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Original Scientific Paper

Complementary Degradation of Fuel Oil in Superficial Waters and in Axenic Cultures of Aerobic Gram-negative Bacteria Isolated from Venice Lagoon*

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The percentages of bacteria degrading fuel oil (*n*-paraffins from C_{12} to C_{28}) were determined in three stations in the northern part of Venice Lagoon. Concentrations of paraffin-degrading bacteria ranged from 8 to 70 bacteria per 100 mL in the least polluted station close to the sea, and from 33 to 345 bacteria per 100 mL in the most polluted station near Porto Marghera. Biological oxygen demand with fuel oil additions was higher in this station, where oxygen was totally depleted in 7 and 5 days in November and June respectively. Twenty-five bacterial strains were isolated from agar plates amended with fuel oil as the sole carbon and energy source; only two were *Acinetobacter* spp. strains. Strain VE-C3 grew in the presence of *n*-paraffins. Growth was inducible with a generation time of 2.77 h and an oxygen consumption rate of 53 $\mu L\ h^{-1}\ mg^{-1}$ of cells (d.w.). Five other strains thrived on intermediate oxidation products of *n*-paraffin.

* Dedicated to Marko Branica on the occasion of his 65th birthday.

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INTRODUCTION

Hydrocarbon pollution was studied extensively in the 1970's in the Venice Lagoon (Italy), using the mussel-watch monitoring approach.[1–5] The Venice Lagoon (northeast Italy) is an estuarine system in which water pollution is a serious problem owing to the input of industrial, urban and agricultural effluent. It has been listed as an environmental risk area by the Italian Government: a heavy industrial area is located on the shores of the lagoon with one of the busiest harbours in Italy, Porto Marghera (4.5×10^6 tons $year^{-1}$ of crude oil unloaded).[6] Hydrocarbon pollution of the lagoon is increased by the intense traffic of public and private boats, which spread fuel oil over the lagoon surface.

A hydrocarbon gradient from Porto Marghera (in the inner part of the lagoon) to the harbour entrance at Lido (near the sea) has been demonstrated on the basis of the hydrocarbon burden of mussels (*Mytilus* sp.).[3,7] However, little information exists on hydrocarbon concentrations obtained directly by water analysis.

In two preliminary surveys,[3,5] the presence of crude oil-degrading microorganisms was investigated in water samples collected from the northern and central part of the Venice lagoon. These microorganisms were also investigated in water samples collected throughout the lagoon, together with the effects of phosphate and nitrate concentration on the kinetics of crude oil biodegradation.[8–10] Microorganisms capable of growing with crude oil as the sole source of carbon and energy are well known, and the correlation between the presence of hydrocarbon-oxidizing microorganisms and crude oil pollution suggests that this bacteria can be used as a biological indicator of crude oil pollution in the sea.[11] Crude oil is a mixture of aromatic and aliphatic compounds, and its complete degradation is performed by the complementary activity of different microorganisms with different degradation pathways.[12]

The genetic basis of hydrocarbon degradation by *Pseudomonas oleovorans* is known. These bacteria metabolize *n*-octane and other *n*-alkanes by means of enzymes codified by *alk* genes, harboured on the OCT plasmid.[13] The first oxidation step is the degradation of *n*-alkanes by mono-oxygenase to produce alcohols. The following steps are the oxidation of alcohols to aldehydes and then to fatty acids. The oxidation sequence reactions are carried out respectively by an alcohol dehydrogenase and an aldehyde dehydrogenase coded by chromosomal genes.[14] Fatty acids are used as carbon and energy sources by the β-oxidation pathway).[13,14] In some microbial species that grow with low concentrations of nitrogen, phosphate and magnesium, the *n*-alkanes are partially converted into intracellular granules of polyhydroxyalkanoates.[13]

The aim of this study was to isolate fuel oil-degrading microorganisms from the surface waters of the Venice Lagoon and to evaluate the fuel oil-

degrading potential of different surface waters. A further aim was to study the complementary degradation of fuel oil by strains having the catabolic capacity to degrade *n*-paraffins and by other strains without this degradation pathway, but still capable of growing on agar plates amended with fuel oil.

