HAL
open science

Gasoline and Diesel Oil Biodegradation

R. Marchal, S. Penet, F. Solano-Serena, J. P. Vandecasteele

To cite this version:

R. Marchal, S. Penet, F. Solano-Serena, J. P. Vandecasteele. Gasoline and Diesel Oil Biodegradation. Oil & Gas Science and Technology - Revue d'IFP Energies nouvelles, 2003, 58 (4), pp.441-448. 10.2516/ogst:2003027 . hal-02043884

HAL Id: hal-02043884

https://ifp.hal.science/hal-02043884v1

Submitted on 21 Feb 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Oil & Gas Science and Technology – Rev. IFP, Vol. 58 (2003), No. 4, pp. 441-448
Copyright © 2003, Éditions Technip

Gasoline and Diesel Oil Biodegradation

R. Marchal[1], S. Penet[1], F. Solano-Serena[1] and J.P. Vandecasteele[1]

1 Institut français du pétrole, 1 et 4, avenue de Bois-Préau, 92852 Rueil-Malmaison Cedex - France
e-mail: remy.marchal@ifp.fr - sophie.penet@ifp.fr - leglise@ensil.unilim.fr - j-paul.vandecasteele@ifp.fr

Résumé — Biodégradation de l'essence et du gazole — Les carburants constituent des polluants organiques importants des sols et des eaux souterraines. La persistance de ces polluants dans l'environnement dépend de la biodégradabilité intrinsèque des hydrocarbures constitutifs, de la présence de microflores actives sur les sites pollués et des facteurs environnementaux.

La biodégradabilité intrinsèque de carburants tels que l'essence ou le gazole a été déterminée en utilisant une microflore aérobie standard provenant d'une boue activée de station d'épuration d'eau urbaine. L'essence présente un taux de biodégradabilité intrinsèque élevé (96 %), mais celui du gazole commercial est plus faible (entre 60 et 73 % selon la microflore utilisée). Les hydrocarbures récalcitrants à la biodégradation sont des cycloalcanes et des alcanes ramifiés, notamment ceux qui comportent des atomes de carbone quaternaires ou des groupements substituants consécutifs sur la chaîne carbonée principale. Dans le cas de gazoles de types particuliers, la composition en classes structurales d'hydrocarbures explique les taux de dégradation variables qui ont été observés. En particulier, le taux de dégradation peut être proche de 100 % lorsque les alcanes linéaires sont abondants (gazole Fischer-Tropsch).

La capacité de dégradation des carburants est largement répandue parmi les microflores de l'environnement. Les microflores des sols pollués présentent en général une capacité de dégradation légèrement supérieure à celle des sols non pollués. Plusieurs mécanismes peuvent rendre compte de l'efficacité des microflores provenant d'environnements pollués :

– la présence de microorganismes ayant un métabolisme spécialisé ;
– l'existence de cométabolisme ;
– des interactions positives entre souches (coopération).

Les mécanismes intervenant dans la dégradation des hydrocarbures récalcitrants sont illustrés dans le cas de la dégradation du cyclohexane par des souches pures.

Abstract — Gasoline and Diesel Oil Biodegradation *— Fuels are major organic pollutants of soils and ground waters. Persistency of pollutants in the environment depends on the intrinsic biodegradability of constituting hydrocarbons of fuels, on the presence of active microflorae at the polluted areas and on local environmental factors.*

The intrinsic biodegradability of fuels such as gasoline or diesel oil was determined by using a reference aerobic microflora taken from an urban waste water treatment plant. Gasoline exhibited a high intrinsic biodegradability (96%) but that of a commercial diesel oil was significantly lower (between 60 and 73%). The recalcitrant hydrocarbons of fuels were cycloalkanes and branched alkanes, in particular those having quaternary carbon atoms or consecutive substituting groups on the main carbon chain. In the case of various types of diesel oil, the composition in terms of hydrocarbon structural classes

accounted for the diverse biodegradation rates observed. In particular, the biodegradation rate was close to 100% when linear alkanes were most abundant (Fischer-Tropsch diesel oil).

