Bioremediation of methyl tertiary-butyl ether (MTBE) by three pure bacterial cultures

Zabihollah Yousefi1*, Zeinab Tahernezhad1, Seyed Noroddin Mousavinasab2, Reza Safari1, Ahmadreza Bekhradnia2

1Department of Environmental Health Engineering, School of Public Health, Mazandaran University of Medical Sciences, Sari, Iran
2Department of Biostatistics, School of Public Health, Mazandaran University of Medical Sciences, Sari, Iran
3Iranian Fisheries Science Research Institute (IFRSI), Caspian Sea Ecology Research Center, Agricultural Research Education and Extension Organization (AREEO), Sari, Iran
4Department of Medicinal Chemistry, Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences, Sari, Iran

Abstract
Background: Bioremediation of groundwater and soil contamination is more economical than physicochemical remediation. The present study focused on the bioremediation capability of two bacterial species (Klebsiella planticola and Enterobacter cloacae) from the family Enterobacteriaceae. These bacteria have been identified as new species with capability of degrading methyl tertiary-butyl ether (MTBE). In order to enhance their degradation capability, selected concentrations and retention time were investigated.

Methods: The bacteria were cultured on the nutrient agar (NA) medium at room temperature. pH of the medium was adjusted to 7. The medium was autoclaved at 121°C for 15 minutes and incubated for 24 hours at 35°C. After 24 hours, the mixture was inoculated into 50 mL of Luria Bertani (LB) liquid medium containing 50 and 150 ppm MTBE. The cultures were incubated for 2 and 5 days at 35°C and shaken on a shaker at 150 rpm. Cell concentrations of the bacteria in pure culture were determined from the optical density at 600 nm using a UV–VIS spectrophotometer. Then, the culture was centrifuged at 3800 rpm for 20 minutes. In the next step, the MTBE concentration in the supernatant was measured by gas chromatography/mass spectrometry (GC/MS, Agilent Technologies, 5975, US10304411, 5.02.07).

Results: The results showed that both strains are able to grow in the presence of 50 and 150 ppm MTBE. In the best conditions, when cell density was 3×108 CFU/mL during 5 days, the highest rate of MTBE degradation for K. planticola and E. cloacae, was 43% and 40%, respectively. It was also revealed that Escherichia coli can degrade 50 and 150 ppm MTBE about 19.8% and 13.65%, respectively.

Conclusion: It seems that E. coli can be a good candidate for MTBE degradation at high concentrations for a time longer than that in the present study. It was also found that the species have high performance at 50 ppm than 150 ppm. So, these bacteria can remove MTBE from the environment.

Keywords: Biodegradation, Klebsiella planticola, Enterobacter cloacae, Escherichia coli, methyl tertiary-butyl ether

Citation: Yousefi Z, Tahernezhad Z, Mousavinasab SN, Safari R, Bekhradnia A. Bioremediation of methyl tertiary-butyl ether (MTBE) by three pure bacterial cultures. Environmental Health Engineering and Management Journal 2018; 5(2): 123–128. doi: 10.15171/EHEM.2018.17.

