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EFFECTS OF STIMULATOR SUBSTANCES ON AEROBIC METHYL *TERT*-BUTYL ETHER BIODEGRADATION BY MICROBIAL CONSORTIUM

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ABSTRACT

In this study dissolved humic substances and yeast extract were tested in different concentrations for enhancing methyl *tert*-butyl ether mineralization by isolated microorganisms from a variety of sources. All experiments were conducted at a constant temperature of 25ºC. Vials of 50 mL and 125 mL volume sealed with Teflon-lined Mini-Nert caps was used for microcosm experiments. In all experiments 1% sodium azide were used as control. Samples of bacterial cultures that metabolize methyl *tert*-butyl ether have been analysed by direct GC analysis using flame ionization detector. Cultures able to metabolize have been found in activated sludge and soils. These microorganisms were gram-positive bacterium. An aerobic microbial consortium was enriched in laboratory for four months. Methyl *tert*-butyl ether has been shown to biodegrade under aerobic and co-metabolic conditions. A microbial consortium isolated from activated sludges was identified as *Cocobacillus*. The concentration of the initial attached biomass was about 0.11 g/L of dry weight. The maximum mineralization rate and beneficial effects of stimulator substances on aerobic biodegradation of methyl *tert*-butyl ether occurred with the culture by combined concentrations of 500 mg/L of yeast extract and 20 mg/L of peat humic growth support of microbial consortium within 216 h and in presence of high oxygen levels and well mixing conditions. It was shown that adding, peat humic and yeast extract together, had better stimulatory effect on methyl *tert*-butyl ether biodegradation. Results clearly showed a stimulatory effect on methyl *tert*-butyl ether consumption higher than 20%. Consortium was capable of degrading concentrations of ≤1000 mg/L, whereas concentrations of >1000 mg/L, were not degraded.

Key words: Methyl *tert*-butyl ether (MTBE), Biodegradation, Enhancement, Microbial, Peat humic, Yeast extract

INTRODUCTION

Methyl *tert*-butyl ether (MTBE) has been incorporated in reformulated gasoline at concentrations up to 15% (v/v) (Burbano *et al*., 2002). It is a synthetic additive originally introduced to replace lead as an octane-enhancer and anti-knocking agent in gasoline to reduce the polluting emissions in exhaust gases (Schirmer *et al*., 2003).

This compound is water soluble (48,000 mg/L) and one of the most common pollutants of groundwater and surface water resources. Because of its undesirable effects on drinking water and ecologically harmful effects, MTBE removal has become a public health and environmental concern (Corcho *et al*., 2000). Biodegradation of MTBE can offer an efficient and low-cost method of treating MTBE contaminated groundwater (Francois *et al*., 2002).

Bacterial degradation of MTBE may provide an attractive solution to groundwater contamination. Such treatment can be performed *in-situ* at the site of contamination or *ex-situ* with biological water treatment reactors (Salanitro *et al*., 2000 ; Stringfellow *et al*., 2002). With *in-situ* bioremediation bacterial cultures naturally existing are metabolically stimulated with substrate addition to degrade the contaminant to non-toxic compounds. Numerous laboratory studies have shown that humic matter has

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significant impact on the mineralization and aiding the biodegradability of some organic pollutants (Pruden *et al*., 2001).

Humic acids

The fraction of humic substances that is not soluble in water under acidic conditions (pH<2) but is soluble at higher pH values and they can be extracted from soil by various reagents and which is insoluble in dilute acid (Somsanak *et al*., 2001). Humic acids (HA_S) are the major extractable component of soil humic substances and they are dark brown to black in color. Humic acids are biopolymers that are formed beside other humic substances (fulvic acids, humin) during the degradation of biological material with high molecular weight. In fact humic acids are thought to be complex aromatic macromolecules with amino acids, amino sugars, peptides, aliphatic compounds involved in linkages between the aromatic groups (Garnier *et al*., 1999).

