



ENHANCED REMOVAL OF METHYL TERT-BUTYL ETHER BY A BACTERIAL CONSORTIUM SUPPLEMENTED WITH YEAST EXTRACT

ELIMINACIÓN MEJORADA DE METIL TERC-BUTIL ÉTER POR UN CONSORCIO BACTERIANO SUPLEMENTADO CON EXTRACTO DE LEVADURA

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Abstract

Methyl tert-butyl ether (MTBE) is highly soluble in water and considered a contaminant. Although MTBE can be removed by microbial consortia its removal efficiency is low due to its toxic effect on microbial growth. An alternative to enhance the MTBE degradation efficiency is by cometabolism. Yeast extract (cometabolite) was studied in the EB-MTBE-24 consortium to enhance the MTBE degradation efficiency in cultures at 105 mg L⁻¹ of MTBE and in cultures at 105 mg L⁻¹ of MTBE supplemented with 100 and 500 mg L⁻¹ of yeast extract (100YE and 500YE, respectively). The 100YE culture presented higher values of $\mu_{max} = 0.07 \pm 0.02 \text{ h}^{-1}$, degradation efficiency of 77.63 \pm 1.16% and a global degradation rate 0.90 \pm 0.05 h⁻¹ than the culture without YE addition. The 500YE culture presented the lowest MTBE degradation efficiency. The kinetics of MTBE degradation for the 100YE culture is described by first-order and pseudo-first-order reaction models. The cultivable fraction of the EB-MTBE-24 consortium is constituted by *Sphingobium barthaii* C1, *Gordonia polyisoprenivorans* C2, and *Ochrobactrum anthropi* C3. The 100YE culture of the EB-MTBE-24 consortium possess a greater potential to degrade high MTBE concentrations at a high rate suggesting that the YE minimizes the MTBE inhibitory effect on microbial growth.

Keywords: MTBE removal, bacterial consortium, cometabolism.

Resumen

El metil tert-butil éter (MTBE) es un contaminante altamente soluble en agua, cuya eliminación mediante consorcios microbianos es baja por su toxicidad sobre el crecimiento microbiano. Una alternativa para mejorar la eficiencia de degradación del MTBE es por cometabolismo. El extracto de levadura (cometabolito) se empleó en el consorcio EB-MTBE-24 para mejorar la eficiencia de degradación en cultivos a 105 mg L⁻¹ de MTBE y en cultivos a 105 mg L⁻¹ de MTBE suplementado con 100 y 500 mg L⁻¹ de extracto de levadura (100YE y 500YE, respectivamente). El cultivo 100YE presentó mayor $\mu_{max} = 0.07 \pm 0.02 \text{ h}^{-1}$, eficiencia de degradación=77.63 \pm 1.16% y tasa de degradación global=0.90 \pm 0.05 h⁻¹ que el cultivo sin adición de YE. El cultivo 500YE presentó la menor eficiencia de degradación de MTBE. La cinética de degradación del MTBE del cultivo 100YE se describe mediante los modelos de reacción de primer-orden y pseudo-primer-orden. La fracción cultivable de EB-MTBE-24 la integran *Sphingobium barthaii* C1, *Gordonia polyisoprenivorans* C2 y *Ochrobactrum anthropi* C3. El cultivo 100YE del consorcio EB-MTBE-24 tiene potencial para degradar altas concentraciones de MTBE a velocidad alta, sugiriendo que el YE minimiza el efecto inhibitor del MTBE sobre el crecimiento microbiano.

Palabras clave: eliminación de MTBE, consorcio bacteriano, cometabolismo.

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1 Introduction

Methyl tert-butyl ether (MTBE) is a compound that has been used for several purposes, the most important application of this compound is within the gasoline industry since it has the function of being an octane-booster with the objective of reducing automotive carbon monoxide (CO) emissions and other air pollutants (Hyman, 2013), for this reason there is a great interest in its production. MTBE is a synthetic compound produced from methanol and isobutylene and it presents favorable properties such as an easy and low-cost production and the ability to be miscible with other gasoline components.

The Henry's law partitioning coefficient of MTBE is $5.87 \text{ \AA} \sim 10^{-4} \text{ atm m}^3 \text{ mol}^{-1}$, which indicates that is a water-soluble compound that can move rapidly through soil and aquifers, therefore, the leaking of underground gasoline storage tanks contaminates surface and groundwater (Malandain *et al.*, 2010) with this compound. It was reported that concentrations exceeding 20 \mu g L^{-1} of MTBE in groundwater are a factor of global concern (Levchuk *et al.*, 2014). Several toxic effects of MTBE to human and animals have been reported such as neurological disorders, respiratory problems and allergic diseases. Furthermore, MTBE could cause the induction of neoplasms in various animal species (Benson *et al.*, 2011; Mehlman 2002), damage DNA by causing single-strand breaks or double-strand breaks (Burns and Melnick, 2012; Chen *et al.*, 2008). According to the criteria applied by the International Agency for Research on Cancer, National Toxicology Program and US Environmental Protection Agency, there is sufficient scientific evidence to classify MTBE as a potential carcinogenic compound for humans (Burns and Melnick, 2012).

Due to the harmful effects that MTBE presents to the environment and mainly to human health, it is necessary to eliminate this compound from surface and underground waters that contain it even at concentrations as low as a few \mu g L^{-1} . Strategies to treat MTBE-contaminated waters include traditional and biological methods (biodegradation). Thus, traditional strategies consider physical and chemical treatments, such as adsorption (Pimentel-González *et al.*, 2008; Corral-Escárcega *et al.*, 2017) and oxidation (Mendoza-Basilio *et al.*, 2017), which are highly expensive methods both, economically and energetically; as well as new strategies such as nano-

contaminants in aerobic bioreactors for wastewater treatment (Cervantes-Avilés *et al.*, 2017). In contrast, biodegradation is economical and, in some cases, it achieves the total mineralization of pollutants while offering an environmental friendly technology, therefore, is not surprising that this is the most used groundwater remediation process (Hyman, 2013).

Several studies on biodegradation of MTBE have been reported. The isolation of a mixed microbial culture obtained from the sludge of a chemical plant's biological treatment process was the first report on the potential removal of MTBE by biological process (Salanitro *et al.*, 1994). Other studies that have involved the utilization of pure bacterial cultures (no mixed microbial culture); such as, *Achromobacter xylosoxidans* (Eixarch and Constantí, 2010), *Enterobacter* sp. (Chen *et al.*, 2011), *Methylibium petroleiphilum* PM1 (Nakatsu *et al.*, 2006), *Ochrobactrum cytisi* (Lin *et al.*, 2007), *Mycobacterium austroafricanum* IFP 2012 (François *et al.*, 2002), *Rhodococcus* and *Arthrobacter* (Mo *et al.*, 1997) have demonstrated the ability of these bacterial genera to remove MTBE.

Based on the above-mentioned bacteria capable of degrading MTBE, several MTBE degradation pathways have been determined, particularly one of these biodegradation pathways has been proposed more frequently, which is described as follows: Firstly, MTBE is oxidized by the MTBE monooxygenase producing tert-butoxy methanol. The tert-butoxy methanol may follow any of these two processes: 1) a spontaneous dismutation to generate tert-butyl alcohol (TBA) and formaldehyde, or 2) an oxidation process to produce tert-butyl formate (TBF). The TBF can generate TBA by hydroxylation and release formic acid by an esterase enzyme. The TBA is hydroxylated to produce 2-methyl-1,2-propanediol (2-M1,2-PD) and this reaction is catalyzed by a second monooxygenase (TBA monooxygenase). Later, the MpdB enzyme oxidizes the 2-M1, 2-PD generating the corresponding hydroxyisobutyraldehyde. Subsequently, the hydroxyisobutyraldehyde undergoes an oxidation process by the MpdC enzyme to produce 2-hydroxyisobutyric acid (2-HIBA). The 2-HIBA can be bifurcated to 3-hydroxybutyryl-CoA by isomerization or to acetone and CO₂ by decarboxylation (Lopes Ferreira *et al.*, 2006).

Despite the isolation and identification of bacterial genera with the potential to degrade MTBE, novel bacterial genera or consortia capable of removing MTBE with a similar or higher efficiency to the

already reported values could be found. The low efficiency of degradation of MTBE by pure cultures or consortia of microorganisms is attributed to the fact that MTBE diminishes the microbial growth because it causes oxidative stress exerting a negative effect on physiological characteristics such as growth, respiration and ATP-synthesis (Krayl *et al.*, 2003).

