

Biodegradation Capacity and Microbial Dynamics in a MTBE and BTEX Biotrickling Filter

林南成、林啟文

E-mail: 9417442@mail.dyu.edu.tw

ABSTRACT

A microbial culture capable of degrading methyl tertiary butyl ether (MTBE) and BTEX from gas stream was enriched in a laboratory-scale biotrickling filter. Removal of MTBE and BTEX vapors from air streams in a biotrickling filter was studied under various operating conditions including various organic loading rates, co-flow type, temperature of recirculation liquid of 28 °C, liquid flow rate of 300 ml/min, and the retention time at 85.6 seconds. The change for microbial community in the reactor was monitored by SSCP under conditions for various organic loading rates and types of carbon sources. In addition, the relationship between removal of the targeted compounds and microbial community was determined. Results of the research indicate: (1) the removal efficiency of the biotrickling filter achieves 98.6-57.3 % with the volumetric MTBE loading rate of 2.67-50.76 g/m³ hr; (2) the MTBE removal increases to 93.5 % as the nitrogen source of (NH₄)₂SO₄ is replaced by NaNO₃; (3) the removals for MTBE and toluene in the biotrickling filter are greater than 85 % with the volumetric loading rates below 22.28 g/m³ hr-carbon; (4) the removal capability for MTBE and BTEX of the biotrickling filter decreases in the order of benzene, toluene, ethylbenzene, xylene, and MTBE; (5) the removal of the biotrickling filter is greater than 75.8 % with the volumetric MTBE and BTEX loading rates below 37.4 g/m³ hr-carbon; (6) in the presence of benzene or toluene in the mixture, the system removal capability was influenced due to the priority of degrading benzene and toluene. the inhibition in degrading other substrates was observed in the bioreactor at high organic loading; (7) under equal amount of carbon condition, the removal efficiency for the BTEX and MTBE mixture is superior to for MTBE only condition in the biotrickling filter.

Keywords : Biodegradation ; Biotrickling filter ; SSCP ; Microbial community ; MTBE ; BTEX

