Methyltert-butyl Ether (MTBE) Degradation by a Microbial Consortium

[1]S.B. Mortazavi, [1]A. Nikpey, [1]A. Rezaee, [1]H. Asilian, [1]A. Khavanin and [2]H. Kazemian
[1]Department of Occupational and Environmental Health, Medical Faculty
Tarbiat Modarres University, Tehran, Iran
[2]Jaber Ibn Hayyan Research lab, Atomic Energy Organization of Iran (AEOI), Tehran, Iran

Abstract: Methyl tert-butyl ether (MTBE) is added to reformulated gasoline to meet the 1990 Clean Air Act directives. Widespread use of MTBE in gasoline has resulted in groundwater contamination. Because of its undesirable effects on drinking water and ecologically harmful effects, MTBE removal has become a public health and environmental concern. In this study, we have isolated a mixed bacterial culture which is capable of degrading the MTBE as a sole carbon and energy source. This consortium was developed from mixed urban and petrochemical activated sludge after 4 month's enrichment. Enrichment was conducted in batch reactor, fitted with a screw cap and butyl rubber septum. MTBE concentration was measured in head space by gas chromatography. Degradation was determined by MTBE removal. MTBE biodegradation was depended to Dissolved Oxygen (DO) concentration and not affected by the changes in concentration of trace element solution or other stimulator Substances. Degradation rates were nearly 1.478 mg MTBE $h^{-1} g^{-1}$ (wet biomass) and didn't change with MTBE concentration (up 500 mg L^{-1}).

Key words: Methyl Tert-butyl Ether (MTBE), Degradation, Microbial Consortium

INTRODUCTION

Methyl tert-butyl ether (MTBE) was introduced in the late 1970s as a gasoline octane enhancer instead of lead tetraethyl. As a result of its low production cost and excellent blending characteristics, its production has been exponentially growing, reaching a value of over 33 million ton per year at the present time [1, 2]. Widespread use of MTBE in gasoline, leaks of fuel underground and aboveground fuel tanks, spill or accidental runoff, from petroleum facilities (e.g. refineries, terminals, pipelines and service stations) and the relative recalcitrance of MTBE to natural attenuation combined with its physico-chemical properties are a threat to Underground water supplies and drinking water wells. Consequently, MTBE is currently, second most frequently detected contaminant in drinking water supply [3, 4].

Starting in 1992 with reports of illnesses associated with exposure to MTBE in U.S, concerns of possible health effects of MTBE have increased. USEPA (1997a) issued a drinking-water advisory for MTBE of 20 to 40 μg L^{-1}, to protect consumer acceptance of water resources and also to provide a large margin of safety from potential toxic effects resulting from exposure to MTBE [5].

The evaluation of innovative and cost-effective treatment such as bioremediation for MTBE spills is warranted. The relatively recalcitrant nature of MTBE to oxidative and/or reductive microbial attack is inherent in its chemical structure, which contains a combination of two bio-recalcitrant organic functional groups: the ether link (C-O-C bonds) and the branched moiety. For example, only 15-65% of added MTBE was mineralized under aerobic conditions in 50 days by diverse sediments [6]. MTBE hardly sustains microbial growth and its biodegradation is characterized by a low biomass yield [1, 7-9].

Although MTBE was once considered recalcitrant, different authors recently reported, that it is biodegradable by an assortment of aerobic microbial cultures from different origins. Cultures able to metabolize MTBE have been found in activated sludge, air and ground water treatment systems, soils, sediments and even Gingko fruit [6, 7, 9-15].

Several pure strains of bacteria able to degrade MTBE have been described [7, 9, 10, 13]. In such strains as PM1 that are able to utilize MTBE as the sole carbon source, with very slow growth rate [9].

This report describes the isolation of mixed bacterial population based on its ability to grow on MTBE as the sole carbon and energy source and effect of oxygen, trace element and other bio stimulator on the MTBE biodegradation rate.

MATERIALS AND METHODS

Chemicals and Reagents: Diluting a GC grade MTBE MERCK (99.9% pure) at the required concentration in aerated sterile Mineral Medium (MM) made the MTBE stock solution. All chemicals and reagents were analytical grade. Humic and fulvic acids were extracted

from a well humified organic soil and purified following a standard procedure of the Canadian society of soil sciences [17].

The mineral medium (MM) consisting of the following components (in g L^{-1}): MgSO4.7H2O, 0.25; KNO3, 0.5; CaCl2.2H2O, 0.009; KH2PO4, 0.5; K2HPO4, 0.5; NaCl, 1.0; and 1.0 mL L^{-1} of trace elements solution were periodically refreshed. The trace elements solution contained (in g L^{-1}): FeCl2·4H2O, 1.5; CuCl2·2H2O, 0.015; NiCl2·6H2O, 0.025; MnCl2·4H2O, 0.1; CoCl2·6H2O, 0.12; ZnCl2, 0.07; NaMoO4·2H2O, 0.025; H3BO3, 0.06; EDTA·4H2O, 5.2; the final pH was 4.2 [18, 19].