Introduction
Unleaded gasoline consists hydrocarbons and different chemical compounds such as methyl tertiary-butyl ether (MTBE) (1,2). MTBE (C8H18O) is an oxygenate organic compound, that has been used as additive in gasoline since the late 1970s, to replace tetraethyl lead (TEL) and other toxic chemicals (3-5). MTBE is a persistent compound in the environment because it is highly soluble in water, poorly adsorbed by soil and is biologically and chemically stable against degradation (6). Accidental fuel leakage during storage or transportation is the main source of environmental contamination with MTBE (7). Therefore, the presence of MTBE in water is responsible for taste and odor related issues, genotoxicity and skin and eye irritation. Taste and odor thresholds for MTBE are 20-40 ppb (8,9). Generally, MTBE is the most common oxygenate compound, because it is cost-effective and easy-to-use (7). Due to its economic benefits, bioremediation with 99% efficiency, is a more attractive option than physicochemical remediation technologies such as ozone utilization, activated carbon, vaporization extraction and other methods (3,7,10-12). All microorganisms are not able to degrade MTBE easily (13). Some bacteria such as Pseudomonas, Rhodococcus, Mycobacterium, Enterobacter,
and Achromobacter are capable of degrading MTBE co-
metabolically but not tert-Butyl Alcohol (TBA) (1). A
few bacterial strains such as Methylibium petroleiphilum
PM1, Hydrogenophaga flava ENV735, Achromobacter
xyllosodans MCM 1/11, Pseudomonas sp., Bacillus sp.,
and Streptococcus sp. can utilize MTBE as the sole source
of carbon and energy (14-18). Two bacterial isolates
(ISO11 and ISO2A) degraded MTBE in both nutrient-rich
and nutrient-limited media. The highest rate of MTBE
degradation was reported 29.6% and 27.8%, respectively,
in 28 days (19). Researchers reported that bacteria
such as A. xyllosodans MCM 1/11 can use MTBE in 7
days (17). In this study, bioremediation of MTBE by
Klebsiella planticola and Enterobacter cloacae at laboratory
conditions was investigated. On the other hand, according
to the abundance of Escherichia coli and its capability to
utilize a wide range of hydrocarbons while the engineered
E. coli was used for bioremediation, therefore, MTBE-
degrading capacity of this bacterium was also compared.
MTBE is one of the gasoline components that, nowadays,
is spreading in the environment and can pollute soil, water,
and groundwater. The influence of microbial degradation
of organic substances and MTBE is well known (17-19).
Many studies have been conducted on the MTBE
biodegradation by pure bacterial cultures such as Bacillus
cereus and Klebsiella terrigena, Enterobacter sp. NKNUO2,
and other microorganisms (7,14-17,20). The first step in
bioremediation is selecting the best bacteria because only
some bacteria can use MTBE as a source of carbon and
energy. A few pure or mixed bacterial cultures can grow
on MTBE and use it as a carbon and energy source and
some strains grow slowly on this oxygenated compound
with low cell yields (21). Due to the complex molecular
structure of MTBE, this compound is resistant to
biodegradation because its ether bond and tertiary carbon
atom are relatively unreactive (21-23). The toxic effects of
products produced during metabolism can cause it.
The intermediate products of MTBE biodegradation are
tert-butoxy methanol (TBM), formaldehyde and TBA,
respectively (23-25). Steffan et al demonstrated that the
growth rates of propane-oxidizing bacteria on MTBE is
very slow. Numerous microorganisms including EVN 735,
Variorovax paradoxus CL-8, Chryseobacterium sp. A-3,
B. cereus, K. terrigena, Enterobacter sp., and NKNUO2,
have also the capability to remove MTBE from the
environment (12,20,26-30). The biological degradation of
MTBE and most of the organic matter is nowadays known
in science (31,32). In a study by Salanitro et al, biomass
yields (gram of dry weight cells per gram of MTBE) were
0.21 to 0.28 (32). Some studies have also investigated
MTBE biodegradation by Mycobacterium (33,34). But so
far, no study has been conducted to investigate the role
of K. planticola in bioremediation of MTBE, while some
species of Klebsiella have been found to be capable of
utilizing MTBE, n-hexadecane, and other hydrocarbons
contaminating soil (20,35,36).

Materials and Methods
Materials
MTBE (GC purity ≥98%) was purchased from Persian
Type Culture Collection (PTCC). Other chemicals were
analytical grade and purchased from Merck (Darmstadt,
Germany).
After sterilization and passing through a 2-mm mesh
sieve, specific concentrations of MTBE were added to 10
g of soil, and the growth rate of microorganisms and
the concentration of MTBE were measured.

Microorganisms and incubation conditions
In order to determine a strain capable of growing on
MTBE, two concentrations and two retention time were examined. K. planticola and E. cloacae were purchased from the PTCC. Then, these bacteria were cultured on the nutrient agar (NA) medium at room temperature. The composition of NA was as follows (gr\(^{-1}\)): 0.5% Peptone, 0.3% beef extract/yeast extract, 1.5% agar, 0.5% sodium chloride, and distilled water. pH of the medium was adjusted to 7. The medium was autoclaved at 121°C for 15
minutes. In the next step, the cultures were incubated for
24 hours at 35°C. After 24 hours, the inoculum density at the beginning of the test were 1.5×10\(^{5}\) CFU/mL and 3×10\(^{5}\)
CFU/mL, the mixture were then inoculated into 50 mL
of Luria Bertani (LB) liquid medium containing 50 and
150 ppm MTBE (based on pretest) (12). LB medium was
composed of (g/L): 10 g/L Trypton, 5 g/L yeast extinction,
and 10 g/L NaCl. Then, the medium was autoclaved at
121°C for 15 minutes at pH 7. The cultures were incubated
2-5 days at 35°C in a shaker incubator at 150 rpm. Cell
centrifugations of K. planticola and E. cloacae in pure
culture were determined from the optical density at 600
nm using a UV–VIS spectrophotometer (Hach model).
Then, the culture was centrifuged in sealed tubes and cells
were harvested from the medium by centrifugation at 3800
rpm for 20 minutes. Afterwards, the MTBE concentration
in the supernatant was measured by gas chromatography/
mass spectrometry (GC/MS, Agilent Technologies, 5975,
US10304411, 5.02.07). The conditions for the GC analysis
were as follows: 45°C held for 4 minutes, temperature
ramped from 16°C/min to 70°C for 4.37 minutes, from
22°C/min to 100°C for 1.36 minutes, and 28°C/min to
220°C for 4.28 minutes (1,18,22). Helium was used as the
carrier gas with an approximate flow rate of 1.10 mL/
min. The sample without microorganisms was applied as
blank in all tests. All specimens were tested two times. In
this study, 38 samples were evaluated. Data were analyzed
using statistical tests such as ANOVA, correlation,
regression, and etc.