EXPERIMENTAL

Sampling

In November 1990 and June 1991 water samples from three different stations in the northern part of Venice Lagoon (A, B, C, from outer to inner part) were taken aseptically from the upper layer (1 cm) with a Shomaker sampler (Hydro-Bios) (Figure 1). The stations were representative of different pollution loads, as selected previously for the mussel »watch monitoring« in the lagoon.[1,3] Both samplings were performed under similar tidal conditions so that the results could be compared. A composite 5-liters sample was taken, consisting of repeated 200 mL samples. For microbiological analyses subsampling was performed under sterile conditions in each station.

Bacteria Count

Total bacteria (TB) were counted in the water samples by direct epifluorescence microscopy.[15] Counts of aerobic heterotrophic bacteria (AHB) were determined by the spread plate method. An aliquot of 0.1 mL from serial dilutions (from 10^{-1} to 10^{-5}) of each sample was streaked on Zobell Bacto Marine Agar (Difco). Two plates were used for each dilution and these were incubated at 22 °C. Colonies were counted after 21 days. Fuel oil-degrading bacteria were also enumerated by the most probable number technique (MPN) and by the spread plate method. As regards MPN counting, four dilution series from 10^{-1} to 10^{-5} were set up in quintuplicate, adding 0.2% fuel oil (mixture of *n*-paraffins from C_{12} to C_{28}) as the sole carbon and energy source to a liquid mineral medium containing (per liter): 24 g NaCl, 1 g $MgSO_4 \cdot 7H_20$, 0.7 g KCl, 2 g KH_2PO_4, 3 g Na_2HPO_4, 1 g NH_4NO_3.[16] The MPN was determined after 8 weeks of incubation at 22 °C. For the count plate method appropriate dilutions of the samples (from 10^{-1} to 10^{-5}) were streaked on duplicate plates with mineral medium amended with 0.2% fuel oil and solidified with 2% of agar (Bacto-agar, Difco). The colony forming units (CFU) were determined after 8 weeks of incubation at 22 °C.

Isolation of n*-Paraffin Degrading Strains on Agar Plates*

Colonies of *n*-paraffin-degrading strains were isolated on agar plates with mineral medium[16] amended with 0.2% fuel oil. Colonies were isolated in terms of shape, colour and morphology and streaked at least twice on fresh agar plates. Isolates were stored at –80 °C in cryovials with 20% glycerol.

Biological Oxygen Demand

The B.O.D. of lagoon water was determined by oxygen consumption after fuel oil additions to the samples. At each station, six 200-mL bottles were filled with 200 mL of surface water. Three of these were spiked with 0.2% of fuel oil (filtered

