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Limitations in MTBE Biodegradation

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Résumé — Étapes limitantes dans la biodégradation du MTBE — La voie métabolique de dégradation du méthyl *tertio* butyl éther ou MTBE chez *Mycobacterium austroafricanum* IFP 2012 a été partiellement élucidée par identification des intermédiaires de dégradation. Elle nécessite l'induction de différentes activités enzymatiques. Au cours des premières étapes de la dégradation du MTBE en *tertio* butyl alcool (TBA), une même monooxygénase est responsable de l'oxydation du MTBE et du TBA avec une faible affinité pour le TBA (Km = 1,1 mM) et une estérase est impliquée dans l'hydrolyse du *tertio* butyl formiate (TBF). La lenteur de la dégradation du MTBE chez *M. austroafricanum* IFP 2012 semble due à un processus complexe combinant principalement un effet négatif du TBF formé au cours de l'oxydation du MTBE sur la MTBE/TBA monooxygénase et l'absence de la dégradation du TBA par la monooxygénase en présence de MTBE. Dans les étapes ultérieures de la dégradation, un besoin spécifique en cations Co^{++} intervient au cours de la dégradation de l'acide 2-hydroxyisobutyrique (HIBA), la croissance sur HIBA étant très faible en absence de cette supplémentation.

Abstract* — *Limitations in MTBE Biodegradation — *The methyl* tert*-butyl ether (MTBE) metabolic pathway was partially elucidated in* Mycobacterium austroafricanum *IFP 2012 by identifying the degradation intermediates. Several enzymatic activities were specifically induced during growth on MTBE. Among those required for the first steps of MTBE degradation to* tert*-butyl alcohol (TBA), the same monooxygenase was responsible for the oxidation of both MTBE and TBA, with a low affinity for TBA (Km = 1.1 mM). An esterase was involved in the hydrolysis of* tert*-butyl formate (TBF). The slowness of the degradation of MTBE by* M. austroafricanum *IFP 2012 was the result of complex interactions, especially the negative effect of TBF formed during MTBE oxidation on the MTBE/TBA moonoxygenase and the absence of TBA oxidation in the presence of residual MTBE. Moreover, concerning the downstream steps of MTBE metabolism,* Co^{++} *ions were required for the degradation of 2-hydroxyisobutyric acid (HIBA) formed by oxidation of TBA as shown by the low growth yield on HIBA in the absence of cobalt.*

INTRODUCTION

Fuel oxygenates have been largely used for the twenty past years as additives to gasoline to increase their octane index and to limit the carbon monoxide in exhaust pipe gases. The compounds of interest are ethers such as methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE) and *tert*-amyl methyl ether (TAME), or related alcohols such as *tert*-butyl alcohol (TBA) and *tert*-amyl alcohol (TAA). Table 1 summarizes data concerning two compounds, MTBE and ETBE. Their properties are so interesting that MTBE became the fourth organic chemicals produced in United States (Johnson *et al.*, 2000). However, the frequent occurrence of leakage of fuel tanks led to the contamination of numerous aquifers by MTBE because of its high solubility in water, its low retention on organic matter and its low biodegradability. The first case of contamination of aquifers by MTBE (MTBE concentration up to 600 $\mu g \cdot l^{-1}$) was reported in 1996 in Santa Monica (CA) (USEPA, 1999). In the United States, 250 000 leaking underground fuel tank sites have shown different levels of MTBE contamination (Johnson *et al.*, 2000; Squillace *et al.*, 1996). In Germany, Achten *et al.* (2002a and 2002b) detected traces of MTBE in rivers and in influents and effluents of waste water treatment plants, thus confirming the low biodegradability of this compound. Actually, the environmental impact of the massive use of such ethers was not studied in advance, except their effect on gaseous emission after combustion of MTBE-containing gasoline.

TABLE 1

Main physicochemical characteristics of MTBE and ETBE

Characteristics	MTBE	ETBE
Molecular weight ($g \cdot mol^{-1}$)	88.15	102.18
Boiling point (°C)	55.3	72.8
Density$_{20°C}$ ($kg \cdot l^{-1}$)	0.74	0.74
Log K_{oc} (1)	0.55< >0.91	Nd
Water solubility ($g \cdot l^{-1}$)	48	12
RON(2) index	118	117
MON(3) index	101	101
Variation of HC emitted in gaseous emission (%)	–6.5*	–5.2**
Variation of CO emitted in gaseous emission (%)	–9.3*	–14.6**

Nd: not determined; HC: hydrocarbons; CO: carbon monoxyde.

* when MTBE is added to a final concentration of 15% (v/v).
** when ETBE is added to a final concentration of 17% (v/v).

(1) K_{oc}: soil adsorption coefficient.
(2) RON: research octane number.
(3) MON: motor octane number.

