

Dynamics of activated sludge degradation of 2,4-Dichlorophenoxyacetic and its intermediates

蔡旭清、張玉明；魏漣邦

E-mail: 9221187@mail.dyu.edu.tw

ABSTRACT

This study was to determine how an activated sludge and a pure culture acclimate and degrade the persistent organic, 2,4-dichlorophenoxyacetic acid (2,4-D), and many intermediates from its known metabolic pathways. The time curves of degradation, and biomass yield after degradation, were observed for all the substrates for the purposes of determining (1), if the intermediates restrict 2,4-D degradation; (2), the biomass yielding process in the course of 2,4-D degradation. Both cultures degrade 2,4-D readily when they had acclimated and degraded 2,4-D previously. The 2,4-D acclimated cultures, however, degrade the intermediate, 2,4-DCP at a slower rate than for 2,4-D itself. Although no evident that 2,4-DCP hindered 2,4-D degradation, the fact that 2,4-DCP was more difficult to degrade held true even for the 2,4-D cultures. When the cultures were placed into reaction with 2,4-DCP, specific mechanism must start from then. The 2,4-D degradation mechanism, once started for 2,4-D, may be useful all the way through. Biomass yields were higher intermediates further downstream. Energies derived from the oxidation were consumed breaking up of the original hard-to-treat substrates, but not for cell growth. 2,4-DCP degradation may require

Keywords : 馴化 ; 中間產物 ; 活性污泥 ; 產值

Table of Contents

授權書.....	iii	中文摘要.....	v	英文摘要.....	vi	致謝.....	vii	目錄.....	viii	圖目錄.....	xiii	表目錄.....	xvi																																																																																																																														
第一章緒論.....	1	1.1 研究緣起.....	1	1.2 研究目標.....	3	第二章文獻回顧.....	5	2.1 2,4-D之生物代謝路徑圖.....	6	2.2 2,4-D之生物代謝路徑圖細則.....	6	2.2.1 2,4-D分解路徑(一).....	6	2.2.2 2,4-D分解路徑(二).....	9	2.2.3 2,4-D分解路徑(三).....	10	2.2.4 2,4-D分解路徑(四).....	10	2.2.5 2,4-D分解路徑(五).....	11	2.2.6 2,4-D分解路徑(六).....	11	2.2.7 2,4-D分解路徑(七).....	12	2.2.8 2,4-D分解路徑(八).....	12	2.3 生物生長動力學.....	12	2.4 基質分解速率與生物產值.....	13	2.5 2,4-D的微生物分解.....	15	2.6 馴化機制(acclimation).....	15	2.7 馴化的變化因子.....	16	2.8 影響微生物分解因素.....	18	2.8.1 目標基質特性與構造.....	18	2.8.2 易分解的基質與目標基質共存.....	19	2.8.3 溫度對微生物生理的影響.....	19	2.8.4 營養鹽類對微生物的影響.....	20	2.8.5 酸鹼度 (pH) 的影響.....	20	2.8.6 氧氣對微生物的活性.....	21	2.9 基質目標物2,4-D.....	21	2.9.1 2,4-D的一般特性.....	22	2.9.2 2,4-D與中間產物之物化化學特性.....	23	2.9.3 2,4-D與中間產物之物化化學特性.....	25	2.9.4 2,4-D在一般的環境特性.....	27	2.9.5 2,4-D的擴散機制.....	30	2.9.6 2,4-D的光分解.....	30	2.9.7 2,4-D的吸附.....	31	2.9.8 2,4-D的化學分解.....	31	2.10 微生物分解除草劑幾種反應之途徑.....	31	第三章 研究方法.....	34	3.1 研究步驟.....	34	3.2 研究架構.....	36	3.3 實驗方法.....	42	3.3.1 活性污泥之培養系統.....	42	3.3.2 純菌培養系統.....	43	3.3.3 懸浮固體物分析.....	43	3.4 馴化實驗.....	44	3.4.1 活性污泥馴化實驗.....	44	3.4.2 活性污泥分解2,4-D與中間產物之馴化實驗.....	45	3.4.3 純菌馴化實驗.....	45	3.4.4 純菌分解2,4-D與中間產物之馴化實驗.....	46	3.5 各基質濃度偵測.....	46	3.5.1 2,4-D濃度偵測與檢量夜製備.....	46	3.5.2 2,4-DCP濃度偵測與檢量夜製備.....	47	3.6 微生物菌量之分析.....	47	3.7 產值(yield)之計算.....	48	3.8 藥品配置.....	49	3.8.1 2,4-D儲備溶液之配置.....	49	3.8.2 2,4-DCP(2,4-Dichlorophenol) 儲備溶液.....	50	3.8.3 Pyruvic acid儲備溶液.....	50	3.8.4 Succinate儲備溶液.....	50	3.8.5 營養鹽儲備液製備.....	50	第四章結果與討論.....	52	4.1 活性污泥分解2,4-D速率與產值.....	52	4.2 活性污泥分解2,4-DCP速率與產值.....	54	4.3 活性污泥分解Pyruvic速率與產值.....	56	4.4 活性污泥分解Succinate速率與產值.....	57	4.5 活性污泥分解Glucose速率與產值.....	59	4.6 活性污泥分解2,4-D後再添加2,4-D之速率與產值.....	60	4.7 活性污泥分解2,4-D後再添加2,4-DCP之速率與產值.....	61	4.8 活性污泥分解2,4-D後再添加Pyruvic之速率與產值.....	63	4.9 活性污泥分解2,4-D後再添加Succinate之速率與產值.....	65	4.10 活性污泥分解2,4-DCP後再	

