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In situ anaerobic degradation of petroleum alkanes in marine sediments: preliminary results

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Abstract

The degradation of acyclic petroleum hydrocarbons was studied during a 24-month experiment in Mediterranean coastal sediments (Gulf of Fos). Sediment cores entirely contaminated with oil (Arabian Light Crude Oil) were incubated in situ. The use of conservative tracers of sediment's particles reworking (luminophores) allowed the distinction of the reworked layer from the anoxic deeper sediments. Using the $17\alpha,21\beta C_{30}$ hopane ($C_{30}H$) as an inert internal reference, we could demonstrate that, after 24 months of experiment, acyclic petroleum hydrocarbons can be degraded under natural anaerobic conditions. The reactivity of individual alkanes appeared to depend on their chemical structure. ***To cite this article: D. Massias et al., C. R. Geoscience 335 (2003).***

Résumé

Dégradation anaérobie d'alcanes pétroliers dans un sédiment marin : résultats préliminaires. Une expérience conduite dans des sédiments côtiers de Méditerranée (golfe de Fos) a permis d'étudier, pendant 24 mois, la dégradation d'hydrocarbures acycliques. Des carottes sédimentaires, entièrement polluées par du pétrole (brut arabe léger), ont été insérées dans les sédiments naturels et incubées in situ. L'ajout de traceurs du remaniement sédimentaire (luminophores) a permis de distinguer la zone remaniée des couches sous-jacentes anoxiques. L'utilisation du $17\alpha,21\beta C_{30}$ hopane ($C_{30}H$) comme étalon interne invariant a permis de déterminer qu'en 24 mois, certains hydrocarbures acycliques pétroliers pouvaient être dégradés en anaérobiose. Leur réactivité est toutefois apparue dépendante de leur structure chimique. ***Pour citer cet article : D. Massias et al., C. R. Geoscience 335 (2003).***

Keywords: petroleum hydrocarbons; anaerobic degradation; in situ experiments; recent sediment; bioturbation; Mediterranean Sea

Mots-clés : hydrocarbures pétroliers ; dégradation anaérobie ; expériences in situ ; sédiment récent ; bioturbation ; mer Méditerranée

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Longtemps controversée, la dégradation anaérobie des hydrocarbures par des cultures pures ou des communautés bactériennes a récemment été démontrée en laboratoire. Les travaux effectués in situ dans les sédiments marins considèrent généralement l'ensemble de la colonne sédimentaire, et ne distinguent pas les processus de dégradation aérobie et anaérobie. Cet article décrit une expérimentation réalisée in situ dans le but de suivre le devenir d'hydrocarbures pétroliers dans des sédiments marins anoxiques. Des carottes de sédiment intégralement contaminées par du pétrole (0–25 cm) ont été implantées dans des sédiments naturels et incubées in situ pendant 24 mois. L'utilisation de luminophores (déposés à la surface des carottes) comme marqueurs inertes de la bioturbation a permis de distinguer la zone d'influence des organismes benthiques de la zone anoxique sous-jacente (Fig. 1). Le suivi des hydrocarbures aliphatiques dans ces zones dépourvues d'oxygène a été réalisé en utilisant un marqueur interne inerte, le $17\alpha,21\beta C_{30}$ hopane. Ceci a permis de démontrer une dégradation significative de certains alcanes linéaires (e.g., n-C_{17}, n-C_{18} et n-C_{30}) et isoprénoïde (pristane) après 24 mois d'incubation (Fig. 2 et Tableau 1). Les pourcentages de (bio)dégradation observés sont de l'ordre de 56% pour le n-C_{17} et n-C_{18}, et de 50% pour le n-C_{30} et le pristane. La bioturbation n'intervenant pas sur le devenir des hydrocarbures à ces profondeurs, cette étude indique l'existence d'une biodégradation anaérobie des alcanes pétroliers dans des conditions naturelles. Les différences de réactivité observées entre les composés montrent, par ailleurs, que cette dégradation est fonction de la structure moléculaire des alcanes (longueur de chaîne carbonée et présence de ramifications).

