BIODEGRADATION OF HYDROCARBON BENZENE, TOLUENE, ETHYLBENZENE AND XYLENE (BTEX) BY CONSORTIUM BACTERIAL CULTURE

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ABSTRACT

The cooperation amongst microbial bacteria community in biodegrading hydrocarbons has been extensively studied particularly in BTEX (benzene, toluene, ethylbenzene and xylene) which is a major component of petroleum products. In this study, the toxicity and the degradation of BTEX by an environmental bacterial consortium culture was determined. Complete growth inhibitions observed for ethylbenzene was at 196.97 mg/L, xylene at 206.11 mg/L, followed by toluene and benzene at 273.11 mg/L and 374.87 mg/L, respectively. Significant decreases in specific growth rate μ (hr^{-1}) of the CC was observed when individual BTEX concentrations were increased from 10 to 500 mg/L ($P<0.05$). The GC-FID chromatographic profile showed that BTEX compounds were reduced ranging from 18-35% after 24 hours and 41-61% after 48 hours. These findings suggest BTEX mineralization by consortium culture has taken place, thus providing an insight that consortium culture utilized BTEX as their sole source of carbon and energy for growth.

Keywords: *Consortium culture, BTEX, biodegradation*

INTRODUCTION

Hydrocarbon benzene, toluene, ethylbenzene and xylene, collectively known as BTEX, are toxic compounds commonly found in petroleum products such as gasoline. BTEX was classified as priority pollutants by the Environmental Protection Agency (USEPA, 1977) as these compounds are carcinogenic and neurotoxic (Dean, 1985). The ubiquitous presence of BTEX in fuel and petroleum products has

contributed to the pollution of the environment wherein BTEX was released into the soil, dissolved and causes groundwater contamination (Rego & Netto, 2007). The efficacy of bioremediation using natural process through the application of microorganisms has shown to be an effective technique to remediate polluted soil and groundwater. Biodegradation of BTEX by pure bacterial culture such as *Pseudomonas species* has been studied, however, biodegradation of BTEX can be enhanced with the use of bacterial consortium (Littlejohns & Daugulis, 2008). The objective of this study is to investigate the capabilities of the consortium culture (CC) developed by Sannasi (2006) to biodegrade hydrocarbon BTEX and using it as their sole source of carbon and energy.

MATERIALS AND METHODS

i. BTEX toxicity to consortium culture

Consortium culture (CC) was obtained from Sannasi (2006) and 5% v/v of CC was exposed to individual BTEX at a final concentration of 10, 25, 50, 80 and 100 mg/L in 250mL of Schott bottle containing 95mL of Bushnell Haas (BH) media. The culture was then incubated for 24 hours at 150rpm in room temperature. The growth of the consortium culture bacterial cells at initial 0h and after 24h was determined spectrophotometrically at 600nm. Percentage of growth inhibition was calculated as:-

$$I\% = (C-N)/C \times 100$$

C = *no of control cells after 24 hours*
N = *no of cells supplemented with individual BTEX after 24 hours*

The specific growth rate, μ (hr^{-1}) of CC exposed to individual BTEX at 10, 50, 100, 250 and 500 mg/L for 48 hours were determined. Bacterial cells at 600nm were taken every 4 hours until the end of the experiment to show growth response of CC towards individual BTEX. The μ value was determined as:-

$$\mu = [(\text{Log}_{10} N - \text{Log}_{10} N_o)2.303]/t\text{-}t_o$$

N=*no of cells at final log phase,*
N_o=*no of cells at the initial log phase,*
t=*time N*
t_o=*time* N_o

ii. BTEX degradation by CC

BTEX degradation was analyzed using GC-FID (HP5890) equipped with a capillary column HP-5 (30mx0.32mmx.25μm). Helium was used as the carrier gas with a flow rate of 2.3ml/min. The

temperature of injector and detector was set at 150°C and 320°C, respectively. The column temperature was initially programmed at 50°C, held for one minute then increased at 20°C/min to 90°C (held for 1 minute) and 120°C (held 10 minutes). Sample preparation was performed by inoculating 5% v/v of CC into 40ml vial containing 28.5 mL of BH media and a final concentration of 50mg/L of individual BTEX. The incubation was performed at 150rpm in room temperature. At 24th and 48th hour, 2mL of sample from each vials were collected and kept in a cooled 4mL vial for headspace extraction using Solid Phase Microextraction (SPME) method. SPME fiber coated with 100µm polydimethylsiloxane (PDMS) was used to trap BTEX residual from the sample. Samples were stirred vigorously at room temperature and the fiber was exposed to the sample to allow absorption of BTEX residues on the headspace for 4 minutes. Subsequently, hydrocarbon trapped in the fiber was injected into GC and left there for 3 minutes for desorption. BTEX degradation by CC was calculated based on peak reduction on chromatogram profile.

RESULTS AND DISCUSSION

Figure 1 shows a growth inhibition of CC after exposing it to a various concentration of individual BTEX for 24 hours while Table 1 shows the specific growth rate µ (hr^{-1}) of CC exposed to individual BTEX for 48 hours.