Cometabolism is defined as the transformation of a non-growth substrate under the obligate presence of a growth substrate or another transformable compound (Dalton and Stirling, 1982). This process has been used in MTBE biodegradation using alkanes as growth substrates with good results, and it was considered as an excellent alternative because alkanes uncouple MTBE biodegradation from growth and consequently the long periods of bacterial adaptation and propagation are reduced (Nava *et al.*, 2007).

Moreover, a previous research has reported that the biodegradation of some xenobiotics can be enhanced by the addition of readily metabolized organic substrates (Ziagova *et al.*, 2007; Chen *et al.*, 2009). In the present work, we used yeast extract as a growth substrate and the MTBE as the non-growth substrate. Such supplementary growth substrates can stimulate the growth of microorganisms, and/or minimize the energy needed for a complete mineralization of the recalcitrant organic compound.

On the other hand, it has also been proposed that the presence of a high concentration of additional organic substrates might decrease the activity of the enzymes responsible for degrading MTBE (Chen *et al.*, 2009). In fact, we think that MTBE is toxic for microorganisms, for that reason microorganisms require metabolites that allow them growing and increasing their biomass along with the diminishing of the toxic effect of MTBE in due to its biodegradation. Thus, the YE supplemented at low concentrations will provide metabolites for the growth of microorganisms, acting as an adjuvant to degrade MTBE and promote microbial growth.

In the present work, a bacterial consortium that exhibits a MTBE biodegradation capability was obtained from gasoline-contaminated soils coming from an ex-refinery. This consortium was analyzed for its MTBE removal capability in a cometabolic culture using yeast extract as co-substrate (cometabolite) and compared with a conventional culture (no-cometabolism). Additionally, members of the bacterial consortium were isolated and identified by using molecular biology tools.

2 Materials and methods

2.1 Soil samples

Soil samples were collected from an ex-refinery located at 19°29'28.73"N, 99°11'25.04"O in Mexico City, Mexico. The samples exhibited a concentration of hydrocarbons corresponding to the light fraction of 195.50 mg kg⁻¹ (dry weight basis) according to the PROY-NMX-AA-105-SCFI-2013 norm (2013). Ten soil samples were taken from an area surrounding the gasoline storage tanks at a depth of 30 cm and to a distance of 1 m between each sample. By mixing uniform weight subsamples (10 g) from each soil sample, a composite sample was formed. Subsequently, the composite sample was sieved through a 2-mm mesh and maintained at 4 °C until utilization.

2.2 Obtaining a bacterial consortium through enrichment culture

The composite soil sample (1 g) was placed in a flask containing 100 mL minimal medium (MM) with the following composition (g L⁻¹): 1 of KH₂PO₄·3H₂O, 1 of Na₂HPO₄, 0.1 of MgSO₄·7H₂O, 1 of NH₄NO₃, 0.001 of CaCl₂·2H₂O, and 0.004 of FeSO₄·7H₂O. MTBE (99.9% purity, Sigma-Aldrich, St. Louis, MO, USA) was added to the MM as the sole carbon and energy source to achieve a final concentration of 1% (v/v).

The flask was incubated at 30 °C and 100 rpm until the culture reached an optical density at 590 nm ranging from 0.2 to 0.4. Enrichment subcultures were performed as follows: 1 mL of the culture was transferred to 100 mL of fresh MM supplemented with 1% (v/v) MTBE and incubated under the conditions described above. This procedure was performed six times successively.

2.3 Metagenomic DNA extraction and evaluation of the consortium stabilization using PCR-DGGE

Metagenomic DNA from enrichment subcultures was extracted using a modified cetyltrimethylammonium bromide extraction protocol described by Murray and Thompson (1980). Briefly, 5 mL of subcultures were incubated at 37 °C during 1 h with 6.25 μL of lysozyme (20 mg mL⁻¹), 2.5 μL of RNase (10 mg mL⁻¹), 1.24 μL of proteinase K (20 mg mL⁻¹)

and 20 μL of 20% SDS. The DNA was obtained by following a sequential extraction procedure with phenol:chloroform:isoamyl alcohol (25:24:1, v/v) and precipitated with isopropanol and washed with ethanol at 70% (v/v). DNA quality was estimated via electrophoresis in 1% (w/v) agarose gel in 1X TAE buffer (40 mmol L^{-1} tris, pH 8.3; 20 mmol L^{-1} acetic acid; 1 mmol L^{-1} ethylenediaminetetraacetic acid) at 80 V. The DNA was stained with a 0.5 $\mu\text{g mL}^{-1}$ ethidium bromide solution and observed under ultraviolet light.

Next, DNA extracted from enrichment subcultures was used as a template to amplify the V6-V8 hypervariable regions of the bacterial 16S rRNA. The PCR reactions were carried out using the forward primer U968-GC [5'-(GC clamp) CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC-3'] and reverse primer L1401 (5'-CGG TGT GTA CAA GAC CC-3') in the following reaction mixture: 50 ng template DNA, 1X reaction buffer, 5 mM MgCl_2 , 2.5 mM of each dNTP, 10 pM of each primer, 1.5 U of Taq DNA polymerase (Invitrogen, Waltham, MA, USA), and adjusted to 25 μL total with water. The PCR conditions were 1 cycle at 95 °C for 10 min, followed by 25 cycles at 95 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min and finally, 1 cycle at 72 °C for 7 min. The expected PCR product sizes were of 433 pb. The PCR products were analyzed using a DCode mutation detection system, following the procedure described by the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA). Products of PCR reaction were applied to 8% (w/v) polyacrylamide gels prepared in 1 x TAE buffer. The denaturing gradients contained 35-65% denaturant [100% denaturant corresponds to 7 M urea and 40% (w/v) formamide]. Electrophoresis was performed at 85 V and 60 °C for 16 h. DGGE gel was fixed and DNA bands were visualized by silver staining with procedures previously reported (Sanguinetti *et al.*, 1994). A 100% match of DGGE band patterns among the enrichment subcultures was used as the criterion to indicate that the consortium had reached a stable state.

2.4 MTBE degradation assays

Consortium was propagated overnight in nutrient broth and the biomass was aseptically separated by centrifugation at 10000 rpm for 15 min. Next, the cell pellets were washed three times with 0.85% saline solution. Finally, the cell suspensions were adjusted to the 0.5 nephelometric McFarland standard with

0.85% saline solution, and 3 mL of the resulting cell suspensions were inoculated into serological vials containing 27 mL of minimal medium (MM) supplemented with MTBE at 105 mg L^{-1} and vials containing 27 mL of MM supplemented with MTBE at 105 mg L^{-1} and 100 or 500 mg L^{-1} of yeast extract (cometabolic cultures). The vials were sealed with PTFE/silicone septa and set on a shaker table at 100 rpm.

During the degradation tests, the biomass and MTBE concentration were monitored periodically every 6 h for a 96 h period. Additionally, experiments without biomass and experiments with heat-inactivated biomass were used as abiotic controls to evaluate MTBE losses by photolysis and/or adsorption to the cells or glass. All experiments were performed in triplicate, and mean values are reported.

2.5 Cell concentration

Biomass was quantified by dry cell weight by filtering 10 mL of the culture samples through previously weighed 0.7 μm filters (Whatman GF/F) and subsequently drying the samples at 105 °C to a constant weight. The cell concentration was obtained by calculating the amount of weight lost during the drying procedure.

2.6 Residual MTBE quantification

MTBE was recovered from each culture sample according to an organic extraction method; briefly, 0.3 mL of a solution containing methanol, 42 μL trichloroethylene and 1 mg L^{-1} of n-hexane were injected rapidly into the sample solution using a 1 mL syringe and the mixture was gently shaken. The mixture was then centrifuged for 3 min at 4500 rpm. The dispersed fine particles of extraction phase were set aside for sedimentation in the bottom of the test tube. 0.5 μL of the sedimented organic phase was removed and quantified. The quantification of MTBE was similar to a method described previously (Kuss *et al.*, 2018), with the modification described as follows: an MTBE standard (99.9% purity, Sigma-Aldrich) was used to construct a calibration curve. MTBE quantification was carried out using a mass spectrophotometer (Agilent Serie 5975C) coupled to a gas chromatograph (Agilent 6850 Series II Network System). The samples were injected into the capillary column (Agilent 19091S-433E; 30 m x 0.25 mm x 0.25 μm) of a gas chromatograph using an automated injector. The split ratio was 100:1. The initial oven

temperature was 40 °C, increased to 50 °C at a rate of 3 °C min⁻¹, and then increased to 58 °C at a rate of 10 °C min⁻¹. The temperature of the injector and detector was set at 250 °C. Helium was used as the carrier gas at a constant flow of 0.7 mL min⁻¹.

