Original article

Oxidation of toxic methyl *tert*-butyl ether (MTBE) by fungi and nanofilter

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How to cite this article:
Emtiazi G, Heydari E, Saleh T. Oxidation of toxic Methyl *tert*-butyl Ether (MTBE) by fungi and nanofilter. Jundishapur J Microbiol. 2010; 3(3): 99-106.

Received: March 2010 **Accepted:** April 2010

Abstract

Introduction and objective: Methyl *tert*-butyl Ether (MTBE) has been used in gasoline as a lead substitute. It is introduced into various environmental compartments during the production, distribution, use and storage of oxygenate-blended fuels. Nanofiltration, widely developed over the past decade, is a promising technology for the treatment of organic and inorganic pollutants. The aim of the present research was to study the efficiency of MTBE removal by collaboration of a nanofilter and fungi.

Materials and methods: In an experimental time of two hours and MTBE initial concentration of 20µl/ml, we investigated the effect of cell biomass, nanofilter and their collaboration on MTBE removal. Removal of MTBE was assayed with UV spectrum at 200-600 nm using chemical oxygen demand (COD) Hach reagent. The obtained blue green colour was measured by a turbidity measurement as (OD at 600 nm) in a UV-visible spectrophotometer against blank. The reduction of blue green colour showed the removal of MTBE.

Results: *Phanerochate chrysosporium* had positive growth on mineral salt media and MTBE as the only carbon sources, but *Aspergillus* did not grow on this media however produced small amount of formaldehyde from MTBE. The results have showed that the MTBE removal by *P. chrysosporium* (5mg/ml), nanofilter (1cm^2/ml) and *P. chrysosporium* with nanofilter were 53%, 47% and 91%, respectively.

Conclusion: The focus of this study was to recommend a new technique based on application of nanotechnology for bioremediation of MTBE as a complementary treatment system after preliminary treatment due to its high MTBE removal efficiency.

Keywords: Methyl *tert*-butyl Ether, Nanofiltration, *Phanerocheate chrysosporium*, Bioremediation, Nanofilter

Introduction

Among fuel oxygenates, methyl *tert*-butyl ether (MTBE) is most commonly used because of its high-octane level, low production cost, ease of blending with gasoline, and ease of transfer and

distribution [1,2]. Currently, an average of 11% MTBE by volume is added to about 30% of the gasoline sold in the United States [3]. Like most other gasoline components, MTBE is introduced into various environmental compartments during the production, distribution, use and storage of oxygenate-blended fuels [4].

Scientific information on the assessment of the carcinogenicity of MTBE in humans comes from animal investigations. However, the potential carcinogenic effect of MTBE on humans remains a matter of debate [5]. MTBE is poorly adsorbed, chemically and biologically stable, and very soluble in water, making it very persistent in the environment. Therefore, effective technologies are in an urgent demand to remove MTBE from contaminated water. Conventional treatment of MTBE-contaminated groundwater is inefficient and unsatisfactory. Air stripping is difficult and requires a high air-to-water ratio (>200/1 l for 95% removal) because of its very low Henry's law constant [6,7].

Several techniques are mainly used for MTBE removal, including physicochemical attenuation mechanisms and biodegradation. It can be treated biologically with special bacterial strains or natural isolates under aerobic condition. However, the bacteria grow slowly with low yields of biomass and are sometimes unstable. As a result, a reliable bioremediation process for MTBE has not been reported until now. In recent years, advanced oxidation processes (AOPs) on the other hand provide promising treatment alternative for MTBE. These processes rely on the generation of highly reactive hydroxyl radicals (OH) that result in oxidation and even complete mineralization of organic species. The chemical oxidation processes by UV/TiO_2 process, UV/H_2O_2 process [8,9], O_3/H_2O_2 process and Fenton's reagent [7] have been proposed for the degradation of MTBE in aqueous solution.

The adsorption process is a proven technology for the removal of synthetic organic compounds from water. Granular activated carbon (GAC), the most popular adsorbent used in water treatment, has been considered a candidate for the removal of MTBE from contaminated water. However, the low affinity of MTBE towards granular activated carbon makes this process undesirable and expensive [10]. Membrane separation technique is a promising technology for the treatment of organic and inorganic pollutants in surface and groundwater resources [11]. This process is utilized to clarify, concentrate and separate continuously molecular or ionic compounds from their solution state.

