

Isolation of the MTBE and BTEX-degrading organisms and their biodegrading kinetics

鄭雅文、林啟文

E-mail: 9417450@mail.dyu.edu.tw

ABSTRACT

The major work for the study is the testing of aromatic compound-degrading cultures and with heavy metals in batch experiments for biodegradation of MTBE, benzene, toluene, ethylbenzene, and xylene. Additionally, batch growth data of four pure cultures (i.e., *Pseudomonas aeruginosa* YAMT521、*Ralstonia* sp. YABE411、*Pseudomonas* sp. YATO411及*Pseudomonas putida* YAEB411) isolated from an industrial wastewater treatment under aerobic conditions has been assessed with nonlinear regression technique. Results of the research show that : (1) In single substrate conditions, relative magnitude of the aromatic compound-degrading rate to *Pseudomonas aeruginosa* YAMT521 can be MTBE > benzene > toluene > p-xylene > ethylbenzene. (2) In two substrate conditions, relative magnitude of the aromatic compound-degrading rate to *Pseudomonas aeruginosa* YAMT521 can be MTBE+benzene > MTBE+toluene > MTBE+ethylbenzene > MTBE+p-xylene. (3) In three substrate conditions, relative magnitude of the aromatic compound-degrading rate to *Pseudomonas aeruginosa* YAMT521 can be MTBE+benzene+p-xylene > MTBE+benzene+toluene > MTBE+benzene+ethylbenzene. (4) In mixed substrate conditions, the aromatic compound-degrading rate to *Pseudomonas aeruginosa* YAMT521 can be debased by increasing the kinds of BTEX. (5) Relative magnitude of the toxic effects of the heavy metals to *Pseudomonas aeruginosa* YAMT521 can be Zn²⁺ > Mn²⁺ > Ni²⁺. (6) μ_m 0.0658 ± 0.04hr⁻¹, KS 14.386 ± 12.04mg/l, Ki 72.47 ± 3.10mg/l, and Y=0.68 ± 0.22 mg/mg for aerobic biodegradation of MTBE by *Pseudomonas aeruginosa* YAMT421; μ_m 0.2927 ± 0.230hr⁻¹, Ki 341.04 ± 15.40mg/l, Y=0.20 ± 0.40 mg/mg, Ks=1.988 ± 0.665 mg/l under substrate concentrations ranging from 9.91 to 94.87 mg/l, and Ks=8.712 ± 1.369 mg/l under substrate concentrations ranging from 115.66 to 178.97 mg/l for aerobic biodegradation of benzene by *Ralstonia* sp. YABE411; μ_m 0.4598 ± 0.0915hr⁻¹, KS 1.788 ± 0.924mg/l, Ki 122.04 ± 5.49mg/l, and Y=0.68 ± 0.07 mg/mg for aerobic biodegradation of toluene by *Pseudomonas* sp. YATO411; μ_m 0.7870 ± 0.0875hr⁻¹, KS 15.631 ± 1.612mg/l, Ki 158.14 ± 3.53mg/l, and Y=0.842 ± 0.159 mg/mg for aerobic biodegradation of ethylbenzene by *Pseudomonas putida* YAEB411. (7) In mixed substrate conditions, the substrate concentration coefficient, Ks, can be used as an indicator of model. (8) The kinetics of heavy metal-induced(Zn²⁺, Mn²⁺, Ni²⁺) repression of *Pseudomonas aeruginosa* YAMT521 can be mathematically described as non-competitive inhibition of a rate-determining enzymatic reaction with fitting values higher than 94%, 92%, and 85%.