Results
During the incubation periods (2 and 5 days), K. planticola
and E. cloacae were capable of growing on MTBE, as the
source of carbon and energy, while initial concentrations of
MTBE were studied. The removal rate of MTBE by two
pure bacterial cultures and the growth rate of two bacterial species at different concentrations of MTBE included in soil samples are shown in Table 1. Biodegradation of 50 ppm MTBE by \( K.\ planticola \) and \( E.\ cloacae \) with inoculum sizes of \( 3 \times 10^8 \) CFU/mL is displayed in Figure 1. As shown in Figure 1, \( K.\ planticola \) and \( E.\ cloacae \) respectively indicated 43% and 40% MTBE degradation, in 120 hours detention time. In addition, the bacteria showed 24.6% and 29% MTBE degradation after 48 hours.

Biodegradation of 150 ppm MTBE by \( K.\ planticola \) and \( E.\ cloacae \) with inoculum size of \( 3 \times 10^8 \) CFU/mL, is displayed in Figure 2. As shown in Figure 2, \( K.\ planticola \) and \( E.\ cloacae \) respectively indicated 30.95% and 33.50% MTBE degradation, in 120 hours detention time. The bacteria also showed 19.95% and 24.30% MTBE degradation after 48 hours.

Biodegradation of 150 ppm MTBE by \( E.\ coli \) with inoculum size of \( 3 \times 10^8 \) CFU/mL, is presented in Figure 3. As presented in Figure 3, \( E.\ coli \) with different inoculum sizes of \( 1.5 \times 10^8 \) and \( 3 \times 10^8 \) CFU/mL showed 13% and 19.8% MTBE degradation, respectively, in 120 hours detention time. The bacteria also showed 9% and 6.5% MTBE degradation after 48 hours.

**Discussion**

The bacteria were capable to grow in all samples while the growth rate of the bacteria and MTBE degradation rate were different. The results showed that both bacterial species, in the similar conditions, could use initial MTBE concentration of 50 ppm better than the concentration of 150 ppm. In a similar study by Okeke et al, concentration

### Table 1. Biodegradation of MTBE by \( Klebsiella\ planticola \) and \( Enterobacter\ cloacae \)

| Bacterial species | Initial concentration of MTBE (ppm) | Initial amount of bacteria (CFU/mL) | Time: 2 days | Time: 5 days |
|-------------------|----------------------------------|-----------------------------------|-------------|-------------|
|                   |                                  | Removal percentage (%) | Residual value of concentration (ppm) | Removal percentage (%) | Residual value of concentration (ppm) |
| \( K.\ planticola \) | 50                               | \( 1.5 \times 10^8 \) | 11.8 | 44.1 | 19 | 40.5 |
|                   |                                  | \( 3 \times 10^8 \) | 29 | 35.5 | 43 | 21.5 |
|                   | 150                              | \( 1.5 \times 10^3 \) | 6.95 | 139.5 | 12.96 | 130.5 |
|                   |                                  | \( 3 \times 10^3 \) | 24.3 | 12.5 | 33.5 | 99.5 |
| \( E.\ cloacae \)  | 50                               | \( 1.5 \times 10^4 \) | 9.1 | 45.45 | 16.3 | 41.85 |
|                   |                                  | \( 3 \times 10^4 \) | 24.6 | 37.7 | 40 | 30.05 |
|                   | 150                              | \( 1.5 \times 10^3 \) | 5.3 | 142 | 11.9 | 132.15 |
|                   |                                  | \( 3 \times 10^3 \) | 15.95 | 126 | 30.95 | 103.5 |
| \( E.\ coli \)    | 50                               | \( 1.5 \times 10^8 \) | 9.1 | 45.45 | 16.3 | 41.85 |
|                   |                                  | \( 3 \times 10^8 \) | 24.6 | 37.7 | 40 | 30.05 |
|                   | 150                              | \( 1.5 \times 10^8 \) | 5.3 | 142 | 11.9 | 132.15 |