1. Introduction

Acyclic petroleum hydrocarbons are well known to be biodegradable in the presence of oxygen, whereas their anaerobic degradation has long been doubted. During the past decade, the anaerobic biodegradation of linear (C_6–C_{34}) and isoprenoid (pristane) alkanes have been demonstrated only under laboratory conditions [7,9]. In coastal marine areas, the upper layers of the sediment are often influenced by the activity of benthic fauna (process known as bioturbation). Bioturbation allows oxygen diffusion deeper into the sediment and oscillating redox conditions to appear [1]. Till now, the study of hydrocarbon (bio)degradation in oil-contaminated sediments considered the totality of the reworked sedimentary column where aerobic and anaerobic processes are integrated [6]. This did not give any information on the potential role of strict anaerobic bacterial communities in the degradation of petroleum hydrocarbons under natural conditions. To this end, we performed an in situ experiment in a marine coastal area. Following the contamination with oil of the first 25 centimetres of the sedimentary column, the fate of petroleum hydrocarbons (n-alkanes and isoprenoids) was followed during a 2-year period. The use of inert tracers of the sediment's solid phase rearrangement (i.e. luminophores [3]) allowed to distinguish the part of the sediment that was reworked from the deeper anoxic layers, and to demonstrate the in situ anaerobic degradation of aliphatic hydrocarbons.

2. Experimental

2.1. Experimental site

Experiments were carried out at 5 m depth in the Carteau Cove (Gulf of Fos, Mediterranean Sea). The experimental site was divided into two similar fields, as described elsewhere [6]. At this site, the temperature varies along the year from 5 °C in winter to 25 °C in summer.

2.2. Sediment contamination

Surface sediments (top 20 cm) were collected with an Orange Peel Grab at the experimental site. After sieving (1 mm), the sediment was mixed with Arabian Light Crude Oil (A.L. Brut) to reach a concentration of 10 g kg^{-1} wet sediment. This increased the hydrocarbon content of the initial sediment by more than 75 times which represented a high level of petroleum contamination. The sediment-oil mixture was then poured into PVC cores (25 cm length, 11 cm diameter). Analysis of the bacterial finger-print in the sediment-oil mixture by molecular techniques (RISA) did not show any change of the major bacterial communities compared with the original sediment (P. Cuny, pers. comm.). Following the addition of luminophores (6 g)

at the surface, the cores were kept frozen until their implantation into the experimental site.

2.3. Incubation and sampling

Four frozen cores were inserted into each field. After 6, 12, 18 and 24 months of in situ incubation, two cores (one per field) were collected by divers. Sediment was extruded from each collected core and sliced as follows: 0–3 cm in 0.5 cm intervals, 3–10 cm in 1 cm intervals and 10 to bottom in 2 cm intervals. Samples were immediately frozen, freeze-dried and carefully homogenised.

2.4. Luminophores and hydrocarbons analyses

The presence of luminophores was determined under UV light in five replicates of each sediment section. Aliphatic hydrocarbons were extracted from a known amount of lyophilised sediment using a Soxhlet apparatus and isolated by column chromatography. Individual alkanes were identified by GC/MS and quantified by GC using two internal standards: hexamethylbenzene (HMB) for C_{14-23} alkanes and squalane (Sq) for C_{24-34} alkanes [6].

3. Results and discussion

In this paper, we present the results obtained for the anoxic part of the sediment after 6 and 24 months of incubation.

3.1. Sediment reworking

Fig. 1 shows a depth profile of luminophores in a core incubated for six months. Since these inert particles were initially deposited on top of the cores, their detection into deeper sediment layers demonstrates the reworking of the sediment. This suggests that the cores were recolonised by benthic macro- and meiofauna during the first six months of the experiment. Indeed, it was previously shown that, in the absence of macrofauna, the luminophores are hardly buried into the sediment [5]. The thickness of the reworked layer varied between the four analysed cores certainly due to differences in macrofaunal composition and/or activity: the luminophores were

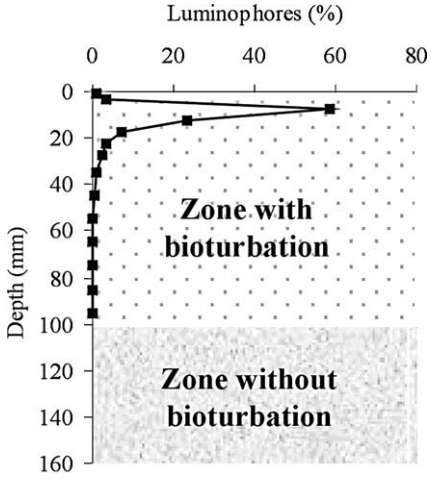

Fig. 1. Example of a depth profile of luminophores after a six-month incubation.