Keywords : MTBE ; inhibition of substrate and heavy metal ; biodegradation ; kinetic

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 vii 目錄 x 圖目錄 xiv 表目錄 xxi 第一章 緒論 1.1 前言
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之使用現況 5 2.1.2 MTBE與BTEX之物理化學性質 6 2.1.3 MTBE與BTEX對人體健康之影響 8 2.2 MTBE與BTEX之生物降解 10 2.3 重金屬對生物降解之影響 19 2.4 微生物反應動力學之相關研究 21 2.4.1 單一基質限制生長 22 2.4.2 單一基質抑制生長 22 2.4.3 基質間之交互作用 23 2.4.4 複合基質抑制方程式 24 2.4.5 重金屬存在下對基質降解之抑制 25 第三章 研究方法 3.1 單一分解菌之分離 26 3.1.1 菌種來源 27 3.1.2 菌群馴化 28 3.1.3 菌株分離 29 3.1.4 菌種篩選 29 3.1.5 菌種保存 32 3.1.6 菌種活化 32 3.2 菌株鑑定 33 3.3 菌種於單一基質存在下之批次降解測試 33 3.3.1 菌量之分析方法 34 3.3.2 碳源消耗之分析 37 3.3.3 基質檢量線之製作 38 3.4 菌種於雙基質共存下之批次降解測試 40 3.5 菌種於三種基質共存下之批次降解測試 41 3.6 重金屬存在下對基質之降解影響探討 41 3.7 單一基質之動力學模式 42 3.7.1 單一基質反應動力學模式 43 3.8 複合基質共存之動力學模式 44 3.9 重金屬存在下對單一基質之降解抑制影響 48 3.10 副產物抑制模式 50 第四章 結果與討論 4.1 菌種鑑定之結果 52 4.2 批次降解試驗 54 4.2.1 基質濃度、降解率及產率之關係 54 4.2.2 基質濃度、碳源及能量源之收率關係 74 4.3 單一基質之動力學參數值 89 4.3.1 *Pseudomonas aeruginosa* YAMT521於單一基質之動力學參數值 90 4.3.2 *Ralstonia* sp. YABE411於單一基質之動力學參數值 97 4.3.3 *Pseudomonas* sp. YATO411於單一基質之動力學參數值 98 4.3.4 *Pseudomonas putida* YAEB411於單一基質之動力學參數值 99 4.4 雙基質之動力學參數值 100 4.4.1 MTBE與苯共存之動力學參數值 100 4.4.2 MTBE與甲苯共存之動力學參數值 103 4.4.3 MTBE與乙苯共存之動力學參數值 105 4.4.4 MTBE與對-二甲苯共存之動力學參數值 107 4.4.5 雙基質共存之動力學參數值小結 108 4.5 三基質之動力學參數值 109 4.5.1 MTBE與苯、甲苯共存之動力學參數值 109 4.5.2 MTBE與苯、乙苯共存之動力學參數值 111 4.5.3 MTBE與苯、對-二甲苯共存之動力學參數值 112 4.5.4 三基質共存之動力學參數值小結 114 4.6 三基質共存之動力學參數值小結 113 4.6 五基質之動力學參數值 115 4.7 金屬存在下對單一基質之降

解抑制影響 116 4.7.1 與鋅共存下對 MTBE 降解之動力學參數值 116 4.7.2 與錳共存下對 MTBE 降解之動力學參數值 119 4.7.3 與鎳共存下對 MTBE 降解之動力學參數值 122 4.8 副產物抑制之動力學參數值 124 第五章 結論與建議 5.1 結論 128 5.2 建議 129 參考文獻 130 圖目錄 圖 2.2-1 於好氧下丙烷氧化菌代謝 MTBE 之途徑 14 圖 2.2-2 於好氧下鄰-二甲苯之代謝途徑 15 圖 2.2-3 於厭氧下五種苯可能的代謝途徑 16 圖 2.2-4 於厭氧下甲苯代謝途徑 17 圖 2.2-5 BTX 共存下 BTX 代謝途徑 18 圖 2.3-1 微生物處理有毒重金屬離子的途徑 20 圖 3.1-1 研究架構流程 27 圖 3.3-1 連續稀釋流程 35 圖 3.3-2(a) 菌量之檢量線(MTBE 分解菌) 35 圖 3.3-2(b) 菌量之檢量線(Benzene 分解菌) 36 圖 3.3-2(c) 菌量之檢量線(Toluene 分解菌) 36 圖 3.3-2(d) 菌量之檢量線(Ethylbenzene 分解菌) 36 圖 3.3-3(a) MTBE 檢量線 39 圖 3.3-3(b) Benzene 檢量線 39 圖 3.3-3(c) Toluene 檢量線 39 圖 3.3-3(d) Ethylbenzene 檢量線 40 圖 3.3-3(e) p-Xylene 檢量線 40 圖 4.2-1(a) Pseudomonas aeruginosa YAMT521 對 MTBE 之降解率與產率的變化趨勢 55 圖 