Microbial Community Dynamics in a Permeable Reactive Barrier using Real-time PCR Technique

葉如芳、林啟文

E-mail: 9708435@mail.dyu.edu.tw

ABSTRACT

This study was conducted with the application of denaturing gradient gel electrophoresis (DGGE), and real-time quantitative polymerase chain reaction (real-time PCR) molecular biotechnology for monitoring the permeable reactive barrier (PRB) in the relation with BTEX decomposition efficiency and the distribution of microbial community.

Various amounts of nitrogen nutrients and BTEX were added to examine the treatment efficiencies. It was shown that the high sodium nitrate amount had improved the BTEX removal, which was an evidence of effects on BTEX treatment. The results of benzene and toluene removal efficiencies revealed that it was completely degraded for both two compounds at the concentrations of 20, 40 and 80 ppm. Increasing the concentrations of these two compounds to 120, 160, 240 and 320 ppm resulted in the decreasing in treatment efficiencies, and the concentrations remained 40, 60, 65, 90 and 100 % for benzene, and 10, 40, 55, 90 % for Toluene, respectively. Furthermore, the microbial variations at various concentrations were consistent via optical density (OD), DGGE analysis and real-time PCR results. Each column test was conducted for 20 days to investigate the effectiveness of oxygen releasing compound (ORC). It was indicated that the highest dissolved oxygen was achieved, which was 5.08 mg/L (equal to 0.25 mg O2/day/g-ORC) at 40 % of CaO2.

The results of long-term stability tests of oxygen releasing from PRB system showed that: (1) Oxygen released from ORC was sufficient for the demand of bacteria. (2) In shock-loading of BTEX tests, the removal efficiencies were reduced by 21%, 19%, 17% and 10 % for benzene, toluene, ethylbenzene and xylene, respectively. (3) Removal efficiencies were then recovered in the ascending order of as follow: xylene> ethylbenzene> benzene> toluene. (4) ORC can be used for 40 days. (5) DGGE analysis showed the changing in the microbial community structure before (13 groups) and after shock-loading (reduced to 9 groups), that implied the shock-loading was harmful to bacteria. (6) The results from real-time PCR in the study of catechol 2,3-dioxygenase gene revealed that the quantification of this gene has been declined after shock-loading, but it was latter well again at the 79th day.

Keywords : denaturing gradient gel electrophoresis、real-time PCR、permeable reactive barrier、bioremediation

Table of Contents

REFERENCES

行政院環境保護署，地下水污染管制標準。URL: http://ww2.epa.gov.tw/SoilGW/0301.asp江姿幸，(2005)，滲透性反應牆對於砷污染土壤進?電動?法?育影響之研究，國立中山大學環境工程研究所，碩士論文。林財富、洪旭文，(1999)，受污染場址現地化學處理方法介紹，工業污染防治，72:178-200。卓坤慶，(2002)，應用慢釋氧物質處理受石油碳氫化合物污染之地下水-現地模場試驗，國立台灣大學環境工程學研究所，碩士論文。翁明章、戴良恭、林裕峰，(1994)，甲苯舖路造成之職業傷害，中華職業醫學雜誌，第1卷，第3期，119-113。陳谷汎，(2001)，以生物復育法整治2,4-二氯酚污染之地下水，國立中山大學環境工程研究所，碩士論文。陳郁旋，(2004)，

