Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 151 (2008) 658–661
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Identification of metabolites during biodegradation of pendimethalin in bioslurry reactor

M. Ramakrishna [a], S. Venkata Mohan [a,*], S. Shailaja [a], R. Narashima [b], P.N. Sarma [a,*]

[a] *Bioengineering and Environmental Center, Indian Institute of Chemical Technology, Hyderabad 500007, India*
[b] *Analytical Division, Indian Institute of Chemical Technology, Hyderabad 500007, India*

Received 21 December 2006; received in revised form 11 June 2007; accepted 11 June 2007
Available online 15 June 2007

Abstract

Bioslurry phase reactor was used for the degradation of pendimethalin, a pre-emergence herbicide in the contaminated soil under aerobic environment. More than 91% degradation of pendimethalin was observed for 5 days of reactor operation augmented with sewage from effluent treatment plant (ETP). The performance of the reactor was monitored regularly by measuring pH and colony forming units (CFU). The metabolites of pendimethalin formed during degradation were identified using various analytical techniques, viz., thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and liquid chromatography–mass spectroscopy (LC–MS/MS). Four metabolites were formed and identified as *N*-(1-ethylpropyl)-3,4-dicarboxy 2,6-dinitrobenzenamine-*N*-oxide, *N*-(1-ethylpropyl)-3,4-dimethoxy-2,6-dinitrobenzenamine and benezimadazole-7-carboxyaldehyde. The reactions involved were monohydrolysis of 2-methyl groups followed by dihydrolysis. Further oxidation of amine groups and hydroxylation of propyl groups produced the above said metabolites. Degradation pathway of pendimethalin has been proposed in the bioslurry phase reactor.

Keywords: Pendimethalin; Slurry phase bioreactor; Metabolites; Bioaugmentation; HPLC and LC–MS/MS

1. Introduction

Pendimethalin is a pre-emergence herbicide, used to destroy or prevent the growth of weeds. Its widespread usage led to its detection as contaminant in soil, ground water, surface water and air [1–5]. Pendimethalin is normally degraded through photo-degradation, volatilization or by biodegradation [6–10]. Several soil fungi were found to degrade pendimethalin as a sole carbon source in mineral solution [11–13]. In this direction pendimethalin bioremediation was successfully reported in soil slurry system [14]. Bioslurry phase systems utilize naturally occurring bacteria (native soil microflora) or inoculated strain having special metabolic property to convert hazardous organic compounds to CO_2 and water present in solid, liquid or solid–liquid interface [14–16]. In this communication we report the results pertaining to the investigations carried out on the biodegradation of pendimethalin using bioslurry reactor to evaluate the metabolites formed during the degradation process.

2. Materials and methods

Pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenenamine) with more than 98% purity was obtained from M/S Shogan Organics Ltd., Mumbai. Pendimethalin has a molecular weight of 281 and soil log K_{oc} is 4.39. The aqueous solubility of pendimethalin at 20 °C is 0.5 mg/l. All solutions were prepared with double glass distilled water (pH 7.02 ± 0.1; CFU-nil; metals-absent). Thin layer chromatography (TLC) plates coated with silica gel having a particle size of 5–7 μm and pore size 60A (Sigma–Aldrich) were used in the experiments to identify the pendimethalin and its metabolites. Soil sample was collected from our institute garden (Indian Institute of Chemical Technology, IICT), which was normally used for horticultural activities. The properties of the selected soil and reactor operation details were reported [14]. Air-dried soil was spiked with pendimethalin dissolved in acetone to impregnate the soil. Soil particles were soaked in acetone containing

* Corresponding authors. Tel.: +91 40 27160123.
E-mail addresses: vmohan_s@yahoo.com (S. Venkata Mohan), sarma1950@yahoo.com (P.N. Sarma).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.06.039

