

MTBE污染地下水之生物復育影響因子及其生物相研究

吳志鴻、林啟文

E-mail: 9314394@mail.dyu.edu.tw

摘要

本研究之目的為探討MTBE污染地下水之生物復育影響因子及其生物相研究，期能藉由研究結果進行分析比較，以瞭解在不同的環境因子參數下之污染物去除效率及微生物菌群結構變化情形。本研究主要以實驗室之密閉瓶進行批次試驗方式測試混合菌群對MTBE之降解效率，以及採用分子生物技術中之聚合?鏈鎖反應(polymerase chain reaction, PCR)與單股DNA構形多型性(single strand conformation polymorphism, SSCP)分析技術來鑑別混合菌群間之生物相結構。研究結果顯示：(1)混合菌群於不同酸鹼度(pH 4.04?pH 10.05)條件下仍可降解MTBE，其分解能力以酸性環境(pH 4.04?pH 6.77)優於中至鹼性環境(pH 7.00?pH 10.05)，且菌群亦可區分為酸性與鹼性環境之兩大微生物結構；(2)單一基質(MTBE)之濃度添加範圍(3.6 ppm?1440 ppm)若在混合菌群可承受之濃度範圍內，其降解效率將隨基質之降解而使生質量增加，反之若高於菌種之忍受範圍，則菌群將無法降解MTBE；(3)雙基質與多重碳源共存下，醚類或芳香烴化合物均可被菌種降解完畢，而所呈現之菌群結構亦將隨基質種類之變動而有消長現象；(4)菌群結構之消長狀態隨溫度之變化而產生微小之差異；(5)進行SSCP跑膠時，將PCR產物濃度控制在15 ng/?l?24 ng/?l之間可呈現最佳圖譜；(6)混合菌群中若含有已篩選或已確認之菌種時，則可以引子方式添加於混合菌群中，並由SSCP凝膠之跳躍位置得知此已知菌種是否存在於系統中；(7)MTBE分解菌(#3菌株)降解單一基質之MTBE時，所產生之中間代謝物(TBA)亦可被MTBE分解菌降解完畢。

關鍵詞：甲基第三丁基醚；聚合?；鏈鎖反應；單股DNA構形多型性分析；菌群社會結構；生物分解

目錄

目錄 封面內頁 簽名頁 博碩士論文電子檔案上網授權書.....	iii	博碩士論文授權書.....	iv							
中文摘要.....	v	英文摘要.....	vii							
誌謝.....	ix									
目錄.....	x	圖目錄.....	xiii	表目錄.....	xv					
第一章 緒論 1.1 前言.....	1	1.1.2 研究目的.....	4	第二章 文獻回顧 2.1 現階						
段MTBE使用現況.....	6	2.2 MTBE與其常見共存污染物之物理化學性質.....	8	2.3 MTBE對人體健康之影響.....						
之影響.....	13	2.4 MTBE物化處理程序.....	14	2.5 MTBE之生物分解特						
性.....	2.6 應用分子生物技術於菌群結構分析之相關研究.....	18	2.6.1 聚合?鏈鎖反應原							
.....	19	2.6.2 單股DNA構形多型性分析.....	20	2.6.3 16S rRNA基因放大與定						
序.....	21	2.6.4 微生物種類辨識方法.....	21	第三章 材料與方法 3.1 研究材料與儀器設						
備.....	30	3.1.1 菌種來源.....	30	3.1.2 藥品種類.....	30	3.1.3 其他相關使用材料.....	3.1.4 儀器設備.....	35	3.2 研究方法與步驟.....	37
3.2.1 菌種篩選方式.....	37	3.2.2 批次分解評估方式.....	38	3.2.3 分子生物技術建						
立.....	40	3.2.4 環境因子對MTBE之去除效率與菌群結構變化之影響.....	55	第四章 結果與討論 4.1 酸鹼值對						
混合菌種降解MTBE效率之影響.....	61	4.2 單一基質(MTBE)濃度變化對菌群降解MTBE效率之影響.....	63	4.3 雙基質(碳源)下對菌群降解MTBE效率之影響.....						