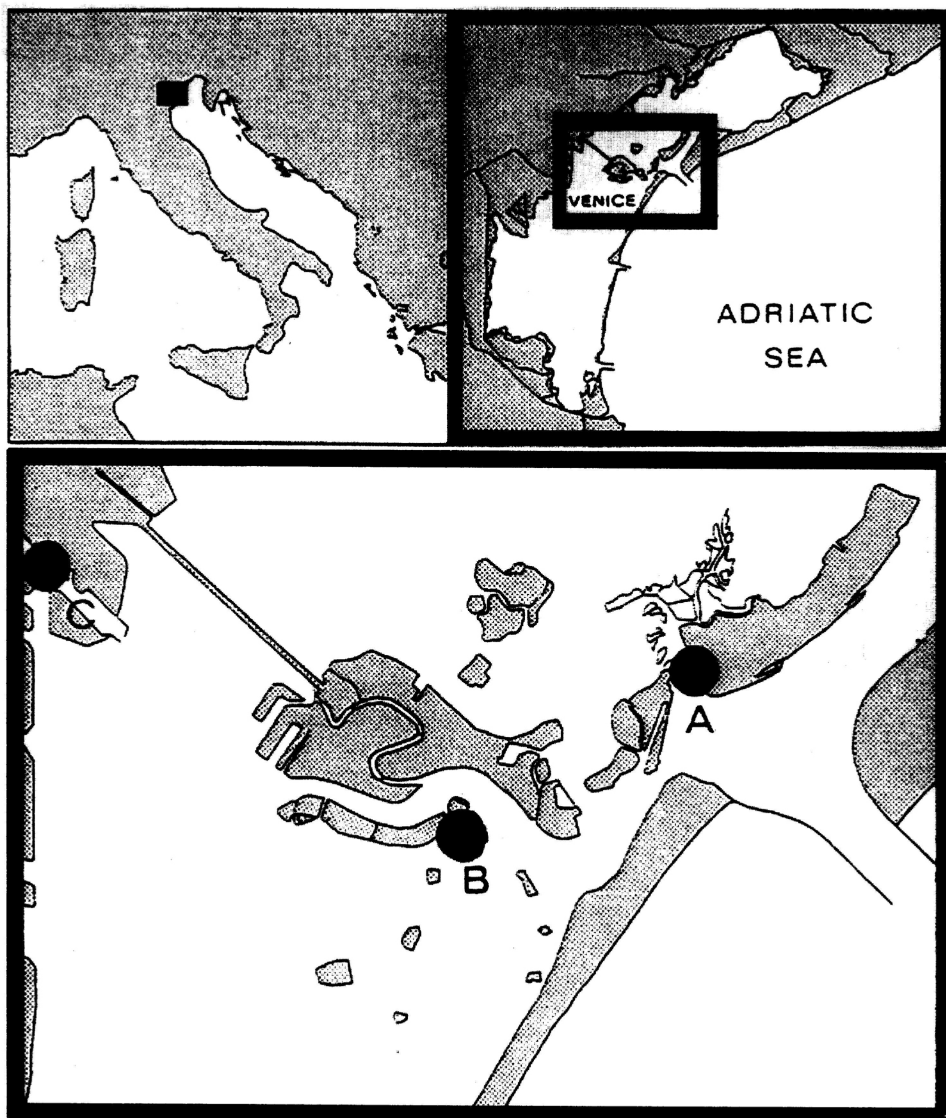

Figure 1. Water sampling sites in the northern part of Venice Lagoon.
Station A: site out of the city; Station B: urbanized area; Station C: industrial site near Porto Marghera.

through 0.2 μm Sartorius filters). The oxygen content of bottles with and without fuel oil was determined by Winkler's method after 7 days and 14 days of incubation at 22 °C for samples collected in November 1990 and after 5, 10, 15 days for those collected in June 1991.

Oxygen Consumption Rate by an Axenic Culture

Conical flasks (1 L) were filled with 300 mL of mineral medium plus 0.2% fuel oil[16] and then inoculated with *Acinetobacter* sp. strain VE-C3. The flask was incubated at 28 °C and shaken at 150 r.p.m. An aliquot of 6 mL was sampled at different times and introduced in the respiratory cell unit of a Biological Oxygen Monitor equipped with a Clark's probe (YSI Model 5300) to determined oxygen consumption rate. Calculations were made on the basis of oxygen concentrations in air saturated water at 28 °C.