**Figure 1.** Biodegradation of 50 ppm MTBE by \( Klebsiella\ planticola \) and \( Enterobacter\ cloacae \) with inoculum size of \( 3 \times 10^8 \) CFU/mL. White column, optical density of \( K.\ planticola \) after 48 and 120 hours and black column, optical density of \( E.\ cloacae \) after 48 and 120 hours.

**Figure 2.** Biodegradation of 150 ppm MTBE by \( Klebsiella\ planticola \) and \( Enterobacter\ cloacae \) with inoculum size of \( 3 \times 10^8 \) CFU/mL. White column, optical density of \( K.\ planticola \) after 48 and 120 hours and black column, optical density of \( E.\ cloacae \) after 48 and 120 hours.
E. cloacae species play an important role in the MTBE biodegradation. They also showed that E. cloacae MCM2/1 has a high potential for utilizing MTBE (39). In the first hours, degradation rate by K. planticola was higher than other strains, and it seems that E. coli needs more time for degrading MTBE (Figure 3), it can be due to the complex molecular structure of MTBE, while the cultures containing yeast extract, this compound makes a good condition for better growth of the strains (19). Generally, there was no difference in the growth rate of both strains (K. planticola and E. cloacae) at different concentrations of MTBE. Besides, degradation rate was lower when the inoculum size was 1.5×10⁶ CFU/mL (Table 1). The results of this study show that there is a direct relation between inoculum size and MTBE degradation. It should be noted that when the inoculum size increases, it uses more oxygen (12), which is consistent with the study by Rui-Ling et al. They reported that when cell density was 2×10⁸ CFU/mL, the species had better performance than when it was 4.5×10⁶ CFU/mL.

Conclusion
Klebsiella planticola, E. cloacae and E. coli could degrade MTBE at different concentrations and different cell densities in different times. Therefore, these strains are good candidates for removing MTBE from the environment. However, further studies using optimized media with other compounds for MTBE removal by these strains are suggested.

Acknowledgments
The authors would like to gratitude the Research Deputy of Mazandaran University of Medical Sciences for funding this study. And special thanks to P. Gil, Masoumeh Eslamifar and Masoumali Movahedi who helped us as research co-operators to perform this research.

Ethical issues
It is confirmed that this manuscript is the original work of the authors. The authors certify that all data collected during the study are presented in this manuscript, and no data from the study has been or will be published separately.

Competing interests
The authors declare that they have no conflicts of interests.

Authors’ contribution
All authors contributed in data collection, analysis, and interpretation. All authors reviewed, refined, and approved the manuscript.

References
1. Guisado IM, Purswani J, Gonzalez-Lopez J, Pozo C. Physiological and genetic screening methods for the isolation of methyl tert-butyl ether-degrading bacteria for
Yousefi et al

bioremediation purposes. Int Biodeterior Biodegradation 2015; 97: 67-74. doi: 10.1016/j.ibiod.2014.11.008.

2. Schmidt TC, Haderlein SB, Pfister R, Forster R. Occurrence and fate modeling of MTBE and BTX compounds in a Swiss Lake used as drinking water supply. Water Res 2004; 38(6): 1520-9. doi: 10.1016/j.watres.2003.12.027.

3. Levchuk I, Bhatakar A, Sillanpaa M. Overview of technologies for removal of methyl tert-butyl ether (MTBE) from water. Sci Total Environ 2014; 476-477: 415-33. doi: 10.1016/j.scitotenv.2014.01.037.

4. The U.S. Environmental Protection Agency. MTBE in Fuels [cited 2017 Oct 23]. Available from: http://www.epa.gov/mtbe/gas.htm.

5. Morse PM. Producers brace for MTBE Phaseout. Chem Eng News 1999; 77(15): 26-7. doi: 10.1021/cen-v077n015.p026.

6. Soltani B, Moheb A. Ultrasound irradiation facilitated adsorption of MTBE from aqueous solution using exfoliated graphite. Chem Eng Technol 2010; 33(7): 1107-11. doi: 10.1002/ceat.200900345.