pendimethalin to induce homogeneous sorption over the soil particles. Soil was subsequently evaporated at room temperature for 10 h. Pendimethalin impregnated soil was made into slurry with soil to water ratio of 1:15 (w/v) for control reactor and soil to ETP outlet in the ratio of 1:15 (w/v) for other reactors. Three slurry reactors, viz. A, B, C (control, soil native microflora and augmented with ETP microflora respectively) were operated. Reactor A was used as control wherein the natural soil microflora present in the soil was sterilized by autoclaving at 120 °C for 20 min. This reactor served to understand the volatile nature of the substrate (if any) and sorption–desorption phenomenon of the substrate during slurry phase operation. The reactors were monitored regularly for every 24 h of the reactor operation for slurry phase pH, oxidation–reduction potential (ORP), dissolved oxygen (DO) and oxygen consumption rate (OCR). The presence of the pendimethalin and metabolites was monitored regularly by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Twenty-five milligrams of soil sample was collected from each reactor and the substrate was extracted five times with 5 ml of acetonitrile. The extracts were pooled and concentrated in a rota-vapor (Buchi-170 model, water-bath at 40 °C temperature) to dryness and brought to a final volume of 2 ml. The final samples were subjected to HPLC after filtration with 0.45 μm nylon membrane. The analysis was done in triplicate to avoid the error and the average value was taken for final result. The coefficient of variance was found to vary between 1.85% and 2.38%.

The extracts from the slurry reactor C were analyzed 3 days for every 8 h by TLC. TLC was run using a mobile phase containing ethyl acetate and hexane in the ratio of 70:30. Two percent of H_2SO_4 in methanol was sprayed on the plate as developing reagent and charred over the hot plate for identification of the spots. The concentration of pendimethalin and the formation of metabolites were monitored for every 24 h by HPLC. Shimadzu LC-10A model equipped with SPD 10 AVP UV–vis detector was used. Kromacil C18 (250 mm × 4.6 mm i.d., 5 μm particle size) column (reverse phase) was used for the analysis with a flow rate of 1 ml/min and injection volume 20 μl and recorded at the wavelength of 225 nm. Ninety percent of acetonitrile in water was used as mobile phase and external standard method was used for calibration. Structural elucidation of the metabolites was carried out by LC–MS/MS.

Mass spectral analysis of pendimethalin and its metabolites were performed using Quattro LC triple-quadrupole mass spectrometer (micromass, Manchester, UK), with electorspray ionization (ESI) source. The data acquisition was under the control of Masslynx software Analysis was performed under positive mode ESI conditions to determine the pendimethalin degradation products. Nitrogen was used as a nebulizer and dissolvation gas, the other instrumental parameters were as follows: nebulizer gas flow 0.9 l/min, desolvation gas flow 9.5 l/min, capillary voltage 3.88 kV, cone voltage 25 V, Source block temperature 80 °C and desolvation temperature 250 °C. Collision-induced dissociation (CID) spectra were obtained selecting the precursor ion of interest with MSI and scanning MS2. Argon was used as the collision gas and the pressure in the collision cell was maintained at 3.0×10^{-4} mbar. All the CID spectra were recorded at 10 EV collision energies.

3. Results and discussions

3.1. Degradation of pendimethalin in bioslurry system

The performance of the bioslurry phase reactors was monitored by assessing the degradation of substrate in the slurry phase. Twenty-one percent of pendimethalin degradation was observed in the reactor A (control reactor) for 3 days, which could be attributed to the abiotic process [14]. It was observed that reactor B, which was operated with native soil microflora, showed 36% degradation of pendimethalin. The augmented reactor (reactor C) showed more than 91% degradation, which might be reasoned to the supplementation of ETP microflora to the native soil microflora in the reactor. The metabolic pathway studies were carried out for the degradation products obtained in reactor C. In reactor C augmented with ETP microflora showed higher CFU values (from 2.4×10^7 CFU/ml to 21.8×10^7 CFU/ml) and relatively less inhibition indicated the enhanced degradation of pendimethalin.

3.2. Identification of metabolites in the slurry phase reactor

Four metabolites were identified during the degradation of pendimethalin in slurry phase reactor by HPLC and LC–MS/MS in reactor C. During the reactor operation pendimethalin degradation was monitored by TLC and HPLC. The degradation products were analyzed for every 24 h by HPLC for a period of 5 days. A typical chromatogram is shown in Fig. 1. Four peaks were observed along with the parent peak (pendimethalin) in the slurry sample taken from reactor C after 48 h of cycle period. The peak obtained at 6.3 min was identified as pendimethalin by injecting a standard sample and comparing the retention time. Other peaks observed were at 1.7 min, 2.3 min, 4.8 min and 5.5 min denoted as M1, M2, M3 and M4 respectively. Studies were carried out by LC–MS/MS to identify the major peaks obtained.