Fig. 1. Exemple de distribution de luminophores après six mois d'incubation.

detected down to 160- and 100-mm depth respectively in the two cores after six months, and down to 100- and 120-mm depth respectively after 24 months. The absence of luminophores below these reworked layers indicated that the deeper sediments were not reworked and thus were never in contact with oxygen throughout the 24-month experiment. These were accordingly considered as anoxic zones.

3.2. Petroleum hydrocarbons

Fig. 2 shows examples of chromatograms of the saturated hydrocarbon fraction (F1) of the oil-sediment mixture at the beginning of the experiment and below the reworked layer in a core incubated in situ for 24 months. The hydrocarbons distribution showed a marked decrease in 'short-chain' alkanes ($< n$-C_{25}) after 24 months. The depletion of individual hydrocarbons was followed through the study of C_{17}, C_{18} and C_{30} n-alkanes and of isoprenoid hydrocarbons (pristane and phytane). In order to demonstrate the degradation of these hydrocarbons, the $17\alpha,21\beta C_{30}$ hopane ($C_{30}H$) was used as an inert internal reference ([8]; Table 1). The degradation of the individual alkanes seemed to depend on their chain length and on the presence of branching. A statistical comparison (Student's t-Test, $n = 5$ or 6, $\alpha = 0.05$) of the averages of degradation observed for different anoxic

Table 1
Relative indexes to $17\alpha,21\beta C_{30}$ hopane of individual linear and isoprenoid alkanes (Pr = pristane and Ph = phytane) at the beginning of experiment (T_0) and after 6 and 24 months of experiment in the sediment layers without luminophores (average of replicate samples ±SD)

Tableau 1
Indices relatifs au $17\alpha,21\beta C_{30}$ hopane pour des alcanes individuels linéaires et isoprénoïdes (Pr = pristane et Ph = phytane) au début de l'expérience (T_0) et après 6 et 24 mois d'expérience dans les couches sédimentaires sans luminophores (moyenne des échantillons ± déviation standard)

Time	Replicate samples	$C_{17}/C_{30}H$	$C_{18}/C_{30}H$	$C_{30}/C_{30}H$	$Pr/C_{30}H$	$Ph/C_{30}H$
T_0	$n=6$	26.8 ± 2.6	26.5 ± 2.4	4.7 ± 0.2	5.2 ± 0.4	9.8 ± 0.9
6 months	$n=5$	22 ± 2.7	22.1 ± 2.8	4.4 ± 0.7	4.2 ± 0.2	9.6 ± 0.5
24 months	$n=6$	13.8 ± 2.8	14.5 ± 2.7	3.1 ± 0.5	3.6 ± 0.6	8.4 ± 1.4

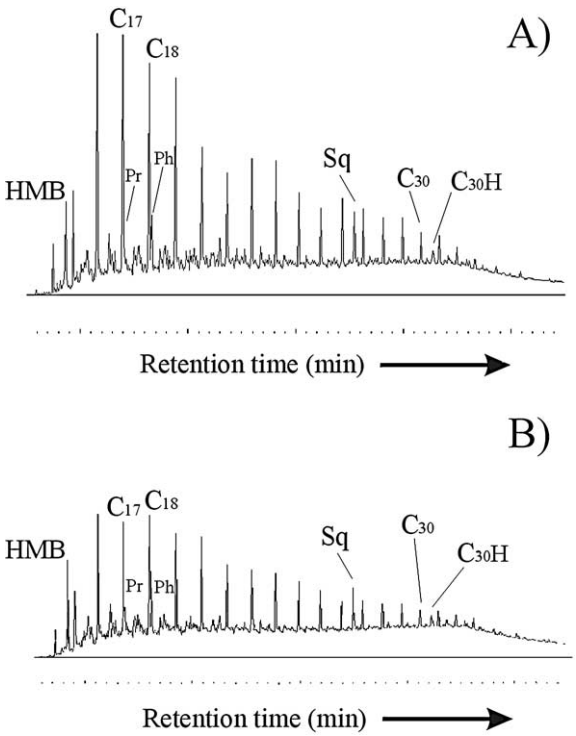

Fig. 2. Chromatograms of the saturated hydrocarbon fraction (**A**) in the oil-sediment mixture at the beginning of the experiment and (**B**) in a sediment layer without luminophores (100–120 mm) after 24 months of in situ incubation. HMB (hexamethylbenzene) and Sq (squalane) = internal standards; Pr = pristane; Ph = phytane; Cn = *n*-alkanes with *n* carbon atoms. Same vertical scale.