4.2-1(b) Pseudomonas aeruginosa YAMT521 對苯之降解率與產率的變化趨勢 56 圖 4.2-1(c) Pseudomonas aeruginosa YAMT521 對甲苯之降解率與產率的變化趨勢 56 圖 4.2-1(d) Pseudomonas aeruginosa YAMT521 對乙苯之降解率與產率的變化趨勢 57 圖 4.2-1(e) Pseudomonas aeruginosa YAMT521 對二甲苯之降解率與產率的變化趨勢 57 圖 4.2-2(a) Ralstonia sp. YABE411 對苯之降解率與產率的變化趨勢 58 圖 4.2-2(b) Ralstonia sp. YABE411 對甲苯之降解率與產率的變化趨勢 58 圖 4.2-2(c) Ralstonia sp. YABE411 對乙苯之降解率與產率的變化趨勢 59 圖 4.2-3(a) Pseudomonas sp. YATO411 對甲苯之降解率與產率的變化趨勢 60 圖 4.2-3(b) Pseudomonas sp. YATO411 對乙苯之降解率與產率的變化趨勢 60 圖 4.2-4(a) Pseudomonas aeruginosa YAMT521 於 MTBE 與苯共存之降解率與產率的變化趨勢 62 圖 4.2-4(c) Pseudomonas aeruginosa YAMT521 於 MTBE 與乙苯共存之降解率與產率的變化趨勢 63 圖 4.2-4(d) Pseudomonas aeruginosa YAMT521 於 MTBE 與對-二甲苯共存之降解率與產率的變化趨勢 64 圖 4.2-5(b) Pseudomonas aeruginosa YAMT521 於「MTBE、苯、甲苯」共存之降解率與產率的變化趨勢 65 圖 4.2-5(c) Pseudomonas aeruginosa YAMT521 於「MTBE、苯、對-二甲苯」共存之降解率與產率的變化趨勢 65 圖 4.2-6 Pseudomonas aeruginosa YAMT521 於「MTBE 與 BTEX」共存之降解率與產率的變化趨勢 66 圖 4.2-7(a) 與鋅(1mg/l)共存之降解率與產率的變化趨勢 67 圖 4.2-7(b) 與鋅(5mg/l)共存之降解率與產率的變化趨勢 68 圖 4.2-7(c) 與鋅(10mg/l)共存之降解率與產率的變化趨勢 68 圖 4.2-7(d) 與鋅(20mg/l)共存之降解率與產率的變化趨勢 68 圖 4.2-7(e) 不同鋅濃度下平均降解率與產率 69 圖 4.2-8(a) 與錳(1mg/l)共存之降解率與產率的變化趨勢 70 圖 4.2-8(b) 與錳(5mg/l)共存之降解率與產率的變化趨勢 70 圖 4.2-8(c) 與錳(10mg/l)共存之降解率與產率的變化趨勢 70 圖 4.2-8(d) 與錳(20mg/l)共存之降解率與產率的變化趨勢 71 圖 4.2-8(e) 不同錳濃度下平均降解率與產率 71 圖 4.2-9(a) 與鎳(1mg/l)共存之降解率與產率的變化趨勢 72 圖 4.2-9(b) 與鎳(2mg/l)共存之降解率與產率的變化趨勢 72 圖 4.2-9(c) 與鎳(3mg/l)共存之降解率與產率的變化趨勢 73 圖 4.2-9(d) 與鎳(4mg/l)共存之降解率與產率的變化趨勢 73 圖 4.2-9(e) 不同鎳濃度下平均降解率與產率 73 圖 4.2-10(a) MTBE 轉換為細胞生長能量與生質量之比率關係 75 圖 4.2-10(b) 苯轉換為細胞生長能量與生質量之比率關係 75 圖 4.2-10(c) 甲苯轉換為細胞生長能量與生質量之比率關係 76 圖 4.2-10(d) 乙苯轉換為細胞生長能量與生質量之比率關係 76 圖 4.2-10(e) 二甲苯轉換為細胞生長能量與生質量之比率關係 76 圖 4.2-11(a) 苯轉換為細胞生長能量與生質量之比率關係 77 圖 4.2-11(b) 甲苯轉換為細胞生長能量與生質量之比率關係 78 圖 4.2-11(c) 乙苯轉換為細胞生長能量與生質量之比率關係 78 圖 4.2-12 甲苯轉換為細胞生長能量與生質量之比率關係 79 圖 4.2-13 乙苯轉換為細胞生長能量與生質量之比率關係 79 圖 4.2-14(a) M 與 B 共存之總碳轉換為細胞生長能量與生質量之比率關係 81 圖 4.2-14(b) M 與 T 共存之總碳轉換為細胞生長能量與生質量之比率關係 81 圖 4.2-14(c) M 與 E 共存之總碳轉換為細胞生長能量與生質量之比率關係 82 圖 4.2-15(a) MBT 共存之總碳轉換為細胞生長能量與生質量之比率關係 83 圖 4.2-15(b) MBE 共存之總碳轉換為細胞生長能量與生質量之比率關係 83 圖 4.2-16 MBTEX 共存之總碳轉換為細胞生長能量與生質量之比率關係 84 圖 4.2-17(a) 與鋅(1mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 85 圖 4.2-17(b) 