Studies on natural attenuation showed very different half-life values depending on the polluted environment: in surface water (lakes, etc.) the MTBE half-life was estimated to 14 days, 3 days in atmosphere but, at least 2 years in most polluted groundwaters (in comparison, the half-life of benzene in the same environment was 2 or 3 months). Moreover, the presence of MTBE in drinkable water was easily detected by consumers and the USEPA standard for MTBE in water was set at 20-40 $\mu g \cdot l^{-1}$ based on that detection level. In the absence of clear data on the effect of MTBE on human health, USEPA (1999) classified MTBE as a potential carcinogen.

Due to its physical characteristics, *i.e.* high water solubility and low retention on organic matter, the usual physicochemical techniques for the clean-up of polluted aquifers (air injection, activated carbon filtering, etc.) are relatively inefficient regarding MTBE. That is the reason why bioremediation processes should be particularly interesting.

The initial question about MTBE persistence was: is it due to the scarcity of MTBE-degrading microorganisms with MTBE degradation activity or is it due to the incapacity of microorganisms to use MTBE as a carbon and energy source?

Different authors recently reported the biodegradation of MTBE by microcosms from different origins (Bradley *et al.*; 1999, Bradley *et al.*, 2001; Fortin *et al.*, 2001; Kane *et al.*, 2001; Landmeyer *et al.*, 2001; Park and Cowan, 1997; Salanitro *et al.*, 1994). Moreover, the cometabolic degradation of MTBE was shown to be an important mechanism for MTBE biodegradation using microrganisms able to grow on gaseous alkanes (Hardison *et al.*, 1997; Steffan *et al.*, 1997; Hyman and O'Reilly, 1999; Liu *et al.*, 2001; Hyman *et al.*, 2000), pentane (Garnier *et al.*, 1999), camphor (Steffan *et al.*, 1997), ethanol (Piveteau *et al.*, 2000) or cyclohexane (Corcho *et al.*, 2000).

Finally, three bacterial strains able to grow on MTBE were isolated, two gram-negative strains, *Rubrivivax gelatinosus* PM1, (Hanson *et al.*, 1999; Deeb *et al.*, 2001) and *Hydrogenophaga flava* ENV735 (Steffan *et al.*, 2000; Hatzinger *et al.*, 2001) and a gram-positive one, *Mycobacterium austroafricanum* IFP 2012 (François *et al.*, 2002).

1 GROWTH OF *M. AUSTROAFRICANUM* IFP 2012 ON FUEL OXYGENATES

M. austroafricanum IFP 2012 was able to grow on MTBE *(Fig. 1)* and TAME. In contrast, ETBE was degraded very slowly without production of biomass. During growth on MTBE, some degradation intermediates were detected, *tert*-butyl formate (TBF), *tert*-butyl alcohol (TBA), 2-hydroxyisobutyric acid (HIBA) and acetone. Since TBF was formed during MTBE degradation, it was assumed that the attack of the ether bond led to the formation of an hemiacetal. From these results, the metabolic pathway of

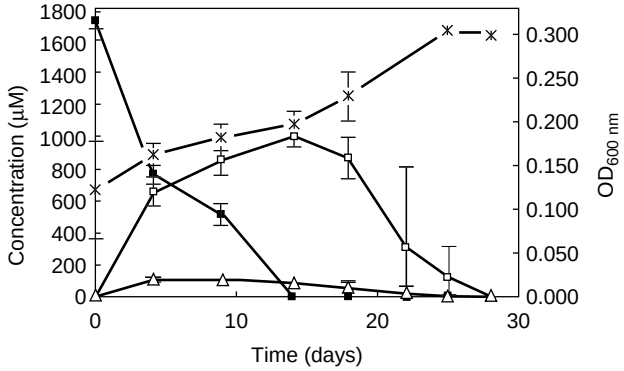

Figure 1

Growth kinetics of *M. austroafricanum* IFP 2012 on MTBE. Growth was estimated by measuring optical density (OD) at 600 nm (×–×). MTBE (■-■) and its degradation intermediates, TBF (Δ-Δ) and TBA (□-□) were measured on filtered samples using GC.

MTBE was partially elucidated *(Fig. 2). M. austroafricanum* IFP 2012 grew 6 times faster on TAME than on MTBE.

Several enzymatic activities involved in the MTBE degradation pathway were shown to be induced during growth on MTBE or TBA. These enzymatic activities were studied at the physiological level: an MTBE/TBA monooxygenase (François *et al.*, 2002), a TBF esterase and an acetone monooxygenase (François *et al.*, accepted for publication). The involvement of a cobalt-dependent HIBA degradation activity (a putative decarboxylase) and of a 2-propanol dehydrogenase in the metabolism was also demonstrated *(Table 2)*.