62	4.3.1 雙基質醚類添加劑共存下對MTBE去除效率之影響.....	66	4.3.2 雙基質芳香烴化合物共存下對MTBE去除效率之影響.....							
66	4.4 多重碳源添加下對菌群降解MTBE效率之影響.....	72	4.5 PCR引子選用與連續稀釋下對產物之影響.....							
72	4.5.1 PCR引子選用.....	73	4.5.2 連續稀釋下對產							
75	4.6 額外添加引子對純菌與混合菌群生物相結構之影響.....	77	4.7 環境因子變動對菌群結構與							
79	4.7.1 酸鹼度變化下對菌群親源相異度分析.....	80	4.7.2 雙基質共存與單一基質濃度變化							
82	4.7.3 溫度變化下對菌群之親源相異度分析.....	87	4.8 MTBE分解菌之代謝產物分析與其菌							
89	4.9 菌種定序比對分析.....	94	第五章 結論與建議 5.1 結							
97	5.2 建議	98	參考文獻.....	100						
圖目錄 圖3-1 整體研究架構流程圖.....	29	圖3.2-1 棕色密閉試驗瓶示意圖.....	40	圖3.2-2 分子生物技						
術分析流程圖.....	41	圖3.2-3 SSCP流程圖.....	46	圖3.2-4 硝酸銀染色流程						
圖.....	49	圖3.2-5 純培養序列比對流程圖.....	51	圖3.2-6 混合菌落序列比對流程						
圖.....	52	圖3.2-7 菌群群集分析流程圖.....	54	圖3.2-8 pH變化對菌群結構與去除效率影響之測試						
56	圖3.2-9 溫度變化對菌群結構與去除效率影響之測試流程圖.....	58	圖3.2-10 基質濃度變化對菌群結構與去除效							
59	率影響之測試流程圖.....	60	圖4.1-1 菌種於中至鹼性環境下							

對MTBE之降解曲線圖.....	62	圖4.1-2 菌種於酸至中性環境下對MTBE之降解曲線圖.....	63
圖4.2-1 單一基質(MTBE)濃度變化對菌種降解曲線圖.....	65	圖4.2-2 菌體生質量於單一基質(MTBE)濃度下之變化圖.....	65
圖4.3-1 雙基質(MTBE與ETBE)共存下之降解曲線.....	68	圖4.3-2 雙基質(MTBE與TAME)共存下之降解曲線.....	68
圖4.3-3 雙基質(MTBE與benzene)共存下之降解曲線.....	69	圖4.3-4 雙基質(MTBE與toluene)共存下之降解曲線.....	69
圖4.3-5 雙基質共存下對MTBE之降解曲線.....	71	圖4.3-6 雙基質共存下對不同碳源之降解曲線.....	71
圖4.4-1 多重基質環境下之降解曲線.....	72	圖4.5-1 PCR產物之圖譜(選用EUB1-1與UNV-2).....	74
圖4.5-2 PCR產物之圖譜(選用EUB3與EUB4).....	75	圖4.5-3 tDNA連續稀釋下對單股DNA變化之影響.....	76
圖4.6-1 不同引子對Tol菌種(<i>Pseudomonas sp. PH1</i>)之影響.....	78	圖4.6-2 不同引子種類添加對混合菌群之影響.....	78
圖4.6-3 U2引子對菌種之影響.....	79	圖4.7-1 酸鹼度(pH)之菌群結構圖譜.....	81
圖4.7-2 不同酸鹼度對菌群相異度之群集分析樹形圖.....	82	圖4.7-3 雙基質共存與單一基質(MTBE)下對菌群結構之圖譜.....	85
圖4.7-4 MTBE濃度變化下對菌群相異度之群集分析樹形圖.....	86	圖4.7-5 雙基質共存下對菌群相異度之群集分析樹形圖.....	86
圖4.7-6 溫度變化對菌群生物相結構圖譜.....	88	圖4.7-7 溫度對菌群相異度之群集分析圖.....	89
圖4.8-1 #3菌種降解MTBE與中間產物曲線(殘存率表示).....	91	圖4.8-2 #3菌種降解MTBE與中間產物曲線(訊號值表示).....	91
圖4.8-3 MTBE菌種篩選後之生物相結構圖譜(#3菌種篩選).....	91	圖4.8-4 #3菌種連續添加MTBE與TBA基質之菌相圖譜.....	92
圖4.8-5 #3菌種連續添加MTBE與TBA基質之親源相似樹形圖.....	92	圖4.8-6 溫度變化對菌群生物相結構圖譜.....	92
93 表目錄 表2.2-1 醣類化合物之物化特性.....	9	表2.2-2 醇類化合物之物化特性.....	9
10 表2.2-3 芳香烴化合物之物化特性.....	11	表2.2-4 美國California地下飲用水所偵測之MTBE佔有比率.....	12
表2.5-1 MTBE生物分解之可行性相關研究.....	12	表2.6-1 傳統方法對環境中微生物之培養可能率.....	23
表2.6-2 分子生物應用技術之相關文獻(1/3).....	25	表2.6-3 分子生物應用技術之相關文獻(2/3).....	26
表2.6-4 分子生物應用技術之相關文獻(3/3).....	27	表3.1-1 碳源與培養基之藥品清單.....	31
表3.1-2 低限營養鹽藥品清單與配比.....	31	表3.1-3 PCR與SSCP相關藥品清單.....	32
表3.1-4 寡核?酸引子種類.....	32	表3.1-5 分子生物技術之相關藥品種類.....	33
表3.1-6 本研究使用相關材料清單.....	35	表3.1-7 儀器設備清單.....	36
表3.2-1 PCR升溫程式.....	43	表3.2-2 PCR藥品與比例.....	44
表3.2-3 SSCP膠片配比(8% Acrylamide/Bis).....	47	表3.2-4 SSCP變性劑>Loading dye成分與配比.....	47
表3.2-5 硝酸銀染色成分與配比.....	48	表4.9-1 甲苯菌(Toluene)與 <i>Pseudomonas sp. PH1</i> 序列比對結果..95 表4.9-2 苯菌(Benzene)與 <i>Ralstonia sp. P-10</i> 序列比對結果....96	95

參考文獻