添加2,4-D之速率與產值.....	66	4.11 活性污泥分解2,4-DCP後再添加2,4-DCP之速率與產值...	68	4.12 活性污泥分解2,4-DCP後再添加Pyruvic之馴化試驗.....	69
4.13 活性污泥分解2,4-DCP後再添加Succinate之速率與產值...	70	4.14 純菌分解2,4-D速率與產值.....	72	4.15 純菌分解2,4-DCP速率與產值.....	73
4.16 純菌分解Pyruvic速率與產值.....	75	4.17 純菌分解Succinate速率與產值.....	77	4.18 純菌分解Glucose速率與產值.....	78
4.19 純菌分解2,4-D後再添加2,4-D之速率與產值.....	79	4.20 純菌分解2,4-D後再添加2,4-DCP之產值.....	81	4.21 純菌分解2,4-D後再添加Pyruvic之速率與產.....	82
4.22 純菌分解2,4-D後再添加Succinate之速率與產值.....	87	4.23 純菌分解2,4-DCP後再添加2,4-D之速率與產值.....	85	4.24 純菌分解2,4-DCP後再添加2,4-DCP之速率與產值.....	87
4.25 純菌分解2,4-DCP後再添加Pyruvic之速率與產值.....	88	4.26 純菌分解2,4-DCP後再添加Succinate之速率與產值.....	89	4.27 活性污泥分解2,4-D其下游基質之動態.....	91
4.28 純菌Arthrobacter sp.分解2,4-DCP其下游基質之動態...	94	4.29 活性污泥與純菌Arthrobacter sp.分解各基質動態比較..	97	4.30 活性污泥分解2,4-D後菌體再添加2,4-D與2,4-DCP之比較..	97
4.31 純菌分解2,4-D後菌體再添加2,4-D與2,4-DCP之比較.....	98	4.32 活性污泥分解各基質之產值.....	99	4.33 純種菌株Arthrobacter sp.分解各基質之產值.....	102
第五章結論.....	105	參考文獻.....	106		