Enrichment of Culture: Activated sludges from urban and petrochemical and a biotrickling filter, which was prepared for removal of MTBE from contaminated air, were used as microbial sources for isolating MTBE-degrading enrichments. In this concern, 300 mL of supernatant wastewater were added to 1L of mineral medium. Then fully flushed with air for 30 min and MTBE was added at the concentration of 100-200 mg L^{-1}. Enrichment was conducted in 1500 mL PVC bottles fitted with a screw cap and butyl rubber septum. In each flask, 300 mL of above mentioned solution was added. The 1200 mL of headspace guaranteed sufficient air for aerobic degradation. After inoculation, all flasks were incubated at room temperature (25°C) on a rotary shaker (130 rpm) in a dark environment. The ability of samples; to degrade MTBE was evaluated by head space analysis by (GC) at different time. MTBE concentrations and TBA (tert butyl alcohol) were determined using a PHILIPS PU-4410 gas chromatograph equipped with a flame ionization detector as possible compounds from MTBE biodegradation in the headspace of the vials. The compounds were separated on a %10 SE30 packed column (1.5 meter. 4mm ID). Column temperature was adjusted isothermally at 50° C ,injector at 180° C and detector at 200° C. Nitrogen gas (30 mL min^{-1}) was used as the carrier gas. Sample (100 µl) injected into the chromatograph, without further treatment. Dissolved Oxygen in MM was measured by DO_2 Meter (JENWAY, 9200). Six flasks were prepared in the same way. A similar flask, which was contained 1% of NaCN as a microbial respiration inhibitor was used as a control medium, to monitor any MTBE loss from volatilization and diffusion from septum. Bacterial cultures were maintained in MM for enrichments and provided with sufficient MTBE to maintain active growth. After the removal of MTBE, culture was aerated to achieve a dissolved–oxygen level of 5-7 mg L^{-1}, then MTBE was added to the bottles. Every 10 days, half part of MM was filtered through 0.22-µm-pore-size Cellulose Acetate filters (Sartorius GmbH-Germany) and a new MM was added to the bottles. After 4 months, one enrichment derived from mixed

petrochemical and urban activated sludge, consistently degraded MTBE.

MTBE Removal Experiments: Consortium grown on MM containing MTBE (200 mgL^{-1}) were harvested by centrifugation at 4000 rpm for 15 min. The biomass were resuspended to obtain an initial concentration of 5 mg wet biomass in 1 mL of mineral medium which containing about 80 µg MTBE mL^{-1}. Experiments were performed in 115 mL bottles fitted with a Teflon lined silicon septum (La-Pha-Pack). In each sterile bottle, 20 mL of above mentioned mixture was added. A 95 mL of headspace volume was guaranteed sufficient air for aerobic degradation. Eight sets of duplicate bottles were amended with above mentioned mixture and respectively, i) 20 µl and 100 µl of trace elements solution, which was prepared as described before ii) 20 µl and 100 µl of Fe2+ solution (FeCl2 4H2O: 1.5 g L^{-1}); iii) 20 and 100 µl of Cu2+ solution (CuCl2 2H2O: 0.015 g L^{-1}); iv) no addition of any trace elements; v) 100 µl humic acid and 20 µl of trace elements solution(Humic acid: 0.1 g L^{-1}); vi) 100 µl fulvic acid and 20 µl of trace elements solution(fulvic acid: 0.1 g L^{-1});vii) 100 µl fulvic acid and100 µl humic acid with 20 µl of trace elements solution(fulvic acid and humic acid: 0.1 g L^{-1}) viii) 100 µl yeast extract and 20 µl of trace elements solution(yeast extract: 0.1 g L^{-1}).

To evaluate the biodegradation ratio under anaerobic conditions, first of all, the MM containing biomass was added to the bottle, sealed and purged with a continues nitrogen gas with a flow rate of 30 mL min^{-1} for 30 min and finally MTBE was added. MTBE was added to flasks at different concentration in order to investigate the effect of concentration on the biodegradation rate. Two un inoculated bottles served as control medium. All flasks were incubated at room temperature (25°C) on a rotary shaker in a dark environment [19]. MTBE degradation was monitored and measured over the whole experiment period by injection of a 100 µl portion of the vials headspace into a chromatography column.

RESULTS AND DISCUSSION

Bioremediation of recalcitrant compounds requires the enrichment of a suitable microbial culture [12]. The consortium was enriched in batch reactor from a mixture of urbane and petrochemical waste waters in a period of 4 month and consistently degraded MTBE (Fig. 1).