- Ahmed, F.E., (2001) " Toxicology and human health effects following exposure to oxygenated or reformulated gasoline ", *Toxicology Letters*, Vol. 123, pp. 89-123. Almeida, T.A., V.M. Cabrera, and J.G. Miranda, (1998) " Improved detection and characterization of mutations by primer addition in nonradioisotopic SSCP and direct PCR sequencing ", *BioTechniques*, Vol. 24(2), pp. 220-221. Amann, R.I., W. Ludwig, and K.H. Schliefer, (1995) " Phylogenetic identification and in situ detection of individual microbial cells without cultivation ", *Microbiological Reviews*, Vol. 59(1), pp. 143-169. Balcke, G.U., L.P. Turunen, R. Geyer, and D.F. Wenderoth, (2004) " Chlorobenzene biodegradation under consecutive aerobic- anaerobic conditions ", *FEMS Microbiology Ecology*, Vol. 49(1), pp. 109-120. Baldrian, P., C. Wiesche, J. Gabriel, F. Nerud, and F. Zadrazil, (2000) " Influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil ", *Applied and Environmental Microbiology*, Vol. 66(6), pp. 2471-2478. Barreto, R.D., K.A. Gray, and K. Anders, (1995) " Photocatalytic degradation of methyl tert-butyl ether in TiO₂ slurries, a proposed reaction scheme ", *Water Research*, Vol. 29(5), pp. 1243-1248. Bassam, B.J., G. Caetano-Anolles, and P.M. Grosshoff, (1991) " Fast and sensitive silver staining of DNA in polyacrylamide gels ", *Analytical Biochemistry*, Vol. 196(1), pp. 80-83. Bolton, J.R., A.A. Safarzadeh, S.R. Cater, B. Dussert, M. Stefan, and J. Mack, (1998) " Mechanism and efficiency of the degradation of MTBE in contaminated groundwater by the UV/H₂O₂ process ", *The southwest focused ground water conference-discussing the issue of MTBE and perchlorate in ground water*, Anaheim, California, National Ground Water Association, pp. 36-39. Bruins, M.R., S. Kapil, and F.W. Oehme, (2000) " *Pseudomonas pickettii*: A common soil and groundwater aerobic bacteria with pathogenic and biodegradation properties ", *Ecotoxicology and Environmental Safety*, Vol. 47(2), pp. 105-111. Cai, Q.Q., and I. Touitou, (1993) " Excess PCR primers may dramatically affect SSCP efficiency ", *Nucleic Acids Research*, Vol. 21(16), pp. 3909-3910. Cha, G.C., and T. Noike, (1997) " Effect of rapid temperature change and HRT on anaerobic acidogenesis ", *Water Science and Technology*, Vol. 36(6-7), pp. 247-253. Converse, B., and E.D Schroeder, (1999) " Biodegradation of methyl tertiary butyl ether(MTBE) using a granular activated carbon trickling filter ", *Proceedings of the 92nd Annual Meeting & Exhibition of the Air & Waste Management Association*, St Louis, Missouri, USA. Davidson, J.M., and D.N. Creek, (2000) " Using the gasoline additive mtbe in forensic environmental investigations ", *Environmental Forensics*, Vol. 1(1), pp. 31-36. Drogos, D.L., (2000) " MtBE v. other oxygenates ", presented at MEALY's MtBE Litigation Conference, May 11-12. Duffy, J.S., J.A. Del-Pup, and J.J. Kneiss, (1992) " Toxicological evaluation of MTBE testing performed under TSCA consent agreement ", *Journal of Soil Contamination*, Vol. 1(1), pp. 27-37. Espejo, R.T., C.G. Feijoo, J. Romero, and M. Vasquez, (1998) " PAGE analysis of the heteroduplexes formed between PCR-amplified 16S rRNA genes: estimation of sequence similarity and rDNA complexity ", *Microbiology*, Vol.