利用Real-time PCR偵測油污中芳香烴開環酵素catechol 2,3-dioxygenase之研究，國立中興大學生命科學系，碩士論文。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，國立彰化師範大學生物學系，碩士論文。張育傑、張怡塘、黃心怡、郭旭瓊、鐘敦仁、葉蕙萱，(2001)，釋氧物質之釋氧反應特性研究，NSC-90-2211-E-236-005。莫展民，Miao Zhang and Scott D. Warner, (2005)，滲透性反應牆在地下水處?上的應用，台灣土壤及地下水環境保護協會簡訊，第15期，7-10。經濟部工業局，(2004)，土壤與地下水整治技術相關研究彙整-BTEX，環保技術e報，第21期。謝昶毅，(2003)，以PCR-DGGE技術分析石油碳氫化合物污染地下水之微生物相，國立中山大學生物科學研究所，碩士論文。羅致良，(2003)，高濃度石油碳氫化合物污染地下水層添加釋氧物質之生物復育，國立台灣大學環境工程學研究所，碩士論文。Andersen, B., J. Smedsgaard, I. Jorring, P. Skouboeb, and L.H. Pedersen, (2006)“ Real-time PCR quantification of the AM-toxin gene and HPLC qualification of toxigenic metabolites from Alternaria species from apples ” International Journal of Food Microbiology, 111: 105-111.Amann, R.I., W. Ludwig, and K.H. Schliefer, (1995)“ Phylogenetic identification and in situ dectection of individual microbial cells without cultivation ” Microbiology and Molecular Biology Reviews, 59: 143-169.Barbara, T., K. Wonerow, K. Paschke, (1999)“ DGGE is more sensitive for the detection of somatic point mutations than direct sequencing ” BioTechnioues, 27: 266-268.Borden, R.C., R.T. Goin, C.M. Kao, (1997)“ Control of BTEX migration using a biologically enhanced permeable barrier ” Ground Water Monitoring and Remediation, 17: 70-80.Chae, J.C., E. Kim, E. Bini, G.J. Zylstra, (2007)“ Comparative analysis of the catechol 2,3-dioxygenase gene locus in thermoacid- ophilic archaeon Sulfolobus solfataricus strain 98/2 ” Biochemical and Biophysical Research Communications, 357: 815-819.Chapman, S.W., B.T. Byerley, D.J.A. Smyth, and D.M. Mackay, (1997)“ A pilot test of passive oxygen release for enhancement of in situ bioremediation of BTEX-contaminated ground water ” Ground Water Monitoring Remediation, 17: 93-105.Chapman, S.W., B.T. Byerly, D.J. Smyth, R.D. Wilson, D.M. Mackay, (1997)“ Semi-passive oxygen release barrier for enhancement of intrinsic bioremediation ” In Situ and On-Site Bioremediation, Battelle Press, Columbus, OH. 4: 209-214.Drogos, D.L., (2000)“ MTBE v. other oxygenates ” Presented at Mealy ’ s MTBE Litigation Conference, 11-12.Filion, M., M. St-Arnaud, and S.H. Jabaji-Hare, (2003)“ Direct quantification of fungal DNA from soil substrate using real-time PCR ” Journal of Microbiological Methods, 53: 67-76.Gachon, C., A. Mingam, and B. Charrier, (2004)“ Real-time PCR: what relevance to plant studies? ” Journal of Experimental Botany, 55: 1445-1454.Gavaskar, A.R., (1999)“ Design and construction techniques for permeable reactive barriers ” Journal of Hazardous Materials, 68: 41-71.Gillham, R.W., (1989)“ Selection of casing materials for groundwater monitoring wells—Sorption processes ” Landfills into the Next Decade, National Solid Wastes Management Assoc, October 23-24, pp. 108-122.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, 35: 1-21.Hendrickx, B., H. Junca, J. Vosahlova, A. Lindner, I. Ruegg, M. Bucheli-Witschel, F. Faber, T. Egli, M. Mau, M. Schlomann, M. Brennerova, V. Brenner, D. H. Pieper, E. M. Top, W. Dejonghe, L. Bastiaens, D. Springael, (2006)“ Alternative primer sets for PCR detection of genotypes involved in bacterial aerobic BTEX degradation: Distribution of the genes in BTEX degrading isolates and in subsurface soils of a BTEX contaminated industrial site ” Journal of Microbiological Methods, 64: 250-265.Hristova, K., B. Gebreyesus, D. Mackay, and K. M. Scow, (2003)“ Naturally occurring bacteria similar to the methyl tert-butyl ether (MTBE)-degrading strain PM1 are present in MTBE- contaminated groundwater ” Applied and Environmental Microbiology, 69: 2616-2623.Kao, C.M., S.C. Chen, J.Y. Wang, Y.L. Chen, and S.Z. Lee, (2003)“ Remediation of PCE-contaminated aquifer by an in situ two-layer biobarrier: laboratory batch and column studies ” Water Research, 37: 27-38.Kao, CM., (1993)“ Bioremediation of BTEX contaminated aquifers using biologically active barriers ” Ph.D. dissertation, North Carolina State University, Raleigh, NC.Kim, D., J.C. Chae, J.Y. Jang, G..J. Zylstra, Y.M. Kim, B.S. Kang, E. Kim, (2005)“ Functional characterization and molecular modeling of methylcatechol 2,3-dioxygenase from o-xylene-degrading Rhodococcus sp. strain DK17 ” Biochemical and Biophysical Research Communications, 326: 880-886.Kovalick, W.W., (1999)“ Field applications of in situ remediation technologies: permeable reactive barriers ” United States Environmental Protection Agency, EPA 542-R-99-002.Labrenz, M., I. Brettar, R. Christen, S. Flavier, J. B?tel, and M.G. H?fle, (2004)“ Development and application of a real-time PCR approach for quantification of uncultured bacteria in the central baltic sea ” Applied and Environmental Microbiology, 70: 4971-4979.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, 59: 695-700.Odziemkowski, M.S., T.T. Schuhmacher, R.W. Gillham, and E.J. Reardon, (1998)“ Mechanism of oxide film formation on iron in simulating groundwater solutions: raman spectroscopic studies ” Corrosion Science, 40: 371-389.Pael, J., and S. Shelton, (1995)“ Permeable barriers to remove benzene: candidate media evaluation ” Journal of Environmental Engineering ASCE, 121: 411-415.Renella, G., M. Mench, A. Gelsomino, L. Landi, and P. Nannipieri, (2005)“ Functional activity and microbial community structure in soils amended with bimetallic sludges ” Soil Biology & Biochemistry, 37: 1498-1506.Ritter, K., M.S. Odziemkowski, R.W. Gillham, (2002)“ An in situ study of the role of surface films on granular iron in the permeable iron wall technology ” Journal of Contaminant Hydrology, 55: 87-111.USEPA, (2002)“ Field applications of in situ remediation technologies: permeable reactive barriers ” Office of Solid Waste and Emergency Response Technology Innovation Office Washington, DC, USA.USEPA, (1998)“ Carcinogenic effects of benzene-an EPA/600/P-97/ 001F ” National Center for Environmental Health of research and Development, Washington, DC, USA.Vezzulli, L., C. Pruzzo, and M. Fabiano, (2004)“ Response of the bacterial community to in situ bioremediation of organic-rich sediments ” Marine Pollution Bulletin, 49: 740-751.Vidic, R.D., and F.G. Pohland, (1996)“ Treatment walls ” Technology Evaluation Report, Ground-Water Remediation Technologies Analysis Center, Pittsburgh, PA, USA.Ward, D.M., R. Weller, and M.M. Bateson, (1990)“ 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community ” Nature, 345: 63-65.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 oligo- nucleotide probes and confocal scanning laser microscopy ” , Journal of Microscopy, 176: 181-187.Watkin, G.E., E.J. Calabrese, and R.H. Harris, (1991) “ Health risks associated with the remediation of contaminated soil ” Hydrocarbon contaminated soil and groundwater, Lewis publishers, Inc., Michigan, 293-300.