The growth of *M. austroafricanum* IFP 2012 on MTBE as a sole carbon and energy source was characterized by the occurrence of two successive steps:

– a long lag phase corresponding to the conversion of MTBE to TBA without any visible growth ;
– a rapid and significant growth on the TBA produced.

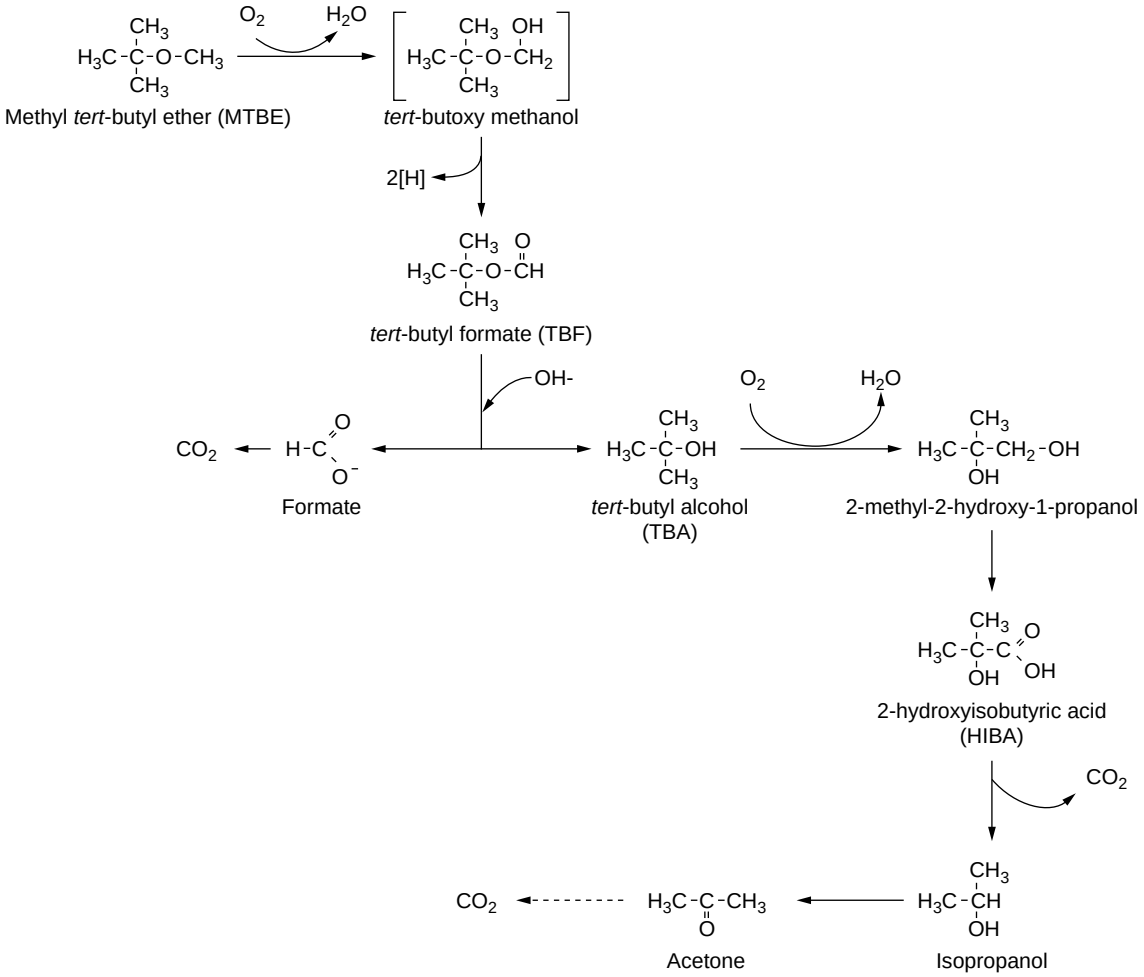

Figure 2

MTBE metabolic pathway of *M. austroafricanum* IFP 2012.

TABLE 2

Enzymatic activities detected during growth of *M. austroafricanum* IFP 2012 on MTBE

Enzyme	Substrate tested	Specific activity of substrate degradation ($\mu mol \cdot h^{-1} \cdot g^{-1}$ biomass)
MTBE/TBA monooxygenase	MTBE	263 ± 21*
TBF esterase	TBF	360 ± 30*
MTBE/TBA monooxygenase	TBA	315 ± 11*
Co^{++}-dependent HIBA decarboxylase	HIBA	498 ± 22*
NDMA: 2-propanol oxydoreductase**	2-propanol	91***
Acetone monooxygenase	Acetone	210 ± 12*

* Activities measured using resting cells after growth of *M. austroafricanum* IFP 2012 on the corresponding substrate. Initial concentrations of MTBE, TBF, TBA, HIBA and acetone were 340, 240, 470, 340 and 222 µM, respectively.
** NDMA: N,N' dimethyl nitrosoaniline.
*** Activity measured with cellular extracts after growth of *M. austroafricanum* IFP 2012 on 2-propanol.