These results (Fig. 1) were shown that macro–nutrient (NPK) limitation; under laboratory conditions (full medium) was very unlikely, because of the low concentrations of MTBE and low biomass yield. Biomass in the microcosm did not show a significant change and fairly constant throughout the duration of the assay, suggesting the organism gains little or no
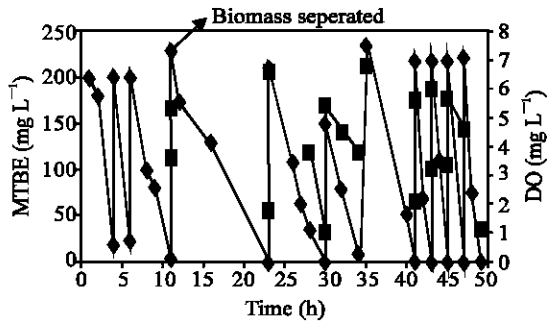
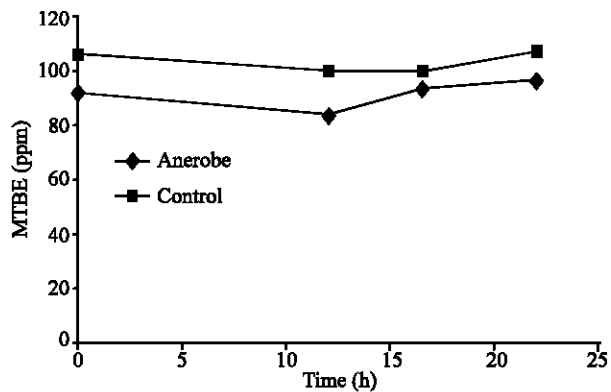Fig. 1: Growth of Microbial Consortium on MTBE (about 200 mg L^{-1}) Concentration of MTBE (◆), Dissolved Oxygen (■)

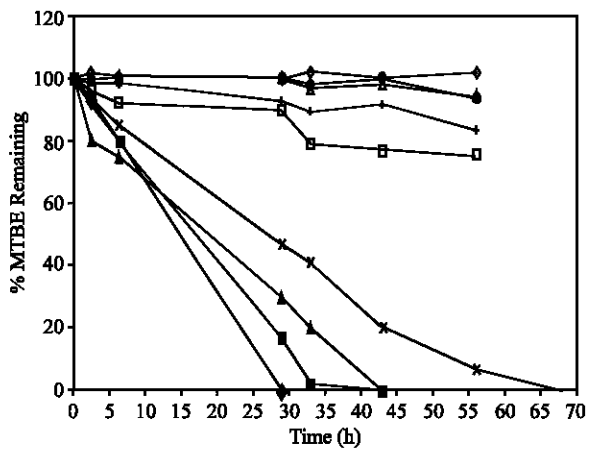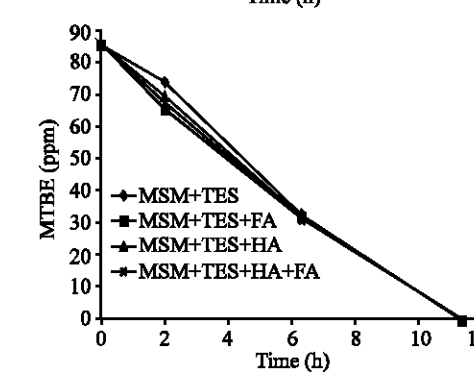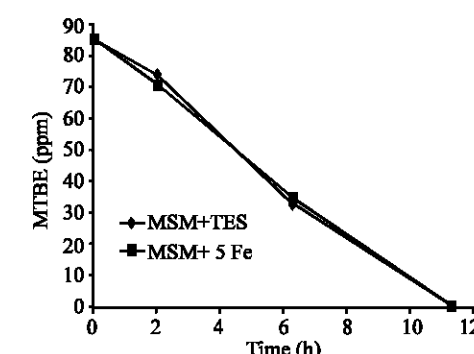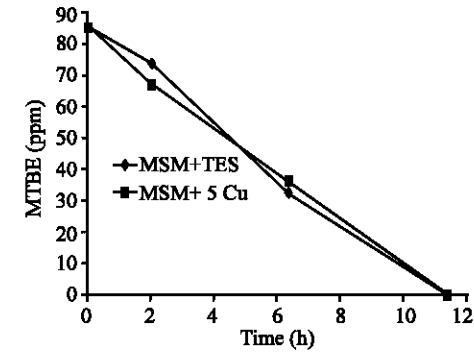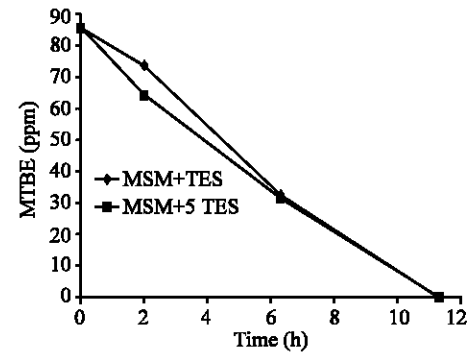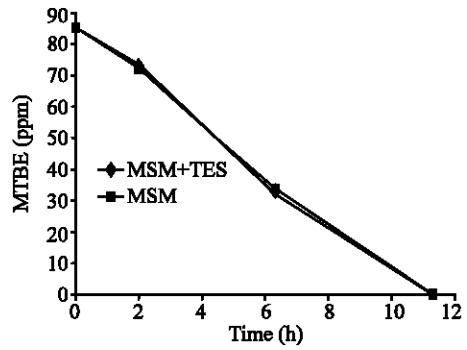Fig. 2: The Effect of Oxygen on MTBE Biodegradation

