

含氧汽油添加劑分解菌之馴化、篩選及生長條件研究

侯松男、林啟文

E-mail: 9121641@mail.dyu.edu.tw

摘要

本研究之目的為探討含氧汽油添加劑分解菌之馴化、篩選及生長條件，期能藉由研究結果之分析比較，以了解MTBE分解菌對MTBE及其他環境中常見之有機污染物分解情形。本研究經由實驗室自行組設氣舉式(Air lift)反應器，馴化(Acclimated)出能以MTBE為利用基質的MTBE分解菌，馴化初期以異丙醇(IPA)與MTBE等比例混合作為其馴化之基質，並隨馴化時間增加而逐漸減少IPA之添加量，最後完全以MTBE為其唯一馴化碳源，待得到具MTBE分解能力之混合菌，再利用批次分解之方式探討該混合菌對MTBE的分解特性、及其他基質(BTEX、TAME、ETBE、Ethyl ether、TBA)存在下之分解情形。最後由此混合菌中進行菌株分離篩選，藉此得到具分解MTBE能力之純菌，再針對分解過程中副產品產生情形及MTBE分解特性進行探討。研究結果顯示：(1)以IPA為輔助碳源進行煉油廠污泥之馴化，可成功地馴化出能以MTBE為利用基質之混合菌，於經過約五個月之馴化後，反應器中菌液之pH呈明顯降低情形；(2)批次分解試驗結果顯示，於約二星期後能完全分解7.2ppm之MTBE，在碳源濃度達288ppm時仍不致造成對MTBE分解的抑制；(3)由馴化所得混合菌進行批次實驗，評估其分解基質能力之結果顯示，此混合菌具有分解多種芳香族化合物之能力，依其分解速率排序由快而慢依序為Toluene、Benzene、Ethyl benzene、p-Xylene、Ethyl ether、MTBE、TAME、ETBE；(4)MTBE與Benzene、Toluene分別共存者，須待Benzene或Toluene分解完畢後才開始分解MTBE，且最後皆仍可完全分解MTBE；(5)MTBE與TBA、TAME、ETBE分別共存者，其添加之複合碳源皆可同時被本土混合菌分解；(6)將本土混合菌以其他碳源馴化培養後之MTBE分解結果顯示，在經過Benzene或Toluene之培養後本土混合菌仍具有分解MTBE之能力；(7)由混合菌中分離所得之純菌，其對MTBE之分解能力優於混合菌，完全分解4.09mg MTBE所需時間約6小時，但其分解MTBE過程中會產生TBA累積之情形；(8)MTBE分解菌於MTBE濃度為30mg/L時具有之最大比生長速率為0.000778hr⁻¹，其飽和常數則為0.029mg/L，但在MTBE濃度增至60mg/L時，其比生長速率會因其濃度的增加而產生抑制。