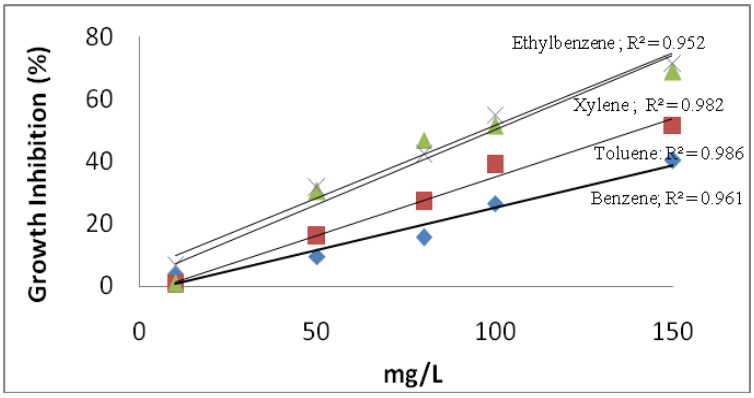

Fig. 1 Growth Inhibition of CC supplemented by BTEX

Table 1 : Specific Growth rate, µ (hr^{-1}) of CC exposed to BTEX for 48 hours

mg/L	Benzene	Toluene	Ethylbenzene	Xylene
10	0.14	0.12	0.11	0.12
50	0.11	0.11	0.11	0.08
100	0.10	0.10	0.07	0.06
250	0.10	0.08	ND	0.05
500	0.06	0.05	ND	ND

Control = 0.24 hr^{-1} ND=Not determined

BTEX showed its toxicity to CC with a complete growth inhibition at 374.87 mg/L of benzene, 273.11 mg/L of toluene, 206.97 mg/L of xylene and 196.97 mg/L of ethylbenzene whilst the specific growth rate, μ of CC decreased significantly when individual BTEX concentrations were increased from 10 to 500 mg/L ($P<0.05$). The specific growth rate of CC exposed to ethlybenzene at ≥250 mg/L and xylene at ≥500 mg/L were unable to be determined due to high toxicity of the hydrocarbons, which caused a complete inhibition to CC. Both experiment showed the same toxicity pattern in increasing order B<T<X<E.

In the study of BTEX biodegradation (Fig.2), it was found that BTEX compounds were reduced by about 18-35% after 24 hours and 41-61% after 48 hours. Based on figure 2, it was observed that CC biodegrades benzene more rapidly followed by toluene, xylene isomers and ethylbenzene (B>T>X>E).

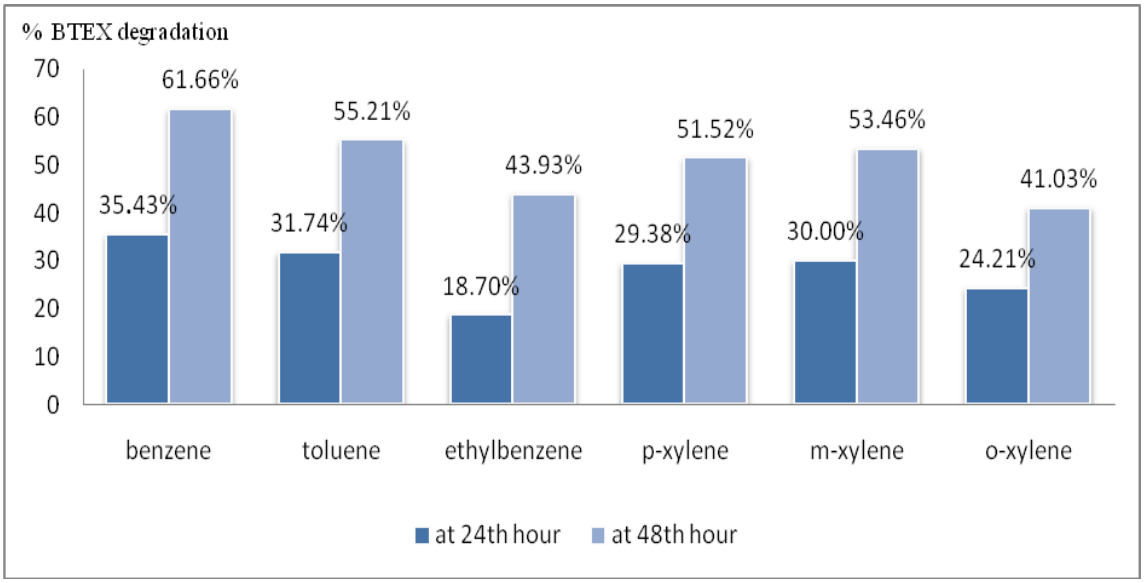

Fig. 2 BTEX degradation by CC after 24 and 48 hours incubation

By comparing the pattern of BTEX toxicity (B<T<X<E) and the percentage of degraded BTEX, we suggests that the ability of CC to biodegrade individual BTEX is influenced by BTEX toxicity exerted towards CC growth. Hence, hydrocarbon that is less toxic to CC will be readily degraded. According to Haigler (1992), a decreased biodegradation rate in hydrocarbon was observed when the hydrocarbon is more toxic to bacterial culture. This showed that hydrocarbon at low toxicity allowed a better microbial population to grow and survive which could in turn enhanced the bacterial capability to degrade hydrocarbons.

CONCLUSION

These findings suggest that the consortium culture is able to grow on individuals BTEX to a relatively high concentration (≈ 200 mg/L). This study showed that benzene is less toxic to CC, followed by toluene, xylene and ethylbenzene. CC was also found capable to biodegrade individual BTEX up to 41-61%. This showed that BTEX mineralization by consortium culture has taken place, thus, we can conclude that consortium culture has utilized individual BTEX as their sole source of carbon for energy.

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