Fulvic acids

The fraction of humic substances that is soluble in water under all pH conditions. Fulvic acids are light yellow to yellow-brown in color and contain both aromatic and aliphatic structures, both extensively substituted with oxygen-containing functional groups. Humic and fulvic acids play an important role for pollution removal. These are predominantly oxygen containing groups, such as carboxyl and phenolic OH groups, that can be easily consume by microorganisms. The quantification of those groups, which is necessary for the investigation of rate of humic acids consumption by microorganisms (Morales *et al*., 2000). The current knowledge of the chemical structure of humic and fulvic acids shows, that the reactive functional groups can be assigned formally to certain low molecular organic compounds.

Hence in this study humic and fulvic acids were extracted from a well humified organic soil and yeast extract as stimulator substances for enhancing MTBE removal.

MATERIALS AND METHODS

Sampling

Samples were separately collected from activated sludges in wastewater treatment plants and oiled soils in Tehran, from Shoush, Shahrak-e-Ghods and Ekbatan wastewater treatment plants, and mixed together.

Laboratory batch microcosm experiments

The ability of an activated sludge sample from Shoush wastewater treatment plant to degrade 1000 mg/L of different fuel oxygenates was studied. A mixed bacterial culture capable of degrading MTBE was obtained from the University of Tarbiat Modarres, Medical Sciences Faculty, Department of Occupational and Environmental Health. Bottles of 50 mL and 125 mL closed with Teflon Mini-Nert valves (Supelco, Buchs; Switzerland) were used for microcosm experiments. In all experiments 1% sodium azide was used as control. All chemicals and reagents were of laboratory research grade. Vials were sealed using Mini-Nert caps and MTBE concentration was monitored by GC/FID headspace analysis.

Analysis of MTBE

Samples of bacterial cultures that metabolize MTBE have been analysed for both MTBE and its metabolite *tertiary*-butyl alcohol (TBA) concentration by direct GC analysis using FID and a Quadrex methyl silicone capillary column (Salanitro *et al*., 2001) were used. For analysis of MTBE (and TBA) in bacterial cultures that degraded TBA, though not MTBE, used a GC capillary column coated with a cross-bound phase and an FID detector (Deeb *et al*., 2000). Cultures were incubated in 50 mL and 125 mL bottles sealed with Teflon-lined Mini-Nert caps (Alltech; Deerfield, Ill.,2003) at 25°C in the dark on an orbital shaker (rotation speed of 150 rpm). MTBE concentrations and TBA possible compounds from biodegradation in the headspace of the vials were determined using a Philips PU-4410 gas chromatograph equipped with a flame ionization detector. Compounds were separated on a %10 SE30 packed column (1.5 m, 0.4 mm ID); the column temperature was isothermally at 50°C, injector at 180°C and detector at 200°C. Nitrogen (30 mL/min) was used as the carrier gas. For volatile/semi-volatile compounds like MTBE we inject headspace of vials was injected in to the gas chromatograph.

All runs were duplicated. Then, 100 µL of microcosm headspace was injected into the chromatograph using a wetted 500 µL gas-tight syringe. Enrichment was conducted in batch reactor, fitted with a screw cap and butyl rubber septum. Static headspace analysis can be used for samples of soil and groundwater. Samples were collected in filled bottles and the bottles were shaken and equilibrated before analysis of the gas phase by the method of GC-FID using a megabore DB-1 capillary column.

Headspace analysis

Static headspace analysis is based on the partitioning of analytes from an aqueous or solid sample to air in a closed system (headspace vials). This method is suitable for compounds that show sufficiently high air-water partitioning (quantified by the Henry's Law constant). Although the Henry's Law constant for MTBE is about one order of magnitude smaller than those for benzene or toluene, analytical methods based on headspace sampling have been developed (Hods *et al*., 2001 ; Centi, 2002).

Bacterial populations and culture conditions

Bushnell-Haas (BH) and MSM basal medium (Difco, Detroit, MI., 2005) were used for batch cultures.