2.7 Cell growth and MTBE degradation parameters

The maximum specific growth rate (μ_{max} , h⁻¹), the efficiency (E, %), and overall rate (V_g, mg L⁻¹ h⁻¹) for the MTBE degradation by the microbial cultures were estimated from the experimental data using [Equation 1], [Equation 2] and [Equation 3], respectively:

$$\mu_{max} = \frac{\ln(X_f - X_i)}{t_f - t_i} \quad (1)$$

$$E = \left(\frac{C_f - C_i}{C_i}\right)(100\%) \quad (2)$$

$$V_g = \frac{C_i - C_f}{T_f - T_i} \quad (3)$$

where: X_i =Biomass concentration at the beginning of the exponential phase; X_f =Biomass concentration at the end of the exponential phase; C_i =Initial MTBE concentration; C_f =Final MTBE concentration; T_i =Initial time of incubation; T_f =Final time of incubation; t_i =Time at the beginning of the exponential phase; t_f =Time at the end of the exponential phase.

2.8 MTBE degradation kinetics modeling

The experimental data of the degradation kinetics of MTBE were analyzed using zero-order [Equation 4], first-order [Equation 5] and pseudo-first-order [Equation 6] models, which have been widely used to understand degradation kinetics of xenobiotics. The kinetic models are expressed as follows:

$$C_t = C_0 - K_0t; T_{1/2} = \frac{C_0}{2K_0} \quad (4)$$

$$\ln C_t = \ln C_0 - K_1t; T_{1/2} = \frac{\ln 2}{K_1} \quad (5)$$

$$C_t = C_0 - e^{-K'_1t}; T_{1/2} = \frac{\ln 0.5}{-K'_1} \quad (6)$$

where C_t =MTBE concentration at time $t = t$; K_0 , K_1 , K'_1 =Apparent rate constants; t =Time; C_0 =Initial concentration of MTBE; $T_{1/2}$ =Half-life period.

2.9 Isolation and identification of the cultivable fraction

Successive decimal dilutions of the stabilized consortium were carried out until 10⁻⁸, and 100 μL of each dilution was spread onto plates containing MM. The plates were incubated under MTBE atmosphere at 28 °C for 24 h, for which MTBE was added daily in the incubation chamber. The isolates were grouped according to their colonial morphology and their microscopic morphology. Each morphotype was grown on nutrient broth overnight. Then, genomic DNA was extracted according to the protocol described previously.

The 16S rRNA gene was amplified by PCR with primers 8 (5'-CCG CGG GCG CCG CTG GAT AGT TTG CAG ATC CTG GCT CAG-3') and 1492 (5'-GGC TCG AGC GGC CGC CCG GGT TAC CTT GTT ACG ACT T-3') (Relman *et al.*, 1993). The reaction mixture and the PCR conditions were similar to those described above, except that the number of amplification cycles was 35 and the amplification stage was 2 min.

The amplified fragments, with sizes of 1484 pb, were purified using the Zymoclean TM Gel DNA recovery kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions, and the integrity was confirmed by electrophoresis in 1% (w/v) agarose gel in 1X TAE buffer. The sequencing of the amplified DNA fragments was performed on an ABI PRISM 310 Genetic Analyzer PE (Applied Biosystems, Foster City, CA, USA).

A collection of taxonomically related sequences obtained from EzBioCloud database (Yoon *et al.*, 2017) using Blast (Camacho *et al.*, 2009) was included in the multiple alignment analyses with CLUSTAL X (Thompson *et al.*, 1997) and was manually edited using SEAVIEW software (Galtier *et al.*, 1996). Similarity analysis was estimated using nucleotide sequences with MEGA (Tamura *et al.*, 2011). Phylogenetic affiliations were based on the limits proposed by Rosselló-Mora and Amann (2001), where < 95, 95-97.5 and > 97.5% define the taxonomic levels of family, genus and species, respectively. The sequences of the 16S rRNA genes reported in this work have been deposited in the GenBank database under accession numbers from MH384879-MH384881.

2.10 Statistical analysis

Statistical analyses of the experimental data were performed using ANOVA, and significant differences ($p < 0.05$) were determined using a Bonferroni test. All statistical analyses were conducted using GraphPad Prism® software version 5.03.

3 Results and discussion

3.1 Obtainment of the bacterial consortium adapted to MTBE

In order to obtain an adapted bacterial consortium using MTBE as the sole source of carbon and energy, an enrichment process was carried out through successive subcultures in minimal medium supplemented with 1% of MTBE as the selective agent. The high concentration of MTBE added to the initial culture was decided because we think that at this stage, there is a high population of microorganisms that could obstruct or mask the enrichment of the MTBE-degrading bacteria. The MTBE could adhere to or internalize in the bacteria of the consortium, causing the decrease of the effective concentration of MTBE that would be needed for the enrichment. Thus, six enrichment subcultures using MTBE as the sole carbon and energy source were carried out and analyzed by PCR-DGGE profiles in order to determine the stabilization of the bacterial consortium. Based on the PCR-DGGE profiles of the V6-V8 region from the 16S rRNA genes, it was evident that stabilization was reached in the 6th transfer because most of the bands were conserved in the last three subcultures (Fig. 1). In addition, a less complex bacterial community was observed in the last subcultures in comparison with the first subculture, in which a major number of bands were presented (bands A to F). While some bands were present in all subcultures (band G, H and I), other bands disappeared from the third subcultures (bands A, B, C, D, E and F); this suggests that only a few bacteria are the main members of the consortium that was adapted to subculture conditions. The enrichment process was successful and adequate, since bands G, H and I were not observed in the first subcultures and their presence was evident in subsequent subcultures, until they remained stable in the 6th subculture.

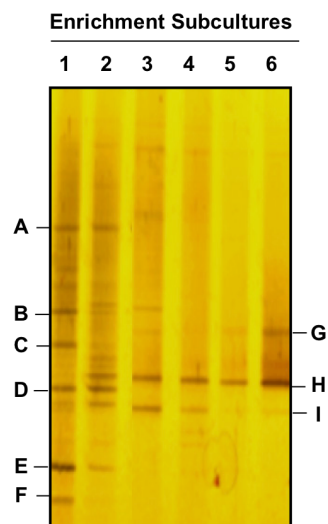


Fig. 1. Stabilization of the enrichment subcultures by DGGE profiles of the 16S rDNA gene. 1-6 indicates the numbers of the successive enrichment subcultures. The stabilization of the bacterial consortium was reached in the sixth (6th) subculture. A - I show representative bands.

Previous studies have used the PCR-DGGE profiles to determine the stability of subcultures and the success of the enrichment process, indicating that this molecular technique is efficient for determining with certainty the stability and complexity of bacterial communities or consortia (Ortega-González *et al.*, 2013; Alfonso-Gordillo *et al.*, 2016). These results indicate that the stabilization and adaptation of the bacterial consortium was obtained in the 6th subculture in MM supplemented with 100 mg MTBE L⁻¹. The obtained bacterial consortium was labeled as the EB-MTBE-24 consortium.

3.2 Removal capability of MTBE by a conventional culture

In order to evaluate the ability of the stabilized EB-MTBE-24 consortium to degrade MTBE, the remaining MTBE concentration was determined in a period of 96 h from an initial concentration of 105 mg L⁻¹ of MTBE in a conventional culture. In Fig. 2 is observed that over time, the concentration of MTBE decreases. During the first 24 h, a slight decrease was observed; however, in the period from 24 to 48 h, the greatest degradation of the contaminant was observed, beyond that period there was no significant decrease.