關鍵詞：甲基第三丁基醚；馴化；氣舉式反應器；批次分解；篩選

目錄

封面內頁 簽名頁 授權書	iii	中文摘要	v	英文摘要	vii	致謝	ix	目錄	x	圖目錄	xv	表目錄	xviii	第一章 緒論	1.1 前言	1.1.2 研究目的																																						
2.1.3 內容結構	3	第二章 文獻回顧	2.1	MTBE對環境之危害及使用現況	5	2.2 MTBE之背景資料	12	2.3 MTBE之生物分解	15	2.3.1 生物分解之原理	15	2.3.2 MTBE之生物分解可行性	15	第三章 研究方法	3.1 研究材料及儀器設備	23	3.1.1 菌種來源																																					
23	3.1.2 藥品	23	3.1.3 研究使用材料	25	3.1.4 研究使用儀器設備	26	3.2 MTBE分解菌之馴化	27	3.2.1 基質	27	3.2.2 低限營養鹽配比	27	3.2.3 馴化實驗碳源之添加時程	30	3.2.4 反應槽低限營養鹽更換時程	30	3.2.5 氣舉式馴化反應器對菌種之增富效率評估																																					
33	3.3 MTBE分解菌效能評估方法	34	3.3.1 菌株消耗碳源之評估方式	34	3.3.2 碳源分析	35	3.3.3 GC-FID及熱脫附吹氣捕集裝置分析條件	35	3.3.4 MTBE檢量線製作	36	3.3.5 批次分解實驗之氣/液MTBE濃度換算方法	39	3.3.6 菌體乾重分析方法	40	3.3.7 菌體於懸浮液之定量	41	3.3.8 殘存率計算方式	41	3.4 環境因子對MTBE分解菌之影響																																			
41	3.4.1 MTBE分解菌對不同碳源之分解情形	42	3.4.2 複合基質共存下對MTBE分解菌之影響	43	3.4.3 MTBE分解菌經其他碳源馴化後之影響	43	3.5 菌種之純化、篩選及保存	44	3.5.1 純化方法	45	3.5.2 具MTBE分解能力菌株之篩選	46	3.5.3 菌株之保存	48	3.6 代謝產物分析方法	48	3.7 MTBE分解菌之動力學特性探討	49	3.7.1 微生物動力實驗	49	3.7.2 微生物生長實驗	50	3.7.3 微生物動力參數求取																															
50	第四章 結果與討論	4.1 本土MTBE分解菌馴化結果	54	4.1.1 經馴化之MTBE分解菌分解效率評估	55	4.1.2 菌種之增富效率評估	59	4.2 MTBE分解菌分解其他碳源之情形	60	4.2.1 Benzene、Toluene、Ethyl Ether之分解情形	61	4.2.2 Ethylbenzene、Xylene、TBA之分解情形	62	4.2.3 ETBE與TAME之分解情形	64	4.3 於複合碳源共存下對MTBE分解菌之影響	65	4.3.1 MTBE與苯二種碳源共存時之基質分解情形	65	4.3.2 MTBE與甲苯二種碳源共存時之基質分解情形	66	4.3.3 MTBE、苯及甲苯三種碳源共存時之基質分解情形	66	4.3.4 MTBE與TAME二種碳源共存時之基質分解情形	67	4.3.5 MTBE與ETBE二種碳源共存時之基質分解情形	67	4.3.6 MTBE與TBA二種碳源共存時之基質分解情形	68	4.3.7 混合碳源共存時之基質分解比較	68	4.4 MTBE分解菌經其他碳源馴化後之影響	75	4.5 純菌株篩選純化分離結果及分解效率	78	4.6 純菌株分解MTBE之TBA產生情形	81	4.7 MTBE分解菌之動力參數	84	第五章 結論與建議	5.1 結論	86	5.2 建議	87	參考文獻	89	附錄一 第26屆廢水處理技術研討會論文(MTBE分解菌於連續回分式批次反應系統之馴化研究)	94	附錄二 第26屆廢水處理技術研討會論文(MTBE分解菌之動力參數研究)	100	附錄三 第七屆生化工程研討會論文(苯與甲苯對甲基第三丁基醚分解菌之抑制研究)	107	附錄四 第七屆生化工程研討會論文(MTBE與芳香化合物共存環境下之基質抑制效應研究)	114