The mineral medium consisted the following components (in g/L): $MgSO_4.7H_2O$, 0.25; KNO_3, 0.5; $CaCl_2.2H_2O$, 0.009; KH_2PO_4, 0.5; K_2HPO_4, 0.5; NaCl, 1.0; and 1.0 mL/L of trace elements solution were periodically refreshed. The trace elements solution contained (in g/L): $FeCl_2.4H_2O$, 1.5; $CuCl_2.2H_2O$, 0.015; $NiCl_2.6H_2O$, 0.025; $MnCl_2.4H_2O$, 0.1; $CoCl_2.6H_2O$, 0.12; $ZnCl_2$, 0.07; $NaMoO_4.2H_2O$, 0.025; H_3BO_3, 0.06; $EDTA.4H_2O$, 5.2; the final pH was 4.2, (Salanitro *et al*., 2001).

Carbon sources were added to BH according to the protocol. The pH was adjusted with NaOH to 7.0 and checked with litmus paper or pH meter. Pure cultures were tested MTBE degradability in 50 mL and 125 mL vials (Fisher Scientific Inc. New Brunswick, NJ., 2003) containing the liquid mineral medium.

MTBE stock solution was made by serially diluting research grade MTBE from MERCK (99.9% purity) at the required concentration in aerated sterile Mineral Salts Medium (MSM) and a final concentration of 76.4 mg/L in de-ionized water. All chemicals and reagents were obtained from assure source. Cultures were passed from Tryptone Soya Agar (TSA) to MTBE-containing Mineral Salts Medium (MSM) a minimum of four times to establish cell line purity (Salanitro *et al*., 2001; Morales *et al*., 2000). Soils were filtered with 100 mL of de-ionized water. Ten milliliters of soil filtrate was added with the minimal salts solution to a final volume of 250 mL in an Erlenmeyer flask with 0.5 mL of enrichment substrate. Water lost in autoclaving was replaced by bringing the flask up to volume with sterile water. All culture medias were prepared with distilled and deionized water (American Society for Testing and Materials type 1 quality, 2005).

Isolation of MTBE-degrading microorganisms

Bacterial populations were plated on agar with MTBE vapors as the only carbon source, and independent colonies were transferred to liquid mineral salts medium. After three weeks growth to saturation, colonies were diluted into fresh mineral salts medium. In the second step, the cultures were transferred on plates and individual colonies were patched on plates and MTBE-containing mineral plates, including wild type controls. Finally, after 8 to 10 days, the colonies showing markedly reduced growth on MTBE plates were selected.

Extraction of humic substances

Humic and fulvic acids were extracted from a well humified organic soil. The Humic substances (HSs) which were derived from four types of soils (peat, tropical peat, brown forest and ando soils) were extracted and purified according to the protocol of the International Humic Substances Society (IHSS., 2004).The humic and fulvic acids (HAs and FAs) from garden soil were purchased and dissolved in aqueous 0.1M NaOH; then the solution was treated with a mixture of HF and HCl acids. The resulting precipitate was transferred to a dialysis tube. After dialysis, the slurry was freeze-dried, resulting in the formation of powders.

RESULTS

Fig. 1 shows the results of MTBE-biodegradation by microbial consortium in presence of different concentrations and yeast extract. The results of MTBE-biodegradation in the presence of different combinations of peat humic concentrations are summarized in Fig. 2.

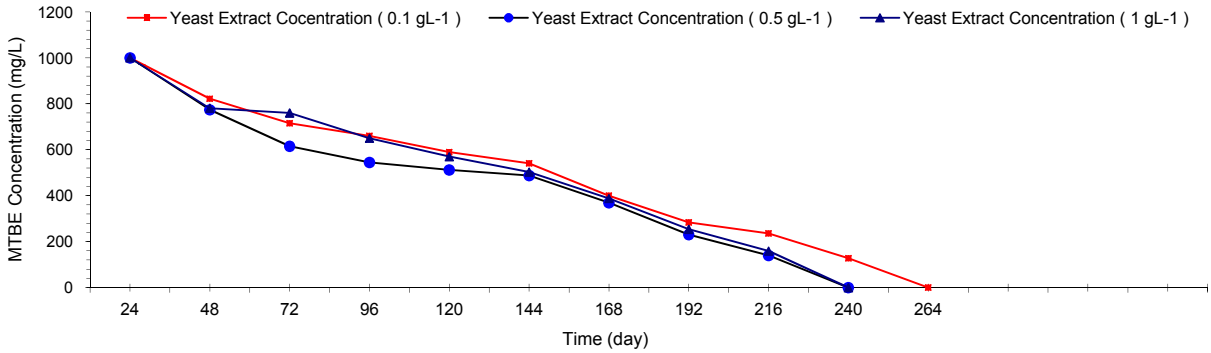

Fig .1: MTBE biodegradation by microbial consortium in the presence of different concentrations of yeast extract