Nanofiltration (NF)-membranes separates nano-ranged solute particles. In the membrane classification, NF-membranes are to be positioned between reverse osmosis (RO) and ultrafiltration (UF) membranes. NF needs relatively tight skin membranes with very small pores (~0.2μm). Organic compounds in the 200 to 2000 molecular weight ranges can be separated [11]. Recently, nano zeolite composites, an inorganic porous material with good mechanical and hydrothermal stability, were used as one of the most competitive adsorbents for adsorbing MTBE from the aqueous phase [12].

The white-rot fungus *P. chrysosporium* has been shown to degrade a wide variety of xenobiotic compounds in addition to its natural substrate, lignin. Recalcitrant xenobiotics that *P. chrysosporium* can degrade include aromatic compounds, polycyclic aromatics, chlorinated aromatics, polycyclic chlorinated aromatics, and non-aromatic chlorinated compounds, as well as some naturally occurring biopolymers [13]. The purposes of this research were to: (i) investigate biodegradation of MTBE by *P. chrysosporium* and *Aspergillus niger*, (ii)

survey the effect of a commercial nanofilter (Nanopac, Iran) on MTBE removal and (iii) collaboration of both *P. chrysosporium* and nanofilter for MTBE removal.

Materials and methods

Microorganisms and culture condition

Phanerocheate chrysosporium and *A. niger* were used for MTBE removal. The cell biomass was obtained from inoculation of fungi on solid media, Yeast potato dextrose agar (YPD-agar, Merck, Germany). The spore harvested, washed and used for MTBE removal. In addition, these fungi were inoculated to potato dextrose broth (PDB, Merck, Germany) and incubated on shaker (INFORS AG, Switzerland) for three days at 37°C operated at 150rpm. The mycelium was centrifuged and 5mg/ml wet cells biomass were used for MTBE removal and compared with spores obtained on solid media.

Growth of fungi on MTBE

The basal medium contained (gl^{-1}) KH_2PO_4, 2; K_2HPO_4, 1; NH_4Cl 1; $MgCl_2$, 0.2; $CaCl_2$, 0.001 and $FeCl_3$, 0.004 and was supplemented with 20µl/ml MTBE as the sole source of carbon and energy. The pH of the medium was adjusted to 7. Basal media were sterilized at 110°C for 10min and then MTBE was added to sterile basal media. *P. chrysosporium* and *A. niger* were inoculated to solid media and incubated at 37°C for seven days.

Batch adsorption of MTBE by nanofilter

The adsorption capacities of $1cm^2$ of nanofilter (Nanopack, Iran) were studied in the batch adsorption experiments. To obtain isotherm data, the initial concentrations of the aqueous phase MTBE included the 20µl/ml, which might be encountered in the event of a major spill. The solution with nanofilter pad was incubated for six hours and compared with the blank without addition of nanofilter. Each sample was measured three times.

MTBE removal assay

Removal of MTBE was assayed with UV spectrum at 200-600nm and reaction with chemical oxygen demand (COD) Hach reagent (4.913g) $K_2Cr_2O_7$ was added to 500ml water with 167ml H_2SO_4 and 33.3g $HgSO_4$. This reagent was dissolved and diluted to 1000ml). 1ml of the cells grown on MTBE were added to 1ml digestion solution (COD Hach reagent), the obtained blue green color was measured by a turbidity measurement as (OD. at 600nm) in a UV-visible spectrophotometer (Shimadzu UV-160, Japan) against blank. The reduction of blue green colour showed the removal of MTBE.

Determination of formaldehyde production

The concentration of produced formaldehyde by studied fungi was measured by Hantzsch method [14]. Equal volumes of Hantzsch reagent (2M ammonium acetate, 50mM acetic acid, 20mM acetyle acetone) were added to 2ml of centrifuge cell biomass grown on nutrient broth and induced with MTBE (4000ppm) for 2h, this mixed incubated at 60°C for 10min. The obtained yellow colour of 3,5 diacetyl 1,4 dihydrolotidin which produced from reaction between formaldehyde and pentane 2,4 dion (acetyle acetone) in the present of ammonium acetate was centrifuged and measured at 412nm against blank.