REFERENCES

- 王俊欽、李季眉，「固定化微生物對2,4-DCP及2,4,6-DCP之分解」，第二十屆廢水處理研討技術論文集，第1-9/11-15頁，1995。王三郎，1994「應用微生物學」，高立圖書有限公司，台北
- Aiking H., and G.Sojka.1979.Response of Rhodospseudomonas capsulate to illumination and growth rate in light-limited continuous culture. *Journal of bacteriology*. Vol.139,pp.530~536. Allan E.K. 1993. Distribution and activity of microorganisms in lake effect of physical processes. pp.47~68. in Ford Y. E. eds. *Aquatic Microbiology*. Blackwell Scientific Publication, Inc., Boston.
- Aly, O. M., and S. D. Faust. (1964). Studies on the fate of 2,4-D and ester derivatives in natural surface waters *Agric. Food Chem.*12(6):541-546. Audus, L. J. (1960) Herbicide behaviour in the soil. Chapter 5 in *Physiology biochemistry of herbicides*. L. J. Audus. Academic Press, New York, N. Y. 555
- Chong, N. M. (1988). Characteristics of xenobiotics Biodegradation. PhD. Dissertation, State University of New York at Buffalo, Buffalo, New York. CCME (1995) 2,4-D. In: Canadian water quality guidelines. Ottawa, Ontario, Canadian Council of Ministers of the Environment. Crosby, D. G., and H. O. Tutass. (1996). Photodecomposition of 2,4-Dichlorophenoxyacetic acid. *J. Agr. Food Chem.* 14(6):596-599. Concha, M., and K. Shepler. (1994). Aerobic Soil Metabolism of [¹⁴C]2,4-Dichlorophenoxyacetic Acid. PTRL Project number 391W. Unpublished study conducted by PTRL West, Inc., Inc. for Industry Task Force II on 2,4-D Research Data. 95 pages. FAO. "Pesticide residues in food (1996). Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues". Rome, Italy 16-25.
- Fukumori F. and R. P. Hausinger (1993) Purification and Characterization of 2,4-Dichlorophenoxyacetate/a-Ketoglutarate Dioxygenase, *J Biol Chem.* 268(32):24311-7. Foster. R. K and R. B. Mckercher. (1973). Laboratory incubation studies of chlorophenoxyacetic acids in chemozemic soils. *Soil Biol. Biochem.* 5:333-337. Foster P.L. and J.M. Trimarchi, (1994). Adaptive reversion of a frameshift mutation in *Escherichia coli* by small base deletion in homopolymeric runs. *Science*, Vol. 265, pp.407-409.
- Gaudy, A.F., and Gaudy, E.T. (1980) *Microbiology for Environmental Scientists and Engineers*. McGraw-Hill New York. Geating, J. (1981). Literature study of biodegradability of chemicals in water. Vol.1. Biodegradability prediction advances in the chemical interferences with wastewater treatment. Municipal Environmental Research Laboratory. Cincinnati, OH.
- Halter, M. (1980). 2,4-D in the aquatic environment. Section II in *Literature Reviews of Four Selected Herbicides: 2,4-D, dichlobenil, diquat & endotall*. Shearer R., and M. Halter, eds. Han, S. O. and P.B NEW. (1994). Effect of water availability on degradation of 2,4-Dichlorophenoxyacetic acid (2,4-D) by soil microorganisms. *Soil Biol. Biochem.* 26(12):1689-1697. Helling, C. C., P. C. Kearney, and M. Alexander. (1971). Behavior of pesticides in soil. *Adv. Agron.* 23:147-240. Hemmett, R. B. and S. D. Faust. (1969). Biodegradation Kinetics of 2,4-dichlorophenoxyacetic acid by aquatic microorganisms. *Residue. Rev.* 29:191-207.
- Heukelejian, H., H. E. Orford, and R. Manganelli. (1951). "Factors Affecting the Quantity of Sludge Production in the Activated Sludge Process", *Sewage and Industrial Wastes*, vol. 23, no. 8.
- Hu, H.Y., Nozawa, M., Fujie, K. Makabe, T. and Urano K. (1998) Analysis of microbial acclimation to refractory chemicals in wastewater using respiratory quinone profiles. *Wat. Sci. Tech.* Vol.37, pp.407~411. Hollander, J., Hopp, W. (1997), "Degradation of 4-chlorophenol via the meta cleavage pathway by *Comamonas testosteroni*". *Appl. Environ. Microbiol.*, 63 : 4567-4572
- Johnson. W.G., T.L. Lavy, and E.E. Gbur. (1995a). Persistence of Triclopyr and 2,4-D in Flooded and Non-Flooded Soil. *Journal of Environmental Quality*, 24(3) pp493-497. Johnson. W. G., T. L. Lavy, and E. E. Gbur. (1995b) "Sorption mobility, and degradation of triclopyr and 2,4-D and four soils". *Weed Sci.* 43:678-684. Lavy T. L., W. Roeth, and C. R. Fenster. (1973). Degradation of 2,4-D and atrazine at three soil depths in the field. *J. Environ. Quality* 2(1):132-137. Kaschabek S.R., Reineke W. (1995). Maleylacetate Reductase of *Pseudomonas* sp. Strain B13: Specificity of Substrate Conversion and Halide Elimination, *J Bacteriol.* 177 (2), 320—325. McCall, P. J., S. A. Vrona. And S. S. Kelley. (1981). Fate of uniformly carbon-14 ring labeled 2,4,5-Trichlorophenoxyacetic acid and 2,4-Dichlorophenoxyacetic acid. *J. Agric. Food Chem.* 29:100-107.
- McCall, P. J., S. A. Crona, and S. Kelley. (1980). Aerobic Soil Degradation and Metabolism of Uniformly ¹⁴C Ring-Labeled 2,4-D Revised report. Project number GH-C 1299R. Unpublished study conducted by Dow Chemical U.S.A. 33 pages. Mahlborg, W. (2000). Personal Communication. Nufarm, Inc. Product Manager. Nufarm, (1999). "Material Safety Data Sheet--Aqua-Kleen", Nufarm, Inc. St. Joseph, MO. 64506 Oh, K-H, and O. H. Tuovinen. (1991). Bacterial degradation of phenoxyherbicide mixtures 2,4-D and MCPP. *Bull. Euvirion. Contum. Toxicol.* 47:222-229. Prescott L.M., J.P. Harley and D.A. Klein. (1999). *Microbiology*, 4th ed. McGraw-Hill. Parker, L.W. and K. G. Doxtader. (1983). kinetics of the