2 ENERGY LOSS DURING MTBE METABOLISM

The biodegradation of MTBE by *M. austroafricanum* IFP 2012 and by the other microorganisms able to grow on MTBE as a sole carbon and energy source, is poorly documented. The study of these newly isolated bacteria could allow the identification of the limitations in MTBE biodegradation.

The MTBE molecule is characterized by the presence of an ether bond and a high steric hindrance of the carbon of the *tert*-butyl group bound to the methoxy group. Alkyl ethers are stable molecules ($\Delta G'_0$ of the ether bond formation = 360 kJ/mole) (White *et al.*, 1996). In the case of MTBE and TAME, a methoxy group is present in both molecules but growth was faster on TAME. However, the specific MTBE and TAME degradation activities measured using resting cells of *M. austroafricanum* IFP 2012 were in the same range, 263 and 327 µmol of ether$\cdot h^{-1} \cdot g^{-1}$ biomass, respectively, suggesting that the initial oxidation of the methoxy group was not responsible for the difference of growth kinetics observed on these two compounds. So, the presence of the ether bond does not seem to be the cause of the recalcitrance of the molecule.

That there is a high energy demand for MTBE degradation is reflected by the low efficiency of biomass production on MTBE. Fortin *et al.* (2001) pointed out the low MTBE biomass yield obtained using consortia (Fortin and Deshusses, 1999; Salanitro *et al.*, 1994) and even using the pure MTBE-growing strain *R. gelatinosus* PM1 (Hanson *et al.*, 1999). These authors proposed to calculate the energy discrepancy index, δ_e, during MTBE metabolism which corresponds to the ratio of the theoretical to the experimental biomass yields ($\delta_e = Y^{theo}_{biomass}/Y^{exp}_{biomass}$). The δ_e value was 1.06 for *P. aeruginosa* on glucose, values greater than 1 being an indication of an inefficient carbon assimilation pathway due to energy loss. Such a calculation provided interesting results as shown in Table 3.

TABLE 3

Growth efficiency of MTBE- and TBA-degrading strains on MTBE or on its degradation intermediates

Microorganism	Substrate	$Y^{exp}_{X/S}$ ($g \cdot biomass \cdot mol^{-1}$)	Av e^- (1)	$Y^{theo}_{X/S}$ ($g \cdot biomass \cdot mol^{-1}$) (2)	δe (3)
M. austroafricanum IFP 2012 (François *et al.*, 2002)	MTBE	33.4*	30	92.1	2.8
	TBA	45.1*	24	73.7	1.6
	HIBA	43.9*	18	55.3	1.3
R. gelatinosus PM1 (Hanson *et al.*, 1999)	MTBE	15.8	30	92.1	5.8
H. flava ENV735 (Hatzinger *et al.*, 2001)	MTBE	35.2	30	92.1	2.6
B. cepacia IFP 2003 (Piveteau *et al.*, 2001)	TBA + Co^{++}	40.0	24	73.7	2.0
	TBA – Co^{++}	13.3	24	73.7	6.0

(1) Av e^- corresponds to the number of electrons available through hydrolysis of substrates: substrate + x H_2O ----> y CO_2 + (Av e^-/2) H_2
(2) $Y^{theo}_{X/S} = 3.07$ Av e^-
(3) $\delta e = Y^{theo}_{X/S} / Y^{exp}_{X/S}$
* All experimental measurements were performed in the presence of $CoCl_2$.

TABLE 4

Characteristics of monooxygenases induced on MTBE, TBA or acetone using resting cells of *M. austroafricanum* IFP 2012

	Oxygenase activity of *M. austroafricanum* IFP 2012 measured on:		
	MTBE	**TBA**	**Acetone**
Oxygenase activity under anaerobiosis	No	No	No
Inducers of the oxygenase activity	MTBE TBA	MTBE TBA	MTBE TBA Acetone
Induction of a peak at 450 nm*	No	No	No
Effect of acetylene addition	Inactivation	Inactivation	No inactivation
Activity of propylene epoxidation	179 ± 4 µmol·h^{-1}·g^{-1} biomass	303 ± 14 µmol·h^{-1}·g^{-1} biomass	69.1 ± 4.3 nmol·h^{-1}·g^{-1} biomass

* The presence of a cytochrome P 450 can be detected by spectral determination of a peak at 450 nm in the presence of CO in cellular extracts.

The δ_e value was the highest for *R. gelatinosus* PM1 on MTBE (5.8), it was 2 fold lower for *H. flava* ENV735 and *M. austroafricanum* IFP 2012 on MTBE (2.6 and 2.7, respectively). Even though the biomass productions were better on MTBE with those two latter strains, energy losses occurred during MTBE metabolism since δ_e was largely superior to 1.