Results

MTBE was assayed by dichromate (COD hach) reagent and the results of calibration line are shown in figure 1. 10-40µl of MTBE in reaction mixture (2ml) can be analyzed by dichromate reagent. *P. chrysosporium* (5mg/ml) was added to scaled cap tube with 20µl/ml MTBE. After 1h MTBE is removed by *P. chrysosporium*

(Fig. 2), this has been extended and after 4h, 50% of MTBE was removed by the studied fungi. The removal of MTBE was temperature dependent and at 4°C, the concentration of MTBE was constant during 6h (Fig. 3). MTBE is toxic to most bacteria and previous work showed none of isolated bacteria from MTBE enrichment soil had any significant growth on MTBE (unpublished data). Here the growth of two fungi on MTBE was tested on MTBE agar. Since MTBE is volatile, 20µl were added to paper filter (UV sterilized and placed on agar medium) every two days. The colony of *P. chrysosporium* was appeared after seven days; however, *A. niger* did not have any growth on MTBE media, we did not continue experiments on this fungus and quitted it. There was no growth for *P. chrysosporium* without addition of MTBE in the blank plate. Biomass of *P. chrysosporium* (5mg/ml) produced formaldehyde from MTBE (Fig. 4).

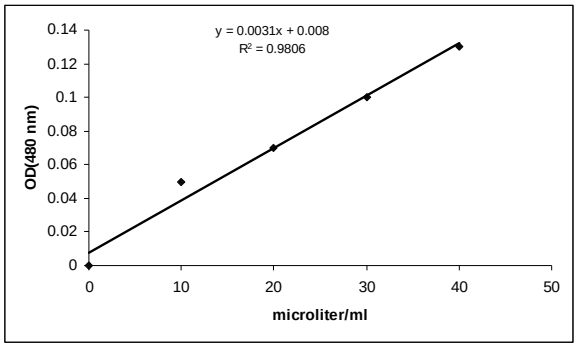

Fig. 1: Calibration line of MTBE assay carried out with dichromate COD reagent

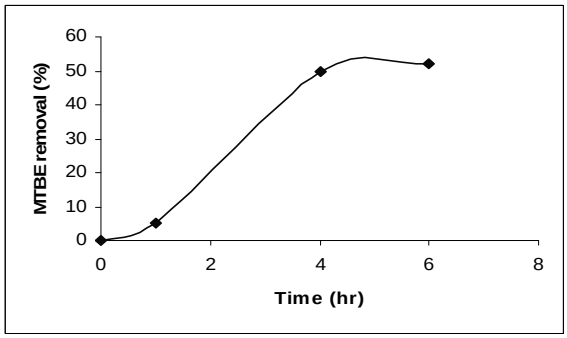

Fig. 2: The removal of MTBE by *P. chrysosporium* (5mg/ml)

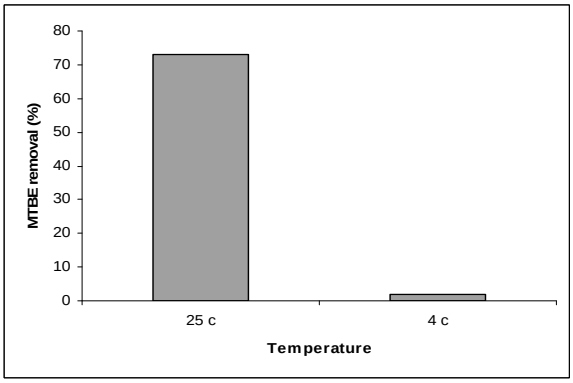

Fig. 3: The effect of temperature on MTBE removal by *P. chrysosporium*

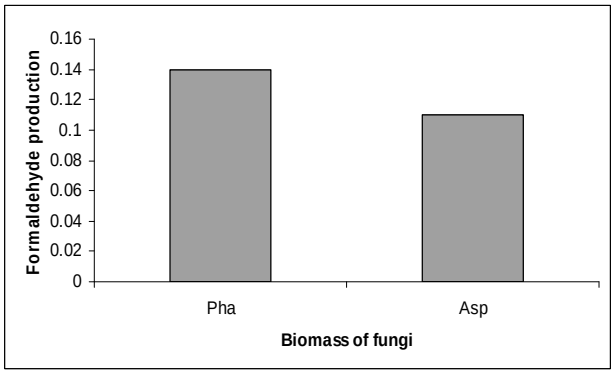

Fig. 4: Production of formaldehyde by *P. chrysosporium* and *A. niger* biomass cells from MTBE