The fuel degradation capability was widespread among the environment microflorae tested. Microflorae from polluted soils displayed in general a slightly higher degradation capacity than that of nonpolluted soils. Several mechanisms are involved in the efficiency of microflorae taken from polluted environments:

- *the presence of microorganisms with specialised metabolic capacities;*
- *the occurrence of cometabolism;*
- *some positive interactions between strains (cooperation).*

The mechanisms involved in the degradation of recalcitrant hydrocarbons were illustrated in the case of cyclohexane degradation by pure strains.

INTRODUCTION

Fuels are significant pollutants of soils and groundwater because of leaks of underground storage tanks and defectiveness of transfer lines (*Council on Environmental Quality*, 1981). In case of pollution, the fate of the fuels released is crucial for the environment protection. Biodegradation studies are of major interest since they allow to foresee the possible risks and to define the most appropriate remediation strategy.

The persistency of fuels released in the environment depends on the biodegradability of the constituting hydrocarbons, on the presence of microflorae suitable for biodegradation and on various environmental factors. In this context, biodegradation studies are quite useful since they allow to assess the natural degradation potential of microflorae on polluted sites. At present, natural attenuation is regarded as a really efficient bioremediation approach. It is no longer considered as a no-action cleanup alternative since it requires a rigorous site evaluation to demonstrate that contaminant migration towards sensitive receptors such as wells or nearby wetlands can be prevented (Chapelle, 1999).

The evaluation of the bioremediation potential of a polluted site is essential to legitimate the choice of natural attenuation which is the cheapest remediation strategy. Several criteria are commonly used to evaluate the possibilities of natural attenuation at a site. The first criterion related to the properties of the pollutant is its intrinsic biodegradability. For commercial compounds, intrinsic biodegradability is commonly assessed with standard tests involving microflorae having wide biodegradation spectra. The microflorae used are usually taken from an activated sludge at urban wastewater treatment plants. In these tests, the biodegradation rate of products is evaluated by O_2 consumption or by CO_2 production. The data obtained can interestingly be compared to biodegradation rates of readily biodegradable products, for example glucose, or a *n*-alkane such as hexadecane for petroleum products. The second criterion which is useful for the evaluation of the natural attenuation potential is the biodegradation capacities of the local native microflora of the polluted site. An interesting comparison of the local microflora with a reference microflora, *i.e.* an activated sludge, can be made. Finally, a third criterion of evaluation is the impact of the local conditions on the degradation activity of the indigenous microflora. In this respect, several parameters must be taken into account: pH, temperature, water content of soil, nutrient and electron acceptor availability, transfer limitations in the soil matrix, in particular for dioxygen and pollutant (Freijer *et al.*, 1996).

The aerobic biodegradation properties of two main types of fuels, gasoline and diesel oil have been incompletely evaluated. Only the most water-soluble components of gasoline, such as benzene (Paje *et al.*, 1997)), toluene (Laehy and Olsen, 1997), ethylbenzene and xylene isomers (Di Lecce *et al.*, 1997), hydrocarbons usually termed as BTEX, have been clearly assessed using pure strains. With complex microflorae, the biodegradability of BTEX has been also confirmed (Mallakin and Ward, 1996; Matteau and Ramsay, 1997). Nevertheless, little information is available about the biodegradation properties of other gasoline components excepted for some polyalkylated benzenes (Lang, 1996; Rozkov *et al.*, 1998) and some linear and branched alkanes (Schaeffer *et al*, 1979). Furthermore, interactions between individual strains may modify the kinetics of degradation as pointed out for instance by Alvarez and Vogel (1991) for BTEX.

The degradation of commercial diesel oils has been studied by several authors. Many of these studies performed with soil microcosms reported the incomplete degradation of diesel oil. However, it is not clear whether the partial resistance to biodegradation was accounted for by the intrinsic biodegradability of the complex petroleum cut or by the incubation conditions creating possible transfer limitation either for dioxygen or hydrocarbons (Seklemova *et al.*, 2001; Marquez-Rocha *et al.*, 2001; Gallego *et al.*, 2001).

In the present study, we focused our investigation on the biodegradability of gasoline and diesel oil in order to get wider information on the fate of identifiable hydrocarbons or structural classes during biodegradation by various microflorae from the environment.

