Regardless the operating procedure, kinetic of oxadiazon degradation in the bioreactor fitted the exponential equation: $C = C_i e^{-kt}$, where C_i is the initial concentration of oxadiazon, t is the time of bioreactor operation (h) and k the rate constant of degradation. The different values of the kinetic parameters corresponding to each operating procedure are shown in Table 1. The bioreactor working by percolating cycles exhibited significantly higher values for the rate constant and the initial degradation rate. In contrast, less oxygen supply occurring under flooding conditions could be a limiting factor of the bacteria activity in the bioreactor. Furthermore, the residual concentration of herbicide in flooding waste water likely contributed to reduce the rate of herbicide degradation. It has been reported that increasing amounts of oxadiazon below the toxic levels, raised the rate of the herbicide removal in pilot-scale bioreactors (Garbi et al., 2006). In spite of the different rates of herbicide degradation depending on the operating procedure, in both cases the bioreactor successfully achieved the complete removal of the varied charge of contamination received. The fact that the whole time the bioreactor operated by percolation to eliminate the highest concentration of oxadiazon in the waste water was close to that necessary for draining a working volume of the bioreactor, pointed at the advantages of this operating procedure for the integration of the remediation system in the water closed circuit.

Besides the water remediation, the balance of nutrients after the bioreactor operation was evaluated (Table 2) in order to assess its quality for fertigation re-use. Likewise other pseudomonades have been described as denitrifying microorganisms (Belloso and Colángelo, 2005), *Pseudomonas fluorescens* strain CG5 metabolized large amounts of nitrates in the bioreactor. The levels of Ca^{2+}, K^{+} and phosphate P also considerably decreased in the bioreactor. Nevertheless, the doses of nutrients in the recycled waste water were close to those in the fertigation water.

Table 1
Kinetic parameters of oxadiazon degradation in the bioreactor fitting exponential equations[a]

Operating procedure	Ci[b] (μg l^{-1})	k[c] (h^{-1})	Initial degradation rate (μg l^{-1} h^{-1})
Percolation	46.5	0.0992	4.6
Flooding	16.1	0.0083	0.1

[a] $r^2 = 0.8804$; $r^2 = 0.9569$ for percolation and flooding procedures, respectively.
[b] C_i is the initial concentration of oxadiazon.
[c] k the rate constant of degradation.

Table 2
Balance of nutrients in the bioreactor operating under flooding conditions and nutrient supply in the fertigation water

	mg/l					
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Nitrate-N	Phosphate-P
Input	239	99	120	240	263	100
Output	157	138	127	161	149	14
Fertigation	142	70	98	135	130	14

4. Discussion

Despite the excess of water used in overhead irrigation was avoided by the trickle irrigation of the containers, the percentage of drained water still represented a considerable loss that in turns, involved the leaching of plant nutrients. Phosphorus was found in large amounts in leachates as already reported in the case of bedding plant substrates including peat in their composition (Juntunen et al., 2002). In addition, a significant amount of the nitrate N supplied as the main source of nitrogen for plant nutrition was mobilized from the containers in accordance with the data of nitrate exported from a rhododendron nursery (Colangelo and Brand, 2001).

In the ornamental conifer nursery the fact that herbicides were applied on the top of the bed surface without incorporation could help the leaching of herbicides shortly afterwards, whereas it took longer to establish the dynamic adsorption equilibrium LaFleur (1979). Although the herbicide oxadiazon exhibited low mobility through the bedding plant substrate, the remediation of the drainage water is required to control the fate of herbicides and their potential detrimental effects on non-target organisms.

By incorporating a bioreactor for the degradation of the herbicides mobilized in the leachates, the recycled waste water could be reused for fertilization without increasing the conifers cultivation exposure to the herbicide. The bioreactor design based on the immobilized-cell system displayed high adequacy for the waste water treatment at a nursery scale. The enhanced viability and activity of the bacteria obtained by cell immobilization on sepiolite (Martin et al., 2000), permitted to operate under inundation periods until the complete clean-up of leachates. Additionally, the immobilized-cell system prevented large losses of biomass during the continuous re-circulation of waste water through the bioreactor. Thanks to the highest rate constant of herbicide degradation reached in the bioreactor operating by percolation, it seemed to be feasible the time-efficient recycling of waste water in a closed circuit for the conifer plant nursery.

The fact that oxadiazon removal was simultaneous to denitrification of nursery waste water could indicate that the mechanisms of the organic compound transformation were similar to other already described for a different *P. fluorescens* strain (Criddle et al., 1990).

In conclusion, the proposed water closed circuit could represent a system for the rational management of water, mineral fertilizer and herbicides that will allow a sustainable and environmental-friendly intensive agriculture in containerized nurseries.