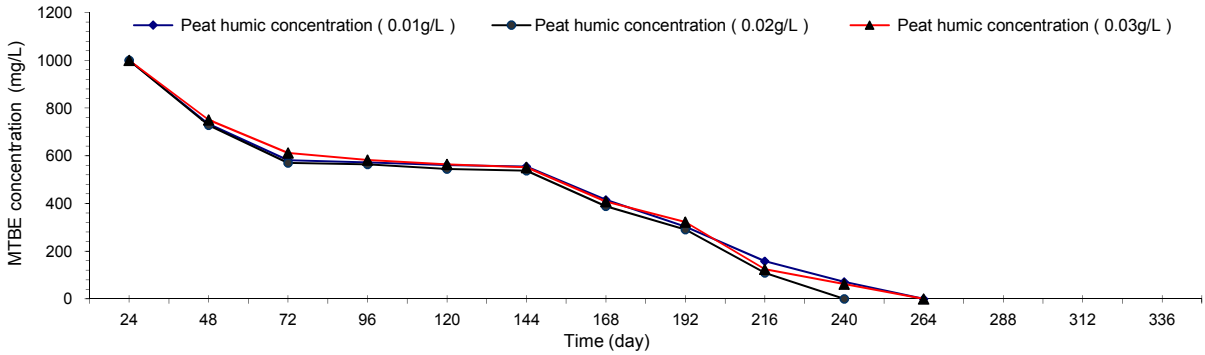

Fig .2: MTBE biodegradation by microbial consortium in the presence of different concentrations of peat humic

Fig. 3 shows the comparative results of MTBE-biodegradation by microbial consortium in the presence of peat humic and yeast extract when added together.

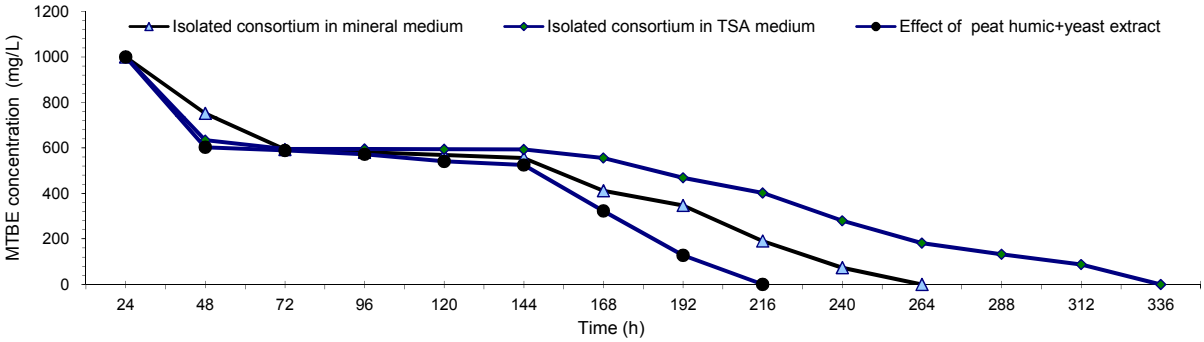

Fig .3: Comparative results of MTBE biodegradation by microbial consortium in the presence of peat humic and yeast extract (added together with best stimulatory effect on MTBE biodegradation)

DISCUSSION

Results indicated that MTBE may slowly be metabolized to CO_2 aerobically in the exerting toxicity to the population of microorganisms capable of degrading it.