The comparison of the δ_e values obtained with *M. austroafricanum* IFP 2012 on MTBE and its degradation intermediates, TBA and HIBA, showed that $\delta_e^{MTBE} > \delta_e^{TBA} > \delta_e^{HIBA}$. This result showed that there was a specific energy loss due to the conversion step from MTBE to TBA.

3 LIMITING STEPS OF MTBE BIODEGRADATION

Salanitro (1995) suggested that the slow growth on MTBE was due to considerable feedback regulation by metabolites on the oxygenase responsible for the ether bond cleavage. This author also considered that TBA and acetone could compete for the same MTBE oxygenase. The necessity of regenerating cofactors, such as NADH, could also have an influence on the rate of MTBE degradation since reduced cofactors are required for several oxidation steps.

3.1 Limiting Factors at the First Oxidation Step (MTBE to TBA)

The possibility of a competition between the oxygenases involved in MTBE metabolism was investigated using *M. austroafricanum* IFP 2012. Oxidation of different products could be involved in MTBE metabolism: MTBE, TBA and acetone. As no activity was detected in disrupted cell extracts, the activity of such oxygenases was studied using resting cells. Different factors were compared, including the involvement of atmospheric oxygen in the reaction, the capacity for epoxide formation (monooxygenase-type activity), the inactivation of the oxidation reaction by acetylene (an inactivator of monooxygenases) and the determination of the substrates inducing the monooxygenase activities. The results obtained are summarized in Table 4. As shown in this table, the same monooxygenase seems to be involved in the oxidation of both MTBE and TBA. On the contrary, the monooxygenase involved in acetone metabolism was clearly different. The simultaneous presence of MTBE and TBA during growth on MTBE could generate a competition between these two compounds. In the case of *M. austroafricanum* IFP 2012, the apparent Km value of the monooxygenase for TBA was 1.1 mM. The affinity of the monooxygenase for MTBE could not be precisely determined but was higher than that for TBA. Moreover, at a molar ratio MTBE/TBA in favor of TBA, the loss in MTBE degradation activity was low *(Table 5)*. So we concluded that competition between MTBE and TBA for the same monooxygenase could not explain the low degradation of

TABLE 5

Effects of TBA and TBF on the enzymatic activities involved in the step of conversion from MTBE to TBA

Mixture of compounds tested	**Molar ratio**	**Effect on MTBE degradation activity* (%)**	**Effect on TBF degradation activity** (%)**
MTBE/TBA	1/7	–18	Nd
MTBE/TBF	3/1	–30	Nd
TBA/TBF	9/1	Nd	–35

Nd: not determined.

* calculated by comparison with the value obtained on MTBE without initial addition of any other compound.

** calculated by comparison with the value obtained on TBF without initial addition of any other compound.

MTBE, which is in agreement with the growth profile observed on Figure 1, *i.e.* successive utilization of MTBE and TBA. As the monooxygenase involved in the degradation of acetone was clearly different *(Table 4)*, acetone should not compete with MTBE and TBA.

The degradation of TBF formed after oxidation of MTBE was previously shown to be a spontaneous hydrolysis at acidic pH (O'Reilly *et al.*, 2001). No data were available on the fate of TBF at neutral pH. Actually, TBF hydrolysis rates (initial concentration about 250 µM) were 8 $\mu M \cdot h^{-1}$ and 48 $\mu M \cdot h^{-1}$ in abiotic conditions and in the presence of *M. austroafricanum* IFP 2012, respectively, showing that TBF hydrolysis was mainly due to an esterase activity in *M. austroafricanum* IFP 2012. Furthermore, TBF affects MTBE degradation activity: a significant inhibitory action of TBF on the monooxygenase activity was shown *(Table 5)* even at a molar ratio in favor of MTBE. Similarly, a negative effect of TBA on the TBF esterase was shown *(Table 5)*, but at a lesser extent. These results are summarized in Figure 3.

3.2 Limiting Factors for TBA Metabolism

Another important point to address was the metabolism of TBA. An inefficient assimilation of TBA could be the reason for the low growth yield observed in some cases.

In the case of *M. austroafricanum* IFP 2012, the addition of TBA did not have any significant effect on MTBE degradation rate by the monooxygenase *(Table 5)*. On the contrary, the presence of MTBE clearly limited TBA degradation since TBA was degraded mainly when no MTBE could be detected *(Fig. 1)*.