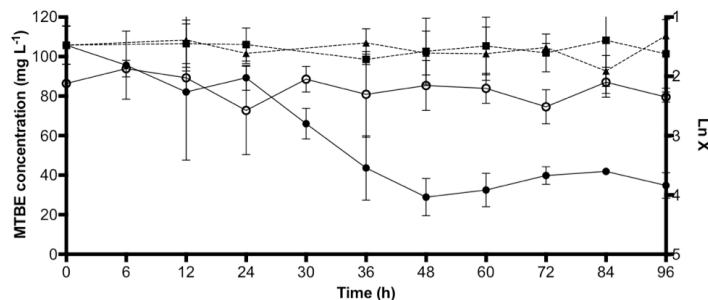


Fig. 2. Kinetics of MTBE degradation by the EB-MTBE-24 consortium in mineral medium without yeast extract. Determination of the kinetics of MTBE degradation by the consortium was carried out at an initial concentration of 105 mg L^{-1} and evaluated every 6 h during a 96 h period at 30°C and 100 rpm. Growth of the consortium (empty circles), X represents biomass concentration (dry weight). Data presented in the graph are the mean and standard error of 3 independent assays. MTBE concentration (mg L^{-1} ; filled circles), sterile control (filled square), abiotic control (filled triangle).

In the controls of biomass inactivated and without biomass, the concentration of MTBE remained constant throughout the kinetics, indicating that the adsorption of MTBE to the bacteria and the elimination of MTBE by photolysis did not occur. Simultaneously, with the monitoring of the MTBE concentration, the cell growth was also evaluated throughout the kinetics, determining that there was no substantial increase; in fact, from 6 and until 24 h, there is a decline in growth, although later it was recovered and practically the cellular biomass remains constant (Fig. 2). The kinetic parameters of cell growth and degradation of MTBE were evaluated. The maximum growth rate (μ_{max}) was of $0.0019 \pm 0.001 \text{ h}^{-1}$, with a degradation efficiency of $65.07 \pm 1.46\%$ and an overall degradation rate of $0.71 \pm 0.02 \text{ h}^{-1}$ (Table 1). In comparison with other studies, the μ_{max} of the EB-MTBE-24 consortium of 0.0019 ± 0.001 is lower than that reported by Nava *et al.* (2007) with a μ_{max} of 0.0083 h^{-1} and the report by Fortin *et al.* (2001) with a μ_{max} of 0.004 h^{-1} for mixed bacterial cultures grown aerobically in a culture medium containing MTBE as the sole source of carbon and energy.

These results suggest that in the EB-MTBE-24 consortium there is no growth during the first hours (6-24 h), and the microorganisms are focusing their metabolism only to eliminate the MTBE, which is being toxic. Although there is a substantial elimination of MTBE from 24 to 48 h, the microorganisms do not have sufficient nutritional requirements to grow. Therefore, these results suggest that the EB-MTBE-24 consortium biotransforms the MTBE to intermediate (s) less toxic to it, which the MTBE is

not used as a source of carbon and energy. Based on these results, we thought about using a substrate for the microbial growth of the consortium and that could improve the biodegradation of MTBE. The yeast extract supplemented at low concentrations has been used as a cometabolic substrate to improve the degradation of toxic compounds (Ziagova *et al.*, 2007, Chen *et al.*, 2009).

3.3 Removal capability for MTBE by the cometabolic culture

The MTBE - ether bond can be difficult to cleave, therefore, under these conditions, MTBE may not be adequate as a sole carbon and energy source (Muñoz-Castellanos *et al.*, 2006). It has been suggested that MTBE has a negative effect on growth, respiration and ATP-synthesis in bacteria (Krayl *et al.*, 2003). Thus, the low efficiency of degradation of MTBE by the EB-MTBE-24 consortium could be due to the fact that MTBE was toxic for bacterial cells, since there was no growth of the EB-MTBE-24 consortium.

A strategy to minimize the toxic effect of this oxygenating compound is to add a metabolizable substrate first (i.e., glucose or yeast extract); to generate biomass and then to stimulate the degradation of the compound (Ziagova *et al.*, 2007). Taking into consideration the aforementioned, the culture of the EB-MTBE-24 consortium was supplemented with yeast extract at two concentrations (100 and 500 mg L^{-1}) to generate a cometabolic culture. Former studies have suggested that the addition of yeast extract contributes to microbial growth (Chen *et al.*, 2009; Xiong *et al.*, 2017b).

Table 1. Growth and MTBE degradation parameters obtained with the IPN-120526 consortium at different concentrations of yeast extract.

	Yeast extract added to the minimum medium		
	0	100	500
V_g	0.71 ± 0.02^b	0.90 ± 0.05^a	0.59 ± 0.04^b
μ_{max}	0.0019 ± 0.001^c	0.076 ± 0.002^a	0.014 ± 0.004^b
E	65.07 ± 1.46^b	77.63 ± 1.10^a	50.04 ± 2.38^c

μ_{max} : maximum specific growth rate (h^{-1}); E : efficiency (%); V_g : overall degradation rate ($\text{mg L}^{-1} \text{h}^{-1}$). Different letters show statistical difference between parameters $p < 0.05$

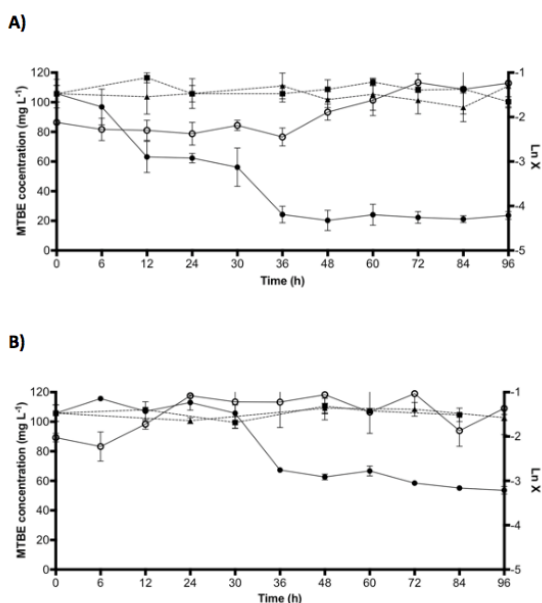


Fig. 3. Kinetics of MTBE degradation by the EB-MTBE-24 consortium in mineral medium supplemented with yeast extract at 100 and 500 mg L^{-1} . Determination of the kinetics of MTBE degradation and growth by the consortium was carried out at an initial concentration of 105 mg L^{-1} and evaluated every 6 h until 96 h at 30 °C and 100 rpm in cometabolic culture at 100 (panel A) and 500 mg L^{-1} (panel B). X represents biomass concentration (dry weight). Data presented in the graph are the mean and standard error of 3 independent assays. MTBE concentration (mg L^{-1} ; filled circles), sterile control (filled square), abiotic control (filled triangle) and growth of the consortium (empty circles).

In addition, it has been suggested that the addition of an organic substrate increases the ability to degrade the contaminant, by the activation of enzymes involved

in the degradation of the recalcitrant compound (Chen *et al.*, 2009). In fact, in some studies it has been mentioned that there are several organic compounds that can contribute to microbial growth such as glucose, yeast extract, tryptone and beef extract (Xiong *et al.*, 2017a; Chen *et al.*, 2009).

It is well known that these substrates can provide not only carbon sources, but also nitrogen sources and growth cofactors for microbial metabolism. Particularly, yeast extract, which is a complex mixture of amino acids, peptides, and proteins (Fava *et al.*, 1995), is often added as an additional alternative of carbon source. It also protects the bacteria, by decreasing the adverse impact of the degradable compounds, and supporting them for a good growth and biodegradation capability.

In Fig. 3A, it was observed that the concentration of MTBE at 36 h decreased substantially when yeast extract (100 mg L^{-1}) was added, however, the microbial growth did not increase until 36 h, after this period, the microorganisms increase their growth exponentially until 72 h. The abiotic controls generated the expected results and no MTBE removal was detected. These results suggest that firstly the microorganisms remove the MTBE from the environment to a concentration lower or sufficient to allow its microbial growth by the nutrients supplied by the yeast extract. In regards to the kinetic parameters, a μ_{max} of $0.076 \pm 0.002 \text{ h}^{-1}$ was obtained, with a degradation efficiency of $77.63 \pm 1.10\%$ and an overall degradation rate of $0.90 \pm 0.05 \text{ h}^{-1}$ (Table 1).