Recent literatures have provided evidences that while single species cultures could not biodegrade MTBE, multiple species growing in a consortium would degrade it to produce CO_2 (Achten *et al*., 2001; Salanitro *et al*., 2001). This finding was elaborated in our study using continuous culture enrichments.

When microbial consortiums were streaked onto 0.13 TSA, three different colony morphologies were observed. Of these, two colonies were purified and tested for the ability to use MTBE as their sole source of carbon and energy. Two strains were able to degrade MTBE as their sole carbon and energy source, one forming large yellow colonies on 0.1 TSA and the other forming white pinpoint colonies on mineral medium. The white colony-forming strain, which had the faster rate of MTBE removal, was selected for further study.

Microscopic analysis of the isolated MTBE-degrading consortium, designated, show this organism is a gram-positive. We found that, oxygen was utilized by a low biomass carbon-limited consortium when small amounts of MTBE were added as the sole carbon source. This provides indirect evidence that MTBE is capable of being metabolized by microbial populations since oxygen is the terminal electron acceptor in aerobic metabolism.

The combined results from the enrichment culture and mineralization experiments indicate that while aerobic metabolism of MTBE may occur to some extent by consortia, it occurs at a very slow rate. Further, MTBE or its metabolites appear to inhibit metabolism of MTBE at concentrations exceeding 1000 mg/L when no other carbon sources are present. Thus it is unlikely that MTBE can serve as a sole carbon source for microorganisms. The adaptation phase was extended by periodic additions of substrate over a two month period but the MTBE-degrading consortium was obtained after four months enrichment.

Our results showed that MTBE had a weak inhibitory effect on the biodegradation of MTBE at a concentration of 1000 mg/L. However, the mechanism of inhibition is not clear.

In summary, when MTBE was added to a carbon-limited enrichment consortium, oxygen levels in the culture vessel decreased, providing indirect evidence of MTBE metabolism. After the substrate was exhausted, this microbial consortium was a gram-positive, strictly aerobic, and formed yellow and white colonies on solid medium and 10 days was necessary for degradation and mineralization of MTBE.

Bacterial populations incubated in MTBE at 1000 mg/L were found to biodegrade 99% of the MTBE present within 240 h(10 days). Simultaneous production of TBA, the primary metabolite of MTBE, was also observed. Mass balances of MTBE and TBA showed that TBA accumulated at a rate slower than that at which MTBE was being biodegraded. This observation suggested that a portion of the TBA being produced was simultaneously being degraded along with MTBE. MTBE at 1000 mg/L was completely degraded within 250 h (10.5 days) in presence of high oxygen levels and well mixing condition. It has been suggested that TBA and MTBE are degraded by the same enzyme (Schmidt *et al.,* 2004 ; Prah *et al*., 2004). Consequently, the effect of TBA on the MTBE biodegradation rate is of interest. The selected microbial consortium can also degrade completely TBA as sole source of carbon and energy, without lag period during initial batch cultures. On this substrate, CO_2 was produced from TBA after a long lag which corresponded to the conversion of MTBE to TBA.

In the final step of experiments, in this study humic and fulvic acids were extracted from a well humified organic soil and purified procedure. In fact, humic and fulvic substances work best in conjunction with mineral nutrients, and combined applications of mineral nutrition and humic substances produce synergistic beneficial effects and play a role as bio-catalyst and bio-stimulant. Various mechanisms are apparently responsible for the positive effects that humic and fulvic substances have been proven to have on plant growth by laboratory experiments whereby the

organic substances in soil are rapidly consumed up by microorganisms and mineralized entirely. Therefore, in this study, the influence of the combination of different concentrations of extracted dissolved humic substances and yeast extract for enhancing of MTBE biodegradation was investigated. Stock solutions of yeast extract were prepared by dissolving it with sterile mineral medium. Five-milliliter suspensions containing known amounts of humic fractions and these suspensions were introduced into 10 mL screw-cap tubes and equilibrated overnight at 25°C in the dark on a rotary shaker operating at 150 rpm.