1 CHARACTERISTICS OF PETROLEUM FUELS

The constituting hydrocarbons of commercial of gasoline on the one hand and of diesel oil on the other, are clearly different (Guibet, 1997). The carbon number of gasoline hydrocarbons is between 4 and 10. Its distillation range is from 30-35°C to 180-200°C. In contrast, the carbon number of diesel oil hydrocarbons is between 11 and 25 and the distillation range is between 180 to 380°C.

Gasoline and diesel oil generally contain low amounts of alkenes. They are both composed of four main structural classes of hydrocarbons:

– *n*-alkanes or *n*-paraffins (linear saturated hydrocarbons);
– isoalkanes or isoparaffins (branched saturated hydrocarbons);
– cycloalkanes or naphthenes (saturated cyclic alkanes);
– aromatics.

In gasoline composition, aromatics amount to about 50% of the total hydrocarbon content. Isoalkanes amount to about 35%. Alkanes, alkenes and cycloalkanes are present in minor quantities (*Fig. 1a*). There are about 230 individual hydrocarbons in gasoline composition. The constituting hydrocarbons can be separated and identified by gas chromatography (Durand 1998; Durand et *al.*, 1995).

In contrast, diesel oil contains 2000 to 4000 hydrocarbons, which cannot be totally separated by gas chromatography. In fact, only *n*-alkanes and a few branched hydrocarbons can be identified as separated compounds. However, the separation of the main structural hydrocarbon classes can be carried out by using a standard procedure of liquid chromatography (Olson *et al.*, 1999). The composition of the commercial diesel oil used in this study is indicated in Figure 1b.

2 METHODOLOGICAL APPROACH FOR BIODEGRADATION STUDIES

The biodegradability of commercial unleaded gasoline and diesel oil was evaluated by using biodegradation tests elaborated for this purpose. As recommended by OECD (1993), the tests were performed in conditions supposed to be optimal for the biodegradation process, *i.e.* concerning pH, temperature, substrate concentration, nutrient supply and oxygen availability. The tests were carried out in closed systems in order to allow assessment of the carbon balance at the end of incubation period (Solano-Serena *et al.*, 1999a). The overall kinetics of biodegradation were monitored in order to determine the end point of experiment. This

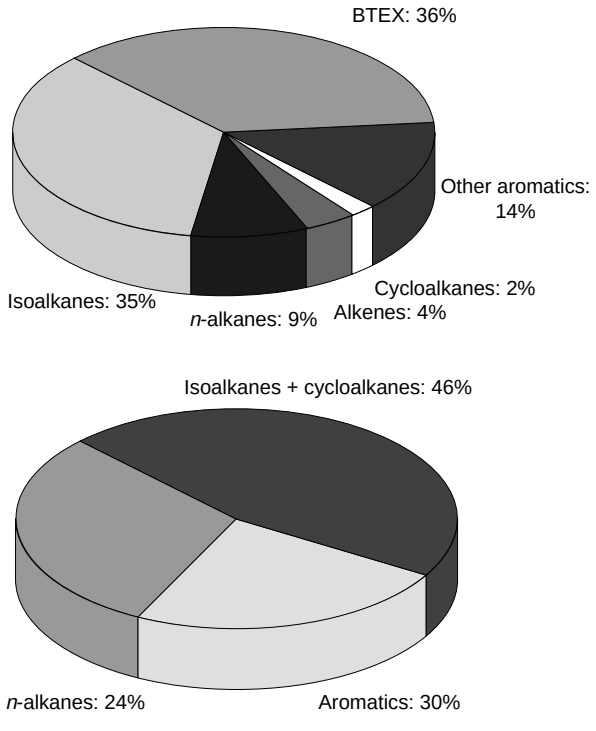

Figure 1

Composition of the commercial gasoline (a) and diesel oil (b) used in the study.