7. Chen CS, Tien CJ, Zhan KV. Evaluation of intrinsic bioremediation of methyl tert-butyl ether (MTBE) contaminated groundwater. Journal of Soil and Groundwater Environment 2014; 19(5): 9-17. doi: 10.7857/jzsche.2014.19.5.009.

8. Moreels D, Bastiaensen L, Ollevier F, Merckx R, Diels L, Springael D. Evaluation of the intrinsic methyl tert-butyl ether (MTBE) biodegradation potential of hydrocarbon contaminated subsurface soils in batch microcosm systems. FEMS Microbiol Ecol 2004; 49(1): 121-8. doi: 10.1016/j.femsec.2004.02.016.

9. Young WF, Horth H, Crane R, Ogden T, Arnott M. Taste and odour concentration thresholds of potential potable water contaminants. Wat Res 1996; 30(2): 331-40. doi: 10.1016/0043-1354(95)00173-5.

10. Nau A, Kohl S, Zanithoff HW, Wierdhold H, Vogel H. Ozonized activated carbon as catalyst for MTBE-cleavage. Appl Catal A Gen 2011; 397: 103-111.

11. U.S. Environmental Protection Agency. Technologies for Treating MTBE and Other Fuel Oxygenates. Washington, DC: Agency Office of Solid Waste and Emergency Response Office of Superfund Remediation and Technology Innovation; 2004.

12. Zhang RL, Huang GQ, Lian JY, Li XG. Degradation of MTBE and TBA by a new isolate from MTBE-contaminated soil. J Environ Sci (China) 2007; 19(9): 1120-4.

13. Davis LC, Erickson LE. A review of bioremediation and a natural attenuation of MTBE. Environmental Progress 2004; 23(3): 243-52. doi: 10.1002/ep.10028.

14. Nakatsu CH, Hristova K, Hanada S, Meng XY, Hanson JR, Scow KM, et al. Methylbium petroleiphilum gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the Betaproteobacteria. Int J Syst Evol Microbiol 2006; 56(Pt 5): 983-9. doi: 10.1099/ijs.0.063524-0.

15. Streger SH, Vainberg S, Dong H, Hatzinger PB. Enhancing transport of hydrogenophaga flav. ENV735 for bioaugmentation of aquifers contaminated with methyl tert-butyl ether. Appl Environ Microbiol 2002; 68(11): 5571-9.

16. Lee EH, Cho KS. Effect of substrate interaction on the degradation of methyl tert-butyl ether, benzene, toluene, ethylbenzene, and xylene by Rhodococcus sp. J Hazard Mater 2009; 167(1-3): 669-74. doi: 10.1016/j.jhazmat.2009.01.035.

17. Eixarch H, Constanti M. Biodegradation of MTBE by Achromobacter xylosidans MCM1/1 induces synthesis of proteins that may be related to cell survival. Process Biochem 2010; 45(5): 794-8. doi: 10.1016/j.procbio.2009.12.015.

18. Okeke BC, Frankenberger WT Jr. Biodegradation of methyl tertiary butyl ether (MTBE) by a bacterial enrichment consortia and its monoculture isolates. Microbiol Res 2003; 158(2): 99-106. doi: 10.1078/0944-5013-00181.

19. Makut MD, Ishaya P. Bacterial species associated with soils contaminated with used petroleum products in Keffi town, Nigeria. Afr J Microbiol Res 2010; 4(16): 1698-702.

20. Nasrollahzadeh HS, Najafpour GD, Aghamohammadi N. Biodegradation of phenanthrene by mixed culture consortia in batch bioreactor using central composite face-entered design. Int J Environ Res 2007; 1(2): 80-87.

21. Talae AR, Jafaarzehe N, Talae MR, Beheshti M. Biodegradation of aromatic compounds in crude oil by isolated microorganisms from environment. J Zanjan Univ Med Sci 2010; 18(70): 68-80. [In Persian].

22. Arabi R, Bemanian SH, Taherzadeh MJ. Rapid biodegradation of Methyl tert – Butyl Ether (MTBE) by pure bacterial cultures. Iran J Chem Chem Eng 2007; 26(1): 1-7.

23. Francois A, Mathis H, Godfroy D, Pivetear P, Fayolle F, Monot F. Biodegradation of methyl tert-butyl ether and other fuel oxygenates by a new strain, Mycobacterium austroafricanum IFP 2012. Appl Environ Microbiol 2002; 68(6): 2754-62.