From the result of the degradation of MTBE that was obtained with YE at 100 mg L^{-1} , we thought that by increasing the concentration of the yeast extract the conditions for the degradation of MTBE could be improved, therefore, we decided to increase the concentration of the yeast extract at 500 mg L^{-1} .

It was observed that there was a change in the performance of the degradation of MTBE compared to the culture added with 100 mg L^{-1} of yeast extract (Fig. 3B), during the first hours of the kinetic and until 30 h, there is no degradation of MTBE compared to the culture added with 100 mg L^{-1} of yeast extract, which in a similar period generated a greater degradation of MTBE (with a remaining concentration of $\text{MTBE}=56.18 \text{ mg L}^{-1}$).

Regarding the microbial growth, the same happens, in the culture added with 500 mg L^{-1} of yeast extract, there was an increase in microbial growth until 24 h and subsequently this growth remains constant; conversely, in the culture added with 100 mg L^{-1} of yeast extract, the increase in microbial growth occurs after 36 h. These results suggest that when the concentration of yeast extract increased, the microorganisms prefer to grow than to degrade the MTBE, after growing, they deviate their metabolism to degrade the MTBE, which is contrary to what occurs at low concentrations of yeast extract (100 mg L^{-1}).

The kinetic parameters that were obtained with the addition of yeast extract at 500 mg L^{-1} were μ_{max} of $0.014 \pm 0.004 \text{ h}^{-1}$, with a degradation efficiency of $50.04 \pm 2.38\%$ and a total degradation rate of $0.59 \pm 0.04 \text{ h}^{-1}$ (Table 1). These results suggest that by increasing the nutrients for microbial growth, the degradation of MTBE tends to decrease since there was no increase in the efficiency of degradation and microbial growth compared to the culture added with 100 mg L^{-1} of yeast extract.

However, the result was similar to that obtained by Li *et al.* 2011, who used single-yeast extract additions at concentrations of 100, 200 and 300 mg L^{-1} in the production of polyvinyl alcohol-degrading enzymes (PVAases). These authors reported that a shot of yeast extract added at a high concentration (300 mg L^{-1}) does not impact as positively on PVAases production as a shot added at a lower concentration (200 mg L^{-1}). Thus, excessive yeast extract may inhibit PVAases synthesis. Similarly, the concentration of 500 mg L^{-1} of yeast extract, as cometabolic substrate, is an excess and does not promote a higher efficiency of MTBE degradation, this may be due to the fact that the microorganisms have sufficient nutritional requirements to grow and tolerate the presence of MTBE, inhibiting the synthesis of the enzymes involved in the degradation of MTBE, which does not occur with the addition of 100 mg L^{-1} of yeast extract.

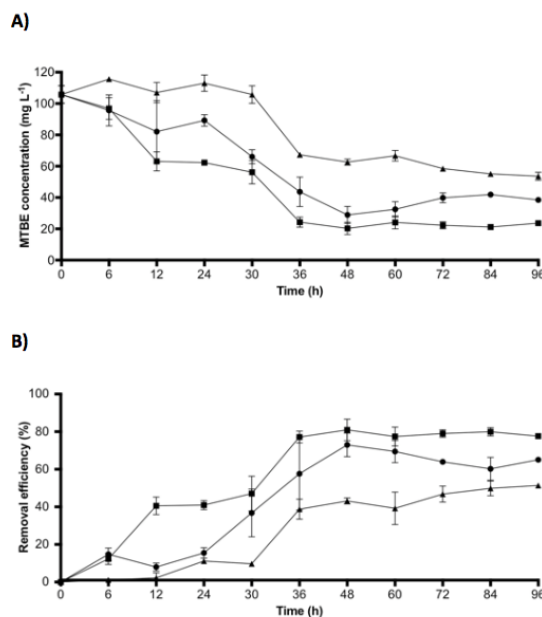


Fig. 4. Comparison of MTBE removal by the EB-MTBE-24 consortium under 3 different conditions. a) Residual concentrations of MTBE in mineral medium at an initial concentration of 105 mg L^{-1} of MTBE, without and with yeast extract addition at 100 and 500 mg L^{-1} . b) Percentage of removal efficiency, evaluated every 6 h at 30°C and 100 rpm . Conventional culture without yeast extract addition (filled circle), culture supplemented with 100 mg L^{-1} yeast extract (filled square) and culture added with 100 mg L^{-1} yeast extract (filled triangle). Each point on the graph represents the mean and standard error of 3 independent assays.

3.4 Comparison of conventional culture and cometabolic culture

To compare the removal of MTBE by the EB-MTBE-24 consortium, the three different conditions of MTBE degradation are exhibited. The average remaining concentration of MTBE was lower in the cometabolic culture using 100 mg L^{-1} of yeast extract (47.28 mg L^{-1}), than the conventional culture (59.68 mg L^{-1} ; Fig. 4A; $p < 0.05$), the results obtained with the conventional culture were lower than the obtained with the culture added with 500 mg L^{-1} of yeast extract (84 mg L^{-1} $p < 0.05$).

Regarding the degradation efficiency of MTBE, it was always higher throughout the degradation kinetics in the cometabolic culture supplemented with 100 mg L^{-1} of yeast extract (55.4%) than in

the conventional culture (43.45%; $p < 0.05$). In the cometabolic culture supplemented with 500 mg L⁻¹ of yeast extract, the degradation efficiency (26.10%) was the lowest compared to the other two cultures ($p < 0.05$; Fig. 4B).

To evaluate the potential of the consortium EB-MTBE-24 to degrade MTBE in aqueous solutions, its overall degradation efficiencies and rates of MTBE degradation achieved were compared with other studies reported on microbial cultures degrading MTBE. The degradation efficiency of MTBE from the conventional culture supplemented with 105 mg L⁻¹ of MTBE, by the consortium EB-MTBE-24 (65.07 ± 1.46%) or with the cometabolic culture supplemented with 100 mg L⁻¹ yeast extract (77.63 ± 1.10%) was higher than those reported for another mixed bacterial culture, which was of 38.59 ± 2.17 (Alfonso-Gordillo et al., 2016). Furthermore, the overall degradation rate reached by the bacterial consortium EB-MTBE-24 in conventional culture (0.71 ± 0.02 mg L⁻¹ h⁻¹) or with cometabolic culture added with 100 mg L⁻¹ yeast extract (0.90 ± 0.05 mg L⁻¹ h⁻¹) was higher than that reported for mixed cultures that was 0.06 mg L⁻¹ h⁻¹ by Raynal and Pruden et al. (2008), 0.01 mg L⁻¹ h⁻¹ by Volpe et al. (2009) and 0.43 mg L⁻¹ h⁻¹ by Pimentel-González et al. (2008). The biodegradation percentage obtained with the consortium EB-MTBE-24 in the present work is higher (77.63%) than the values obtained during growth in the presence of MTBE. Mo et al. (1997) obtained a biodegradation of 28-29% after 2 weeks of incubation with the biodegradation rate being the highest during the first 3 days. Muñoz- Castellanos et al. (2006) obtained similar values (22-37% biodegradation) after 5 days of incubation with *Rhodococcus*, *Bacillus* and *Aureobacterium*. The biodegradation can increase up to 51% if cells are previously induced with dicyclopropylketone for the case of *Pseudomonas*

putida Gpo1 (Smith and Hyman, 2004).

When resuspended cells are used, Pimentel-Gonzalez et al. (2008) reported that 85% MTBE was consumed during the first 5 days of incubation of a microbial consortium. In this last case there are more interactions among the bacteria in a mixed culture, which may improve metabolism and consequently the biodegradation efficiency (Eixarch and Constanti, 2010). Few reports on the biodegradation of MTBE using yeast extract as a co-substrate have been reported (Chen et al., 2009), most studies have focused on the use of toxic compounds such as alkanes (Nava et al., 2007) or pentane (Li et al., 2016). It is important to notice that yeast extract is not a pollutant for the environment such as alkanes or pentane, it is also easy to obtain and improves the degradation efficiency of MTBE. Co-metabolic culture with yeast extract could be an attractive strategy for the bioremediation of MTBE-contaminated wastewater.