Table of Contents

封面內頁	簽名頁	博碩士論文授權書	iii	中文摘要	iv	英文摘要	vi	誌謝	viii	目錄	x	圖目錄	xiv	表目錄	xvi	第一章 緒論	1.1	前言	1.1	1.2 研究目的	2	1.3 研究內容	3	第二章 文獻回顧	2.1	MTBE與BTEX之簡介	5	2.1.1	MTBE與BTEX之物化性質	6	2.1.2	MTBE與BTEX之使用及對人體之危害	8	2.1.3	MTBE與BTEX之生物降解	10	2.2	生物反應器介紹	12	2.2.1	生物濾床	15	2.2.2	生物滴濾塔	16	2.2.3	生物洗滌塔	17	2.3	生物滴濾塔之相關處理技術與研究	17	2.3.1	生物滴濾塔處理廢氣之研究	18	2.4	生物滴濾塔之操作參數	20	2.4.1	氣-液接觸方式	21	2.4.2	溫度	21	2.4.3	pH值	21	2.4.4	填充材料	22	2.4.5	其它輔助碳源之添加	23	2.4.6	有機負荷	23	2.4.7	循環水流量	24	2.4.8	空塔停留時間	24	2.5	應用分子生物技術於菌群結構分析之相關研究	25	2.5.1	聚合?鏈鎖反應原理	25	2.5.2	單股DNA構形多型性分析	26	2.5.3	微生物種類辨識方法	27	第三章 材料與方法	3.1	菌種來源	34	3.2	生物滴濾塔之材料與設備	34	3.2.1	生物滴濾塔之儀器設備	34	3.2.2	其它儀器設備	39	3.2.3	生物滴濾塔試驗之相關藥品	40	3.3	分子生物技術之材料與設備	41	3.3.1	分子生物技術之相關藥品	41	3.3.2	分子生物技術之儀器設備	43	3.3.3	其它相關使用材料	43	3.4	研究方法與步驟	45	3.4.1	生物滴濾塔之去除效率評估方法與步驟	45	3.4.2	生物滴濾塔分解MTBE與BTEX實驗	50	3.4.3	分子生物技術建立	52	第四章 結果與討論	4.1	探討氮源成份與穩定期對生物滴濾塔之影響	56	4.1.1	氮源成份對生物滴濾塔之影響	56	4.1.2	氮源成分變化對菌群結構差異之分析	60	4.1.3	穩定期菌群結構差異之分析	63	4.2	探討基質濃度對生物滴濾塔之影響	68	4.2.1	MTBE進氣濃度對分解效率影響	68	4.2.2	基質濃度對菌群結構差異之分析	70	4.2.3	更改基質濃度後其穩定期菌群結構 差異之分析	73	4.3	循環水溫對生物滴濾塔之影響	76	4.3.1	循環水溫度對分解效率之影響	76	4.3.2	更換溫度後之穩定期其菌群結構差異分析	79	4.4	以生物滴濾塔處理複合碳源MTBE+Toluene	82	4.4.1	MTBE與Toluene廢氣之去除效率評估	82	4.4.2	MTBE與Toluene試程塔內之菌群結構變化	85	4.5	以生物滴濾塔處理複合碳源MTBE+Toluene	89	4.5.1	MTBE與Toluene廢氣之去除效率評估	90	4.5.2	MTBE與Toluene廢氣之去除效率評估	93	第五章 結論與建議	5.1	結論	96	5.2	建議	99	參考文獻	100
------	-----	----------	-----	------	----	------	----	----	------	----	---	-----	-----	-----	-----	--------	-----	----	-----	----------	---	----------	---	----------	-----	--------------	---	-------	----------------	---	-------	---------------------	---	-------	----------------	----	-----	---------	----	-------	------	----	-------	-------	----	-------	-------	----	-----	-----------------	----	-------	--------------	----	-----	------------	----	-------	---------	----	-------	----	----	-------	-----	----	-------	------	----	-------	-----------	----	-------	------	----	-------	-------	----	-------	--------	----	-----	----------------------	----	-------	-----------	----	-------	--------------	----	-------	-----------	----	-----------	-----	------	----	-----	-------------	----	-------	------------	----	-------	--------	----	-------	--------------	----	-----	--------------	----	-------	-------------	----	-------	-------------	----	-------	----------	----	-----	---------	----	-------	-------------------	----	-------	--------------------	----	-------	----------	----	-----------	-----	---------------------	----	-------	---------------	----	-------	------------------	----	-------	--------------	----	-----	-----------------	----	-------	-----------------	----	-------	----------------	----	-------	-----------------------	----	-----	---------------	----	-------	---------------	----	-------	--------------------	----	-----	--------------------------	----	-------	-----------------------	----	-------	-------------------------	----	-----	--------------------------	----	-------	-----------------------	----	-------	-----------------------	----	-----------	-----	----	----	-----	----	----	------	-----