The LC–MS/MS spectra of degradation products of pendimethalin are shown in Fig. 2. Four degradation products were detected and the structures of the metabolites were identified. The mass spectrum of M1 shows a prominent molecular ion at *m/z* 342 in addition to other characteristic ions at *m/z* 301, 270 and 100 (base peak). On the basis of this result the compound is identified as *N*-(1-ethylpropyl)-3,4-dicarboxy 2,6-dinitro benzenamine. For M2 a prominent molecular ion at *m/z* 355 was obtained in addition to other characteristic ions at *m/z* 311, 173 and 100 (base peak). The mass spectrum M3 showed a molecular ion at *m/z* 371 in addition to other characteristic ions at *m/z* 329, 313 and *m/z* 295. Similarly for M4 a molecular ion at *m/z* 146 was observed in addition to the characteristic ions at *m/z* 105 and 64. Based on the above results the compounds were assigned the structures of *N*-(1-ethyl-1-propyl)-3,4 dicarboxy-2,6-dinitrobenzenamine-*N*-oxide, *N*-(1-ethylpropyl)-3,4 dimethoxy-2,6-dinitrobenzenamine and

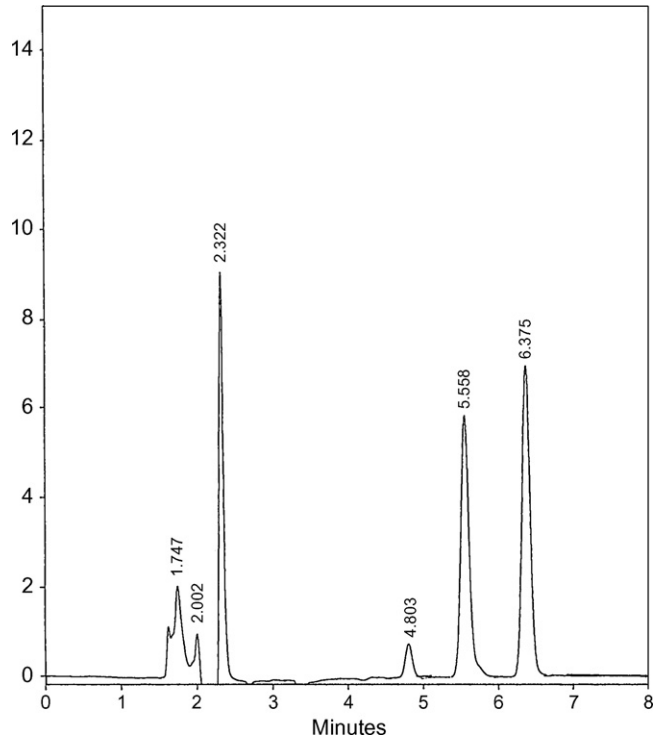

Fig. 1. HPLC chromatogram of reactor C. RT (1.7 min): *N*-(1-ethylpropyl)-3'4-dicarboxy 2'6-dinitro benzenamine. RT (2.3 min): *N*-(1-ethyl-1-propyl)-3,4 dicarboxy-2'6-dinitrobenzenamine-*N*-oxide. RT (8 min): *N*-(1-ethylpropyl)-3,4 dimethoxy-2,6-dinotrobenzenamine. RT (5.5 min): benzimadazole-7-carboxyaldehyde.

Table 1
Mass fragmentation pattern of pendimethalin and its metabolites

S. no.	Compound	RT (min)	Base peak	*m/z*
1	Pendimethalin	6.3	–	–
2	M1	1.7	301	342 and 270
3	M2	2.3	355	311 and 173
4	M3	4.8	329	371, 313 and 295
5	M4	5.5	105	146 and 64

benzimadazole-7-carboxyaldehyde respectively. The fragmentation pattern of pendimethalin and its metabolites by HPLC and LC–MS/MS is summarized in Table 1.

3.3. Metabolic pathway

The proposed metabolic pathway (Fig. 3) of pendimethalin in bioslurry reactor was derived by consolidating the analytical data. Since the bioslurry reactor was operated in aerobic environment. Two methyl groups attached to the benzene ring were subjected to hydrolysis forming M1 and M2 respectively (alcohol and acid). Oxidation of the amine groups led to formation of M3 and finally the hydroxylation of propyl groups gave benzimadazole-7 carboxyaldehyde (M4) with a molecular weight of 146. The metabolites, which are formed, were observed to be different from the literature [12]. The pathway proposed was in accordance with the results reported by Walker and Bond [16], in solid phase degradation.