It was previously reported that a strain able to grow on TBA but not on MTBE, *Burkholderia cepacia* IFP 2003, required the presence of Co^{++} for its growth on TBA (Piveteau *et al.*, 2001). We showed that Co^{++} were also required for growth on TBA of *M. austroafricanum* IFP 2012 (François *et al.*, 2002). The δ_e values for *B. cepacia* IFP 2003 growing on TBA were calculated in the presence and in the absence of cobalt. The δ_e value obtained on TBA in absence of Co^{++} was 3 fold lower than that obtained on TBA in presence of such cations *(Table 3)* confirming the positive effect of cobalt. TBA was also degraded via HIBA (result not shown) by *B. cepacia* IFP 2003. We showed that Co^{++} cations were specifically required in HIBA catabolism by both strains *(Figs 4a and b)*. The degradation of HIBA most probably occurred via a decarboxylation step and some metalloenzymes, including decarboxylases, specifically requiring Co^{++} in their active site were recently rewieved by Kobayashi and Shimidzu (1999).

CONCLUSIONS

Ethers are generally reported as compounds being highly resistant to biological mineralization due to the presence of an ether bond. In the case of MTBE, the complete understanding of its poor biodegradability would require the isolation of specialized microorganisms, the determination of the MTBE metabolic pathway, the characterization of the genes and enzymes involved in the degradation and their mode of regulation. In spite of the isolation of some rare microorganisms able to grow on MTBE as a sole source of carbon and energy, we are still far from understanding all the reasons for its low biodegradability. The identification of MTBE degradation intermediates during growth of *M. austroafricanum* IFP 2012 led to the partial determination of the MTBE metabolic pathway. Using this strain, we demonstrated that the cleavage of the ether bond involved

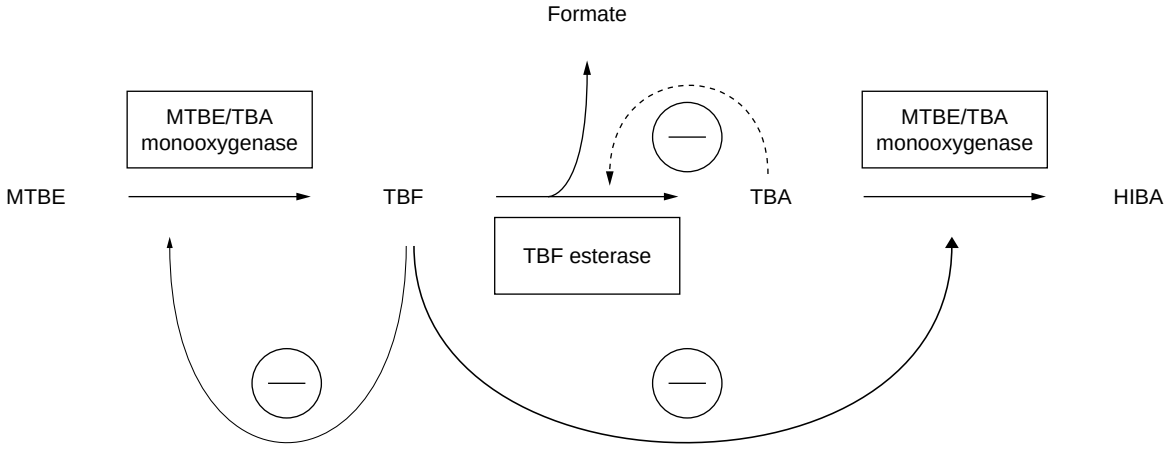

Figure 3

Regulation effects during the early steps of MTBE metabolism in *M. austroafricanum* IFP 2012.

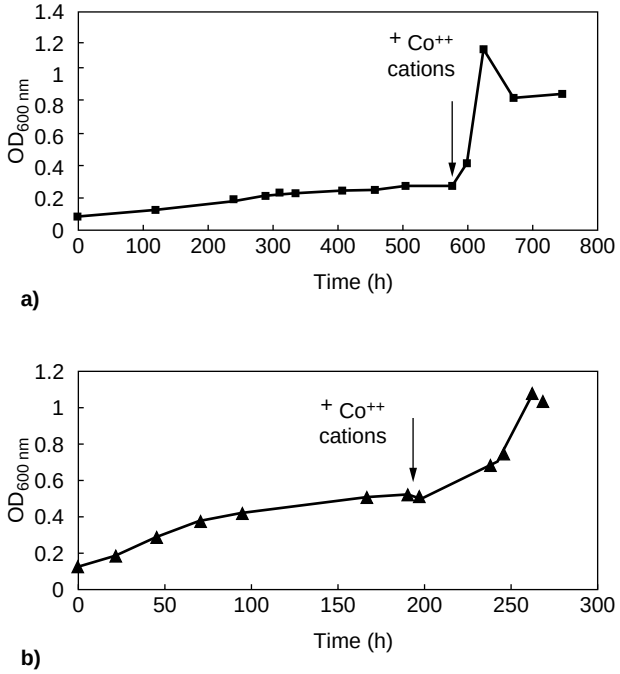

Figure 4

Effect of cobalt addition on growth on HIBA of (a) *M. austroafricanum* IFP 2012: (■-■) and (b) *B. cepacia* IFP 2003 (▲-▲). The arrow corresponded to the addition of $CoCl_2$ at a final concentration of 1 $mg \cdot l^{-1}$.

two successive steps: the oxidation of the ether to the corresponding ester, *tert*-butyl formate (TBF) and the hydrolysis of TBF. A complex mechanism of regulation at these early steps of MTBE degradation in relation to products inhibition seems to be responsible for the slow MTBE degradation. Moreover, the metabolism of TBA was slowed down by the reduced availability of the MTBE/TBA monooxygenase and was including a step requiring cobalt ions. The study of the different enzymes involved in the pathway and the determination of the corresponding gene sequences and their distribution among degrading strains would provide keys to understand the whole process.