microbialdegradation of 2,4-D in soil: effects of temperature and moisture. J Environ. Qual 12(4).pp.555-558. Qu, L-T. (1984). 2,4-D degradation and 2,4-D degrading microorganisms in soils. Soil Sci. 137(2).pp.100-107. Que Hee, S. S., and R. G. Sutherland. (1981). The phenoxyalkanoic Herbicides, Volume I : Chemistry, Analysis, and Environmental Pollution Press. Inc., Boca Raton, Florida 319 pgs. Rheinheimer G.(1992). " The influence of environmental factord on the development of microorganisms " .pp.111~147. in Rheinheimer G.ed.s. Aquatic Microbiology 4 th ed. Baffins Lane, England. Roelof O., Lucy K. Arat L. Janssen D. B. and Bernard W. (1989), " Degradation of Chlorinated and Non-chlorinated Aromatic Solvents in Soil Suspensions by Pure Bacterial Cultures. " Appl Microbiol Biotechnol, 30:211 Sandmann, E. R. I., M. A. Loos, and L. P. Van Dyk .(1988). The microbial degradation of 2,4-Dichlorophenoxyacetic acid in soil. Reviews Environ. Contam. Toxicol. 101:1-53. Stanier,R.Y, Ornston,L.N. (1973).The -keto adipate pathway. Adv Microbial Physiol. NO.1,pp.89-149. Saroski, R.A. and Stannard, M.C. (1974). Controlling preharvest drop of citrus. New South Wales Agricultural Gazette, pp.3-5. Shaw, L. J., and R. G. Burns. (1998). Biodegradation of 2,4-D in a noncontaminated grassland soil profile J. Environ. Qual 27.pp.1464-1471. Smith, A.E. (1989). Degradation, Fate, and Persistence of Phenoxyalkanoic Acid Herbicides in Soil. Reviews of Weed Science, 4:1-24. Smith,A.E., and A.J. Aubin.(1991) Transformation of C-14 2,4-Dichlorophenol in Saskatchewan Soils. Journal of Agriculturaland Food Chemistry, 39(4).pp.801-804. Stanier, R. Y., M. Doudoroff, and E. A. Adelberg: (1970)The Microbial World, 3d ed., Prentice-Hall, Englewood Cliffs, N.J., The University of Minnesota Biocatalysis/Biodegradation Database(UM-BBD, <http://umbbd.ahc.umn.edu/>) provides. Vollmer MD, Stadler-Fritzsche K, Schlomann M. (1993) Conversion of 2-chloromaleylacetate in Alcaligenes eutrophus JMP134. Arch Microbiol. 1993;159(2):182-8. Veeh, R.H., W.P. Inskeep, and A.K Camper.(1996). Soil Depth and Temperature Effects on Microbial Degradation of 2,4-D. Journal of Environmental Quality, 25(1).pp.5-12. Wang,Y.et.al.,(1994).Accumulation of 2,4-D and Glyphosate in Fish and Water Hyacinth. Water,Air and Soil Pollution,Kluwer Academic Publishers.74:pp397-403 Winteringham, F.P.W.(1985). Environment and Chemicals in Agriculture. Elsevier Applied Sc. Pub., London. WHO (1984) 2,4-Dichlorophenoxyacetic acid (2,4-D). Geneva, World Health Organization (Environmental Health Criteria 29).