Gas Chromatography Determination of Hydrocarbons in Lagoon Water

The concentration of total hydrocarbons in lagoon water was determined in 5 L of water. The sample was consecutively extracted three times with 50 mL *n*-hexane. The three solvent fractions were pooled, dehydrated with anhydrous sodium sulfate, concentrated in a rotary evaporator and purified by elution on a Florisil column (2 g), then concentrated to 1.0 mL and analyzed by gas-chromatography. Gas-chromatography conditions were: injection port and flame ionization detector (FID) at 320 °C; nitrogen flow 30 mL min^{-1}; column temperature ramp from 100 °C to 300 °C at 8 °C min^{-1} rate; dual packed glass columns 200 cm × 4 mm i.d. packed with 5.0% SE-30 on 70–80 mesh Chromosorb W AW-DMCS. The identity of *n*-paraffins was determined from their retention times. Quantification was based on peak-height measurement and comparison with a reference standard of *n*-paraffins in the range C_{12}–C_{28}. The C.V. for five replicate analyses was 6.3%.

n-*Paraffin Degradation in Lagoon Water under Laboratory Conditions*

The *n*-paraffin-degrading potential of surface water was determined in laboratory experiments. In November, four 5-L flasks were filled with lagoon surface water from station C. The flasks were spiked with 0.2% fuel oil and two of these were sterilized (autoclaved) as controls. After 30 days of incubation at 22 °C, the percentage of fuel oil consumed was determined for *n*-paraffins residues by GC analyses, after *n*-hexane extraction. The same experiment was repeated in June.

Acinetobacter *sp. Growth on Fuel Oil*

Growth of the strain VE-C3 (previously identified as an *Acinetobacter* sp.) in the presence of fuel oil was studied. Two conical flasks (1 L) containing 250 mL mineral medium[16] plus 0.2% fuel oil were inoculated 1:100 (*v*:*v*) with induced exponential cultures of the strain. Growth was measured by counting bacterial cells by epifluorecence light microscopy (Axiophot, Zeiss), during 24 h incubation at 28 °C, in aliquots previously fixed with formalin and then stained with acridine orange. The generation time (t_{gen}) of the strain was calculated on the basis of the doubling of cell numbers in 6 h of incubation.

Inducibility of Fuel Oil Degradation

Studies of inducible growth were performed with *Acinetobacter* strain VE-C3, which was transferred 5 times consecutively to mineral medium amended with 0.2% fuel oil as the sole carbon source. The non induced strain was grown by transferring it 5 times to fresh complex medium, without fuel oil, containing 5 g Tryptone (Difco), 2.5 g yeast extract (Difco) and 1 g D-glucose per liter of distilled water. The fuel oil-induced and non induced cultures were grown simultaneously in two one-litre flasks with 300 mL of mineral medium and 0.2% of fuel oil. The flasks were incubated at 22 °C for two days. Aliquots of 3 mL were collected at different times, and cell growth was measured with an UV-visible spectrophotometer (Shimadzu mod. UV-160) in term of absorbance at 600 nm.

Nutrient and Dissolved Oxygen Analysis

Dissolved nutrients were determined with a Technicon Autoanalyzer according to a standard method.[17] pH was measured with a Beckman Research pH-meter equipped with a glass combined electrode. Dissolved oxygen concentration was determined by Winkler's method and percentage saturation values were then calculated.[18]

Growth of Microorganisms in Paraffins and Their Oxidation Products

Twenty-five isolated bacteria were screened by genetic methods.[19,20] The 25 strains were grouped genetically into 9 clusters by means of ARDRA (amplified ribosomal DNA restriction) and RAPD (random amplified polymorphic DNA) analyses. Only seven strains belonging to different groups were identified on the basis of rDNA sequences: *Acinetobacter* sp. VE-C3, *Acinetobacter* sp. VE-B1/II°, *Pseudomonas fluorescens* VE-A1/I°, *P. flavescens* VE-B2/I°, *Ochrobactrum antropi* VE-A1/IV°, *Alcaligenes faecalis* VE-B3/I° and *A. faecalis* VE-C5/I°.[21]