REFERENCES

Achten, C., Kolb, A. and Puttmann, W. (2002a) Methyl *tert*-butyl ether (MTBE) in River and Wastewater in Germany. *Environ. Sci. Technol.*, **36**, 3652-3661.

Achten, C., Kolb, A. and Puttmann, W. (2002b) Occurrence of methyl *tert*-butyl ether (MTBE) in Riverbank Filtered Water and Drinking Water Produced by Riverbank Filtration. *Environ. Sci. Technol.*, **36**, 3662-3670.

Bradley, P.M., Landmeyer, J.E. and Chapelle, F.H. (1999) Aerobic Mineralization of MTBE and *tert*-butyl alcohol by Stream-Bed Sediment Microorganisms. *Environ. Sci. Technol.*, **33**, 1877-1879.

Bradley, P.M., Landmeyer, J.E. and Chapelle, F.H. (2001) Widespread Potential for Microbial MTBE Degradation in Surface-Water Sediments. *Environ. Sci. Technol.*, **35**, 658-662.

Corcho, D., Watkinson, R.J. and Lerner, D.N. (2000) Cometabolic Degradation of MTBE by a Cyclohexane-Oxidising Bacteria. In: Wickramanayake, G.B., Gavaskar, A.R., Alleman, B.C. and Magar, V.S. (eds) *Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds*, Battelle, Columbus, Ohio, 183-189.

Deeb, R.A., Hu, H.Y., Hanson, J.R., Scow, K.M. and Alvarez-Cohen, L. (2001) Substrate Interactions in BTEX and MTBE Mixtures by an MTBE-Degrading Isolate. *Environ. Sci. Technol.*, **35**, 312-317.

Fortin, N. and Deshusses, M.A. (1999) Treatment of methyl *tert*-butyl ether Vapors in Biotrickling Filters. 1. Reactor Startup, Steady State Performance and Culture Characteristics. *Environ. Sci. Technol.*, **33**, 2980-2986.

Fortin, N., Morales, M., Nakagawa, Y., Focht, D.D. and Deshusses, M.A. (2001) Methyl *tert*-butyl ether (MTBE) Degradation by a Microbial Consortium. *Environ. Microbiol.*, **3**, 407-416.

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 2012. *Appl. Environ. Microbiol.*, **68**, 2754-2762.

François, A., Garnier, L., Mathis, H., Fayolle, F. and Monot, F. Roles of *tert*-butyl Formate, *tert*-butyl alcohol and Acetone in the Regulation of methyl *tert*-butyl ether Degradation by *Mycobacterium austroafricanum* IFP 2012. Accepted for publication in *Appl. Microbiol. Biotechnol.*

Garnier, P.M., Auria, R., Augur, C. and Revah, S. (1999) Cometabolic biodegradation of methyl *t*-butyl ether by *Pseudomonas aeruginosa* grown on pentane. *Appl. Microbiol. Biotechnol.*, 51, 498-503.

Hanson, J.R., Ackerman, C.E. and Scow, K.M. (1999) Biodegradation of methyl *tert*-butyl ether by a Bacterial Pure Culture. *Appl. Environ. Microbiol.*, 65, 4788-4792.

Hardison, L.K., Curry, S.S., Ciuffetti, L.M. and Hyman, M.R. (1997) Metabolism of Diethyl Ether and Cometabolism of methyl *tert*-butyl ether by a Filamentous Fungus, a *Graphium* sp. *Appl. Environ. Microbiol.*, **63**, 3059-3067.

Hatzinger, P.B., McClay, K., Vainberg, S., Tugusheva, M., Condee, C.W. and Steffan, R.J. (2001) Biodegradation of methyl *tert*-butyl ether by a Pure Bacterial Culture. *Appl. Environ. Microbiol.*, **67**, 5601-5607.

Hyman, M. and O'Reilly, K. (1999) Physiological and Enzymatic Features of MTBE-degrading Bacteria. In: Alleman, B.C. and Leeson, A. (eds) In situ *Bioremediation of Petroleum Hydrocarbon and Other Organic Compounds*, Batelle, Columbus, Ohio, 7-12.