144, pp. 1611-1617. Eweis J., E. Schroeder, D. Chang, K. Scow, R. Morton, and R. Caballero, (1998) "Meeting the challenge of MTBE biodegradation", Presented at the 90th annual meeting and exhibition, Air and Waste Management Association, Toronto, Ontario, Canada, June 8-13. Ferguson, R.L., E.N. Buckley, and A.V. Palumbo, (1984) "Response of marine bacterioplankton to differential filtration and confinement", Applied and Environmental Microbiology, Vol. 47(1), pp. 49-55. Fortin, N.Y. and M.A. Deshusses, (1999) "Treatment of methyl tert-butyl ether vapors in biotrickling filters. 1. Reactor startup, steady-state performance, and culture characteristics", Environmental Science and Technology, Vol. 33(17), pp. 2980-2986. Foucher, S., F. Battaglia-Brunet, P. d'Hugues, M. Clarenz, J.J. Godon, and D. Morin, (2003) "Evolution of the bacterial population during the batch bioleaching of a cobaltiferous pyrite in a suspended-solids bubble column and comparison with a mechanically agitated reactor", Hydrometallurgy, Vol. 71(1-2), pp. 5-12. Futamata, H., S. Harayama, and K. Watanabe, (2001) "Diversity in kinetics of trichloroethylene-degrading activities exhibited by phenol-degrading bacteria", Applied Microbiology and Biotechnology, Vol. 55(2), pp. 248-253. Fujiwara Y., T. Kinoshita, H. Stao, and I. Kojima, (1984) "Biodegradation and bioconcentration of alkylethers", Yukagaku Japan oil chemists' society, Vol. 33, pp. 111-115. Garrett P., M. Moreau, and J.D. Lowry, (1986) "MTBE as a groundwater contaminant", Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection and Remediation Conference. Happel A.M., E.H. Beckenbach, and R.U. Halden, (1998) "An evaluation of MTBE impacts to California groundwater resources", Lawrence Livermore National Laboratory, Environmental Protection Department, Environmental Restoration Division, University of California, UCRL-AR-130897. Hardison, L.K., S.S. Curry, L.M. Ciuffetti, and M.R. Hyman, (1997) "Metabolism of diethyl ether and cometabolism of methyl tert-butyl ether by a filamentous fungus, a Graphium sp.", Applied and Environmental Microbiology, Vol. 63(8), pp. 3059-3067. Hatzinger, P.B., K. McClay, S. Vainberg, M. Tugusheva, C.W. Condee, and R.J. Steffan, (2001) "Biodegradation of methyl tert-butyl ether by a pure bacterial culture", Applied and Environmental Microbiology, Vol. 67(12), pp. 5601-5607. Head, I.M., J.R. Saunders, and R.W. Pickup, (1998) "Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultured microorganisms", Microbial Ecology, Vol. 