參考文獻

- 1.中國石油公司汽油規範，1996，1997。2.平成股份有限公司，1999，工業區土壤及地下水質污染全面性調查工作，新竹市。3.李家偉，2000，甲基第三丁基醚(MTBE)吸入後在人體之分布及排泄特性研究，第十七屆空氣污染控制技術研討會，國立雲林科技大學環境與安全工程技術系。
- 4.林啟文等，2000，MTBE分解菌之動力參數研究，中華民國環境工程學會第二十六屆廢水處理技術研討會，高雄縣。
- 5.曹以會，1998，無鉛汽油添加劑MTBE致癌，中國石油公司汽油規範。
- 6.張全勝，1994，MTBE及TAME製程與觸媒發展及其反應原料之取得，觸媒與製成，第三卷第三期，pp54-58。
- 7.張怡塘等，1999，環境微生物學，中華民國環境工程學會。
- 8.Amy Pruden, Makram T Suidan, Albert D Venosa, Gregory J Wilson, 2001, " Biodegradation of methyl tert-butyl ether under various substrate conditions." Environ. Sci. Technol, 35, :4235—4241
- 9.Church CD, Tratnyek P, and Scow K (2000) " Pathways for the degradation of MTBE and other fuel oxygenates by isolate PM1." Preprints of Extended Abstracts, Am. Chem. Soc. 40: 261—263
- 10.Chun J.S., Burleigh-Flayer H.D., Kintigh W.J., 1992, " Methyl tertiary ether: vapor inhalation oncogenicity study in Fisher 344 rats." Bushy Run Research Center Report No. 91N0013B.
- November 13. Union Carbide Chemicals and Plastics Company, Inc. submitted to the U.S. EPA under TSCA Section 4 Testing Consent Order 40 CFR 799.500 with cover letter dated November 19, 1992. EPA/OPTS#42098. Export, PA: Bushy Run Research Center.
- 11.Burleigh-Flayer HD, Neptun DA, Dodd DE Garman RH, Bird MG, Keiss JJ, 1993, " Methyl Tertiary Butyl Ether Vapor Inhalation Oncogenicity Study in Mice. Society of Toxicology." Abstracts of the 32Annual Meeting 13(1): 153.
- 12.Deeb RA, Scow KM, and Alvarez-Cohen L (2000) " Aerobic MTBE biodegradation: an examination of past studies, current challenges and future research directions." Biodegradation, 11, 171—186
- 13.Deeb RA & Alvarez-Cohen L (2000) " Aerobic biotransformation of gasoline aromatics in multi-component mixtures." Biorem. J. 4: 171—179
- 14.Duffy JS, Pup JA, and Kneiss JJ (1992) " Toxicological evaluation of MTBE testing performed under ESCA consent agreement." Journal of Soil Contamination, 1, p27-37.
- 15.Eweis J, Schroeder E, Chang D, Scow K, Morton R, and Caballero R (1997) " Meeting the Challenge of MTBE Biodegradation." Proceedings of the 90th Annual Meeting & Exhibition, Air & Waste Management Association, Toronto, Canada, June 8-13
- 16.Fujiwara Y, Kinoshita T, Sato H, and Kojima I (1984) " Biodegradation and bioconcentration of alkylethers." Yugagaku 33: 111—114
- 17.Garnier P, Auria R, Auger C, and Revah S (1999) " Cometary biodegradation of methyl t-butyl ether by *Pseudomonas aeruginosa* grown on pentane." Appl. Microbial. Biotechnol. 51: 498—503
- 18.Garrett P, Moreau M, and Lowry JD (1986) " MTBE as a groundwater contaminant." Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation Conference.
- 19.Hardison LK, Curry SS, Ciuffetti LM, and Hyman MR (1997) " Metabolism of diethyl ether and cometabolism of methyl tert-butyl ether by a filamentous fungus, a *Graphium* sp." Appl. Environ. Microbiol. 63: 3059—3067
- 20.Hanson JR, Ackerman CE, and Scow KM (1999) " Biodegradation of methyl tert-butyl ether by a bacterial pure culture." Appl. Environ. Microbiol. 65: 4788—4792
- 21.Happel AM, Beckenbach EH, and Halden RU (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
- 22.Hernandez-Perez G, Fayolle F, Vandecasteele JP, 2001, " Biodegradation of ethyl t-butyl ether (ETBE), methyl t-butyl ether (MTBE) and t-amyl methyl ether (TAME) by *Gordonia terrae*." Appl. Environ. Microbiol. 55: 117—121
- 23.Hong JY, Wang YY, Bondoc FY, Yang CS, Chung S, Lee M, and Huang WQ (1997) " Rat olfactory displays a high activity in metabolizing methyl tert-butyl ether and other gasoline ethers." Fundam. Appl. Toxicol. 40: 205—210
- 24.Hong J, Yang CS, Lee M, Wang Y, Huang W, Tan Y, Patten CJ, and Bondoc FY (1997) " Role of cytochrome P450 in the metabolism of methyl tert-butyl ether in human liver." Arch. Toxicol. 71: 266-269
- 25.Jensen HM, and Arvin E (1990) " Solubility and degradability of the gasoline additive MTBE, methyl tert.-butyl ether, and gasoline compounds in water." In: Arendt F, Hinsenfeld M & Van den Brink WJ (Eds). Contaminated Soil ' 90, pp 445—448
- 26.Johanson G, Nihlen A, and Lof A (1995) " Toxic kinetics and acute effects of MTBE and ETBE in male volunteers." Toxicol. Lett. 82—83: 713—718
- 27.Joseph, P., 1997, " Changes in disease rates in Philadelphia following the introduction of oxygenated gasoline ", Presented at the Air & Waste Management Association ' s 90th Annual Meeting, Toronto, Ontario, Canada.
- 28.Keller A et al. (1998) " Health and environmental assessment of MTBE." Report to the governor and legislature of the state of California as sponsored by SB 521.
- 29.Mehiman, M. A., 1998, " Dangerous and cancer-causing properties of products and chemicals in the oil-refining and petrochemical industries." Part-XXV: Neurotoxic, allergic, and respiratory effects in humans from water and air contaminated by MTBE in gasoline. J Clean Technol, Environ Toxicol and Occup Med, 7(1), 65-84.
- 30.Miller MJ, Ferdinandij ES, Klan M, Andrews LS, Douglas JF, and Kneiss JJ (1997) " Pharmacokinetics and disposition of methyl tbutil ether in Fischer-344 rats." J. Appl. Toxicol. 17: S3—S12
- 31.Mo K, Lora CO, Wanken AE, Javanmardian M, Yang N, and Kulpa CF (1997) " Biodegradation of ethyl tert-Butyl Ether by pure bacterial cultures." Appl. Microbiol. Biotechnol, 47:69-72.
- 32.Nakamura DN (1994) " MTBE, still the best choice." Hydrocarbon Processing 73: 17
- 33.Nihlen A, Lof A, and Johanson G (1998) " Controlled ethyl tert-butyl ether (ETBE) exposure of male volunteers. II Acute effects." Toxicol. Sci. 46: 143—150
- 34.Park K, and Cowan R (1997) " Effects of oxygen and temperature on the biodegradation of MTBE." Proceedings of the 213th ACS National Meeting: Division of Environmental Chemistry, San Francisco, CA, April 13-17.
- 35.Piel WJ, and Thomas RX (1990) " Oxygenates for reformulated gasoline." Hydrocarbon Processing 69: p68—73
- 36.Salanitro JP, Diaz LA, Williams MP, and Wisniewski HL (1994) " Isolation of a bacterial culture that degrades methyl t-butyl ether." Appl. Environ. Microbiol. 60: 2593—2596
- 37.Salanitro JP, Chou CS, Wisniewski HL, and Vipond TE (1998) " Perspectives on MTBE biodegradation and the potential for in situ aquifer bioremediation." In: Proceedings of the Southwestern Regional Conference of the National Ground Water Association, MTBE and Perchlorate in Ground Water, 3—4 June, Anaheim, CA
- 38.Salanitro J, Spinnler G, Maner P, Wisniewski H, and Johnson P (1999) " Potential for MTBE

