

## THE DEVELOPMENT AND APPLICATIONS OF MOLECULAR TECHNIQUES FOR BTEX TREATMENT

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One benzene and toluene contaminated site was discovered in petroleum plant located in Southern Taiwan. One pilot study was initiated to evaluate the effect of molecular techniques including the DNA analysis method and reporter gene system on bioremediation of BTEX contamination. The bioremediation efficiency of organic pollutant relies on the enzyme activity of microorganisms. Detection of the enzyme DNA in contaminated site is important for bioremediation. The primers for DNA amplifications of toluene dioxygenase, 1, 2-catechol dioxygenase, and 2, 3-catechol dioxygenase were designed for microbial screening. One bacterial strain *Pseudomonas putida* dcblux containing the plasmid, which carries the luxCDABE gene sequence triggered by toluene dioxygenase promoter, was also constructed to detect the concentration and degradation efficiency of BTEX. Once the strain dcblux contacts the pollutant, the tod promoter will be activated and turn on the gene of luxCDABE to emit luminescence light. While the concentration is increased or the detection condition is optimized, strain dcblux will produce more luminescence activity. The more luminescence activity also indicates higher degradation capability.

The whole monitoring system of bioremediation established in this research consisted three parts: (1) the bacterial strain screening by DNA analysis, (2) bacterial population quantification and gene copy number analysis by real time PCR, and (3) the enzyme activity assay and the pollutant concentration monitoring through the reporter gene system. Using this system, two bacterial strains have been found to metabolize the benzene and toluene at the rate of 8 ppm/mg dry cell weight. Although the concentration of benzene and toluene was near 100 ppm, which had a little inhibition effect on the degradation, the degradation efficiency was still reach 40%. The temperature and pH of the underground water in contaminated site were near optimal conditions for biological treatment. One bioremediation strategy, which was to provide the bacterial strains containing the tod dioxygenase gene at the concentration of 10<sup>6</sup> C FU/g soil and supply enough oxygen, was applied in this site. After six-month treatment, the pollutant was cleaned up, but the degradation efficiency was only increased from 40 to 60%. It implies that the geochemistry will affect the enzyme activity. Based on the reporter gene system, we can measure the degradation efficiency and the pollutant concentration. Combined the degradation efficiency data with the bacterial population, it is possible to calculate how long it will take for bioremediation. These useful techniques will give the clear information for bioremediation.

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