與鋅(5mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 85 圖 4.2-17(c) 與鋅(10mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 85 圖 4.2-17(d) 與鋅(20mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 86 圖 4.2-18(a) 與錳(1mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 86 圖 4.2-18(b) 與錳(5mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 86 圖 4.2-18(c) 與錳(10mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 87 圖 4.2-18(d) 與錳(20mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 87 圖 4.2-19(a) 與鎳(1mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 88 圖 4.2-19(b) 與鎳(2mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 88 圖 4.2-19(c) 與鎳(3mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 89 圖 4.2-19(d) 與鎳(4mg/l)共存之碳轉換為細胞生長能量與生質量之比率關係 89 圖 4.3-1(a) 實驗與模式預估比較圖 94 圖 4.3-1(b) 實驗與模式預估比較圖 94 圖 4.3-1(c) 實驗與模式預估比較圖 94 圖 4.3-1(d) 實驗與模式預估比較圖 95 圖 4.3-1(e) 實驗與模式預估比較圖 95 圖 4.3-2 實驗與模式預估比較圖 97 圖 4.3-3 實驗與模式預估比較圖 99 圖 4.3-4 實驗與模式預估比較圖 100 圖 4.4-1 實驗與模式預估比較圖 102 圖 4.4-2 實驗與模式預估比較圖 103 圖 4.4-3 實驗與模式預估比較圖 105 圖 4.4-4 實驗與模式預估比較圖 106 圖 4.4-5 實驗與模式預估比較圖 108 圖 4.5-1 實驗與模式預估比較圖 111 圖 4.5-2 實驗與模式預估比較圖 112 圖 4.5-3 實驗與模式預估比較圖 114 圖 4.6 實驗與模式預估比較圖 116 圖 4.7-1(a) 不同鋅濃度下實驗與模式預估比較圖 117 圖 4.7-1(b) 不同鋅濃度下實驗與模式預估比較圖 118 圖 4.7-1(c) 不同鋅濃度下實驗與模式預估比較圖 118 圖 4.7-1(d) 不同鋅濃度下實驗與模式預估比較圖 119 圖 4.7-1(e) 不同鋅濃度下實驗與模式預估比較圖 119 圖 4.7-2(a) 不同錳濃度下實驗與模式預估比較圖 121 圖 4.7-2(b) 不同錳濃度下實驗與模式預估比較圖 121

圖4.7-2(c) 不同錳濃度下實驗與模式預估比較圖 122 圖4.7-2(d) 不同錳濃度下實驗與模式預估比較圖 122 圖4.7-3(a) 不同鎳濃度下實驗與模式預估比較圖 124 圖4.7-3(b) 不同鎳濃度下實驗與模式預估比較圖 124 圖4.8-1 副產物累積圖 126 圖4.8-2 副產物累積實驗與模式預估比較圖 127 表目錄 表2.1-1 MTBE與BTEX之物理化學性質 7 表2.2-1 比較各種混合菌與純菌株分解MTBE之情形(1/2) 12 表2.2-1 比較各種混合菌與純菌株分解MTBE之情形(2/2) 13 表3.1-1 無機營養鹽配比 29 表4.1 各菌種比對結果與特性 53 表4.3-1(a) *Pseudomonas aeruginosa* YAMT521於不同MTBE濃度之動力學參數值 92 表4.3-1(b) *Pseudomonas aeruginosa* YAMT521於不同苯濃度之動力學參數值 92 表4.3-1(c) *Pseudomonas aeruginosa* YAMT521於不同甲苯濃度之動力學參數值 93 表4.3-1(d) *Pseudomonas aeruginosa* YAMT521於不同乙苯濃度之動力學參數值 93 表4.3-1(e) *Pseudomonas aeruginosa* YAMT521於不同對-二甲苯濃度之動力學參數值 93 表4.3-1(f) 比較菌種於不同基質種類之動力學參數值 96 表4.3-2 *Ralstonia* sp. YABE411於不同苯濃度之動力學參數值 98 表4.3-3 *Pseudomonas* sp. YATO411於不同甲苯濃度之動力學參數值 99 表4.3-4 *Pseudomonas putida* YAEB411菌於不同乙苯濃度之動力學參數值 