bioremediation — in situ inoculation of specialized cultures. ” In: Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation Conference, 17—20 November, Houston, TX 39.Steffan RJ, McClay K, Vaiberg S, Condee CW, and Zhang D (1997) “ Biodegradation of the gasoline oxygenates Methyl tert-Butyl Ether, Ethyl tert-Butyl Ether, tert-Amyl1 Ethyl Ether by propane-oxidizing bacteria. ” Applied Environ. Microbiology 63(11):4216-4222. 40.Stocking AJ, Deeb RA, Flores AE, Stringfellow W, Talley J, Brownell R, and Kavanaugh MC (2000) “ Bioremediation of MTBE: a review from a practical perspective. ” Biodegradation, 11, p187—201 41.Suflita JM, and Mormile MR (1993) “ Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. ” Environ. Sci. Technol. 27: 976—978 42.U.S. Environmental Protection Agency, 1996, “ Drinking water regulations and health advisories ” , Washington, D.C., Office of Water 43.Werner I, Koger CS, Deanovic LA, and Hinton DE (1999) “ Toxicity of methyl-tert-butyl ether to freshwater organisms. ” Environmental Pollution, 111, p83-88. 44.White, M.C., Johnson, C.A., Ashley, D.L., et al., 1995, “ Exposure to methyl tertiary-butyl ether from oxygenated gasoline in Stamford. ” Connecticut, Arch. Env. Health 50(3): 183-189. 45.Yeh CK (1992) “ Degradation of gasoline oxygenates in the subsurface. ” PhD Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA 46.Zogorski J, Morduchowitz A, Baehr A, Bauman B, Conrad D, Drew R, Korte N, Lapham W, Pankow J, and Washington E (1997) “ Fuel oxygenates and water quality coordinated by the interagency oxygenated fuel assessment ” Office of Science and Technology Policy, Executive Office of the President, Washington, DC