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Biodegradation of Methyl tert-butyl ether by isolated bacteria from contaminated soils to gasoline

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ABSTRACT: Methyl tert-butyl ether (MTBE) was originally introduced as an additive to increase octane number into gasoline in the 1970s. Its use was primarily as an octane enhancer to replace lead in gasoline. Later on, it was also used as an oxygenate, up to 15% v/v, to accomplish a cleaner burning fuel with reduced emissions of carbon monoxide and hydrocarbons. The problem with MTBE is that it has caused wide-scale contamination of groundwater supplies from accidental releases and leaking underground fuel tanks. In this study, we have isolated three bacterial species from contaminated soils with gasoline which were capable of degrading of MTBE as sole carbon and energy source .The degradation rates of MTBE in 500ppm concentrationafter20 days about *Micrococcus luteus, Bacillus subtilis* and*Bacillusmegaterium*was93.2%, 60%and 97.97% respectively. These findings indicated that these bacteria were successfully adapted on MTBE and can be potentially offer to suitable and efficient method to treat MTBE contaminated environments.
Key words: Biodegradation, Mtbe, Bacteria

INTRODUCTION

MTBE (Methyl tert-butyl ether) is a clear liquid with a strong odor and it is result of reaction between methanol and isobutylene(Hao et al., 2012).MTBE initially introduced in the 1970s .The low cost, easy production, favorable transfer, and blending feature made it a most common oxygenate added to gasoline formulation as octane enhancer, commutation efficiency and to reduce the emissions of airborne toxics , carbon monoxide and volatile organic agents(Bradley et al., 1999; Fiorenza and Rifai, 2003; Atienza et al., 2005).This compound has been applied as the primary oxygenate in 30% of all gasoline and constituted 11% by volume of oxygenated fuels in both the US and Europe to replace organic-lead compounds (Deeb et al., 2000; Squillace et al., 1996,1996).In Iran it has been used since 2001 and more than half million tons are mixed with gasoline annually (Nikpay et al., 2006).There are many problems with the storage and distribution of MTBE (Salanitro et al., 1994; Ji et al., 2009).MTBE may cause headache, dyspnea, asthma, dizziness, insomnia and rash, and the United States Environmental Protection Agency has classified it as a possible human carcinogen (Kamalan et al., 2009; Liang et al., 2010; Aghaie et al., 2012). MTBE is biologically and chemically stable. Unique physical and chemical properties of MTBE like high solubility in water, poorly adsorbed by soil, low viscosity and high half-life making it very persistent in the environment and a threat to underground water supplies and drinking water wells(Bradly et al., 2001; Emtiazi et al., 2010; Blazo et al., 2012).MTBE has since been cited as the current second most abundant ground water contaminant in shallow drinking water wells(Acuna-Askar et al., 2000; Kamalan et al., 2009; Liang et al., 2010).There are few reports that some microorganisms were used to degrade MTBE. The first time, Salanitro et al 1994 reported degradation of MTBE by bacteria(Salanitro et al., 1994). *Mycobacterium* , *Pseudomonas* , *Arthrobacter* , *Rhodococcus* , *Flavobacterium*can degrade MTBE in aerobic conditions(Deeb et al., 2000; Lawrence and Larry, 2004; Ferreira et al., 2006; Ohkubo et al., 2009).The first time ,Yeh and Novak (1994) showed anaerobic degradation of MTBEO (Yeh and ovak, 1994). Iron-reducing bacteria(Landmeyer et al., 1998; Bradley et al., 2001) , Nitrate reducing bacteria(Bradley et al., 2001) and methanogenic organisms(Mormile et al., 1994)in bed sediments of fresh water streams can degrade MTBE in anaerobic conditions as well . The main scope of this work because of the increased contamination of soils and water by MTBE due to using gasoline additiveswas to look for some soil local bacteria which are able to degrade MTBE in aerobic conditions.

MATERIALS AND MEHODS

Sampling and Isolation of bacteria

Soil samples were separately collected from differential local gas stations in Kerman, Iran. For enrichment and isolation of bacteria the minimal salt medium broth was used with the following components (in g/L): MgSO4.7H2O, 0.25; CaCl2.2H2O, 0.009; KH2PO4, 0.5; K2HPO4, 0.5; NaCl, KNO3, 0.5; 1.0; and 1.0 ml/L of trace elements solution were periodically refreshed. The trace elements solution contained (ing/L):CuCl2.2H2O, 0.015; NiCl2.6H2O, 0.025; MnCl2.4H2O, 0.1; CoCl2.6H2O, 0.12; ZnCl2, 0.07; FeCl2.4H2O, 1.5; H3BO3, 0.06; EDTA.4H2O, 5.2; NaMoO4.2H2O, 0.025; (Alimohammadi et al., 2005; Farrokhi and Ahmadizad, 2009).

This medium supplemented and enriched with 0.1 percent yeast extract. After sterilization of culture media by autoclave, MTBE (98% purity, Aldrich chemical Co. Inc., Milwaukee, WI) was added to a final concentration of 500 ppm. 5 gram of each soil sample at the depth of 50 cm were inoculated in 500 ml flasks with 250 ml of minimal media containing 500 ppm of MTBE and shacked at 150rpm for 5 days and then were cultured into solid media (mentioned media with 1.5% agar) . The cultured plates were incubated for 48h at 28ºC. Isolated colonies were purified and tested with 100 and 250 ppm of MTBE. Pure cultured were identified by gram stain reaction, biochemical tests like sugar fermentation, motility and oxidize test(Bradly et al., 2001; Muñoz-Castellanos et al., 2006).

