

# Acclimation, Isolation, and Growth Conditions for Gasoline Oxygenate Degrading Culture

侯松男、林啟文

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

## ABSTRACT

The objectives of the research are to investigate the acclimation, isolation, and growth conditions for gasoline oxygenate degrading culture. Specially, the experiments are to study the biodegradation of MTBE and other organic contaminants in the environment. The MTBE degrading mixed culture was derived from an acclimated sludge, which was originally maintained on isopropanol and MTBE at equal amount, and finally shifted to on MTBE as the sole carbon source in an air-lift reactor. After yielding the MTBE degrading mixed culture, the experiments to study MTBE degradability in the presence of other substrates including BTEX, TAME, ETBE, Ethyl ether, and TBA were conducted in 250 ml amber glass bottles. The final stage of the research is to develop MTBE degrading pure cultures through isolating and screening procedures from the MTBE degrading mixed culture. In addition, the accumulation of reaction by-products and MTBE biodegradability by the isolated pure culture were examined. Results of batch substrate removal experiments shown that: (1) An aerobic mixed culture capable of degrading MTBE as a sole carbon and energy source was developed from an acclimated activated sludge, initially using isopropanol as a co-substrate. It was also found the solution pH in the air-lift reactor consistently decreased after five months acclimation; (2) MTBE with concentration of 7.2ppm was completely removed within two weeks, and there is no inhibition occurred for the initial MTBE concentration as high as 288ppm; (3) Biodegradation of the targeted compounds by the mixed culture in order of removal rate was: toluene, benzene, ethyl benzene, p-xylene, ethyl ether, MTBE, TAME, and ETBE; (4) Mixtures of MTBE and benzene, or toluene showed inhibited the removal of MTBE. It was not degraded for MTBE until benzene or toluene were degraded completely, and MTBE was degraded completely in the final; (5) Biodegradation experiments with mixtures of MTBE and TBA, TAME, or ETBE showed good removals; (6) The mixed cultures still can degrade MTBE after the incubation of benzene or toluene; (7) The MTBE biodegradation rate for a pure culture is higher than for a mixed culture, and the pure culture was shown to degrade up to 4.09mg MTBE within a 6-hour period, whereas TBA was formed as a transient metabolic intermediate during the breakdown of MTBE; (8) The maximum specific growth rate and the saturation constant are 0.000778hr<sup>-1</sup> and 0.029mg/L at MTBE concentration of 30mg/L. However, high MTBE concentration (60 mg/L) was slightly inhibitory to growth of the mixed culture.

Keywords : MTBE ; acclimation ; air-lift reactor ; batch biodegradation ; screening

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## REFERENCES

- 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, Nepton DA, Dodd DE Garman RH, Bird MG, KeissJJ, 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) " Cometabolic biodegradation of methyl t-butyl ether by Pseudomonas aeruginosa grown on pentane. " Appl. Microbiol. 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, Hinsenveld 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 tbutyl 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