한국생물공학회지 제 11 권 제 5 호
Korean J. Biotechnol. Bioeng.
Vol. 11. No. 5. 524-528(1996)

컬럼반응조내에서의 고정된 생물막에 의한 농약 4-chloro-2-methylphenoxyacetic acid의 분해

†오 계 헌 · 차 민 석
순천향대학교 생명과학과

Degradation of a Pesticide, 4-Chloro-2-methylphenoxyacetic Acid by Immobilized Biofilm in Bench-scale Column Reactors

Kye-Heon Oh† and Min-Seok Cha
Department of Life Science, Soonchunhyang University, Asan, Chungnam 336-600, Korea

ABSTRACT

Bacterial degradation of 4-chloro-2-methylphenoxyacetic acid (MCPA) was studied in column reactors under conditions approximating a fluidized bed system, with granular activated carbon (GAC) as a support matrix. A mixed bacterial culture of MCPA-degrading bacteria was used as an inoculum to develop a biofilm on GAC. Initially, adsorption of MCPA by GAC and biofilm formation on GAC were examined. MCPA degradation was evaluated with a batch and continuous mode of operation of the GAC fixed-film column reactors. In the batch operations, complete degradation of MCPA was achieved during the incubation period. Partial degradation of MCPA occurred in the continuous operations and MCPA degradation was dependent on the feeding rate of MCPA solution.

INTRODUCTION

4-Chloro-2-methylphenoxyacetic acid (MCPA) is extensively used as a broad-leaved weed controller in cereal crops as well as grass land (1). Disposal of MCPA and solutions from cleaning of equipment may result in localized land pollution and also pollution of water supplies through direct contamination or leaching from soil(2). MCPA is readily susceptible to the microbiological degradation(3-5). The microbiological degradation of MCPA has been demonstrated in several soil and laboratory studies (6-8).

In our previous work, we derived mixed bacterial cultures from a rice field. The cultures were initially selected for MCPA as the sole substrate and some of the test cultures were able to degrade MCPA under aerobic conditions(8). GAC has been previously used as a solid support matrix for fixed-film applications to provide high cell density systems for the biodegradation of aromatic compounds(9-13). In this study, a biodegradative fixed-film pro-

† Corresponding Author

cess was investigated for the treatment of MCPA-containing waste solutions in a bench-scale column reactor with GAC as the solid support matrix for bacterial immobilization.

MATERIALS AND METHODS

Bacterial culture and growth conditions

The test culture SM1 of MCPA-degrading bacteria was derived from a soil sample which was enriched with MCPA as a sole source of carbon and energy. The medium contained (per liter)(14); 0.5 g $(NH_4)_2SO_4$, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 10 mg $FeCl_3 \cdot 6H_2O$, 0.1 mg $CaCl_2 \cdot 2H_2O$, 0.1 mg $MnCl_2$, 0.01 mg $ZnSO_4$ and 500-2,000 mg MCPA. The medium was adjusted to pH 7.0 with NaOH before autoclaving. The culture was grown in shake flasks at 160 rpm and at 24℃. Isolates, capable of utilizing MCPA as a sole carbon and energy, were derived from the test culture with MCPA -containing solid media. The isolates were Gram-negative and rod-shaped. Diagnostic testing with API Rapid NFT strips and fatty acid profiles keyed the isolates to *Pseudomonas aeruginosa, Alcaligenes,* and *Flavobacterium* spp. Confirmatory taxonomic work was not within the scope of this study.

Operation of column reactor

The column reactor design was based on a glass column (internal radius 40 mm × height 330 mm) with inlets for fresh medium and air at the bottom. The total volumn of the column was 180 mL. The working volume of the column was 150 mL for batch mode and 180 mL for continuous, respectively. The outlets for effluent and exhaust air were at the top of the column. The reactor was aerated at 120 mL/min, and operated at 24℃. During the continuous flow mode, the flow rates of fresh media were regulated with a peristaltic pump shown in Fig. 1.