Acknowledgements

Funding for this study was provided by the Comunidad de Madrid Project GR/AMB/0073/2004.

References

Belloso, C.O., Colángelo, I., 2005. Nitrate removal from groundwater by in situ denitrification, In: Alleman, B.C., Kelley, M.E. (Conference Chairs). Proceedings of the English International In Situ and On-site Bioremediation Symposium, Battelle Press, Columbus, OH, paper O-03.

Chakraborty, S.K., Chowdhury, A., Bhattacharyya, A., Ghosh, S., Pan, S., Waters, R., Adityachaudhury, N., 1995. Microbial degradation of oxadiazon by soil fungus *Fusarium solani*. J. Agric. Food Chem. 43, 2964–2969.

Colangelo, D.J., Brand, M.H., 2001. Nitrate leaching beneath a containerized nursery crop receiving trickle or overhead irrigation. J. Environ. Qual. 30, 51–59.

Criddle, C.S., DeWitt, J.T., Grbic-Galic, D., Mc Carty, P.L., 1990. Transformation of carbon tetrachloride (CT) by *Pseudomonas fluorescens* strain KC under denitrification conditions. Appl. Environ. Microbiol. 56, 3240–3246.

European Community, 2000. UE Water Framework Directive 2000/60/EC. Off. J. Eur. Commun. L 327: pp. 1–21.

Eymar, E., Cadahía, C., López-Vela, D., Martín, I., 2005. Fertirrigación de plantas ornamentales. In: Cadahía, C. (Ed.), third ed., Ferirrigación. Cultivos hortícolas, frutícolas y ornamentales Mundi-Prensa, Madrid.

Garbi, C., 2002. Sistema biológico de descontaminación y reciclado de las aguas de drenaje de un vivero de plantas ornamentales: desarrollo, monitorización y modelización. PhD thesis. Universidad Complutense de Madrid, Madrid, Spain.

Garbi, C., Casasus, L., Martinez-Alvarez, R., Robla, J.I., Martin, M., 2006. Biodegradation of oxadiazon by a soil isolated *Pseudomonas fluorescens* strain CG5: implementation in an herbicide removal reactor and modelling. Water Res. 40, 1217–1223.

Gerhardt, P., Murray, R.G., Costilow, R.N., Nester, E.W., Wood, W.A., Krieg, N.R., Phillips, G.B., 1995. Manual of methods for general and molecular bacteriology. The American Soc. Microbiol., Washington, DC.

Goodwin, P.B., Beach, S., 2001. Oxadiazon, oryzalin, and oxyfluorfen residues in container plant nurseries. HortScience 36, 900–904.

Harris, G.L., Hodgkinson, R.A., Scott, M., Mason, D.J., Pepper, T.J., 1997. Impact of hardy ornamental nursery stock (HONS) systems on the environment: losses of nutrients and agrochemicals. Agricultural Water Manag. 34, 95–110.

Hrenovic, J., Tibljas, D., Orhan, Y., Büyükgüngör, H., 2005. Immobilisation of Acinetobacter calcoaceticus using natural carriers. Water SA. 31, 261–266.

Juntunen, M-J., Hammar, T., Rikala, R., 2002. Leaching of nitrogen and phosphorus during production of forest seedlings in containers. J. Environ. Qual. 31, 1868–1874.

LaFleur, K.S., 1979. Sorption of pesticides by model soils and agronomic soils: rates and equilibria. Soil Sci. 127, 94–101.

Lubello, C., Gori, R., Nicese, F.P., Ferrini, F., 2004. Municipal-treated wastewater reuse for plant nursery irrigation. Water Res. 38, 2939–2947.

Martin, M., Mengs, G., Plaza, E., Garbi, C., Sánchez, M., Gibello, A., Gutierrez, F., Ferrer, E., 2000. Phropachlor removal by *Pseudomonas* strain GCH1 in an immobilized-cell system. Appl. Environ. Microbiol. 66, 1190–1194.

Runes, H.B., Jenkins, J.J., Moore, J.A., Bottomley, P.J., Wilson, B.D., 2003. Treatment of atrazine in nursery irrigation runoff by a constructed wetland. Water Res. 37, 539–550.

USEPA, 2004. EPA 822-R-04-005. 2004 Edition of the drinking water standards and health advisories. Office of water USEPA, Washington, DC.

Wehtje, G.R., Gilliam, C.H., Hajek, B.F., 1993. Adsorption, desorption, and leaching of oxadiazon in container media and soil. HortScience 28, 126–128.

Ying, G.-G., Williams, B.D., 2000. Mobility and persistence of four herbicides in soil of a South Australian vineyard. Pest. Manag. 56, 277–283.