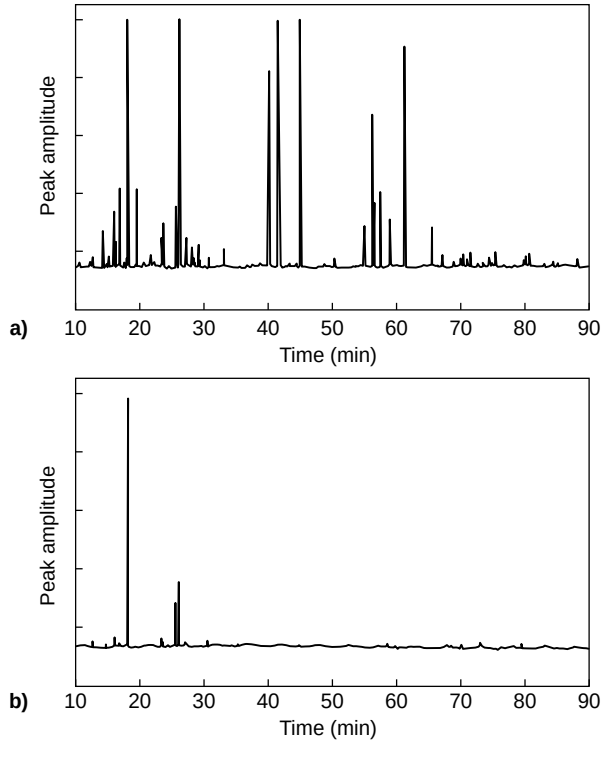

Figure 2

Chromatographic profiles of residual hydrocarbons of gasoline in abiotic (a) and test flasks (b).

monitoring was based either on dioxygen consumption by using electrolytic respirometry or on carbon dioxide production by using gas chromatography. At the end of the incubation period, the residual hydrocarbons were quantified and the degradation rates were determined by comparison with hydrocarbons of abiotic control flasks in which microbial growth was inhibited by $HgCl_2$ addition *(Fig. 2).*

3 BIODEGRADATION OF FUELS BY A MICROFLORA FROM AN ACTIVATED SLUDGE

As previously indicated, the biodegradation extent of a commercial compound like gasoline by a microflora of an activated sludge from a urban waste water treatment plant, gives a good preliminary approximation of its intrinsic biodegradability. In this respect, the activated sludge microflora can be considered as a reference microflora (Solano-Serena *et al.*, 1999b and 2000b).

TABLE 1

Biodegradation of gasoline by the reference microflora

Hydrocarbon class	Degradation rate*(%)	Resistant fraction**(%)
n-alkanes	100	0
Methyl- and dimethylalkanes	98.3	0.5
Trimethylalkanes	51.3	2.8
Cycloalkanes	99.7	0
BTEX	99.9	0
Other aromatics	98.8	0.2
Alkenes	100	0
Total gasoline	96.5	3.5

* with respect to the corresponding hydrocarbon class.
** with respect to initial gasoline.

Table 1 shows the degradation rates of the main hydrocarbon classes of gasoline by the reference microflora from activated sludge. This microflora extensively degraded gasoline (up to 96%). All *n*-alkanes, cycloalkanes, aromatics were totally consumed. The main recalcitrant hydrocarbons belonged to the class of methylated alkanes. In particular, about 50% of initial trimethylalkanes resisted to microbial degradation. Gas chromatographic identification indicated that the molecular recalcitrant structures were either branched hydrocarbons with a quaternary carbon or hydrocarbons with methyl groups on consecutive carbon atoms *(Fig. 3).*

Concerning diesel oil degradation, the major problem is the analytical complexity related to the huge number of components. As an example, the chromatographic profile of the commercial diesel oil used is shown in Figure 4a. Such a profile displayed a quite satisfactory resolution for all *n*-alkanes, which could be easily identified. Some other branched alkanes such as farnesane, pristane and phytane could also be detected. Nevertheless, the major fractions of the crude oil still were not characterized because the majority of the components could not be resolved and appeared as a "hump" or "unresolved complex mixture (UCM)" on chromatograms. At the end of the incubation period, the profile of hydrocarbons showed that all *n*-alkanes were totally consumed by the reference microflora. The UCM was clearly reduced (*Fig. 4b).* Actually, the lightest hydrocarbons of the initial UCM were degraded but the heaviest ones were not.