3.5 Mathematical models of the cometabolic culture

Furthermore, the mathematical behavior that fits the MTBE degradation of the cometabolic culture supplemented with 100 mg L⁻¹ of yeast extract was determined. The apparent kinetic rate constants (K_0 , K_1 , K_1'), half-lives ($T_{1/2}$) and the regression equations (R^2) under given conditions for each reaction model are calculated. Based on the aforementioned parameters, the degradation kinetics of MTBE by the consortium EB-MTBE-24 are described better by first-order and pseudo-first-order reaction models. This implies that the biodegradation capability presented by the consortium EB-MTBE-24 is a time-dependent process. Additionally, the half-life of the first-order and pseudo-first-order degradation reaction does not depend on the initial MTBE concentration.

Table 2. Phylogenetic affiliation of bacteria isolated from the EB-MTBE-24 consortium.

Isolate name	^a Best match database (GenBank Accession number)	^b Similarity (%)	Microbial affiliation group
C1	<i>Sphingobium barthaii</i> (NHQ830159)	99.93 (1/1426)	<i>Sphingobium barthaii</i>
C2	<i>Gordonia polyisoprenivorans</i> (BAEI01000017)	99.93 (1/1426)	<i>Gordonia polyisoprenivorans</i>
C3	<i>Ochrobactrum anthropi</i> (CP000758)	100 (0/1426)	<i>Ochrobactrum anthropi</i>

^aThe best match was identify by using the EzBioCloud server on the basis of 16S rRNA sequence data (Yoon et al., 2017)

^bPercentage of similarity was estimated by considering the number of nucleotide substitutions between a pair of sequences divided by the total number of compared bases × 100%. Taxonomic limits to define family, genera and species: $x < 95$, $95 < x < 97.5$ and $x > 97.5$, respectively (Rosselló-Mora and Amann, 2001).

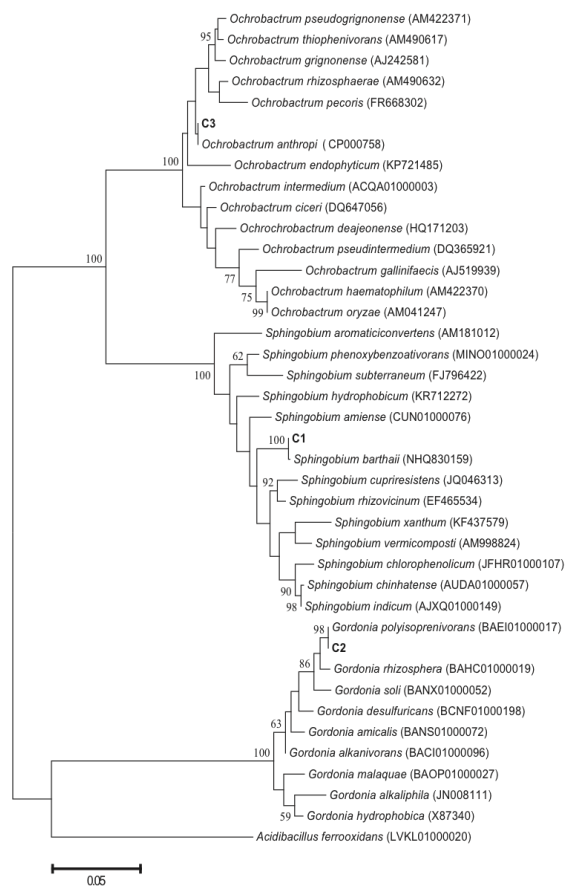


Fig. 5. Phylogenetic tree of sequences of the 16S rRNA gene of the isolates obtained from the EB-MTBE-24 consortium. The tree was constructed using the neighbor-joining method; where *Acidibacillus ferrooxidans* (LVKL01000020) served as outgroup. The numbers at the branches indicate the bootstrap values of 1000 re-samplings. The bar at the bottom indicates the number of substitutions per site.

3.6 Isolation and identification of the culturable bacterial fraction

In order to know which members belong to the EB-MTBE-24 consortium, we conducted the isolation and identification of the culturable fraction. Only three bacterial morphotypes were isolated (C1, C2 and C3). The isolate C1 presented a similarity of 99.93% with *Shingobium barthaii* (NHQ830159), C2 presented a similarity of 99.93% with *Gordonia polyisoprenivorans* (BAEI01000017) and C3 presented a similarity of 100% with

Ochrobactrum anthropi (CP000758). Therefore, the three bacteria isolated from the consortium were identified as *Shingobium barthaii* C1, *Gordonia polyisoprenivorans* C2 and *Ochrobactrum anthropi* C3 (Fig. 5 and Table 2).

Previous studies with *A. xylooxidans*, *E. cloacae* and *O. anthropi* have already demonstrated the ability of these bacteria to degrade a wide range of compounds such as chlorophenols, polycyclic aromatic hydrocarbons, and endosulfan (Müller *et al.*, 1998; Gómez-Acata *et al.*, 2018; Maeda *et al.*, 2015); also, they have been detected in environments contaminated with petroleum (Jiao *et al.*, 2016).

Particularly, it has been reported that *O. anthropi* degraded 50% of MTBE in 5 days out of 70 mg L⁻¹ of MTBE in minimum medium supplemented with yeast extract at a concentration of 50 mg L⁻¹ (Barberà *et al.*, 2011); while *O. cytisi* completely degraded the compound in 80 h from an initial concentration of 75 mg L⁻¹, with a $\mu = 0.04 \pm 0.022$ h⁻¹ (Lin *et al.*, 2007). In addition, Key *et al.* (2013) detected the presence of *Shingobium* sp., by ribosomal gene library, as a member of a bacterial community capable of removing MTBE from an MTBE-contaminated groundwater. However, in their study the authors did not demonstrate the ability of *Shingobium* to remove MTBE. While the genus *Gordonia* has been linked to the degradation of long-chain n-alkanes (Hao *et al.*, 2008), particularly *Gordonia amicalis* T3, this bacterium has been isolated from a hydrocarbon-polluted soil site and the report indicates that it reached a degradation of 14 mg L⁻¹ of MTBE after 21 days of incubation in a mineral salts medium amended with the fuel oxygenates MTBE (Purswania *et al.*, 2011).

On the other hand, the present work was focused on isolating and identifying the cultivable fraction, and probably the non-cultivable fraction also contributes to the degradation of MTBE. It should also be considered that only the residual MTBE was determined here during the degradation kinetics and that the intermediate metabolites were not evaluated.

Conclusions

The results suggest that the consortium EB-MTBE-24 exhibits the ability to remove high MTBE concentrations at a high rate using yeast extract at 100 mg L⁻¹ as a cometabolic substrate, which makes these microbial cultures an attractive alternative for the bioremediation of MTBE-contaminated wastewater.

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Abbreviations

MTBE	Methyl tert-butyl ether
YE	yeast extract
100YE	supplementation of 100 mg L ⁻¹ of yeast extract
500YE	Supplementation of 500 mg L ⁻¹ of yeast extract
CO	carbon monoxide
TBA	tert-butyl alcohol
TBF	tert-butyl formate
2-M1,2-PD	2-methyl-1,2-propanediol
2-HIBA	2-hydroxyisobutyric acid
MM	minimal medium
<i>E</i>	Efficiency, %
<i>V_g</i>	overall rate, mg L ⁻¹ h ⁻¹
<i>Greek symbols</i>	
μ_{max}	specific growth rate, h ⁻¹