These seven microorganisms were cultured in 10 mL of mineral medium in 20 mL tubes capped with radial cups. The sole carbon and energy source consisted of *n*-paraffins with different numbers of carbon atoms: *n*-decane (C_{10}), *n*-tetradecane olefine free (C_{14}), and *n*-eicosane (C_{20}); or alcohols: *n*-decanol (C_{10}), *n*-tetradecanol (C_{14}) and *n*-eicosanol (C_{20}); or aldehydes: *n*-decanal (C_{10}) and *n*-tetradecanal (C_{14}), (the C_{20}-aldehyde is unstable and not available); or fatty acids: capric acid (C_{10}), myristic acid (C_{14}) and arachidic acid (C_{20}). All the chemical products were provided by Fluka. The tubes were inoculated 1:100 (*v:v*) and then incubated at 28 °C in a rotary drum. Positive (+) and negative (–) cell growth was observed after 24 hrs.

RESULTS AND DISCUSSION

Environmental Studies

The sampling sites were chosen according to previous monitoring results using mussels as bioindicators for hydrocarbon pollution (Figure 1). Station A, towards the lagoon entrance, was chosen as control, Station B repre-

sented an urbanized part of the lagoon, and Station C near Porto Marghera an industrial site, the most polluted of the three. Values of the chemical and physical parameters of surface water samples in the three stations are reported in Table I.

TABLE I

Basic chemical and physical parameters of surface water from three sampling stations in Venice Lagoon.

Station	T(°C)	Sal (%)	pH	DO mg L^{-1}	%DO sat	NH_3 μmol L^{-1}	NO_2^{-1} μmol L^{-1}	NO_3^{-1} μmol L^{-1}	PO_4^{-3} μmol L^{-1}	SiO_2 μmol L^{-1}	n-alkanes μg L^{-1}	UCM μg L^{-1}
November, 1990												
A	16	34.2	8.18	7.64	95.2	4.40	0.61	10.9	0.32	6.72	–	–
B	16.5	34.1	8.17	7.85	97.9	6.82	0.91	15.2	0.35	19.9	–	–
C	18	29.3	7.96	6.47	81.5	28.4	2.93	42.2	3.43	19.9	–	–
June, 1991												
A	24	32.4	8.76	8.19	117	5.44	0.52	2.95	0.53	–	1.12	12.8
B	27	30.1	8.04	7.28	109	6.26	0.53	3.80	0.43	–	0.99	9.9
C	28	27.7	8.42	7.51	112	9.93	1.07	3.25	2.65	–	1.11	12.3

Abbreviations: Sal: salinity; DO: dissolved oxygen; UCM: unresolved complex oil mixture. (–) not determined.

Hydrocarbon contamination was low (0.99–1.12 μg L^{-1}) and similar in the three stations, even station C. Similar levels of aliphatic hydrocarbons have been reported in surface water samples from the Strait of Messina (De Domenico, 1975). An inverse correlation between temperature and salinity was found because of the inputs of heated cooling water from six electric power plants: two at Marghera (140 MW) and four at Fusina (960 MW). Moreover, the water at station C has less turnover with the open sea. The water at stations A and B had a lower temperature and higher salinity.

Dissolved oxygen concentrations ranged from 6.47 to 7.64 mg L^{-1} in November and from 7.28 mg L^{-1} to 8.19 mg L^{-1} in June. In June oxygen saturation was more than 100%, clearly due to higher photosynthetic activity, particularly at station A, the least polluted site. Nutrient concentrations showed marked seasonal variations, especially in station C, with lower values in June consistent with the highest primary production.[22]

The number of fuel oil-degrading bacteria ranged from 8 mL^{-1} to 3.45×10^2 mL^{-1} (Table II). These concentrations reflected the relatively low hydrocarbon load of the surface water layer (about 1 μg L^{-1}). The percentage of fuel oil-degrading bacteria (0.028% to 0.23%) was two-three orders of magnitude lower than in chronically polluted areas in the world. At »Laguna de Terminos«, in the southern part of the Gulf of Mexico, the percentage of hy-

TABLE II

Counts of aerobic heterotrophic bacteria and fuel oil-degradaing bacteria determined by epifluorescence, most probable number and plate count, in surface water from three sampling stations in Venice Lagoon.