100 表4.4-1(a) 雙基質動力學參數值(M+B競爭型) 102 表4.4-1(b) 雙基質動力學參數值(M+B非競爭型) 102 表4.4-2(a) 雙基質動力學參數值(B+M競爭型) 103 表4.4-2(b) 雙基質動力學參數值(B+M非競爭型) 103 表4.4-3(a) 雙基質動力學參數值(M+T競爭型) 104 表4.4-3(b) 雙基質動力學參數值(M+T非競爭型) 104 表4.4-4(a) 雙基質動力學參數值(M+E競爭型) 106 表4.4-4(b) 雙基質動力學參數值(M+E非競爭型) 106 表4.4-5(a) 雙基質動力學參數值(M+X競爭型) 108 表4.4-5(b) 雙基質動力學參數值(M+X非競爭型) 108 表4.4-6 單一與雙基質共存動力學參數值 109 表4.5-1(a) 三基質動力學參數值(M+B+T競爭型) 110 表4.5-1(b) 三基質動力學參數值(M+B+T非競爭型) 110 表4.5-2(a) 三基質動力學參數值(M+B+E競爭型) 112 表4.5-2(b) 三基質動力學參數值(M+B+E非競爭型) 112 表4.5-3(a) 三基質動力學參數值(M+B+X競爭型) 113 表4.5-3(b) 三基質動力學參數值(M+B+X非競爭型) 113 表4.5-4 單一與三基質共存動力學參數值 114 表4.6(a) 五基質動力學參數值(M+BTEX競爭型) 115 表4.6(b) 五基質動力學參數值(M+BTEX非競爭型) 115 表4.7-1 與鋅共存下對MTBE降解之動力學參數值 117 表4.7-2 與錳共存下對MTBE降解之動力學參數值 120 表4.7-3 與鎳共存下對MTBE降解之動力學參數值 123 表4.8(a) 副產物累積對MTBE之動力學參數值(競爭型) 127 表4.8(b) 副產物累積對MTBE之動力學參數(非競爭型) 127

REFERENCES

- 中國石油公司，(2004)，95無鉛汽油物質安全資料表，<http://www.cpctmtd.com.tw/U002%2095%B5L%B9%5D%A8T%AAo-safety.htm>
- 方瑋寧，(2002)，MTBE好氧分解之可行性研究，國立中山大學環境工程研究所，碩士論文。行政院環境保護署，(2001)，飲用水與甲基第三丁基醚相關資料，<http://www.epa.gov.tw/waterpollution/drinkwater/mains/m10.htm> 行政院環境保護署，(2003)，車用汽柴油成分及性能管制標準，http://www.epa.gov.tw/gaiscqi/query_run.exe 林秋裕，(1990)，衛工微生物學，大學圖書供應社，台中。吳智輝，(2004)，*Pseudomonas aeruginosa*與*Ralstonia taiwanensis*對重金屬與酚之毒理學與生物復育研究，國立成功大學化學工程學系，碩士論文。吳美慧，(2003)，芳香族碳氫化合物在非水相液體與水中分佈行為之研究，國立高雄師範大學生物科學研究所，碩士論文。許盈志，(2002)，使用高效益固定生物反應器處理系統(FFR)處理地下水之可行性評估試驗研究，國立高雄第一科技大學環境與安全衛生工程系，碩士論文。趙雅鈴，(2003)，加油站作業人員暴露於危害性有機物質之健康風險評估研究，中國文化大學勞工研究所，碩士論文。盧重興、林明瑞、李弘志、謝潮明，(1995)，厭氧旋轉生物盤法溫度效應、圓盤轉速及圓盤浸水率之研究，第20屆廢水處理技術研討會論文集，3-27。Ahemd, F.E., (2001) " Toxicology and human health effects following exposure to oxygenated or reformulated gasoline," *Toxicology Letters*, 123: 89-113. Alberto, C., F. Sara, M. Fernando, and G. Julian, (1998) " Effects of copper and zinc on the activated sludge bacteria growth kinetics," *Water Research*, 32: 1355-1362. Amor, L., C. Kennes, and M.C. Veiga, (2001) " Kinetics of inhibition in the biodegradation of monoaromatic hydrocarbons in presence of heavy metals," *Bioresource Technology*, 78: 181-185. Andrews, J.F., (1968) " A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates," *Biotechnology and Bioengineering*, 10: 707-723.