24. Smith CA, O’Reilly KT, Hyman MR. Characterization of the initial reactions during the cometabolic oxidation of methyl tert-butyl ether by propane-grown Mycobacterium vaccae JOB5. Appl Environ Microbiol 2003; 69(2): 796-804.

25. H.Hatzinger PB, McClay K, Vainberg S, Tugusheva M, Condee CW, Steffan RJ. Biodegradation of Methyl tert – Butyl Ether by a pure bacterial culture. Appl Environ Microbiol 2001; 67(12): 5601-7. doi: 10.1128/AEM.67.12.5601–5607.2001

26. Zaitsev GM, Uotila JS, Haggblom MM. Biodegradation of methyl tert-butyl ether by cold-adapted mixed and pure bacterial cultures. Appl Microbiol Biotechnol 2007; 74(5): 1092-102. doi: 10.1007/s00253-006-0737-3.

27. Chen SC, Chen CS, Zhan KV, Yang KH, Chien CC, Shieh BS, et al. Biodegradation of methyl tert-butyl ether (MTBE) by Enterobacter sp. NKKNU02. J Hazard Mater 2011; 186(2-3): 1744-50. doi: 10.1016/j.jhazmat.2010.12.079.

28. Hanson JR, Ackerman CE, Scow KM. Biodegradation of methyl tert-butyl ether by a bacterial pure culture. Appl Environ Microbiol 1999; 65(11): 4788-92.

29. Hatzinger PB, McClay K, Vainberg S, Tugusheva M, Condee CW, Steffan RJ. Biodegradation of methyl tert-butyl ether by a pure bacterial culture. Appl Environ Microbiol 2001; 67(12): 5601-7. doi: 10.1128/AEM.67.12.5601–5607.2001

30. Hansson JR, Ackerman CE, Scow KM. Biodegradation of methyl tert-butyl ether by bacterial pure culture. Appl Environ Microbiol 1999; 65(11): 4788-92.
31. Mo K, Lora CO, Wanken AE, Javanmardian M, Yang X, Kulpa CF. Biodegradation of methyl t-butyl ether by pure bacterial cultures. Appl Microbiol Biotechnol 1997; 47: 69–72.

32. Salanitro JP, Diaz LA, Williams MP, Wisniewski HL. Isolation of a bacterial culture that degrades methyl t-Butyl ether. Appl Environ Microbiol 1994; 60(7): 2593-6.

33. Fayolle F, Vandecasteele JP, Monot F. Microbial degradation and fate in the environment of methyl tert-butyl ether and related fuel oxygenates. Appl Microbiol Biotechnol 2001; 56(3-4): 339-49.

34. Alfonso-Gordillo G, Flores-Ortiz CM, Morales-Barrera L, Cristiani-Urbina E. Biodegradation of methyl tertiary butyl ether (MTBE) by a microbial consortium in a continuous up-flow packed-bed biofilm reactor: kinetic study, metabolite identification and toxicity bioassays. PLoS One 2016; 11(12): e0167494. doi: 10.1371/journal.pone.0167494.

35. Nduka JK, Umeh LN, Okerulu IO, Umedum LN, Okoye HN. Utilization of different microbes in bioremediation of hydrocarbon contaminated soils stimulated with inorganic and organic fertilizers. J Pet Environ Biotechnol 2012; 3(2): 1-9. doi: 10.4172/2157-7463.1000116.

36. Dutra ES, Pascon RC, Vallim MA. Sao Paulo Zoo composting as a source of bacteria with bioremediation potential. Afr J Microbiol Res 2013; 7(45): 5200-6. doi: 10.5897/AJMR2013.5874.

37. Lalevic B, Raicevic V, Kikovic D, Jovanovic L, Surlan-Momirovic G, Jovic J, et al. Biodegradation of MTBE by bacteria isolated from oil hydrocarbons contaminated environments. Int J Environ Res 2012; 6(1): 81-6. doi: 10.22059/ijer.2011.474.

38. Abbaspour M, Javid AH, Jalilzadeh Yengjeh R, Hassani AH, Ghavam Mostafavi P. The Biodegradation of methyl tert-Butyl ether (MTBE) by indigenous Bacillus cereus strain RJ1 isolated from soil. Petroleum Science and Technology 2013; 31(18): 1835-41. doi: 10.1080/10916466.2011.611562.

39. Jose Barbera M, Mateo E, Monkaityte R, Constanti M. Biodegradation of methyl tert-butyl ether by newly identified soil microorganisms in a simple mineral solution. World J Microbiol Biotechnol 2011; 27(4): 813-21. doi: 10.1007/s11274-010-0522-4.