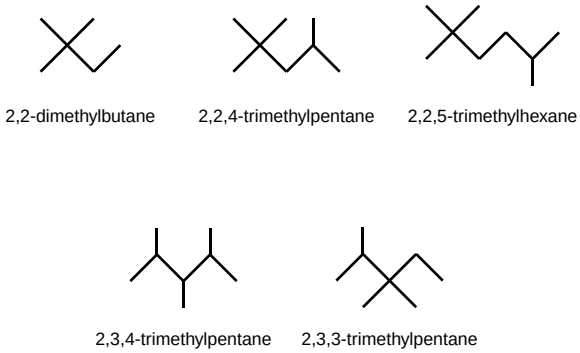

Figure 3

Main recalcitrant molecular structures of gasoline.

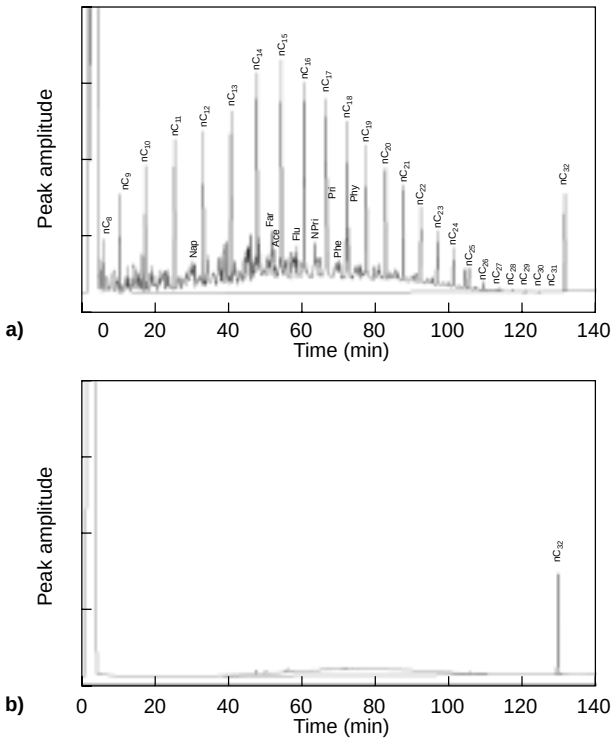

Figure 4

Chromatographic profiles of residual hydrocarbons of diesel oil in abiotic (a) and test (b) flasks.

The procedure defined for commercial diesel oil was then used to evaluate the degradation rates of several types of diesel oils *(Table 2)*. The commercial diesel oil was not totally biodegradable. Furthermore, the susceptibility to biodegradation of other diesel oils was found dependent on the molecular structure like in the case of gasoline. Linear alkanes of the Fischer-Tropsch or linear fatty acid methyl esters of rapeseed were quite extensively biodegraded. Hydrocracking diesel oil was found slightly less biodegradable than straight run diesel oil probably because of the branched structures of the constituting hydrocarbons.

TABLE 2

Degradation of various types of diesel oil by the reference microflora

Diesel oil type	Degradation rate (%)	Mineralisation rate** (%)
Commercial	73	62
Straight run	83	47
Hydrocracking	77	55
Fischer-Tropsch	98	60
Rapeseed methyl esters	100	79
Synthetic*	99	62

* tetradecane plus 1-methylnaphthalene 50/50 (w/w).
** ratio (in moles of carbon) of CO_2 produced to diesel oil supplied.

4 DEGRADATION CAPACITIES OF MICROFLORAE FROM THE ENVIRONMENT

The gasoline degradation capacities of various microflorae of the environment were examined in comparison with those of the reference microflora. Two nonpolluted-soil microflorae and 6 polluted-soil microflorae were tested *(Table 3)*. The hydrocarbon degradation rates measured with nonpolluted soils were found slightly lower than with the reference microflora, in particular, for cycloalkanes that displayed incomplete biodegradation. The degradation rates measured with polluted soils were generally higher than that of the reference microflora. Even trimethylalkanes were consumed by the polluted-soil microflorae.

The degradation capacities of microflorae from the environment were similarly assayed on diesel oil *(Table 4)*. Two microflorae (microflorae 1 and 2) from distinct wastewater treatment plants exhibited different degradation capacities. The degradation capacities of the refinery sludge where close to those of microflora 2 whereas those of the nonpolluted soil were close to those of microflora 1. With the polluted soil, the level of biodegradation was higher than with the nonpolluted soil. In this case, a quite moderate mineralization rate was calculated. This rate seemed to indicate that intermediary metabolites could accumulate as a result of an incomplete degradation of the substrate.