Chromatographic analysis

A Gas Chromatograph (GC) is an instrument that separates and identifies chemical compounds. The basic principle of a GC involves the injection of a sample into a mobile phase. This phase is generally a gas and its purpose is to carry the sample through the stationary phase of a column. The components that exit the column enter the detector and are then mixed with hydrogen gas that causes it to burn creating charged ions. A high electrical potential is applied to these charged ions allowing them to be collected by the electrode. The current is then measured, amplified and sent to an external data capturing system. The used FID system in this study is a widely used detector in the analysis of volatile hydrocarbons. It displays high sensitivity for compounds containing only hydrogen and carbon atoms. In the presence of oxygen, the sensitivity is slightly compromised. The response created by the FID is independent of volume and therefore the sensitivity is not affected by the flow rate. In this process comparison of the peak area between different concentration of MTBE and media containing MTBE without bacteria as control was tested(Hong et al., 1999).

Bacterial isolates were inoculated on Muller Hinton Agar (Merck , Germany) media andafter incubation for 24h at 30ºC , one colony inoculated to Muller Hinton Broth media (Merck , Germany) to obtain 1.5×10^8 cells/ml based on turbidity measurements at 550 nm O.D . From the bacterial suspension, 100μl were separately added to vials containing 0.1ml of minimal salt medium with 250 and 500 ppm of MTBErespectively. The vials sealed with a Teflon-Lind septum and incubated at 30ºC for 5 days in shaker incubator (150rpm). A control vial without bacteria was inoculated at same conditions. 2 μl from each sample wereanalyzedin an auto system XL (Perkin Elmer) gas chromatograph with a splatters injector and an FID detector equipped with a PE-WAX column (N931-b403 , 30m×0.25 I.D. ×0.25μm) . Nitrogen was applied as a carrier gas and the conditions mentioned below were set for the analysis : injector at 100ºC , detector at 250ºC , the initial oven temperature was 25ºC held for 3 min , followedbya ramp-upof 7ºC/minup to 90ºC with a total run time of 15 min(Deeb et al., 2000; Muñoz-Castellanos et al., 2006) .

RESULTS

From the 10 enrichment culture flasks with MTBE as carbon source 12 bacterial species were obtained. The results demonstrated that all of bacterial isolates had potential to biodegrade MTBE aerobically in 100ppm and three of them could growth in 500ppm of MTBE.All of them were gram positive. Based on biochemical tests, the bacteriacapable to growth in high concentration of MTBEwere:*Bacillus megaterium, Bacillus subtilis* and *Micrococcus luteus*. The availability of dissolved O2 and incubation in shaking conditions in 150rpm affect MTBE biodegradation significantly incomparison to static incubation .Chromatography experiments done in order to determine the rate of degradation. Comparison between peak area of control samples and bacterialspecies showed that *Micrococcus luteus* in 500ppm concentration, after 10 and 20 days degrade 92.4 and 93.2 percent of this compound, *Bacillussubtilis* in500 ppm concentrations, after 10 and 20 days decomposed 35.34 and 60 percent of MTBEand *Bacillusmegaterium* in 500 ppm concentration, after 10 and 20 days decomposed 75.96 and 97.97 percent of MTBE respectively.

DISCUSSION

The tertiary structure of MTBE leads to a satiric hindrance to an enzymatic attackon the molecule(White et al., 1996). Compounds with ether bonds are also generallyrelatively stable (Fayolle et al., 2001). For these reasons, MTBE is a rather difficultcompound to degrade. There are also naturally occurring microorganisms which have been shown to completely mineralize MTBE under aerobic conditions. Several pure strains that have been isolated and studied can mineralize MTBE. They do so by direct metabolism, whereby, MTBE is used as the sole carbon and energy source(Hristova et al., 2003; Francois et al., 2012).Munoz- Caste llanos et al. (2006) isolated 59 different bacterial strains include*Bacillus, Rhodococcus, Micrococcus, Aerobacterium* and *Proteus* and 6 strains could completely reduce MTBE(Muñoz-Castellanos et al., 2006). Youngster et al. (2010) analyzed somebacterial species in anaerobic enriched cultures originating from three different contaminated sediments(Youngster et al., 2010). In addition, Arabi et al.(2007) reported3 bacterial strains that could degrade MTBE rapidly under aerobic conditions(Arabi, 2007).These strains were identified as Bacillus cereus and *Klebsiella terrigena.* Both strains could be able to grow in the presence of high concentration of MTBE (e.g. 48 g L-1) in water. Both thestrains were aerobic, and cannot able to grow and degrade MTBE anaerobically(Arabi, 2007). In another study, Chang et al., 2001 showed that the butane-utilizing microorganisms could co-metabolically degrade MTBE and other gasoline oxygenates compounds(Chang et al., 2001). Compared to aerobic MTBE degradation, removal rates under anaerobic conditions are extremely slow, and long acclimatization periods are required(Waul et al., 2009; Youngster et al., 2010). It cannot be considered as a feasible remediation option until further research is carried out.

CONCLUSION

This research indicates the need for more study on MTBE for industrial application. Additionally, the research demonstrates the need to treat not only the fuel oxygenate, or other main environmental contaminant, but also its breakdown components because the secondary, or break down, compounds may play as large a part in the safety of the environment and human health as the contaminant itself. Findings from this study also suggest that these cultures are able to adapt to high MTBE concentrations if maintained on them as the sole carbon and energy source. This tolerance, coupled with MTBE affinity for water, indicates that this culture may be a good candidate to biologically regenerate activated carbon saturated in MTBE.

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