

活性污泥分解2,4-D中間產物之動態

蔡旭清、張玉明；魏漣邦

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

摘要

本研究用活性污泥，及純種菌株分別對持久性有機物2,4-Dichlorophenoxyacetic acid (2,4-D)，與生化代謝之中間產物分別作馴化與分解。本研究是要探討各基質之分解趨勢，及測出各基質分解後菌體所產出的產值。上下游基質的分解趨勢，可用來觀察2,4-D分解是否受到下游何者之限制；而各個產值則是要瞭解分解2,4-D的菌體，是由下游物產出，還是上游物產出者。活性污泥分解上下游物可由不同菌種來完成，純菌則一路完成，故兩者在分解及產值上的異同要作實驗觀察，實驗結果發現，下游物並不會限制上游物馴化分解時間。已經馴化過2,4-D之菌體，再添加2,4-D分解快速。而已經馴化後2,4-D，再添加2,4-DCP，分解還是緩慢；但是分解過2,4-DCP的菌體再添加2,4-DCP，分解也是不快，因此不能斷言2,4-DCP有所限制，而可能是分解2,4-DCP的機制不易起動。本實驗使用的純種菌株是Arthrobacter sp，結論是活性污泥與 Arthrobacter sp.在分解2,4-D及中間產物時，兩者的結果都是類似的，只有分解2,4-D與2,4-DCP之馴化時間不同。Arthrobacter sp. 分解2,4-D比活性污泥來的快；而分解2,4-DCP比活性污泥來的慢，可能原因是菌種分解動力有所差異。實驗結果發現下游物質的產值較大，因而可以推論前面難分解有機物之所以消失，只是打破，只消耗能量而已，不產細胞。難分解物的產值，大部分是由下游物所產出者。

關鍵詞：馴化；中間產物；活性污泥；產值

目錄

授權書.....	iii	中文摘要.....	v	英文摘要.....	vi																																																										
致謝.....	vii	目錄.....	viii	圖目錄.....	xiii																																																										
錄.....	xvi	第一章緒論.....	1.1.1 研究緣起.....	1.1.2 研究	1																																																										
目標.....	3	第二章文獻回顧.....	5 2.1 2,4-D之生物代謝路徑圖.....	2,4-D之生物代謝路徑圖細則.....	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 膽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與中間產物之物化學特性.....	23 2.9.3 2,4-D與中間產物之物化學特性.....	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 膽養鹽儲備液製備.....	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 性污泥分解2,4-DCP後再添加Succinate之速率與產值...	70	
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	
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
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	

參考文獻

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?1-15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 Rhodopseudomonas capsulata to illumination and growth rate in light-limited continuousculture.Journal of bacteriology. Vol.139,pp.530~536. Allan E.K. 1993. Distribution and activity of microorganisms in lakeeffect 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 andester derivatives in natural surface waters Agric. Food Chem.12(6):541-546. Audus, L. J.(1960) Herbicide behaviour in the soil. Chapter 5 inPhysiology biochemistry of herbicides. L. J. Audus. Academic Press,New York, N. Y.555 Chong, N. M. (1988). Characteristics of xenobiotics Biodegradaton.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-Dichlorthophenoxyacetic 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 IndustryTask Force II on 2,4-D Research Data. 95 pages. FAO. " Pesticide residues in food (1996). Report of the Joint Meetingof the FAO Panel of Experts on Pesticide Residues in Food and theEnvironment 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 inhomopolymeric runs. Science, Vol. 265,pp.407-409. Gaudy,A.F.,and Gaudy,E.T.(1980) Microbiology for Envirnom ental Scienctists and Engineers. Mc.Graw-Hill Now York. Geating,J.(1981). Literature study of biodegradability of chemicals inwater.Vol.1.Biodegradability prediction advances in the chemical interferences with wastewater treatment.Municipal Environmental Reserch Laboratory.Cincinnati,OH. Halter, M.(1980). 2,4-D in the aquatic environment. Section II inLiterature 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). Bechavior 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. Heukelekian, 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 quinine profiles. Wat.Sci. Tech. Vol.37,pp.407~411. Hollander, J., Hopp, Dott, W.(1997), " Degradation of 4-chlorophenol via the meta cleavage pathway by Comamonas testosterone. " 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 qtrazine at three soil depths in the field. J. Environ. Quality2(I):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 uniformlycarbon-14 ring labeled 2,4,5-Trichlorophenoxyacetic acid and2,4-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-DRevised report. Project number GH-C 1299R. Unpublished study conducted by Dow Chemical U.S.A. 33 pages. Mahlburg, W. (2000). Personal Communication. Nufarm, Inc. ProductManager. 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. Euviron. Contum. Toxicol.47:222-229.

Prescott L.M.,J.P.Harley and D.A.Klein. (1999). Microbioloy,4 th ed.McGraw-Hill . Parker, L.W. and K. G. Doxtader.(1983). kinetics of the microbial degradation of 2,4-D in soil: effects of temperature and moisture. JEnviron. 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 phenoxyalkanic 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. 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 -ketoadipate pathway. Adv Microbial Physiol. NO.1,pp.89-149. Sarooski, 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. Elswevien Applied Sc. Pub., London. WHO (1984) 2,4-Dichlorophenoxyacetic acid (2,4-D). Geneva, World Health Organization (Environmental Health Criteria 29).