In this work, 47% of MTBE was removed by 1cm^2 of nanofilter. Collaboration of *P. chrysosporium* (5mg/ml) and nanofilter (Fig 5) showed that MTBE removal was 91% during 2h (Table 1). Complementary experiments showed that MTBE oxidized to TBA that was probably due to nanosilver particles of nanofilter that could react like Fenton's reagent.

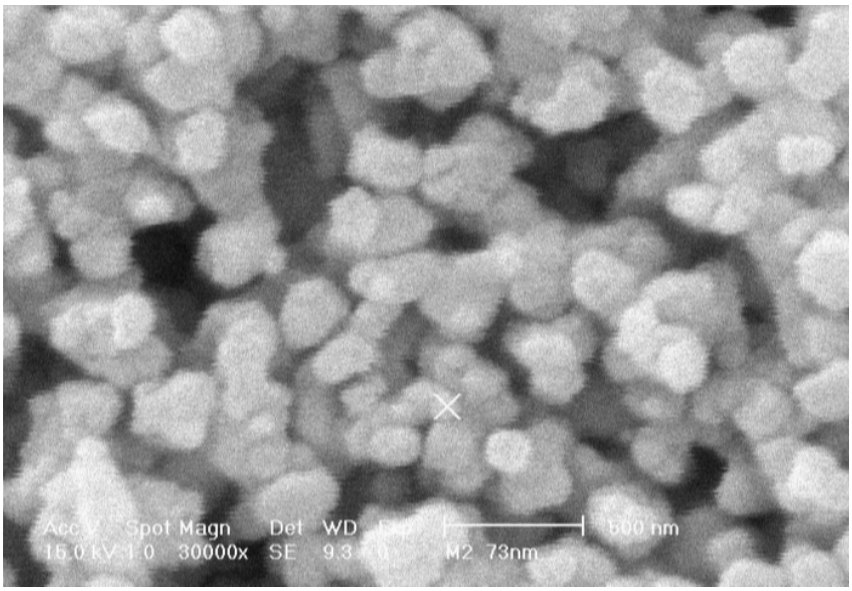

Fig. 5: Scanning electron microscopy image of nano particle sprayed on filter

Table 1: Removal of MTBE (20µl/ml) by fungi (5mg/ml) and nanofilter (1cm^2)

Treatment	MTBE removal (%)
Blank MTBE 40ml	0
MTBE+ *Phanerocheat* (5mg/ml)	53
MTBE+ Spore of *Phanerocheate* (5mg/ml)	51
MTBE+ Nanofilter	47
MTBE+ Nanofilter + fungi (5mg/ml)	91

Discussion

One of the most effective means to eliminate organic compounds and gases is adsorption specifically adsorption using activated carbon. Adsorption is the physics of attracting and retaining molecules of the undesirable compound on the surface. MTBE is solubilized in water and these properties facilitated vapor transfer of MTBE into the aqueous phase. In surface water, volatilization is very fast. Therefore, nanofilters are effective for the control of gases and odors of MTBE. Several techniques are mainly used for MTBE remediation such as physio-chemical and biological mechanisms. Over the past few years, reports discussing the use of advanced oxidation processes (AOPs) as means of remediating organic chemicals, including MTBE, have emerged in the literature [15]. AOPs typically use ozone alone, ozone and ultraviolet (UV) radiation, or ozone and hydrogen peroxide (H_2O_2) [19].