Station	TB mL^{-1} epifluorescence	ODB 100 mL MPN	AHB-CFU mL^{-1} PC	ODB-CFU mL^{-1} PC	%ODB
November, 1990					
A	5.0×10^5	8	–	–	–
B	1.1×10^6	23	–	–	–
C	1.6×10^6	33	–	–	–
June, 1991					
A	6.3×10^5	70	7.0×10^3	1.6×10^1	2.3×10^{-1}
B	1.3×10^6	221	4.5×10^3	5.4×10^2	1.2×10^{-1}
C	3.6×10^6	345	1.3×10^4	3.6×10^2	2.77×10^{-2}

Abbreviations: TB: total bacteria; AHB: aerobic heterotrophic bacteria; ODB: fuel oil-degrading bacteria; MPN: most probable number; PC: plate count; %ODB: percentage of fuel oil-degrading bacteria; (–) not determined.

drocarbon-degrading bacteria is reported to vary from 6% to 49%.[23] In the Basrah Lagoon (Iraq), northwestern Arabian Gulf, the percentage of oil-degrading bacteria has been estimated to vary from 1.6% to 2.2%.[24] High percentages of hydrocarbon-degrading bacteria have also been found in the Bohai Sea (China),[25] the Baltic Sea,[26] and the northwestern Atlantic.[27]

In the Mediterranean Sea (UNEP/IOC, 1988) there have been several studies on the isolation and distribution of hydrocarbon-degrading bacteria in water and sediment.[11,28–33] The percentage of hydrocarbon-degrading bacteria varies from 1.4% to 5.1% close to crude oil refineries and along crude oil tanker routes off southern Italy.[28,29,31] The percentage of hydrocarbon-degrading bacteria found in the northern part of Venice Lagoon is similar to values found in the strait of Messina,[28] where surface water has similar hydrocarbon contents. The percentage of hydrocarbon-degrading bacteria is generally considered a good indicator of crude oil pollution,[34] but in the present study the percentage was not well correlated with hydrocarbon content. The concentrations in the surface water of the Venice Lagoon were probably not high enough to induce a large population of hydrocarbon-degrading bacteria.[35] With regard as temporal variations, the concentrations of total bacteria and hydrocarbon-degrading bacteria were higher in June than November, probably due to more favourable temperature conditions.[35]

Temperature and nutrient concentrations are the most important environmental factors influencing hydrocarbon oxidation. Studies in Lake Mendota have shown that the optimum temperature for crude oil biodegradation ranges from 20 °C to 25 °C throughout the year. In summer, when tempera-

tures were optimal, nutrient deficiencies limited crude oil biodegradation.[36] In our case, the stimulating effect of temperature in June was probably counterbalanced by the adverse effect of lower concentrations of nitrogen and phosphorus.

In our laboratory experiments, the biological oxygen demand determined in four bottles of fresh surface water, varied between the three sites and was enhanced by fuel oil additions, particularly in station C, where eutrophication was highest. This was true for samples taken both in November (Figure 2A) and June (Figure 2B), in which all the oxygen was consumed in 7 and 5 days respectively. In station C, we also found the highest number of fuel oil--degrading bacteria (33 × 100 mL in November and 345 × 100 mL in June). The oxygen consumption rate varied in the three stations even without fuel oil additions. Again station C showed the highest oxygen consumption rates. About 50% total oxygen was consumed in 7 days in November and in 5 days in June.