References

- Alfonso-Gordillo, G., Cristiani-Urbina, E., Flores-Ortiz, C.M., Peralta, H., Cancino-Díaz, J.C., Cruz-Maya, J.A. and Jan-Roblero, J. (2016). *Stenotrophomonas maltophilia* isolated from gasoline-contaminated soil is capable of degrading methyl tert-butyl ether. *Electronic Journal of Biotechnology* 23, 12-20. <http://dx.doi.org/10.1016/j.ejbt.2016.06.006>
- Barberà, M. J., Mateo, E., Monkaityte R. and Constantí, M. (2011). Biodegradation of methyl tert-butyl ether by newly identified soil microorganisms in a simple mineral solution. *World Journal of Microbiology and Biotechnology* 27, 813-82. <http://dx.doi.org/10.1007/s11274-010-0522-4>
- Benson, J.M., Gigliotti, A.P., March, T.H., Barr, E.B., Tibbetts, B.M., Skipper, B.J., Clark, C.R. and Twerdok, L. (2011). Chronic carcinogenicity study of gasoline vapor condensate (GVC) and GVC containing methyl tertiary-butyl ether in F344 rats. *Journal of Toxicology and Environmental Health Part A* 74, 638-657. <http://dx.doi.org/10.1080/15287394.2011.538837>
- Burns, K.M. and Melnick, R.L. (2012). MTBE: recent carcinogenicity studies. *International Journal of Occupational and Environmental Health* 18, 66-69. <http://dx.doi.org/10.1179/107735212X13293200778947>
- Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. and Madden, T.L. (2009). BLAST+: architecture and applications. *BMC Bioinformatics* 10, 421. <http://dx.doi.org/10.1186/1471-2105-10-421>
- Cervantes-Avilés, P., Souza-Brito, E., Bernal-Martínez, A., Reyes-Aguilera, J., de la Rosa, G., and Cuevas-Rodríguez, G. (2017). Impacto de los nanocontaminantes en biorreactores aerobios para el tratamiento de aguas residuales. *Revista Mexicana de Ingeniería Química* 16, 247-261. <http://www.redalyc.org/articulo.oa?id=62049878024>
- Chen, C.S., Hseu, Y.C., Liang, S.H., Kuo, Y.J., and Chen, S.C. (2008). Assessment of genotoxicity of methyl-tert-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay. *Journal of Hazardous Materials* 153, 351-356. <http://dx.doi.org/10.1016/j.jhazmat.2007.08.053>
- Chen, D., Chen, J. and Zhong, W. (2009). Enhancement of methyl tert-butyl ether degradation by the addition of readily metabolizable organic substrates. *Journal of Hazardous Materials* 167, 860-865. <http://dx.doi.org/10.1016/j.jhazmat.2009.01.065>
- Chen, S.C., Chen, C.S., Zhan, K.V., Yang, K.H., Chien, C.C., Shieh, B.S. and Chen, W.M. (2011). Biodegradation of methyl tert-butyl ether (MTBE) by *Enterobacter* sp. NKNU02. *Journal of Hazardous Materials* 186, 1744-1750. <http://dx.doi.org/10.1016/j.jhazmat.2010.12.079>

- Corral-Escárcega, M.C., Ruiz-Gutiérrez, M.G., Quintero-Ramos, A., Meléndez-Pizarro, C.O., Lardizabal-Gutiérrez, D., Campos-Venegas, K. (2017) Use of biomass-derived from pecan nut husks (*Carya illinoensis*) for chromium removal from aqueous solutions. Column modeling and adsorption kinetics studies. *Revista Mexicana de Ingeniería Química* 16, 939-953. <http://www.redalyc.org/articulo.oa?id=62053304021>
- Dalton, H. and Stirling, D.I. (1982) Co-metabolism. *Philosophical Transactions of Royal Society of London B* 297, 481-496. <http://dx.doi.org/10.1098/rstb.1982.0056>
- Eixarch, H. and Constantí, M. (2010). Biodegradation of MTBE by *Achromobacter xylosoxidans* MCM1/1 induces synthesis of proteins that may be related to cell survival. *Process Biochemistry* 45, 794-798. <http://dx.doi.org/10.1016/j.procbio.2009.12.015>
- Fava, F., Armenante, P.M., Kafkewitz, D. and Marchetti, L. (1995). Influence of organic and inorganic growth supplements on the aerobic biodegradation of chlorobenzoic acids. *Applied Microbiology and Biotechnology* 43, 171-177. <http://dx.doi.org/10.1007/BF00170640>
- Fortin, N.Y., Morales, M., Nakagawa, Y., Focht, D.D. and Deshusses, M.A. (2001). Methyl tert-butyl ether (MTBE) degradation by a microbial consortium. *Environmental Microbiology* 3, 407-416. <http://dx.doi.org/10.1046/j.1462-2920.2001.00206.x>
- François, A., Mathis, H., Godefroy, D., Piveteau, P., Fayolle, F. and Monot, F. (2002). Biodegradation of methyl tert-butyl ether and other fuel oxygenates by a new strain, *Mycobacterium austroafricanum* IFP. *Applied and Environmental Microbiology* 68, 2754-2762. <http://dx.doi.org/10.1128/AEM.68.6.2754-2762.2002>
- Galtier, N., Gouy, M. and Gautier, C. (1996). SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment and molecular phylogeny. *Computer Applications in the Biosciences* 12, 543-548. <http://dx.doi.org/10.1093/bioinformatics/12.6.543>
- Gómez-Acata, S., Vital-Jácome, M., Pérez-Sandoval, M.V., Navarro-Noya, Y.E., Thalasso, F., Luna-Guido, M., Conde-Barajas, E. and Dendooven, L. (2018). Microbial community structure in aerobic and fluffy granules formed in a sequencing batch reactor supplied with 4-chlorophenol at different settling times. *Journal of Hazardous Materials* 342, 606-616. <http://dx.doi.org/10.1016/j.jhazmat.2017.08.073>
- Hao, D.H., Lin, J.Q., Song, X., Lin, J.Q., Su, Y.J. and Qu, Y.B. (2008). Isolation, identification, and performance studies of a novel paraffin-degrading bacterium of *Gordonia amicalis* LH3. *Biotechnology and Bioprocess Engineering* 13, 61-68. <http://dx.doi.org/10.1007/s12257-007-0168-8>
- Hyman, M. (2013). Biodegradation of gasoline ether oxygenates. *Current Opinion in Biotechnology* 24, 443-450. <http://dx.doi.org/10.1016/j.copbio.2012.10.005>
- Jiao, S., Chen, W., Wang, E., Wang, J., Liu, Z., Li, Y. and Wei, G. (2016). Microbial succession in response to pollutants in batch-enrichment culture. *Scientific Reports* 6, 21791. <http://dx.doi.org/10.1038/srep21791>
- Key, K.C., Sublette, K.L., Duncan, K., Mackay, D.M., Scow, K.M. and Ogles, D. (2013). Using DNA-Stable Isotope Probing to identify MTBE- and TBA-degrading microorganisms in contaminated groundwater. *Ground Water Monitoring and Remediation* 33, 57-68. <http://dx.doi.org/10.1111/gwrmr.12031>
- Krayl, M., Benndorf, D., Loffhagen, N. and Babel, W. (2003). Use of proteomics and physiological characteristics to elucidate ecotoxic effects of methyl tert-butyl ether in *Pseudomonas putida* KT2440. *Proteomics* 3, 1544-1552. <https://doi.org/10.1002/pmic.200300477>
- Kuss, K., Alano, C., Arbigaus, A., Rampinelli, J., Silveira, M., Bonatti-Chaves, M. and Furlan, S. (2018). Study of removal of bisphenol-A by *Pleurotus sajor-caju* crude enzyme broth. *Revista Mexicana de Ingeniería Química* 17, 989-998. <https://doi.org/10.24275/uam/izt/dcbi/revmexin/gquim/2018v17n3/Kuss>
- Levchuk, I., Bhatnagar, A. and Sillanpää, M. (2014). Overview of technologies for removal of methyl