Hyman, M., Taylor, C. and O'Reilly, K. (2000) Cometabolic degradation of MTBE by iso-alkane-utilizing bacteria from gasoline impacted soils. In: Wickramanayake, G.B., Gavaskar, A.R., Alleman, B.C. and Magar, V.S. (eds) *Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds*, Battelle, Columbus, Ohio, 149-155.

Johnson, R., Pankow, J., Bender, D., Price, C. and Zogorsky, J. (2000) MTBE. To What Extent Will Past Release Contaminate Community Water Supply Wells? *Environ. Sci. Technol.*, **34**, 210A-217A.

Kane, S.R., Beller, H.R., Legler, T.C., Koester, C.J., Pinkart, H.C., Halden, R.U. and Happel, A.M. (2001) Aerobic Biodegradation of methyl *tert*-butyl ether by Aquifer Bacteria from Leaking Underground Storage Tank Sites. *Appl. Environ. Microbiol.*, **67**, 5824-5829.

Kobayashi, M. and Shimidzu, S. (1999) Cobalt Proteins. *Eur. J. Biochem.*, **261**, 1-9.

Landmeyer, J.E., Chapelle, F.H., Herlong, H.H. and Bradley, P.M. (2001) Methyl *tert*-butyl ether Biodegradation by Indigenous Aquifer Microorganisms under Natural and Artificial Oxic Conditions. *Environ. Sci. Technol.*, **35**, 1118-1126.

Liu, C.Y., Speitel, G.E. Jr and Georgiou, G. (2001) Kinetics of methyl *t*-butyl ether Cometabolism at Low Concentrations by Pure Cultures of Butane-Degrading Bacteria. *Appl. Environ. Microbiol.*, **67**, 2197-2201.

O'Reilly, K.T., Moir, M.E., Taylor, C.D., Smith, C.A. and Hyman, M.R. (2001) Hydrolysis of methyl *tert*-butyl ether (MTBE) in Dilute Aqueous Acid. *Environ. Sci. Technol.*, **35**, 3954-61.

Park, K. and Cowan, R.M. (1997) Effects of Oxygen and Temperature on the Biodegradation of MTBE. In: *Preprints of Extended Abstracts, ACS National Meeting, Am. Chem. Soc., Div. Environ. Chem.*, Washington DC, **37**, 421-424.

Piveteau, P., Fayolle, F., Le Penru, Y. and Monot, F. (2000) Biodegradation of MTBE by Cometabolism in Laboratory-Scale Fermentations. In: Wickramanayake, G.B., Gavaskar, A.R., Alleman, B.C. and Magar, V.S. (eds) *Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds*, Battelle, Columbus, Ohio, 141-148.

Piveteau, P., Fayolle, F., Vandecasteele, J.P. and Monot, F. (2001) Biodegradation of *tert*-butyl Alcohol and Related Xenobiotics by a Methylotrophic Bacterial Isolate. *Appl. Microbiol. Biotechnol.*, **55**, 369-373.

Salanitro, J.P. (1995) Understanding the Limitations of Microbial Metabolism of Ethers Used as Fuel Octane Enhancers. *Curr. Op. Biotechnol.*, **6**, 337-340.

Salanitro, J.P., Diaz, L.A., Williams, M.P. and Wisniewski, H.L. (1994) Isolation of a Bacterial Culture that Degrades methyl *tert*-butyl ether. *Appl. Environ. Microbiol.*, **60**, 2593-2596.

Squillace, P.J., Zogorski S., Wilber, W.G. and Price, C.V. (1996) Preliminary Assessment of the Occurence and Possible Sources of MTBE in Groundwater in the United States, 1993-1994. *Environ. Sci. Technol.*, **30**, 1721-173.

Steffan, R.J., McClay, K., Vainberg, S., Condee, CW. and Zhang, D. (1997) Biodegradation of the Gasoline Oxygenates methyl *tert*-butyl ether, ethyl *tert*-butyl ether, and *tert*-amyl methyl ether by Propane-Oxidizing Bacteria. *Appl. Environ. Microbiol.*, **63**, 4216-4222.

Steffan, R.J., Vainberg, S., Condee, C.W., McClay, K. and Hatzinger, P.B. (2000) Biotreatment of MTBE with a New Bacterial Isolate. In: Wickramanayake, G.B., Gavaskar, A.R., Alleman, B.C., and Magar, V.S. (eds) *Bioremediation and Phytoremediation of Chlorinated and Recalcitrant Compounds*, Battelle, Columbus, Ohio, 165-173.

USEPA (1999) *Achieving Clean Air and Clean Water: the Report of the Blue Ribbon Panel on Oxygenates in Gasoline*, US Government Printing Office, Washington DC, EPA420-R-99-021.

White, G.F., Russell, N. and Tidswell, E. (1996) Bacterial Scission of Ether Bonds. *Microbiol. Rev.*, **60**, 216-232.

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