Adsorption and biofilm development by GAC

GAC was obtained from the Samchully Industrial Co. (size distribution 450-920 μm). Adsorption of MCPA by GAC was estimated in a column reactor which contained 20 mg of the activated carbon in a total of 150 mL. The MCPA medium (500 mg/L) was continuously pumped through the column reactor. After adsorption/desorption equilibrium in GAC was established, the column reactor was inoculated with bacteria previously grown with 500 mg MCPA/L. Scanning electron microscopy (SEM) was used to examine the bacterial immobilization on GAC. Samples of GAC were mounted on aluminum stubs. After drying and gold coating, the samples were examined using a SEM (Joel) at 25 kV.

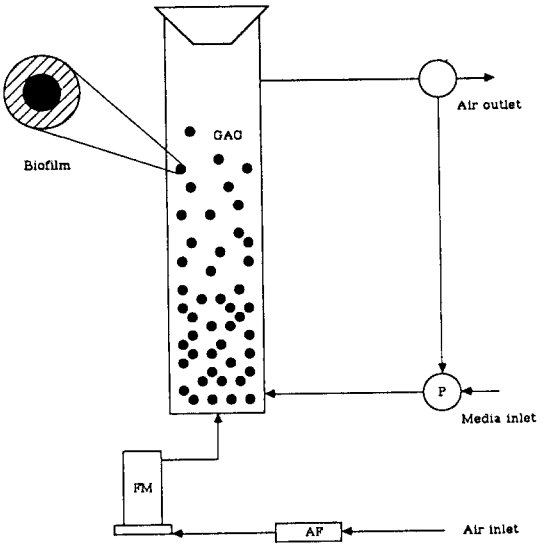

Fig. 1. Schematic diagram of bench-scale fixed-film column reactor. P:peristaltic pump, FM:flow meter, AF:air filter.

Analytical methods

UV-spectrophotometer was used for the determination of maximum peak of adsorption of 279 nm for MCPA. Standard stock solutions of MCPA were prepared by adding 0.1 g of analytical grade compound of interest to a 100 mL volumetric flask containing 10 mL of 0.5 N NaOH. When dissolved completely, the vol-

ume was made up with double-distilled water. The standards were prepared by pipetting 50, 25, 10, 5, 2.5, 1 and 0.5 mL aliquots of the stock solution into separate 100 mL volumetric flasks, and making to the volume with double distilled water. Samples of bacterial cultures were centrifuged at 6000 × g for 15 minute (4℃) before dilution with double distilled water. The UV spectra were recorded from 320 to 230 nm with a Jasco spectrophotometer.

RESULTS AND DISCUSSION

The test culture SM1 used in this study was originally enriched with MCPA as a sole source of carbon under aerobic conditions(8). The culture was used in subsequent studies to evaluate MCPA degradation in fixed-film GAC column reactor. In intial experiments, the adsorption of MCPA by GAC was determined by feeding MCPA medium in continuous flow mode through the column reactor before inoculation. MCPA sorption by GAC was monitored by UV-spectrophotometer (A_{279}) and was determined to 3.17 mg MCPA per mg of GAC. The equilibrium conditions were reached after about 270 hours of column operation at 0.52 mL/min.

A development of biofilm on GAC, with MCPA as a sole carbon and energy source in the column reactor, was observed (Fig. 2). Bacteria attached on GAC were coated with a continuous layer of extracellular material which allowed microbial attachment to the GAC surface as well as other cells. The surface displayed a high degree of porosity, typical of GAC which would be shielded from shear forces, thereby providing a favorable environment for biofilm formation.

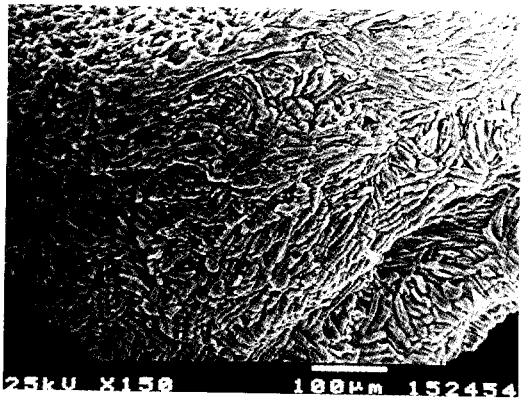

Fig. 2. Scanning electron micrograph of GAC after 168 hours of incubation.