35(1), pp. 1-21. Humphries S.E., V. Gudnason, R. Whittall, and N.M. Ian, (1997) "Single-strand conformation polymorphism analysis with high throughput modifications, and its use in mutation detection in familial hypercholesterolemia", Clinical Chemistry, Vol. 43(3), pp. 427-435. Perez, H.G., F. Fayolle, and J.P. Vandecasteele, (2001) "Biodegradation of ethyl tert-butyl ether(ETBE), methyl tert-butyl ether(MTBE) and tert-amyl methyl ether(TAME) by Gordonia terrae", Applied Microbiology and Biotechnology, Vol. 55(1), pp. 117-121. Jones, C.W., J.M. Brice, and C. Edwards, (1977) "The effect of respiratory chain composition on the growth efficiencies of aerobic bacteria", Archives of Microbiology, Vol. 24, pp. 85-93. Joseph, P.M., (1997) "Changes in disease rates in Philadelphia following the introduction of oxygenated gasoline", Air and Waste Management Association 90th Annual Meeting, Toronto, Ontario, pp. 1-15. Keller, A., J. Froines, C. Koshland, J. Reuter, I. Suffet, and J. Last, (1998) "Health and environmental assessment of MTBE", Report to the Governor and Legislature of the State of California as Sponsored by SB 521, Delivered to the Governor's office on November 12. Kharoune, M., A. Pauss, and J.M. Lebeault, (2001) "Aerobic biodegradation of an oxygenates mixture: ETBE, MTBE and TAME in an upflow fixed-bed reactor", Water Research, Vol. 35(7), pp. 1665-1674. Kutty, R., H.J. Purohit, and P. Khanna, (2000) "Isolation and characterization of a Pseudomonas sp. strain PH1 utilizing meta-aminophenol", Canadian Journal of Microbiology, Vol. 46(3), pp. 211-217. LaPara, T.M., C.H. Nakatsu, L.M. Pantea, and J.E. Alleman, (2001) "Aerobic biological treatment of a pharmaceutical wastewater: Effect of temperature on COD removal and bacterial community development", Water Research, Vol. 35(18), pp. 4417-4425. Lee, D.H., Y.G. Zo, and S.J. Kim, (1996) "Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism", Applied and Environmental Microbiology, Vol. 62(9), pp. 3112-3120. Lim, P.E., S.A. Ong, and C.E. Seng, (2002) "Simultaneous adsorption and biodegradation processes in sequencing batch reactor (SBR) for treating copper and cadmium-containing wastewater", Water Research, Vol. 36(3), pp. 667-675. Liu, W.T., T.L. Marsh, H. Cheng, and L.J. Forney, (1997) "Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA", Applied and Environmental Microbiology, Vol. 63(11), pp. 4516-4522. Nakamura D.N., (1994) "MTBE, still the best choice", Hydrocarbon Processing, Vol. 73, pp. 17. Neefs, J.M., Y.V. Peer, P. Rijk, S. Chapelle, and R. Wachter, (1993) "Compilation of small ribosomal subunit RNA structures", Nucleic Acids Research, Vol. 21(13), pp. 3025-3049. Maidak, B.L., N. Larsen, M.J. McCaughey, R. Overbeek, G.J. Olsen, K. Fogel, J. Blandy, and C.R. Woese, (1994) "The ribosomal database project", Nucleic Acids