Fig. 2. Chromatogrammes de la fraction saturée (**A**) du mélange pétrole–sédiment au début de l'expérience et (**B**) dans une couche sans luminophores (100–120 mm) après 24 mois d'incubation in situ. HMB (hexaméthylbenzène) et Sq (squalane) = standards internes ; Pr = pristane ; Ph = phytane ; Cn = *n*-alcanes avec *n* atomes de carbone. Échelle verticale identique.

layers showed that n-C_{17}, n-C_{18} and Pr were significantly degraded after six months of incubation ($0 < p < 0.016$). Their extents of degradation by the end of the experiment (24 months incubation) were 57, 56.5 and 50%, respectively. n-C_{30} appeared to be significantly degraded (51%) only after 24 months of incubation ($p = 0.0001$), whereas no significant degradation of phytane could be established throughout the experiment (Table 1). Phytane degradation could be observed (up to 38%), however, in some individual anoxic sedimentary layers (results not shown).

This structure-dependent reactivity of alkanes under anaerobic conditions is in good agreement with the results of Giger et al. [4], obtained under laboratory conditions. In addition, a similar scale of reactivity was observed when kinetics of hydrocarbon degradation was studied in the whole reworked layer [6]. Although the pathways for the anaerobic biodegradation of isoprenoid hydrocarbons remain unknown, the lower reactivity of phytane compared to pristane may be due to the ante-iso structure of one end of the carbon skeleton of the phytane molecule. Alternatively, a production of phytane from the phytyl side-chain of chlorophyll-*a* present in the original sediment cannot be ruled out and might have compensated the degradation of this compound in some anoxic sediment layers [2].

The preliminary results of this in situ experiment show that below the bioturbated zone, linear and isoprenoid acyclic petroleum hydrocarbons can be biodegraded under anoxic conditions, after 6 or 24 months of incubation, depending on the compound. Although the individual alkanes showed varying reactivity, it is clear that such compounds cannot be considered as recalcitrant biomarkers in recent marine anoxic sediments.

References

[1] R.C. Aller, Bioturbation and remineralization of sedimentary organic matter: effects of redox oscillation, Chem. Geol. 114 (1994) 331–345.
[2] B.M. Didyk, B.R.T. Simoneit, S.C. Brassell, G. Eglinton, Organic geochemical indicators of palaeoenvironmental conditions of sedimentation, Nature 272 (1978) 216–222.
[3] M. Gerino, R.C. Aller, C. Lee, J.K. Cochran, J.Y. Aller, M.A. Green, D. Hirschberg, Comparaison of different tracers and methods used to quantify bioturbation during a spring bloom: 234-thorium, luminophores and chlorophyll *a*, Estuar. Coast. Mar. Sci. 46 (1998) 531–547.
[4] W. Giger, C. Schaffner, S.G. Wakeham, Aliphatic and olefinic hydrocarbons in recent sediments of Greifensee, Switzerland, Geochim. Cosmochim. Acta. 44 (1980) 119–129.
[5] F. Gilbert, G. Stora, J.-C. Bertrand, In situ bioturbation and hydrocarbon fate in an experimental contaminated Mediterranean coastal ecosystem, Chemosphere 33 (1996) 1449–1458.
[6] V. Grossi, D. Massias, G. Stora, J.-C. Bertrand, Burial, exportation and degradation of acyclic petroleum hydrocarbons following a simulated oil spill in bioturbated Mediterranean coastal sediment, Chemosphere 48 (2002) 947–954.
[7] P. Rueter, R. Rabus, H. Wilkes, F. Aeckersberg, F.A. Rainey, H.W. Jannasch, F. Widdel, Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria, Nature 372 (1994) 455–458.
[8] Z. Wang, M. Fingas, D.S. Page, Oil spill bioremediation, J. Chromatogr. A 843 (1999) 369–411.
[9] F. Widdel, R. Rabus, Anaerobic biodegradation of saturated and aromatic hydrocarbons, Curr. Opin. Biotechnol. 12 (2001) 259–276.