- Annieappleseedproject, " *Pseudomonas putida* cleans organic pollutants in soil," <http://www.annieappleseedproject.org/pseudputclea.html>
- Baehr, A.L., P.E. Stackelberg, and R.J. Baker, (1999) " Evaluation of the atmosphere as a source of volatile organic compounds in shallow groundwater," *Water Resources Research*, 35: 127-136. 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*, 66: 2471-2478. Bielefeldt, A.R. and H.D. Stensel, (1999) " Modeling competitive inhibition effects during biodegradation of BTEX mixtures," *Water Research*, 33: 707-714. Caprino, L. and G.I. Togna, (1998) " Potential health effects of gasoline and its constituents: a review of current literature (1990-1997) on toxicological data," *Environmental Health Perspectives*, 106: 115-125.
- Cavalleri, A., F. Gobba, E. Nicoll, and V. Ficchi, (2000) " Dose-related color vision important in toluene exposed workers," *Archives of Environmental Health*, 55: 399-404. Chang, M.K., T.C. Voice, and C.S. Criddle, (1992) " Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and p-xylene by two *Pseudomonas* isolates," *Biotechnology and Bioengineering*, 41: 1057-1065. Coates, J.D., R. Chakraborty, and M.J. McInerney, (2002) " Anaerobic benzene biodegradation-a new era," *Research in Microbiology*, 153: 621-628.
- Codina, J.C., M.A. Munoz, F.M. Cazorla, P.G. Alejandro, M.A. Morinigo, and A.D. Vicente, (1998) " The inhibition of methanogenic activity from anaerobic domestic sludges as a simple toxicity bioassay," *Water Research*, 32: 1338-1342. Deeb, R.A., K.M. Scow, and L. Alvarez-Cohen, (2000) " Aerobic MTBE biodegradation: an examination of past studies, current challenges and future research directions," *Biodegradation*, 11: 171-186. Dilek, F.B., C.F. Gokcay, and U. Yetis, (1998) " Combined effects of Ni() and Cr() on activated sludge," *Water Research*, 2:

303-312. Drogous, D.L., (2000) " MTBE v. other oxygenates, " presented at Mealey ' s MTBE Conference. Eastern Research Group, (2000) " Summary of workshop on biodegradation of MTBE, " workshop sponsored by the U.S. Environmental Protection Agency and American Petroleum Institute. Eweis, J.B., D.P.Y. Chang, E.D. Schroeder, K.M. Scow, R.L. Morton, and R.C. Caballero, (1997) " 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. Fennouhy S., V. Casimiri, A. Geloso-Meyer, and C. Burstein, (1998) " Kinetic study of heavy metal salt effects on the activity of L-lactate dehydrogenase in solution or immobilized on an oxygen electrode, " Biosensors & Bioelectronics, 13: 903-909. Francois, A., H. Mathis, D. Godefory, P. Piveteau, F. Fayolle, and F. Monot, (2002) " Biodegradation of methyl tert-butyl ether and other fuel oxygenates by a new strain, *Mycobacterium austroafricanum* IFP 2012, " Applied and Environmental Microbiology, 68: 2754-2762. Futamata, H., S. Harayama, and K. Watanabe, (2001) " Diversity in kinetics of trichloroethylene-degrading activities exhibited by phenol-degrading bacteria, " Applied Microbiology and Biotechnoloby, 55: 248-253. Gennaro, P.D., E. Rescalli, E. Galli, G. Sello, and G. Bestetti, (2001) " Characterization of *Rhodococcus opacus* R7, a strain able to degrade naphthalene and o-xylene isolated from a polycyclic aromatic hydrocarbon-contaminated soil, " Research in Microbiology, 152: 641-651. Ghittori, S., M. Imbriani, L. Maestri, E. Capodaglio, and A. Cavalleri, (1999) " Determination of S-phenylmercapturic acid in urine as anindicator of exposure to benzene, " Toxicology Letters, 108: 329-334. Gonzalez-Gil, G., R. Kleerebezem, and G. Lettinga, (1999) " Effects of nickel and cobalt on kinetics of methanol conversion by methanol conversion by methanogenic sludge as assessed by on-line CH₄ monitoring, " Applied and Environmental Microbiology, 65: 1789-1793. Greenberg, M.M., (1997) " The central nervous system and exposure to toluene: a risk characterization, " Environmental Research, 72: 1-7. Han, K. and O. Levenspiel, (1988) " Extended Monod kinetics for substrate, product, and cell inhibition, " Biotechnology and Bioengineering, 32: 430-437. Hanson, J.R., C.E. Ackerman, and K.M. Scow, (1999) " Biodegradation of methyl tert-butyl ether by a bacterial pure culture, " Applied and Environmental Microbiology, 65: 4788-4792. 