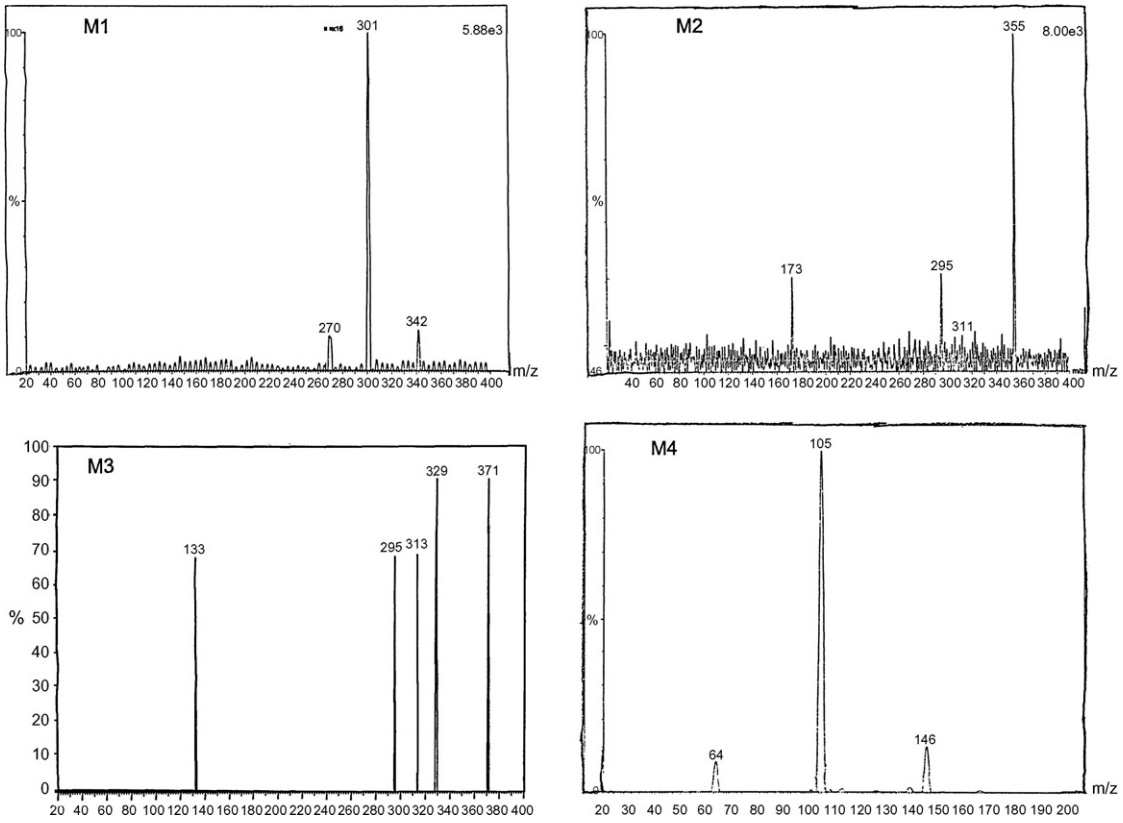

Fig. 2. LC–MS/MS of four metabolites.

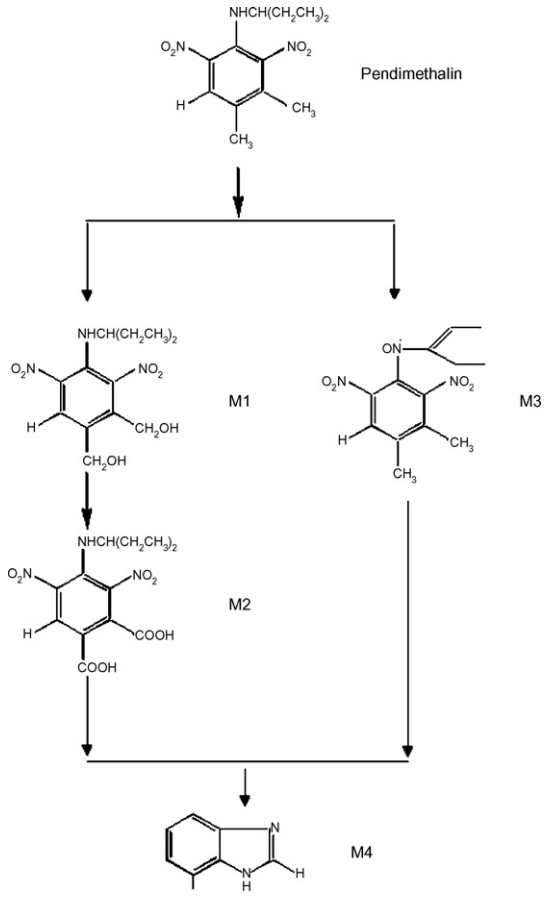

Fig. 3. Degradation pathway of pendimethalin in slurry phase reactor.

