Influence of Trace Element to Benzene, Toluene, Ethylbenzene, o-, m-, p-Xylene Bioremediation using Non-Pathogenic Photosynthetic Bacterial Mixture

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Introduction.

Previously studied BTEX bioremediation candidates are known to have pathogenic characteristics; harmful to the environment and included organisms. A mixture of non-pathogenic photosynthetic bacteria were picked and cultured for the removal of BTEX. By using non-pathogenic bacteria, removal of volatile toxic chemicals from soil or water can occur without producing any negative effects on its surroundings. Throughout this experiment, the use of trace element solution has been identified to further accelerate the biodegradation process. Trace element solution consists of an assortment of different metals. Although the reason is still unclear, continuous molecular level studies can help provide the answer to what enzymatic reaction is the cause of this result. The biological relationship of the mono- and dioxygenase enzyme produced from the non-pathogenic consortia has been identified as the key metabolic pathway for BTEX bioremediation.

The objective of this work is to confirm through molecular studies which specific enzyme is responsible for the biodegradation process, identify their location within a gene, and also their expression time. This study can help pinpoint the exact genes that can efficiently biodegrade volatile toxins.

Methods.

A mixture of 14 different photosynthetic bacteria were grown in 27s media for 3 days and further processed through adaptation with BTEX in several time intervals (10, 20, 30, 40 hrs). 1g/L cell mass was later used in 50ml 27s medium to analyze biodegradation efficiency of total 100ppm of BTEX using GC-FID within 24 hours. 1x amount of trace element was injected after 12 hours in order to improve the enzymatic activity.

Using high efficient bacterial mixture, preparation for real time PCR begins by cDNA synthesis. RNA is extracted and copied into cDNA by reverse transcriptase. 20ul of oligo (dT) as primer and 1ug of RNA; after 1hr incubation diluted to 150ul. 5ul of diluted cDNA used for real-time PCR with necessary primers purchased according to target enzyme. Further work to indicate internal or external expression of specific enzymes and expression time and factors can contribute to the cloning and transformation of the target enzyme.

Results.

Efficient removal of BTEX was observed after 1x trace element was injected upon 12 hours of the experiment. Total 100ppm of BTEX is shown to be biodegraded within the following 12 hours

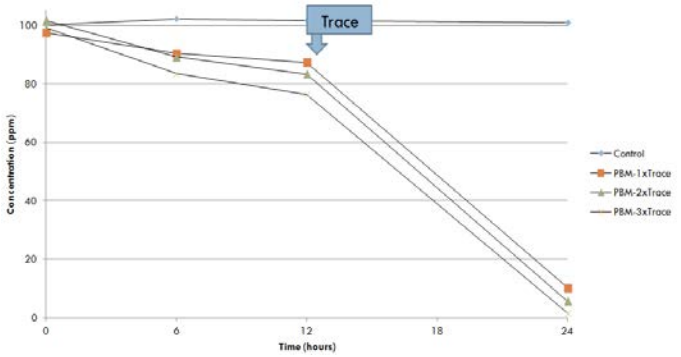

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Fig.1 Biodegradation of BTEX using trace element influenced photosynthetic bacterial mixture under aerobic condition within 24 hours.

Conclusions.

The effect of trace element solution caused some enzymatic reaction to provide the photosynthetic bacterial mixture to produce an enzyme able to efficiently biodegrade 95-99ppm of BTEX within 12 hours. Using this efficient bacterial mixture, molecular level work was performed to identify the specific enzyme and also its location within the gene. An increase expression depending on certain time intervals and factors also indicate the significance of the enzyme and its role in the bio-removal of toxic substances.

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References.

1. Choi EJ, Jin HM, Lee SH, Madsen EL, Jeon CO. (2013). *Appl. Environ. Microbiol.* Vol 79: 663-671

2. Wolicka D, Suszek A, Borkowski A, Bielecka A. (2009). *Biortech*. Vol 100:3221-3227.

3. Hendrickx B, Dejonghe W, Faber F, Boenne W, Bastiaens L, Vertraete W, Top EM, Springael D. (2006). *FEMS Microbiol. Ecol.* Vol 55:262-273