5 MECHANISMS OF RECALCITRANT HYDROCARBONS DEGRADATION

The recalcitrance of some hydrocarbon classes of fuels using nonpolluted-soil microflorae showed the relevance of micro-organisms having specialized metabolic capacities in order to achieve a maximal consumption of fuels. In polluted environments, the selective pressure resulting from pollution appeared to enrich soils with biodegraders of cyclic or branched hydrocarbons. Actually, microflorae specifically degrading cycloalkanes or an isoalkane such as isooctane were obtained from polluted environments. Using an isooctane-degrading microflora, a pure strain was isolated (Solano-Serena *et al.*, 2000a). This strain and its specific properties quite well illustrate the main mechanisms prevailing in the degradation of recalcitrant hydrocarbons in the environment. These mechanisms are the cometabolism and strain cooperation.

TABLE 3

Gasoline degradation capacities of microflorae from the environment

Microflora	Degradation rate (%) for:					
	n-alkanes	Mono- and di-methylalkanes	Trimethylalkanes	Cycloalkanes	Aromatics	Total
Activated sludge	100	100	27	100	100	95
Spruce forest soil	100	92	14	29	98	89
Garden soil	99	100	28	75	100	94
Diesel oil-polluted soil	100	100	100	100	100	100
Jet fuel-polluted soil	100	96	17	85	85	85
Gasoline-polluted soil	100	100	100	100	100	100

TABLE 4

Degradation capacities of various microflorae for commercial diesel oil

Microflora	Biodegradation rate* (%)	Mineralisation** (%)
Urban activated sludge 1	60	57
Urban activated sludge 2	73	62
Refinery activated sludge	76	67
Nonpolluted soil	60	58
Polluted soil	91	55

* ratio of degraded diesel oil to provided diesel oil..
** ratio (in moles of carbon) of CO_2 produced to diesel oil supplied.

TABLE 5

Carbon balance of isooctane degradation by *M. austroafricanum*

Products	Carbon incorporation rate (%)*
Biomass	19
CO_2	49
Dissolved organic carbon	30
Total	98

* on initial isooctane.

The specialized strain, obtained from a gasoline-polluted groundwater, was identified by 16S rDNA as *Mycobacterium austroafricanum*. The substrate used for strain selection was isooctane, a molecule composed of a *tert*-butyl group and of an iso-butyl group. The final balance of isooctane degradation is indicated in Table 5. The degradation data indicate that more than 50% of the initial carbon was recovered in cell biomass plus CO_2. This clearly showed that *M. austroafricanum* had the capacity to degrade the quaternary carbon atoms. Further investigation revealed that isooctane attack probably involved a cytochrome P450.

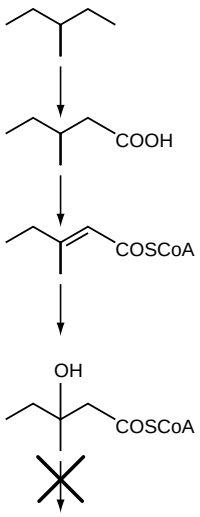

Figure 5

β-oxidation blocking in the degradation of *anteiso*-methylalkanes.

Another interesting capacity of this specialized microorganism is the degradation of *anteiso*-methylalkanes. Substituting groups on carbon 3 of alkanes are known to block the β-oxidation process at the step of the β-hydroxyacyl-CoA dehydrogenase because tertiary alcohols cannot be dehydrogenated *(Fig. 5)*. Since *M. austroafricanum* can grow on *anteiso*-methyl alkanes, it possessed a metabolic pathway allowing β-oxidation to proceed. This pathway could involve a decarboxymethylation step as already demonstrated in other microorganisms (Fall *et al.*, 1979).