- tert-butyl ether (MTBE) from water. *Science of Total Environment*, 476-477, 415-433. <http://dx.doi.org/10.1016/j.scitotenv.2014.01.037>
- Li, M., Liao, X., Zhang, D., Du, G. and Chen, J. (2011). Yeast extract promotes cell growth and induces production of polyvinyl alcohol-degrading enzymes. *Enzyme Research* 2011, 179819. <http://dx.doi.org/10.4061/2011/179819>
- Li, S.S., Wang, S. and Yan, W. (2016). Enhanced cometabolic degradation of methyl tert-butyl ether by a *Pseudomonas* sp. strain grown on n-pentane. *IOP Conferences Series: Earth and Environmental Science* 39, 012012. <http://dx.doi.org/10.1088/1755-1315/39/1/012012>
- Lin, C.W., Cheng, Y. W. and Tsai, S.L. (2007). Influences of metals on kinetics of methyl tert-butyl ether biodegradation by *Ochrobactrum cytisi*. *Chemosphere* 69, 1485-1491. <http://dx.doi.org/10.1016/j.chemosphere.2007.04.057>
- Lopes-Ferreira, N., Malandain, C. and Fayolle-Guichard, F. (2006). Enzymes and genes involved in the aerobic biodegradation of methyl tert-butyl ether (MTBE). *Applied Microbiology and Biotechnology* 72, 252-262. <http://dx.doi.org/10.1007/s00253-006-0494-3>
- Maeda, A.H., Kunihiro, M., Ozeki, Y., Nogi, Y. and Kanaly, R.A. (2015). *Sphingobium barthaii* sp. nov., a high molecular weight polycyclic aromatic hydrocarbon-degrading bacterium isolated from cattle pasture soil. *International Journal of Systematic and Evolutionary Microbiology* 65, 2919-2924. <http://dx.doi.org/10.1099/ijs.0.000356>
- Malandain, C., Fayolle-Guichard, F. and Vogel, T.M. (2010). Cytochromes P450-mediated degradation of fuel oxygenates by environmental isolates. *FEMS Microbiology Ecology* 72, 289-296. <http://dx.doi.org/10.1111/j.1574-6941.2010.00847.x>
- Mehlman, M.A. (2002). Carcinogenicity of methyl-tertiary butyl ether in gasoline. *Annals of the New York Academy of Science* 982, 149-159. <http://dx.doi.org/10.1111/j.1749-6632.2002.tb04930.x>
- Mendoza-Basilio, C., Yee-Madeira, H., Ramírez-Rodríguez, T. and Colindres-Bonilla, P. (2017). Oxidation of textile dye reactive yellow 84 in aqueous solution in order to reuse treated water. *Revista Mexicana de Ingeniería Química* 16, 581-589. <http://www.redalyc.org/articulo.oa?id=62052087022>
- Mo, K., Lora, C.O., Wanken, A.E., Javanmardian, M., Yang, X. and Kulpa, C.F. (1997). Biodegradation of methyl t-butyl ether by pure bacterial cultures. *Applied Microbiology and Biotechnology* 47, 69-72. <http://dx.doi.org/10.1007/s002530050890>
- Müller, R.H., Jorks, S., Kleinstüber, S. and Babel, W. (1998). Degradation of various chlorophenols under alkaline conditions by Gram-negative bacteria closely related to *Ochrobactrum anthropi*. *Journal of Basic Microbiology* 38, 269-281. [http://doi.org/10.1002/\(SICI\)1521-4028\(199809\)38:4<269::AID-JOBM269>3.0.CO;2-P](http://doi.org/10.1002/(SICI)1521-4028(199809)38:4<269::AID-JOBM269>3.0.CO;2-P)
- Muñoz-Castellanos, L.N., Torres-Muñoz, J.V., Keer-Rendon, A., Manzanares-Papayanopoulos, L.I. and Nevarez-Moorillon, G.V. (2006). Aerobic biodegradation of methyl tert-butyl ether (MTBE) by pure bacterial cultures isolated from contaminated soil. *World Journal of Microbiology and Biotechnology* 22, 851-855. <https://doi.org/10.1007/s11274-005-9114-0>
- Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8, 4321-4325. <http://dx.doi.org/10.1093/nar/8.19.4321>
- Nakatsu, C.H., Hristova, K., Hanada, S., Meng, X.Y., Hanson, J.R., Scow, K.M. and Kamagata, Y. (2006). *Methylibium petroleiphilum* gen. nov., sp. nov., a novel methyl tert-butyl ether-degrading methylotroph of the Betaproteobacteria. *International Journal of Systematic and Evolutionary Microbiology* 56, 983-989. <http://dx.doi.org/10.1099/ijs.0.63524-0>
- Nava, V., Morales, M. and Revah, S. (2007). Cometabolism of methyl tert-butyl ether (MTBE) with alkanes. *Reviews in Environmental Science and Biotechnology* 6,

- 339-352. <https://doi.org/10.1007/s11157-006-9119-7>
- Ortega-González, D.K, Zaragoza, D., Aguirre-Garrido, J., Ramírez-Saad, H., Hernández-Rodríguez, C. and Jan-Roblero, J. (2013). Degradation of benzene, toluene, and xylene isomers by a bacterial consortium obtained from rhizosphere soil of *Cyperus* sp. grown in a petroleum-contaminated area. *Folia Microbiologica* 58, 569-777. <http://dx.doi.org/10.1007/s12223-013-0248-4>
- Pimentel-González, D. and Revah, S., Campos-Montiel, R., Monroy-Hermosillo, O., Vernon-Carter, E.J. (2008). A laboratory study of the biodegradation of MTBE solubilised in water by a microbial consortium entrapped in a water-in-oil-in-water double emulsion. *Process Biochemistry* 43, 1239-1243. <http://dx.doi.org/10.1016/j.procbio.2008.07.004>
- PROY-NMX-AA-105-SCFI-2013. Suelos-hidrocarburos fracción ligera por cromatografía de gases con detectores de ionización de flama o espectrometría de masas, published in Diario Oficial de la Federación, August 28th, 2013
- Purswania, J., Juárez, B., Rodelasa, B., Gónzalez-López, J. and Pozo, C. (2011). Biofilm formation and microbial activity in a biofilter system in the presence of MTBE, ETBE and TAME. *Chemosphere* 85, 616-624. <http://dx.doi.org/10.1016/j.chemosphere.2011.06.106>.
- Raynal, M. and Pruden, A. (2008). Aerobic MTBE biodegradation in the presence of BTEX by two consortia under batch and semi-batch conditions. *Biodegradation* 19, 269-82. <http://dx.doi.org/10.1007/s10532-007-9133-7>
- Relman, D.A. (1993). Universal bacterial 16S rDNA amplification and sequencing. In: *Diagnostic Molecular Microbiology: Principles and Applications*, (D.H Persing, T.F. Smith, F.C. Tenover and T.T White, eds.), Pp. 489-95. American Society for Microbiology, Washington.
- Rosselló-Mora, R. and Amann, R. (2001). The species concept for prokaryotes. *FEMS Microbiology Reviews* 25, 39-67. <http://dx.doi.org/10.1111/j.1574-6976.2001.tb00571.x>
- Salanitro, J.P., Diaz, L.A., Williams, M.P. and Wisniewski, H.L. (1994). Isolation of a bacterial culture that degrades methyl t-butyl ether. *Applied and Environmental Microbiology* 60, 2593-2596.
- Sanguinetti, C.J., Dias Neto E. and Simpson A.J. (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17, 914-921.
- Smith, C.A. and Hyman, M.R. (2004). Oxidation of methyl tert-butyl ether by alkane hydroxylase in dicyclopropylketone-induced and n-octane-grown *Pseudomonas putida* GPo1. *Applied and Environmental Microbiology* 70, 4544-4550. <http://dx.doi.org/10.1128/AEM.70.8.4544-4550.2004>
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28, 2731-2739. <http://dx.doi.org/10.1093/molbev/msr121>
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25, 4876-4882. <http://dx.doi.org/10.1093/nar/25.24.4876>
- Volpe, A., Del Moro, G., Rossetti, S., Tandoi, V. and Lopez, A. (2009). Enhanced bioremediation of methyl tert-butyl ether (MTBE) by microbial consortia obtained from contaminated aquifer material. *Chemosphere* 75, 149-155. <http://dx.doi.org/10.1016/j.chemosphere.2008.12.053>
- Xiong, J., An, T., Li, G. and Peng, P. (2017a). Accelerated biodegradation of BPA in water-sediment microcosms with *Bacillus* sp. GZB and the associated bacterial community structure. *Chemosphere* 184, 120-126. <http://dx.doi.org/10.1016/j.chemosphere.2017.05.163>

- Xiong, J., Li, G. and An, T. (2017b). The microbial degradation of 2,4,6-tribromophenol (TBP) in water/sediments interface: Investigating bioaugmentation using *Bacillus* sp. GZT. *Science of Total Environment* 575, 573-580. <http://dx.doi.org/10.1016/j.scitotenv.2016.09.017>
- Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H. and Chun, J. (2017). Introducing EzBioCloud: A taxonomically united database of 16S rRNA and whole genome assemblies. *International Journal of Systematic Evolutionary Microbiology* 67, 1613-1617. <http://dx.doi.org/10.1099/ijsem.0.001755>
- Ziagova, M. and Liakopoulou-Kyriakides, M. (2007). Kinetics of 2,4-dichlorophenol and 4-Cl-m-cresol degradation by *Pseudomonas* sp. cultures in the presence of glucose. *Chemosphere* 68, 921-927. <http://dx.doi.org/10.1016/j.chemosphere.2007.01.039>