In first concentrations, results showed that 1000 mg/L of yeast extract and 20 mg/L of peat humic support growth of microbial consortium by itself, clearly had a stimulatory effect on MTBE consumption. A similar effect of 500 mg/L of yeast extract and 10 mg/L of peat humic was observed too. But in these concentrations, the effect of peat humic was observed only in the length of the acclimation period. In this treatment, the maximum rates and extents of mineralization were not statistically different from those of the control.

Darwin et al found that organic support (peat) and inorganic support (perlite) enhance the activity of pure culture of *mycobacterium austroafricanum* when MTBE is used as the sole source of carbon and perlite has been more effective than peat (Darwin *et al*., 2006).

The maximum mineralization rates was observed in the presence of 500 mg/L of yeast extract and it was added together with 20 mg/L of peat humic and had best stimulatory effects on MTBE consumption. It is shown that adding of peat humic and yeast extract together have better stimulatory effect on MTBE biodegradation. In these concentrations, MTBE was completely degraded within 216 h (9 days) in presence of high oxygen levels and well mixing conditions. In this stage, the acclimation phase was shortened, and the maximum rates and extents of mineralization were statistically higher than those of the control. It clearly had a stimulatory effect on MTBE consumption upper than 20% by decreasing length of the biodegradation period.Nikpey founded that adding of yeast extract and peat humic substances with concentrations of 0.25-0.5mg/L to promote biomass growth in MTBE degradation are unsuccessful (Nikpey *et al*., 2006). In our study no biomass aggregates were observed during all the batch cultures, but the attached biomass was observed (the concentration of the initial attached biomass was about 0.11 g/L of dry weight). Stimulatory effects of humic acid and yeast extract complexes leaded to higher biomass production by accelerating cell division (about 0.135 g/L of dry weight).Humic substances have stimulate enzymes and act as an organic catalyst by increasing vitamins and mineral content and promote the conversion of nutrient elements (N, P, K, Fe, Zn ions and other trace elements) into forms available to microorganisms.

It seems that, stimulator substances act as natural chelator for metal ions too. Humic acids chelate nutrient compounds, especially iron. The stimulation observed may be the result of an increased concentration of substrate in the vicinity of the bacterial cells, caused by the direct contact with humic acid and humic acid-yeast extract complexes. Humic acids, which harbor both hydrophilic and hydrophobic moieties, play a key role in facilitating better access of microbes to MTBE adsorbed to humic acid-yeast extract complexes.

However, current scientific studies show that the MTBE-biodegrading is determined to a very large extent by the content of humic substances. Their high cation-exchange capacity, the oxygen content as well as the above average water holding capacity are the reasons for the high value of using humic substances for improving biodegradability and microbial growth. The most important feature of humic substances lies in their ability to bind insoluble metal ions, oxides and hydroxides, and to release them slowly and continually to plants when required. Due to these properties, humic substances are known to produce three types of effects: physical, chemical and biological.

Similar effects by trace elements such as copper and iron ions on biodegradability enhancement of MTBE have been shown. It is shown that with increasing of iron and copper concentration to 5 times, the biodegradability of MTBE decrease (Deshusses *et al*., 2003). Many studies show that MTBE biodegradation could be inhibited

in the presence of more easily biodegradable compounds (Rula *et al*., 2004).

Although MTBE-degrading bacteria have been isolated, but there are still unanswered questions about which specific members of the microbial community are capable of degrading MTBE, the enzymatic pathways and metabolic pathways involved and most fundamentally, the differences between degradation enzymes that explains the restrictions in substrate utilization in bacteria. Additional research about Effects of other stimulator substances and trace elements is needed to optimize the growth conditions of these organisms by accelerating microbial growth rate so as to obtain the best MTBE bioremediation rates.

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REFERENCES

Achten, C., Kolb, A., Uttmann, W.P., Methyl tert-butyl ether (MTBE) in urban and rural precipitation in Germany. Atmospheric Environment. 2001., **35**: 6337–6345.

Ambrose, D., J. H., Ellemder, C. H. S., Sprake, and R., Townsend, 1997. Thermodynamic Properties of Organic Oxygen Compounds, XLIII. Vapor Pressures of Some Ether. J. Chem. Thermodyn., **8**: 165-178.