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, 63: 3059-3067. Hartley, W.C., and A.J. Englande, (1992) " Health risk assessment of the migration of unleaded gasoline-A model for petroleum products, " Water Science and Technology, 25: 65-72. Hassen, A., N. Saidi, M. Cherif, and A. Boudabous, (1998a) " Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*, " Bioresource Technology, 65: 73-82. Hassen, A., N. Saidi, M. Cherif, and A. Boudabous, (1998b) " Resistance of environmental bacteria to heavy metals, " Bioresource Technology, 64: 7-15. 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, 67: 5601-5607. Hernandez-Perez, G., F. Fayolle, and J.-P. Vandecasteele, (2001) " Biodegradation of ethyl t-butyl ether(ETBE), methyl t-butyl ether(MTBE) and t-amyl methyl ether(TAME) by *Gordonia terrae*, " Applied Microbiology and Biotechnology, 55: 117-121. Hrelia, P., F. Maffei, S. Angelini, G.C. Forti, (2004) " A molecular epidemiological approach to health risk assessment of urban air pollution, " Toxicology Letters, 149: 261-267. 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, 35: 1665-1674. Kutty, R., H.J. Purohit, and P. Khanna, (2000) " Isolation and characterization of a *Pseudomonas* sp. Strain PH1 utilizing metaaminophenol, " Canadian Journaol of Microbiology, 46: 211-217. Lee, J.Y., K.H. Jung, S.H. Choi, and H.S. Kim, (1995) " Combination of the tod and the tol pathways in redesigning a metabolic route of *Pseudomonas putida* for the mineralization of a benzene, toluene, and p-xylene mixture, " Applied and Environmental Microbiology, 61: 2211-2217. Leutwein, C. and J. Heider, (1999) " Anaerobic toluene-catabolic pathway in denitrifying *Thauera aromatica*: activation and b-oxidation of the first intermediate, (R)-(M)-benzylsuccinate, " Microbiology, 145: 3265-3271. Liu, C.Y., G.E. Speitel, Jr., and G. Georgiou, (2001) " Kinetics of methyl t-butyl ether cometabolism at low concentrations by pure cultures of butane-degrading bacteria, " Applied and Environmental Microbiology, 67: 2197-2201. Luong, J.H.T., (1987) " Generalization of Monod kinetics for analysis of growth data with substrate inhibition, " Biotechnology and Bioengineering, XXIX: 242-248. Maliyekkal, S.M., E.R. Rene, L. Philip, T. Swaminathan, (2004) " Performance of BTX degraders under substrate versatility conditions, " Journal of Hazardous Materials, B109: 201-211. Mays, M.A., (1989) " The use of the oxygenated hydrocarbons in gasoline and their contribution to reducing urban air pollution, " Pure and Applied Chemistry, 61: 1373-1578. Miguel, A.L.Z., F. Naoyuki, and T. Tetsuo, (2004) " Modeling of aerobic biodegradation of feces using sawdust as a matrix, " Water Research, 38: 1327-1339. Monod, J., (1949) " The growth of bacterial cultures, " Annual Review of Microbiology, 3: 371-374. Mondal M.S., A.K. Sau, and S. Mitra, (2000) " Mechanism of the inhibition of milk xanthine oxidase activity by metal ions: a transient kinetic study, " Biochimica et Biophysica Acta, 1480: 320-310. Mosche, M. and H.