Cometabolism capacities of *M. austroafricanum* were demonstrated for cyclohexane degradation. *M. austroafricanum* was unable to utilize cyclohexane as a sole carbon source *(Table 6)*. However, when isooctane was provided to the microorganism, cyclohexane was oxidized into cyclohexanone which accumulated. This shows that

TABLE 6

Strain cooperation for mineralisation of cyclohexane

Culture conditions		Substrates or products		
Microrganisms	Carbon sources*	Cyclohexane degraded (μmol)	Cyclohexanone produced (μmol)	CO_2 produced (μmol)
A. lwoffii	Cyclohexane	0	0	0
A. lwoffii	Cyclohexane + isooctane	0	0	0
M. austroafricanum	Cyclohexane	0	1.2	0
M. austroafricanum	Cyclohexane + isooctane	14.8	12.4	89
M. austroafricanum + *A. lwoffii*	Cyclohexane	0	0	2
M. austroafricanum + *A. lwoffii*	Cyclohexane + isooctane	15.4	0	120

* when added, cyclohexane and isooctane amounts were respectively 35 mmol and 24 mmol per flasks.

M. austroafricanum could cometabolize cyclohexane when an energy substrate was available.

A strain of *Acinetobacter lwoffii* was isolated for its capacity to degrade cyclohexane. It did not degrade cyclohexanone. When *M. austroafricanum* and *A. lwoffii* were cultivated simultaneously on cyclohexanone, cyclohexane was degraded but no cyclohexanone accumulated. Measurements of final CO_2 in the culture headspace showed that cyclohexane was totally mineralised. This illustrated the positive cooperative effect between the two strains for cyclohexane mineralisation.

CONCLUSION

A valuable approximation of the intrinsic biodegradability of fuels can be obtained in flask tests using an activated-sludge microflora. In the case of gasoline, the estimation given by an activated sludge is quite pertinent since similar biodegradation rates are determined using various microflorae. However, in the case of diesel oil, the approximation is less adapted since slightly different biodegradation rates were found when using various microflorae. These variations probably arose from the differences in biodegradation capacities for branched alkanes which were present in large amounts in diesel oil.

Gasoline is highly biodegradable. Up to 96% of initial gasoline was degraded during the 28-day tests. Out of the 95 major components, 72 were completely degraded and 23 were partially consumed. Actually, diesel oil is less biodegradable than gasoline. From 60% to 73% of initial diesel oil (according to the activated sludge used), was degraded in the tests. All *n*-alkanes and lighter hydrocarbons were degraded but some heavy branched hydrocarbons were recalcitrant to biodegradation.

Degradation of recalcitrant hydrocarbons by environmental microflorae involves microorganisms having specialized metabolic capacities. In polluted environments, specialized microorganisms are abundant because of the adaptation of the microflorae to pollutant. Their efficiency is still enhanced by two main mechanisms. The first one is the cometabolism by which a strain is able to oxidize recalcitrant hydrocarbons when an energy source is available. The second is cooperation between strains. The two mechanisms explain why soil microflorae have extended capacities to eliminate pollution from contaminated environments.

ACKNOWLEDGEMENTS

We thank Véronique Bardin for helpful advice on gas chromatography of fuels, Vincent Genet, Maud Billon for experimental work and Frédéric Monot for fruitful discussions.

REFERENCES

Alvarez, P.J.J. and Vogel, T.M. (1991) Substrate interaction of benzene, toluene and *para*-xylene during microbial degradation by pure cultures and mixed aquifer slurries. *Appl. Environ. Microbiol.*, **57,** 2981-2985.

Chapelle, F.H. (1999) Bioremediation of petroleum hydrocarbon-contaminated ground water: the perspectives of history and hydrology. *Ground water,* **37**, 122-132.

Council on Environmental Quality (1981) Contamination of ground water by toxic organic chemicals. Washington, D.C. Government Printing Office.

Di Lecce, C., Accarino, M., Bolognese, F., Galli, E. and Barbieri, P. (1997) Isolation and metabolic characterization of a *Pseudomonas stutzeri* mutant able to grow on the three isomers of xylene. *Microbiol*., **63**, 3279-3281.

Durand, J.P. (1998) Le rôle de la CPG dans l'industrie pétrolière et la pétrochimie. De l'analyse détaillée des hydrocarbures à la distillation simulée. *Analusis,* **26**, M17-M21.

Durand, J.P., Béboulène, J.J. and Ducrozet, A. (1995) Detailed characterization of petroleum products with capillary analyzers. *Analusis*, **23**, 481-483.