REFERENCES

- 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. Bassam, B.J., G. Caetano-Anolles, and P.M. Gresshoff, (1991) “ Fast and sensitive silver staining of DNA in polyacrylamide gels ” , Analytical Biochemistry, Vol. 196(1), pp. 80-83. Cai, Q.Q., and I. Touitou, (1993) “ Excess PCR primers may dramatically affect SSCP efficiency ” , Nucleic Acids Research, Vol. 21(16), pp. 3909-3910. 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. Cox, H. H. J. and M. A. Deshusses., (2002) , “ Co-treatment of H₂S and toluene in a biotrickling filter ” , Chem. Engine. Journal ,87:101-110. Diks R.M.M. and S.P.P. Ottengraf, (1991), “ Verification studies of a simplified model for the removal of dichloromethane from waste gases using a biological trickling filter, ” Bioprocess engineering, 6:93-99. Drogos, D.L., (2000) “ MTBE v. other oxygenates ” , presented at MEALY's MtBE Litigation Conference, May 11-12. Eweis, J. B., Chang, P. Y., and Schroeder, E. D., (1997) Meeting the challenge of methyl tertiary butyl ether (MTBE) biodegradation. Presentation at Air & Waste Management Association ' s 90th Annual Meeting & Exhibition, Toronto, Ontario, Canada. Eweis J., E. Schroeder, D. Chang, K. Scow, R. Morton, and R. Caballerp, (1998) “ Meeting the challenge of MTBE biodegradation ” , Presented of 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. Findlay G. Edwards and N. Nirmalakhandan. (1996) “ Biological Treatment of Airstreams Contaminated with VOCs:an Overview ” . Wat. Sci. Tech. 34: 565-571 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. Clarens, 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. 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. 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. 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. Hekmat, D., A. Linn, M. Stephan, and D. Vortmeyer. (1997), “ Biodegradation dynamics of aromatic compounds from waste air in a trick-bed reactor ” Appl. Microbiol.Biotechnol. 48: 129-134 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. 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. 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. Mpanias, C. J., B. C. Baltzis . (1998) “ Biocatalytic removal of mono-Chlorobenzene vapor in trickling filters ” , Catalysis Today. 40: 113-120 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. 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. Sa, C. S. A., R. A. R. Boaventura. (2001) “ Biodegradation of phenol by *Pseudomonas putida* DSM 548 in a trickling bed reactor ” , Biochem. Eng. 9: 211-219 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. 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. Sorial, G.A., F.L. Smith, P. Biswas, and R.C. Brenner, 1993, “ Development of aerobic biofilter design criteria for treating VOCs, ” Proceedings of the 86th Annual Meeting & Exhibition of the Air & Waste Management Association, Denver, CO, USA. 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. Stoffels, M., R. Amann,W. Ludwig, D. Hekmat, and K.-H. Schleife. (1998) “ Bacterial Community dynamics during start-up of a Trickle-bed Bioreactor degrading Aromatic Compounds ” , Appl. Environ. Microbiol.. 64: 930-939 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. 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. 王嘉禧 (2000), 以生物滴濾塔處理排氣中氨之操作性能研究, 碩士論文, 國立中山大學環境工程研究所。王永福 (2002), 應用分子生物學方法研究分析複合基質中之中溫產氫菌群, 第27屆廢水處理技術研討會。朱文昌 (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。朱振華 (1998), 生物濾床法處理含BTEX廢氣程序控制之研究, 碩士論文, 國立中興大學環境工程研究所。吳非隆 (1995), 以生物滴濾塔處理排氣中甲苯成份之操作性能研究, 碩士論文, 國立中山大學環境工程研究所。邱創汎、王耀銘、張坦卿 (1996), 「空氣污染生物處理技術本土化之評析」, *工業污染防治*, 第58期, p111-124。邱仁杰、歐陽嶠暉、林志墩 (1999), 「生物濾床去除營養物質之原理與程序」, *國立中央大學環境工程學刊*第五期。林彥穎 (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 (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。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 (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。涂秀妹 (2001), 以實場生物滴濾塔處理排氣中苯乙炔及丙烯之操作性能研究, 碩士論文, 國立中山大學環境工程研究所。施雅馨 (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。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。陳良誌 (2000), 1.模場生物滴濾處理含異辛醇排氣之操作性能研究; 2.以實場生物滴濾塔處理合成樹脂廠排氣之操作性能研究, 碩士論文, 國立中山大學環境工程研究所。陳信源 (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。許盈志 (2002), 使用高效益固定生物模反應器處理系統 (FFR) 處理地下水之可行性評估試驗研究, 碩士論文, 國立高雄第一科技大學環境與安全衛生工程所。黃志謙 (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。黃忠永 (1996), 以生物滴濾塔及濾床處理煉油廢水場排氣中揮發性有機物之研究, 碩士論文, 國立中山大學環境工程研究所。黃俊霖 (2001), 以分子生物技術探討厭氧生物產氫程序之菌群結構, 碩士論文, 國立中央大學環境工程研究所。黃?琹 (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 (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 (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。鄭建業 (2001), 生物有機化學。俊傑書局股份有限公司。蘇佳慶 (1996), 以生物滴濾塔處理排氣中一氧化氮之操作性能研究, 碩士論文, 國立中山大學環境工程研究所。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)。