The AOP reactions generate the highly reactive hydroxyl radical (•OH), which then oxidizes MTBE [9]. Zang and Farnood [16] showed the photocatalytic degradation of methyl *tert*-butyl ether (MTBE) in the aqueous slurry of titanium dioxide (TiO_2) particles irradiated with xenon lamp in a batch reactor, in the presence and absence of hydrogen peroxide. Another effective means to eliminate organic compounds and gases is adsorption, specifically adsorption using activated carbon. Adsorption processes, moreover, have the advantage of ease in use and are easy to combine with other treatment technologies. Granular activated carbon (GAC), the most popular

adsorbent used in water treatment, has been considered a candidate for the removal of MTBE from contaminated water.

The removal of MTBE by the usual methods such as adsorption by activated carbon (in powdered or granular form) or oxidation has some disadvantages. The limitation of carbon filters is that the filter saturates rapidly. That is why the cost is high due to the frequent change and/or regeneration of the carbon filter. Recently the removal of organic pollutants by membrane retention (reverse osmosis, ultrafiltration and nanofiltration) has become increasingly popular. Since UV radiation, hydrogen peroxide and ozone are expensive and toxic, also have hazardous properties; therefore, nanofilters are low cost, continuous and non toxic could be interesting options for MTBE removal.

Nano zeolite composites of selective supports have been attempted on removing MTBE from water system by Lu *et al*. [12]. In the case of biological degradation of MTBE, many studies have reported that MTBE is recalcitrant under both aerobic and anaerobic conditions [17,18]. However, recent studies have reported the ability of several microorganisms from various environmental sources to degrade MTBE either as the sole source of carbon and energy or cometabolically. Many of these investigations show that the cellular yield of microorganisms utilizing MTBE as the sole organic carbon source can be expected to be very low [3,20].

The same results were obtained in previous study which three isolated bacterial strains have been observed to grow at a relatively very slow rate and did not have any significant growth on MTBE. The present research relates to the degradation of MTBE using the white rot fungus, *P. chrysosporium,* under the nutrient, carbon and nitrogen source rich and non-ligninolytic conditions.

More recently, a number of chloroaromatic pollutants were shown to be degraded by *P. chrysosporium* under nonligninolytic culture conditions (such as in defined high-N medium or nutrient-rich malt extract medium) when lignin peroxidases (LIPs) and Mn (II)-dependent peroxidases (MNPs) are not produced [21]. Kay-Shoemake and Watwood [22] showed that no significant degradation of MTBE was observed under ligninolytic cultures of *P. chrysosporium*. Their results demonstrated the oxidative limitations of the lignin peroxidase enzyme system in which the ether compounds such as MTBE were present as sole carbon source. Therefore, in this research degradation of MTBE was carried out under nutrient rich, non-ligninolytic conditions using *P. chrysosporium.*

Phanerocheate chrysosporium has a good capacity to remove MTBE mostly with co metabolism growth on aromatic compound, but in the nanofilter/*P. chrysosporium* system, nanofilter enhances MTBE remediation by fungus. Our thought is that this nanofilter contains nanosilver particles that can react like Fenton's reagent to oxide the MTBE to TBA. MTBE oxidation has been reported by UV radiation, hydrogen peroxide and ozone but they are toxic, hazardous and also not cost-effective. However application of nanofilter that contains low concentration of nanosilver particles is inexpensive, continuous and non toxic which is an interesting option for MTBE oxidation.

This is the first conclusive demonstration of the growth of *P. chrysosporium* on MTBE as the sole source of carbon and energy. In particular, the present study demonstrated degradation of MTBE by *P. chrysosporium* in higher rate in comparison with previous studied microorganisms, and formaldehyde is the byproduct of MTBE degradation by *P. chrysosporium.* The collaboration of

nanofilter and *P. chrysosporium* on MTBE degradation has not been documented yet.

Conclusion

Therefore, the objective of present work was to study the MTBE degradation rate in the presence of nanofilter, *P. chrysosporium* alone and nanofilter/*P. chrysosporium* system. The focus of this work was to recommend a new technique based on application of nanotechnology for the bioremediation of MTBE as a complementary treatment system after preliminary treatment due to its high MTBE removal efficiency.

Acknowledgment

The financial support from the Pars Oil and Gas Company of Iran is acknowledged (Grant No. 861010).

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