Burbano, A. A., D. D., Dionysiou, T. L., Richardson, and M. T., Suidan, 2002. Degradation of MTBE intermediates using Fenton's reagent. J. Environ. Eng., **128**: 799-805.

Carter, W. P. L., E. C. ,Tuazon, and S. A., Aschmann, 1991. Investigation of the Atmospheric Chemistry of MTBE. Oil Air Quality Improvement Research Program, Atlanta, GA: 88-93.

Centi , G., 2002. Catalytic conversion of MTBE to biodegradation chemicals in contaminated water. Catalysis Today ., **75**(7): 69 – 76.

Church, C.D.P.G., J. F., Tratnyek, J. E., Pankow, A.L., Landmeyer, M. A., Baehr, A. S., Thomas, and M., Schimer,1999. Effects of Environmental Conditions on MTBE Degradation in Model Column Aquifers. Proceeding of a Technical Meeting. Report Number: 99-4018C. USGS Toxic Substances Hydrology Program. Charleston, S.C. **l3**: 93-101.

Corcho, D., R. J., Watkinson, and D. N., Lerner, 2000. Cometabolic degradation of MTBE by a cyclohexane-oxidising culture. Bioremediation and phytoremediation of chlorinated and recalcitrant compounds. In Wickramanayake, G. B., A. R. Gavaskar, B. C. Alleman and V. S. Magar Batelle Press, Columbus, Ohio. pp. 183–189.

Darwin, Lyew, Serge, R., Guiot, Feredric, Monto and Francois Fayolle., 2006. Comparison of different support materials for their capability to immobilize *mycobacterium austroafricanum* IFP 2012 and to adsorb MTME. Enzyme and Microbial Technology. **40** : 1524-1530.

Deeb, R.A.K.M., M., Scow, and L., Alvarez-Cohen, 2000. Aerobic MTBE Biodegradation: Examination of Past Studies. Current Challenges and Future Research Directions. Biodegradation **11**: 171-186.

Delta Environmental Consultants. 2004. Groundwater Oxygenate Cleanup Levels for LUST Sites. http://www.epa.gov/oust/mtbe/mtbetable.pdf.

Eweis, J. B., E. D., Schroeder, D. P.Y., Chang and K.M., Scow, 1998. Biodegradation of MTBE in a pilot-scale biofilter. Proceedings of Natural Attenuation: Chlorinated and recalcitrant Compounds, **1**: 18-21.

Fortin, N.Y., M.A., Deshusses, 1999. Treatment of Methyl tert-Butyl Ether Vapors in Biotrickling filters 2 Analaysis of the rate-limiting step and behavior under transient conditions. Environmental Science and Technology **17**: 2987-2991.

Francois, A., H., Mathis, D., Godefroy, P., Piveteau, F., Fayolle and F., Monot, 2002. Biodegradation of methyl tert-butyl ether and other fuel oxygenates by a new strain, Mycobacterium austroafricanum IFP 2012. App. Environ. Microbial, **68**: 2754- 2762.

Garnier, P.M., R., Auria, C., Augur and S., Revah, 1999. Cometabolism biodegradation of methyl tert-butyl ether by Pseudomonas aeruginosa grown on pentane. App. Microbiol. Biotechnol, **51**: 498–503.

Hanson, J.R., C.E., Ackerman, and K.M., Scow, 1999. Biodegradation of methyl tert-butyl ether by a bacterial pure culture. App. and Environ. Microbio, **65**: 4788-4792.

Hernandez-Perez, G., F., Fayolle, and J.P., Vandecasteele, 2001. Biodegradation of ethyl t-butyl ether (ETBE), methyl t-butyl ether (MTBE) and t-amyl methyl ether (TAME) by Gordonia terrae. App. Microbiol. Biotechnol, **55**: 117–121.

Hods, L . R ., Verstudy, A .W ., 2001 . Selecting Analytical Methods for Determination of Oxygenate in Environmental Samples and Gasoline . Pollution Engineering Online. **2** (1): 5 – 14 .