The degradation of MCPA in batch mode in the column reactor containing GAC-immobilized cells is shown in Fig. 3. Various concentrations of MCPA ranging from 500 to 2,000 mg/L were fed to the immobilized cells and degraded without a discernible lag period. MCPA was degraded completely within 5 to 9 days of contact time. In the batch mode, pH change of the medium was monitored during the degradation of MCPA. The pH profile showed a good agreement with HCl formation due to the dechlorination reaction of MCPA, with final pH 5.54 after 5 days of incubation. UV spectrometry showed the peak of maximum absorption at 279 nm for MCPA (Fig. 4). The test culture displayed no detectable spectral changes or peak shifts in the UV-absorbance.

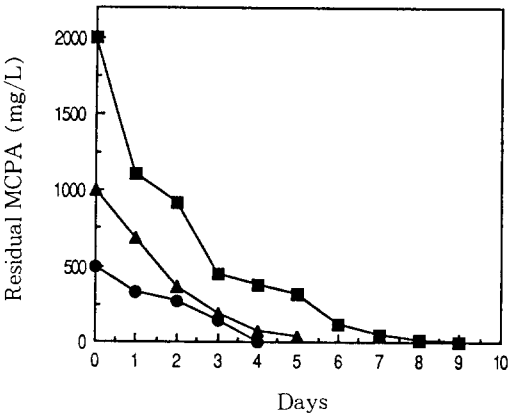

Fig. 3. Degradation of MCPA in fixed-film GAC-column reactor in a batch operation. The initial concentration of MCPA was 500 (●), 1,000 (▲), and 2,000 mg/L (■).

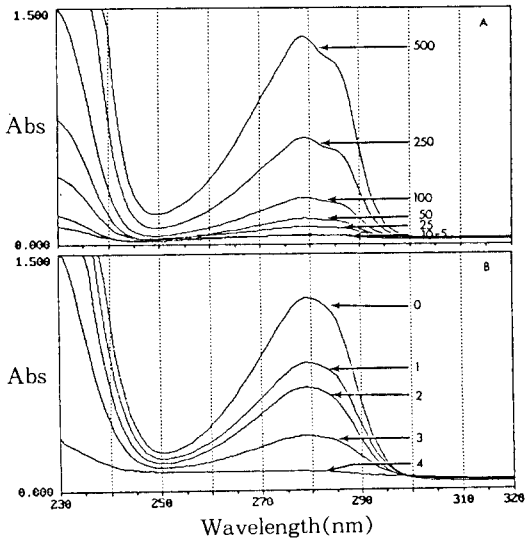

Fig. 4. UV spectral scans of (A) a standard solution containing 5, 10, 25, 50, 100, 250, and 500 mg of MCPA per liter; and (B) supernatants of culture samples. The length of incubation preceding the scan is indicated in days.

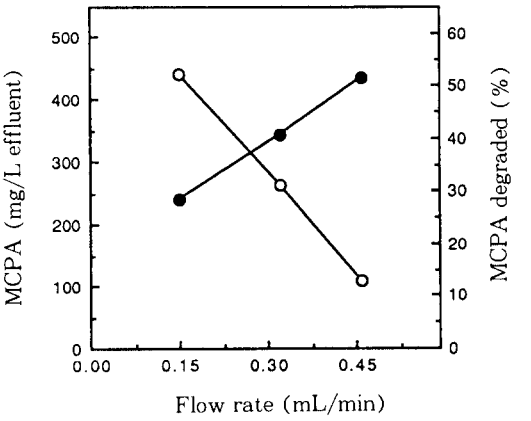

Fig. 5. Changes in the concentration of MCPA (●), % of MCPA degraded (○), depending on the feeding flow rate in a continuous mode operation of the column reactor. The reactor was aerated at a rate of 120 mL/m.

Fig. 5 shows the results of MCPA degradation in the continuous-flow column reactor. The extent of MCPA degradation remained between 13% to 52% during continuous flow operation of the fixed-film GAC-column. Although complete degradation of MCPA was not achieved, the fixed-film GAC-column reactor was proved as a feasible approach for MCPA treatment.