The highest n-paraffin-degradation potential, estimated as the percentage of n-paraffins consumed after 30 days of incubation at 22 °C in the dark, was found again in water samples from station C. In November, the oil-deg-

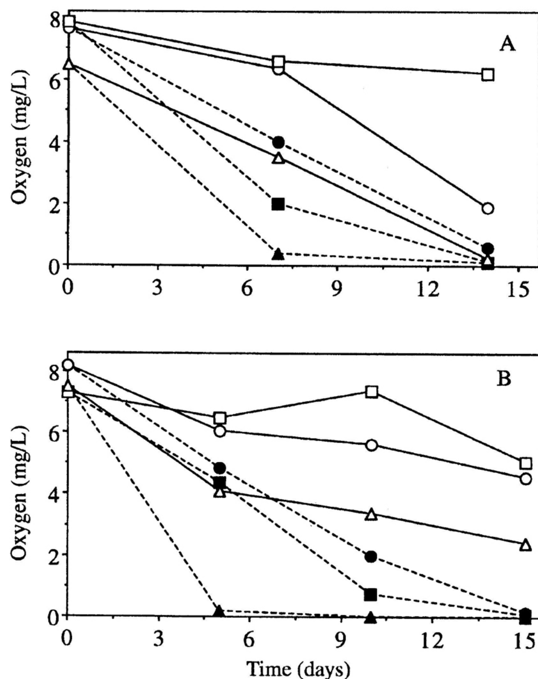

Figure 2. Fuel oil-degrading microbial activities evaluated by B.O.D. measurements in surface water samples from the northern part of Venice Lagoon: in November (A) and June (B). Water samples from stations A (●), B (■) and C (s) amended with 0.2% fuel oil and water samples from stations A (O), B (□), and C (Δ) without added fuel oil.

radation potential resulted in 80% disappearance of *n*-paraffins (Figure 3). In June, it was less than 10%, even though the total number of oil-degrading bacteria was higher (Table 2). When the whole microbial population was incubated at the optimal temperature, 22 °C, in sealed bottles amended with fuel oil, was found that nutrient concentrations was the most important parameter. There were significantly more nutrients in surface water of the Venice Lagoon in November than in June. Consequently the microbial community in November had the higher fuel oil-degrading activity if the temperature was raised to 22 °C.

Microbiological Studies under Laboratory Conditions

Twenty-five Gram-negative heterotrophic aerobic strains were isolated from surface water of the three stations and they grew on agar plates amended with fuel oil. Seven strains were identified but only the *Acineto-*

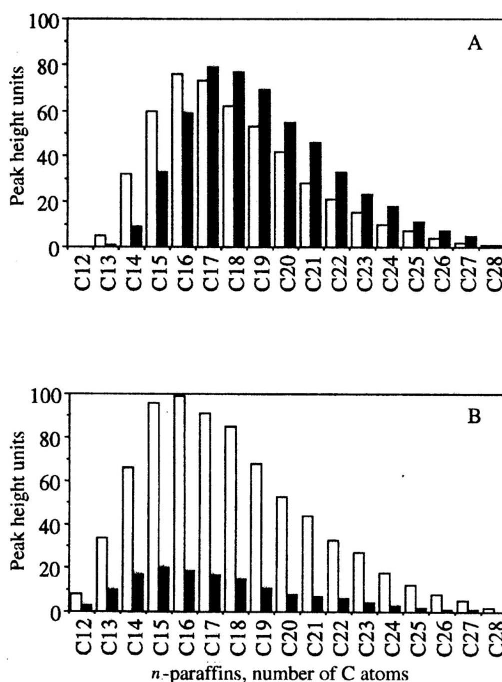

Figure 3. Relative intensity of *n*-parafine peaks determined by gas-chromatography in surface water samples from the northern part of Venice Lagoon (station C) spiked with 0.2% of fuel oil: (A) sterile samples initially as controls (white columns) and after 30 days of incubation at 22 °C in the dark (black columns); (B) natural water samples at the start (white columns) and after 30 days of incubation at 22 °C in the dark (black columns).

bacter sp. VE-C3 thrived with paraffins as the sole carbon and energy source. Another strain of *Acinetobacter* sp. VE-B1/II° grew at a low rate on the same substrate. These strains harbored plasmids of 15 Kb and 10 Kb respectively, whereas the other five strains identified did not.[20] Using *P. oleovorans alk*BFGH genes as probe, hybridization signals were found with both chromosomal and plasmid DNA of both *Acinetobacter* strains.[21] The *n*-alkane oxidation pathway is well known in *Pseudomonas oleovorans*, in which genes coding for enzymes of *n*-octane oxidation harbored in the OCT plasmid.