4. Conclusions

The experimental data obtained in the present study revealed the effectiveness of bioaugmentation technique in the slurry phase reactor for the degradation of pendimethalin in contaminated soil. The metabolic pathway was established during the degradation process. It was concluded from the study that the degradation process started with dihydrolysis of methyl groups.

References

[1] J.E. Barbash, E.A. Resek, Pesticides in ground water distribution, trends and governing factor, in: R.J. Gilliom (Ed.), Pesticides in the Hydrologic System, vol. 2, Ann Arbor (Press) Inc., Chelsea, MI, 1996.
[2] P.D. Capel, M. Lin, P.J. Wotzka, Wet Atmospheric Deposition of Pesicides in Minnesota 1989–94, US Geological Survey, Mounds View, MN, 1998.
[3] S.J. Larson, R.J. Geilliom, P.D. Capel, Pesticides in Streams of the United States. Initial Results from the National Water Quality Assessment Program, US Geological Survey, Sacramento, CA, 1999.
[4] K.D. Racke, Pesticides for Turfgrass Pest Management: Uses and Environmental Issues, ACS Symposium Series 743, 2000, pp. 45–64.
[5] E. Barriuso, S. Houot, C. Serra-Wittling, Influence of compost edition to soil on the behaviour of herbicides, Pestic. Sci. 49 (1) (1997) 65–75.
[6] WHO., Guidelines for drinking-water quality, World Health Org. (1993) 87–88.
[7] P.N. Moza, K. Husterrt, S. Pal, P. Sukul, Photolytic decomposition of pendimethalin and alachor, Chemosphere 25 (11) (1992) 1675–1682.
[8] R.H. Smith, J.E. Oliver, W.R. Lusby, Degradation of pendimethalin and its *N*-nitroso and *N*-nitro derivatives in anaerobic soil, Chemosphere 8 (1997) 855–867.
[9] C.M. Miller, R.L. Valentine, M. Roehl, P.J.J. Alwarez, Chemical and microbial assessment of pendimethalin contaminated soil after treatment with Fenton's reagent, Water Res. 30 (11) (1996) 2579–2586.
[10] S. Piutti, A.L. Marchand, B. Lagacherie, F. Martin-Laurent, G. Soulas, Effect of cropping cycles and repeated herbicide applications on the degradation of diclofop-methyl, bentazone, diuron, isoproton and pendimethalin in soil, Pestic. Manage. Sci. 58 (3) (2002) 303–312.
[11] S. Yoko, S. Mamouri, S. Takayuki, Degradation of pendimethalin by bacteria isolated from soil, Zarso Kenkyu 35 (1) (1990) 61–67.
[12] A.S. Barua, J. Saha, S. Chaudhuri, A. Chowdhury, N. Adityachaudhury, Degradation of pendimethalin by soil fungi, Pestic. Sci. 29 (4) (1990) 419–425.
[13] R.K. Kole, J. Saha, S. Pal, S. Chaudhuri, A. Chowdhury, Bacterial degradation of the herbicide pendimethalin and activity evaluation of its metabolites, Bull. Environ. Contam. Toxicol. 52 (5) (1994) 779–786.
[14] M. RamaKrishna, S. Shailaja, K. Sirisha, S. Venkata Mohan, P.N. Sarma, Bio-remediation of pendimethalin contaminated soil by bio-slurry phase reactor: bio-augmenting with ETP microflora, Int. J. Environ. Pollut. 27 (4) (2006) 374–387.
[15] S. Venkata Mohan, S. Shailaja, M. Rama Krishna, K.B. Reddy, P.N. Sarma, Bioslurry phase degradation of DEP contaminated soil in period discountinous mode operation: influence of bioaugmentation and substrate partition, Process Biochem. 41 (2006) 644–652.
[16] V. Walker, V. Bond, Persistence of herbicide *N*-(1-ethylproply)-2,6-dinitro-3,4-xylidine in soils, Pestic. Sci. 8 (1997) 359–367.