GAC has been used a matrix for fixed film applications to provide high cell density systems for the biodegradation of phenolic and other xenobiotic compounds(9, 11, 15). In the present work, adsorption/desorption equilibrium was initially established after several days of continuous-flow operation in the absence of bacteria. It took several days to reach equilibrium conditions presumably because of slow diffusion caused by the highly microporous interior structure of GAC. After equilibrium, initial colonization upon inoculation and sebsequent formation of a biofilm community were readily accomplished during the batch mode operation. Active bacterial degradation of MCPA took place in batch mode operation but scanning electron microscopic examination of the particles indicated the lack of bacterial colonization(16). The present study demonstrated a feasibility of biological treatment of MCPA in solutions. A mixed culture approach was essential for this objective, because a pure culture approach would be questionable relevance for environmental treatment applications where aseptic conditions and proper selection pressure for pure culture cannot be maintained.

ACKNOWLEDGEMENTS

We thank Mr. Ye-Gyu Lee, Laboratory of Electron Microscope, Korea University, for technical assistance in SEM. This study was supported by the Korea Research Foundation (Non-directed Fund, 1995).

요 약

과립활성탄(GAC)상에 고정된 세균에 의한 4-chloro-2-methylphenoxyacetic acid(MCPA)의 분해가 유동 조건하의 컬럼반응조에서 연구되었다. GAC상에 생물막을 형성시키기 위한 접종으로서 MCPA를 분해하는 세균의 혼합배양이 사용되었다. 접종하기 전에 GAC에 의한 MCPA의 흡착을 조사하였으며 접종후 GAC상에 형성된 생물막을 주사전자 현미경으로 관찰하였다. MCPA 분해는 생물막이 형성된 GAC를 포함하는 컬럼반응조의 회분 및 연속운전 조건하에서 조사되었다. 회분배양에서 MCPA의 완전분해가 이루어졌다. 연속배양상태에서 부분적인 MCPA의 분해가 일어났으나 분해정도는 기질인 MCPA의 컬럼반응조내로 유입속도에 의존되었다.

REFERENCES

1. G. L. Sinton, L. T. Fan, L. E. Erickson, and S. M. Lee(1986), *Enz. Microb. Technol.*, **8**, 395.
2. USEPA(1988), *Rev. Environ. Contam. Toxicol.*, **101**, 1.
3. J. K. Gaunt and W.C. Evans(1971), *Biochem. J.*, **122**, 533.
4. A. E. Smith and B. J. Hayden(1981), *Weed Res.*, **21**, 179.
5. D. H. Pieper, W. Reineke, K.-H. Engesser, and H.-J. Knackmuss(1988), *Arch. Microbiol.*, **150**, 95.
6. S. Kilpi, V. Backstrom, and M. Korhola (1980), *FEMS Microbiol. Lett.*, **8**, 177.
7. E. R. I. C. Sandmann and M. A. Loos (1988), *Can. J. Microbiol.*, **34**, 125.
8. K. H. Oh, S. K. Ahn, K. H. Yoon, and Y. S. Kim(1995), *Bull. Environ. Contam. Toxicol.*, **55**, 539.
9. M. Tsezo and A. Benedek(1980), *J. Water Pollut. Contr. Fed.*, **52**, 578.
10. E. J. Bouer and P. L. McCarty(1982), *Environ. Sci. Technol.*, **12**, 836.
11. H. M. Ehrhardt and H. J. Rehm(1985), *Appl. Microbiol. Biotechnol.*, **21**, 32.
12. K. H. Oh and O.H. Tuovinen(1994), *Intern. Biodeterior. Biodegrad.*, **33**, 93.
13. K. H. Oh and J. C. Kim(1996), *J. Kor. Soc. Environ. Engr.*, **18**, 489.
14. K. H. Oh and O. H. Tuovinen(1990), *J. Ind. Microbiol.*, **6**, 275.
15. L. Z. Hu and W. K. Shieh(1987), *Biotech. Bioeng.*, **30**, 1077.
16. W. J. Weber, M. Pirbazari, and G. L. Melson(1978), *Amer. Chem. Soc.*, **12**, 817.