Fall, R.R., Brown, J.L. and Schaeffer, T.L. (1979) Enzyme recruitement allows the biodegradation of recalcitrant branched hydrocarbons by *Pseudomonas citronellonis*. *Appl. Microbiol. Biotechnol.* **38**, 715-722.

Freijer, J.I., de Jonge, H., Bouten, W. and Verstraten, J.M. (1996) Assessing mineralization rates of petroleum hydrocarbons in soils in relation to environmental factors and experimental scale. *Biodegradation*, **7**, 487-500.

Gallego, J.L.R., Loredo, J., Llamas, J.F., Vazquez, F. and Sanchez, J. (2001) Bioremediation of diesel contaminated soils; evaluation of potential *in situ* techniques by study of bacterial degradation. *Biodegradation*, **12**, 325-335.

Guibet, J.C. (1997) *Carburants et moteurs*, Éditions Technip, Paris, 21-70.

Lang, E. (1996) Diversity of bacterial capabilities in utilizing alkylated benzenes and other aromatics compounds. *Lett. Appl. Microbiol.*, **23**, 257-260.

Leahy, J.G. and Olsen, R.H. (1997) Kinetics of toluene degradation by toluene-oxidizing bacteria as a function of oxygen concentration, and the effect of nitrate. *FEMS Microbiol Ecol*., 23, 23-30.

Mallakin, A. and Ward, O.P. (1996) Degradation of BTEX compounds in liquid media and in peat biofilters. *J. Ind. Microbiol.*, **16**, 309-318.

Marquez-Rocha, F.J., Hernandez-Rodriguez, V. and Lamela, M.T. (2001) Biodegradation of diesel oil in soil by a microbial consortium. *Water Air and Soil pollution,* **128**, 313-320.

Matteau, Y. and Ramsay, B. (1997) Active biofiltration of toluene. *Biodegradation*, **8**, 135-141.

OECD (1993) Guidelines for the testing of chemicals. In: *Les éditions de l'OCDE*, Paris. **2**, 1-13.

Olson, J.J., Mills, G.L., Herbert, B.E. and Morris, P.J. (1999) Biodegradation rates of separated diesel components. *Environ. Toxicol. Chem.*, **18**, 2448-2453.

Paje, M.L.F., Neilan, B.A. and Couperwhite, I. (1997) A *Rhodococcus* species that thrives on medium saturated with liquid benzene. *Microbiology,* **143**, 2975-2981.

Rozkov, A., Käärd, A. and Vilu, R. (1998) Biodegradation of dissolved jet fuel in chemostat by a mixed bacterial culture isolated from heavy polluted site. *Biodegradation*, **8**, 363-369.

Schaeffer, T.L., Cantwell, S.G., Brown, J.L., Watt, D.S. and Fall, R.R. (1979) Microbial growth on hydrocarbons; terminal branching inhibits biodegradation. *Biodegradation*, **9**, 319-326.

Seklemova, E., Pavlova, A. and Kovacheva, K. (2001) Biostimulation-based bioremediation of diesel fuel: field demonstration. *Biodegradation*, **12**, 311-316.

Solano-Serena, F., Marchal, R., Lebeault, J.M. and Vandecasteele, J.P. (1999a) Assessment of intrinsic capacities of microflorae for gasoline degradation. In: *In situ and on site bioremediation, Symp. 5th*, Battelle Press, Columbus, Ohio, 177-182.

Solano-Serena, F., Marchal, R., Ropars, M., Lebeault, J.M. and Vandecasteele, J.P. (1999b) Biodegradation of gasoline: kinetics, mass balance, fate of individual hydrocarbons. *J. Appl. Microbiol.*, **86,** 1008-1016.

Solano-Serena, F., Marchal, R., Casaregola, S., Vasnier, C., Lebeault, J.M. and Vandecasteele, J.P. (2000a) A new *Mycobacterium* strain with extended degradation capacities for gasoline hydrocarbons. *Appl. Environ. Biotechnol.*, **66**, 2392-2399.

Solano-Serena, F., Marchal, R., Huet, T., and Vandecasteele, J.P. (2000b) Biodegradability of volatile hydrocarbons of gasoline. *Appl. Microbiol. Biotechnol*., **54**, 126-132.

Final manuscript received in February 2003