-J. Jordening, (1999) " Comparison of different models of substrate and product inhibition in anaerobic digestion, " Water Research, 33: 2545-2554. Nihle ' n, A., R. Walinder, A. Lof, and G. Johanson, (1998a) " Experimental exposure to methyl tertiary-butyl ether: I. Toxicokinetics in humans, " Toxicology and Applied Pharmacology, 148: 274-280. Nihle ' n, A., R. Walinder, A. Lof, and G. Johanson, (1998b) " Experimental exposure to methyl tertiary-butyl ether: II. Acute effects in humans, " Toxicology and Applied Pharmacology, 148: 281-287. Ogata, M., H. Michitsuji, and Y. Fujiki, (1999) " Estimating amounts of toluene inhaled by workers with protective mask using biological indicators of toluene, " Toxicology and Applied Pharmacology, 108: 223-239. Oh, Y.S., (1994) " Interactions between benzene, toluene, and p-xylene BTX) during their biodegradation, " Biotechnology and Bioengineering, 44: 533. Prenafeta-Boldu, F.X., J. Vervoort, J.T.C. Grotenhuis, and J.W. van Groenestijn, (2002) " Substrate Interactions during the Biodegradation of Benzene, Toluene, Ethylbenzene, and Xylene (BTEX) Hydrocarbons by the Fungus *Cladophialophora* sp. Strain T1, " Applied and Environmental Microbiology, 68: 2660-2665. Ren, S. and P. D. Frymier, (2003) " Kinetics of the toxicity of metals to luminescent bacteria, " Advances in Environmental Research, 7: 537-547. Rothman, N.,

G.-L., M. Dosemeci, W.E. Bechtold, G.E. Marti, Y.-Z. Wang, M. Linet, L.-G. Xi, W. Lu, M.T. Smith, N. Titenko-Holland, L.-P. Zhang, W. Blot, S.-N. Yin, R.B. Hayes, (1996) " Hematotoxicity among Chinese workers heavily exposed to benzene, " American Journal of Industrial Medicine, 29: 236-246. Sponza, D.T. and M. I?ik, (2004) " Decolorization and inhibition kinetic of direct black 38 azo day with granulated anaerobic sludge, " Enzyme and Microbial Technology, 34: 147-158. Smith, C.A., K.T. O ' Reilly, and M.R. Hyman, (2003) " Characterization of the initial reactions during the cometabolic oxidation of methyl tert-butyl ether by propane-grown *Mycobacterium vaccae* JOB5, " Applied and Environmental Microbiology, 69: 796-804. Smith, C.A. and M.R. Hyman, (2004) " Oxidation of methyl tert-butyl ether by alkane hydroxylase in dicyclopropylketone-induced and n-octane-grown *Pseudomonas putida* GPo1, " Applied and Environmental Microbiology, 70: 4544-4550. 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, 63: 4216-4222. Szoboszlay, S., B. Atzel, B. Kriszt, (2003) " Comparative biodegradation examination of *Pseudomonas aeruginosa* (ATCC 27853) and other oil degraders on hydrocarbon contaminated soil, " Commun Agric Appl Biol Sci., 68(2 Pt A): 207-10. The Health Effects Institute, (2004) " Metabolism of ether oxygenates added to gasoline, " Synopsis of Research Report 102. Turkel, B., and U. Egeli, (1994) " Analysis of chromosomal aberrations in shoe workers exposed long term to benzene, " Occupational and Environmental Medicine, 51: 50-53. U.S. Environmental Protection Agency, (1995) " Biostimulation of BTX degradation with environmentally benign aromatic, " EPA-Grant Number: R823420. U.S. Environmental Protection Agency, (1996) " Drinking water regulations and health advisories ", Washington, D.C., Office of Water. U.S. Environmental Protection Agency, (1997) " Consumer acceptability advice and health effects analysis on methyl tertiary-butyl ether(MTBE), " Drinking Water Advisory, EPA-822-F-97-009, Office of Water: Washington, DC. Valls, M., V.d. Lorenzo, (2002) " Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution, " FEMS Microbiology Reviews, 26: 327-338. Wang, S.J. and K.C. Loh, (1999) " Modeling the role of metabolic intermediates in kinetics of phenol biodegradation, " Enzyme and Microbial Technology, 25: 177-184. Yang, R.D. and A.E. Humphrey, (1975) " Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures, " Biotechnology and Bioengineering, 17: 1211. Yeh, C.K. and J.K. Novak, (1995) " The effect of hydrogen peroxide on the degradation of methyl and ethyl tert-butyl ether in soils, " Water Environmental Research, 67: 828-834. Zylstra, G.J., (1994) " Molecular analysis of aromatic hydrocarbon degradation, " In Molecular Environmental Biology, ed. S. J. Garte. Lewis Publishers, Boca Raton.