Research, Vol. 22(17), pp. 3485-3487. Maidak, B.L., J.R. Cole, T.G. Lilburn, C.T. Parker Jr, P.R. Saxman, R.J. Farris, G.M. Garrity, G.J. Olsen, T.M. Schmidt, and J.M. Tiedje, (2001) "The RDP-II (Ribosomal Database Project)", Nucleic Acids Research, Vol. 29(1), pp. 173-174. Mo, K., C.O. Lora, A.E. Wanken, M. Javanmardian, N. Yang, and C.F. Kulpa, (1999) "Biodegradation of methyl t-butyl ether by pure bacterial cultures", Applied Microbiology and Biotechnology, Vol. 47(1), pp. 69-72. Muyzer, G., E.C. Waal, and A.G. Uitterlinden, (1993) "Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA", Applied and Environmental Microbiology, Vol. 59(3), pp. 695-700. Muyzer, G., (1999) "DGGE/TGGE a method for identifying genes from natural ecosystems", Current Opinion in Microbiology, Vol. 2(3), pp. 317-322. Mullis, K.B., (1990) "The unusual origin of the polymerase chain reaction", Scientific American, Vol. 262, pp. 56-65. Ong, S.A., P.E. Lim, and C.E. Seng, (2003) "Effects of adsorbents and copper(II) on activated sludge microorganisms and sequencing batch reactor treatment process", Journal of Hazardous Materials, Vol. 103(3), pp. 263-277. Orita, M., H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekiya, (1989) "Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms", Proceedings of the National Academy of Sciences, Vol. 86, pp. 2766-2770. Park, K.Y., (1999) "Biodegradation of the fuel oxygenate, methyl tert-butyl ether(MTBE), and treatment of MTBE contaminated ground water in laboratory scale reactors", Ph. D.

dissertation, The State University of New Jersey. Said, W.A., and D.L. Lewis, (1991) " Quantitative assessment of the effect on metal on microbial degradation of organic chemicals ", Applied and Environmental Microbiology, Vol. 57(5), pp. 1498-1503. Salanitro, J.P., L.A. Diaz, M.P. Williams, and H.L. Wisniewski, (1994) " Isolation of a bacterial culture that degrades methyl t-butyl ether ", Applied and Environmental Microbiology, Vol. 60, pp. 2593-2596. Sambrook, J., D.W. Russell, and J. Sambrook, (2001) " Molecular cloning: a laboratory manual ", 3rd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. Schirmer, M., B.J. Butler, C.D. Church, J.F. Barker, and N. Nadarajah, (2003) " Laboratory evidence of MTBE biodegradation in Borden aquifer material ", Journal of Contaminant Hydrology, Vol. 60(3-4), pp. 229-249. Schmidt, T.C., (2003) " Analysis of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA) in ground and surface water ", Trends in Analytical Chemistry, Vol. 22(10), pp. 776-784. Schmidt, T.C., M. Schirmer, H. Weis, and S.B. Haderlein, (2004) " Microbial degradation of methyl tert-butyl ether and tert-butyl alcohol in the subsurface ", Journal of Contaminant Hydrology, Vol. 70(3-4), pp. 173-203. Schwieger, F., and C.C. Tebbe, (1998) " A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis ", Applied and Environmental Microbiology, Vol. 64(12), pp. 4870-4876. Sedran, M.A., A. Pruden, G.J. Wilson, M.T. Suidan, and A.D. Venosa, (2002) " Effect of BTEX on degradation of MTBE and TBA by mixed bacterial consortium ", Journal of Environmental Engineering, Vol. 128(9), pp. 830-835. Selvaratnam, S., B.A. Schoedel, B.L. McFarland, and C.F. Kulpa, (1995) " Application of reverse transcriptase PCR for monitoring expression of the catabolic dmpN gene in a phenol-degradation sequencing batch reactor ", Applied and Environmental Microbiology, Vol. 61(11), pp. 3981-3985. Spinnler, G.E., J.P. Salanitro, P.M. Manner, and K.A. Lyons, (2001) " Enhanced bioremediation of MTBE at retail gas stations ", Contaminated Soil Sediment and Water Special Oxygenated Fuel Edition, pp. 47-49. Staley, J.T., and A. Konopka, (1985) " Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats ", Annual Review of Microbiology, Vol. 39, pp. 321-346. Steffan, R.J., K. Mcclay, S. Vainberg, C.W. Condee, and D. Zhang, (1997) " Biodegradation of the gasoline oxygenates methyl tert-butyl ether, ethyl tert-butyl ether, and tert-amyl methyl ether by propane-oxidizing bacteria ", Applied and Environmental Microbiology, Vol. 63(11), pp. 4216-4222. Stocking A.J., R.A. Deeb, A.E. Flores, W. Stringfellow, J. Talley, R. Brownell, and M.C. Kavanaugh, (2000) " Bioremediation of MTBE: A review from a practical perspective ", Biodegradation, Vol. 11(2-3), pp. 187-201. Suflita, J.M., and M.R. Mormille, (1993) " Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface ", Environmental Science and Technology, Vol. 27(5), pp. 976-978. Teske, A., C. Wawer, G. Muyzer, and N.B. Ramsing, (1996) " Distribution of sulfate-reducing bacteria in a stratified fjord (Mariager Fjord, Denmark) as evaluated by most-probable- number counts and denaturing gradient gel electrophoresis of PCR-amplified ribosomal DNA fragments ", Applied and Environmental Microbiology, Vol. 62(4), pp. 1405-1415. Torsvik, V., J. Goks0yr, and F.L. Daae, (1990) " High diversity in DNA of soil bacteria ", Applied and Environmental Microbiology, Vol. 56(3), pp. 782-787. Wagner, M., R. Amann, H. Lemmer, and K.H. Schleifer, (1993) " Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure ", Applied and Environmental Microbiology, Vol. 59(5), pp. 1520-1525. Wagner, M., B. Assmus, A. Hartmann, P. Hutzler, and R. Amann, (1994) " In situ analysis of microbial consortia in activated sludge using fluorescently labelled, rRNA-targeted oligonucleotide probes and confocal scanning laser microscopy ", Journal of Microscopy, Vol. 176, pp. 181-187. Ward, D.M., R. Weller, and M.M. Bateson, (1990) " 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community ", Nature, Vol. 345(6270), pp. 63-65. Wang, X., (2003) " From Microorganisms to engineered systems: A laboratory study on the bioremediation of MTBE contaminated groundwater ", Ph. D. dissertation, University of California, Riverside. Wilhelm, M.J., V.D. Adams, J.G. Curtis, and E.J. Middlebrooks, (2002) " Carbon adsorption and air-stripping removal of mtbe from river water ", Journal of Environmental Engineering, Vol. 128(9), pp. 813-823. Williams, Pamela R.D., (2001) " MTBE in California drinking water: An analysis of patterns and trends ", Environmental Forensics, Vol. 2(1), pp. 75-85. Wilson, R.D., D.M. Mackay, and K.M. Scow, (2002) " In situ MTBE biodegradation supported by diffusive oxygen release ", Environment Science Technology, Vol. 36(2), pp. 190-199. Yeh, C.K., and J.T. Novak, (1995) " The effect of hydrogen peroxide on the degradation of methyl and ethyl tert-butyl ether in soils, " Water Environmental Research, Vol. 67(5), pp. 828-834. Zoeckler, J.R., M.A. Widdowson, and J.T. Novak, (2003) " Aerobic biodegradation of methyl tert-butyl ether in gasoline-contaminated aquifer sediments ", Journal of Environmental Engineering, Vol. 129(7), pp. 642-650. 王永福、鄭幸雄、曾怡禎 (2002)，應用分子生物學方法研究分析複合基質中之中溫產氳菌群族，第27屆廢水處理技術研討會。方瑋寧、高志明 (2002)，MTBE好氧分解之可行性研究，碩士論文，國立中山大學環境工程研究所。朱文昌、盧重興、林明瑞 (1996)，生物濾床法處理含BTEX廢氣之研究，碩士論文，國立中興大學環境工程研究所。林哲昌、林淑滿 (2003)，以薄膜程序處理受MTBE污染之地下水的應用向探討，第1屆土壤及地下水處理技術研討會。林彥穎、劉文佐 (2001)，以葡萄糖為基質的生物除磷系統體積負荷與磷負荷對代謝行為與菌相影響之研究，碩士論文，國立中央大學環境工程研究所碩士班。林依蓉、劉文佐 (2001)，多氯聯苯厭氧馴養降解菌群微生物多樣性解析，碩士論文，國立中央大學生命科學研究所碩士論文。林啟文、吳志鴻、陳立軒、林虹君 (2003)，ETBE、MTBE及TAME之生物降解中間產物探討，第28屆廢水處理技術研討會。林啟文、吳志鴻、陳政遠、鄭雅文、林虹君、陳立軒 (2003)，本土MTBE分解菌對甲基第三丁基醚之動力模式探討，第八屆生化工程研討會論文集。林啟文、鄭雅文、陳信源、洪照先、羅伊翔 (2003)，MTBE分解菌之外加碳源及最適量化研究，第一屆土壤與地下水研討會。侯松男、林啟文 (2002)，含氧汽油添加劑分解菌之馴化、篩選及生長條件研究，碩士論文，大葉大學環境工程研究所碩士論文。施雅馨、賴吉永 (2003)，台灣黑翅土白蟻(*Odontotermes formosanus*)腸道共生菌功能群之研究，碩士論文，國立彰化師範大學生物學系碩士班。姜法清、李季眉 (1996)，生物濾床法處理VOCs廢氣之生物相與動力研究，碩士論文，國立中興大學環境工程研究所。胡苔莉、蔡蘊華 (2002)，以原位雜交技術探討配水系統中之生物膜，第27屆廢水處理技術研討會。高志明、陳谷汎、方瑋寧、陳廷育 (2002)，以好氧生物復育法整治受甲基第三丁基醚 (MTBE)污染場址之評估，第一屆海峽兩岸土壤及地下水污染整治研討會。陳信源、林啟文 (2003)，甲基第三

丁基醚分解菌之分解能力與重金屬抑制效應研究，碩士論文，大葉大學環境工程研究所。張全勝(1994)，MTBE及TAME製程與觸媒發展及其反應原料之取得，觸媒與製程，第三卷第三期第54-58頁。張怡塘、李俊福、洪俊雄、李佳熹、張綺璦、楊子君、周希領、王灝頤、詹惠萍、葉榮美(2003)，應用螢光原位雜交技術(FISH)偵測土壤與地下水微生物族群結構，第一屆土壤與地下水研討會。曾怡禎、張權英(2001)，利用分子生物方法分析微生物社會的結構，環境保護分子生物科技策略論壇會議資料集(III)。黃志謙、賴吉永(2003)，新的電泳技術-改良式DNA單股構型多形及部分雜交方法分析白蟻腸道的細菌，碩士論文，國立彰化師範大學生物學系碩士班。黃俊霖、劉文佐(2001)，以分子生物技術探討厭氧生物產氳程序之菌群結構，國立中央大學環境工程研究所碩士論文 黃?珽、廖述良、劉文佐(2001)，單槽連續進流回分式活性污泥系統微生物菌相變化之研究，碩士論文，國立中央大學環境工程研究所。廖世媚、揚秋和(1997)，利用PCR-SSCP技術分析Rh基因型，碩士論文，中央警察大學警政研究所。廖俊博、曾怡禎(2002)，南仁山古湖底泥甲烷氧化菌社會結構之研究，碩士論文，國立成功大學生物學系碩士班。鄭幸雄、陳乃慈、簡杏純、邱瓊芳、吳坤龍(2003)，污染場址馴化之微生物對柴油降解性之研究，第一屆土壤與地下水研討會。鄭幸雄、吳坤龍、曾怡禎、莊蕙萍(2003)，高溫厭氧流體化床處理PAN人造纖維廢水之微生物族群動態研究，第28屆廢水處理技術研討會。簡青紅、曾怡禎、張權英、劉憶芬(2003)，厭氧生物產氳反應槽的微生物社會結構，第28屆廢水處理技術研討會。美國環保署網站(USEPA) www.epa.gov。中華民國環保署網站www.epa.gov.tw。環保署公告毒性化學物質安全資料表(MSDS) <http://www.epa.gov.tw/J/toxic/> 資料查詢/160-01.doc。中國石油公司會計資料(1996、1997)。