Fig. 3: Degradation of Increasing MTBE Concentration by Consortium. Consortium was Incubated with MTBE at Initial Concentrations of 200(◆), 300(■), 400 (▲), 500 (×), 750 (□), 1150 (●), 2850 (+), 3120 (△). A Representative a Biotic Control (100 mg L^{-1}) is also shown (◇), Control at all Concentration were Similar

metabolic energy from the MTBE molecules. Based on the data obtained from this experiment, the rate of MTBE elimination was depended to the concentration
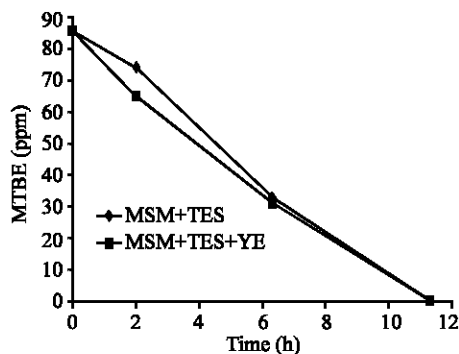
Fig. 4: The Effect of Trace Elements on MTBE Biodegradation. Effect of A) Trace Element Solution (TES) at either the Normal Concentration (×1) or a Five Time Concentrated (×5) or without (TES); B) Iron Addition; C) Copper Addition; D) Humic and Fulvic Acid; E) Yeast Extract

of Dissolved Oxygen (DO),From modest susceptibility to oxygen concentration (less than 1 mg L^{-1}) to serious failure (0 mg L^{-1}) can occur when the oxygen supply is insufficient. No MTBE degradation was observed in the experiments performed under anaerobic condition and inoculated with the MTBE degrading consortium (Fig. 2).

The Consortium was capable to degrade up to concentration 500 µg MTBE mL^{-1} without any remarkable lag period, whereas the concentrations higher the of 500 µg MTBE mL^{-1} was not degraded. The initial linear rates of degradation were not dependents to MTBE concentration. Estimated rates were nearly 1.478 mg MTBE $h^{-1} g^{-1}$ (wet biomass) for all range of MTBE concentration (Fig. 3).

A recent study, reported that Co2+ and yeast extract had a positive effect on MTBE biodegradation by a gram-positive methylotrophic bacterium identified as Mycobacterium austroafricanum IFP 2012 [16]. Another study reported that Peat Humic Substance stimulated environmental bioprocess [1]. Under these circumstances, the impact of particular of trace elements in nutrient Fe2+, Co2+, Cu2+ and other trace ions and some of inducers on the biodegradation of MTBE yeast extract and peat humic substance were investigated by our consortium.

Experimental results obtained of the effect of trace elements on MTBE biodegradation are summarized in Fig.4. Complete degradation of 85 ppm MTBE was observed in approximately 12 hours. MTBE biodegradation occurred in all bottles inoculated with the MTBE degrading consortium. The specific rate of MTBE degradation was 1.478 mg MTBE $h^{-1} g^{-1}$ (wet biomass).

MTBE biodegradation was depended to the Dissolved Oxygen (DO) and not affected by the changes in concentration of iron, copper, cobalt (in trace element

solution) or other stimulator substances. It is not possible to conclude anything on a possible involvement or lack of involvement of a cytochrome P450 in the biodegradation of MTBE using these experiments alone. Interestingly, biodegradation in absence of trace elements proceeded at the same rate as with all amendments (Fig. 4). One possible explanation for this is that the MTBE degrading consortium is growing at such a slow rate, that its requirements in micronutrients are virtually nil, once an effective culture is obtained. This explanation would be consistent with the lack of improvement in biodegradation rate with increasing micronutrients. It suggests that further efforts in optimizing micronutrient supply are unnecessary [19].

ACKNOWLEDGMENTS

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