Hyman, M., P. K., Won, K., Williamson and M., O'Reilly, 1998. Cometabolism of MTBE by alkane-utilizing microorganisms. Natural attenuation: chlorinated and recalcitrant compounds. In Wickramanayake, G. B. and Hinchee R. E. Battelle Press, Columbus, Ohio : 321–326.

Hyman, M., 2000. Microbial co metabolism of MTBE. Presented at the MTBE Biodegradation Workshop, Cincinnati, OH, February 1-3:18-21.

Morales, M., M. A., Deshusses, S., Revah, 2000. Microcosm and Column Studies on the Biodegradation of Methyl Tert-Butyl Ether (MTBE) in Soil-Water Systems: 133-138.

Nikpey, A., Nikpey, M., and Kazemian, H., 2006. Removal of MTBE vapour from contaminated air streams using

different bacterial culture in biotrickling filters. Iranian Journal of Environmental Health, **3** (2) :117-122

Prah, J., EPA, D., Ashley, B., Blount, M., Case, T., Leavens, J., Pleil, and F., Cardinali. 2004. "Dermal, Oral, and Inhalation Pharmacokinetics of Methyl Tertiary Butyl Ether (MTBE) in Human Volunteers". Toxicological Sciences. **77**:195-205.

Pruden, A., M. T., Suidan, A.D., Venosa, G. J., Wilson, 2001. Biodegradation of Methyl Tert-Butyl Ether under Various Substrate Conditions. Environ. Sci. Technol., **35**: 4235– 4241.

Rula, A., Deeb, Kate, M., Scow, and Lisa, Alvarez, Cohen, 2004. Aerobic MTBE degradation: an examination of past studies, current challenges and future research direction. Journal of Biodegradation, **11**(2-3) : 171-185 .

Salanitro, J. P., and H. L., Wisniewski, 1997. Effects of MTBE on BTEX Biodegradation in Groundwater. Abstract. ASM 97TH General Meeting. Miami Beach. Florida.: 97-106.

Salanitro, J.P., L.A., Diaz, M.P., Williams, and H.L., Wisniewski, 2000. Isolation of a Bacterial Culture that Degrades Methyl T-Butyl Ether. App. Environ. Microbial, **60**: 2593-2596.

Schirmer, M., B. J., Butler, C. D., Church, J. F., Barker, and N., Nada rajah, 2003. Laboratory Evidence of MTBE Biodegradation in Borden Aquifer Material. J. Contam. Hydrol, **60**: 229–249.

Schmidt, T.C., M.., Schirmerb, H., Wei, and S. B., Haderleina, 2004. Microbial degradation of methyl *tert* butyl ether and tert-butyl alcohol in the subsurface. Journal of Contaminant Hydrology. **70** (3-4): 173-203.

Solano-Serena, F., R., Marchal, S., Casaregola, C., Vasnier, J., Lebeault, and J., Vandercasteele, 2000. A Mycobacterium strain with extended capacities for degradation of gasoline hydrocarbons. App. Environ. Microbial, **66**: 2392-2399.

Somsanak, P., R. M., Cowan, M. M., Haggblom, 2001. Anaerobic Biotransformation of Fuel Oxygenates Under Sulfate-Reducing Conditions. Fames. Microbiol. Ecol., **37**: 259–264.

Squillace, P. J., J. S., Zogorski, W.G., Wilber and C.V., Price, 1996. Preliminary assessment of the occurrence and possible sources of MTBE in groundwater in the United-States, 1993-1994. Environ. Sci. Technol., **30**: 1721-1730.

Steffan, R.J., K., McClay, S., Vainberg, C.W., Condee, and D., Zhang, 1997. Biodegradation of the gasoline oxygenates methyl tert-butyl ether, ethyl tert-butyl ether, and tert-amyl methyl ether by propane-oxidizing bacteria. App. Environ. Microbial, 63. pp: 4216– 4222.

Stringfellow, W.T., K.C., Oh, 2002. Initiation of MTBE Biotreatment in Fluidized-bed Bioreactors. J. Environ. Eng., **128**: 852–861.