Acinetobacter sp. strain VE-C3 was growth inducible in the presence of fuel oil. Experiments on the inducibility of *n*-paraffin degradation showed that the fuel oil-induced culture started growing three hours after inoculation, while the uninduced culture grew after 24 hrs. The efficiency of *n*-paraffin degradation by this strain was confirmed by various physiological analyses carried out in the laboratory. The strain showed a paraffin degradation activity with a generation time (t_{gen}) of 2.77 h in the presence of fuel oil. The rate of fuel-oil consumption of was 53 μL O_2 h^{-1} mg d.w.

It is known that in *P. oleovorans* genes coding for enzymes for the oxidation of long chain alcohols, aldehydes and fatty acids, are located on the bacterial chromosome.[13,14]

TABLE III

Growth of seven aerobic heterotrophic Gram-negative bacteria strains isolated from Venice Lagoon, in mineral medium with different substrates as the sole carbon and energy source.

Substrates		Strains*						
		1	2	3	4	5	6	7
n-Alkanes	decane (C_{10})	+	+	–	–	–	–	–
	tetradecane (C_{14})	+	+	–	–	–	–	–
	eicosane (C_{20})	+	+	–	–	–	–	–
Alcohols	1-decanol (C_{10})	–	–	+	+	–	–	–
	1-tetradecanol (C_{14})	+	+	+	+	–	–	–
	1-eicosanol (C_{20})	+	+	–	+	–	–	–
Aldehydes	decanal (C_{10})	–	–	+	+	–	–	–
	tetradecanal (C_{14})	+	+	+	+	–	–	–
Fatty acids	capric acid (C_{10})	–	–	–	–	–	–	–
	myristic acid (C_{14})	+	+	+	+	+	+	+
	arachidic acid (C_{20})	+	+	–	+	–	–	–

* 1. *Acinetobacter* sp. VE-C3; 2. *Acinetobacter* sp. VE-B1/II; 3. *Pseudomonas fluorescens* VE-A1/I; 4. *P. flavescens* VE-B2/I; 5. *Ochrobactrum antropi* VE-A1/IV; 6. *Alcaligenes faecalis* VE-B3/I; 7. *A. faecalis* VE-C5/I. Positive growth (+), no growth (–).

The five non-paraffin-degrading bacteria were isolated on the same agar plates amended with fuel oil as the two paraffin-degrading *Acinetobacter* strains. Growth experiments with bacteria without plasmids on long-chain alcohols, aldehyedes and fatty acids were carried out to demonstrate their co-metabolism with the two *Acinetobacter* strains (Table III). The *P. fluorescens* and *P. flavescens* strains grew in the presence of alcohols, aldehydes and fatty acids, but not with *n*-paraffins. The others strains could grow only in the presence of long-chain fatty acids, the last oxidation product of *n*-alkane degradation. The total degradation process appears to involve all the strains; it starts with *Acinetobacter* strains, the only ones harboring plasmids, which first oxidize the *n*-alkanes, providing organic residues suitable for the other bacteria. This could explain why in oil spills in marine environments the number of bacteria harboring plasmids is very limited (37). This aspect is also evident when the number of plasmids is determined in a mixed population isolated from different types of organic pollution. For instance, populations of bacteria isolated from textile industry wastes can have a high frequency of plasmids.[38]

The present results strongly suggest that complete *n*-alkane degradation is carried out by a microbial consortium, the single members of which perform different steps of *n*-alkane degradation in a sort of »functional complementation«.

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SAŽETAK

Usporedna razgradnja naftnih ugljikovodika u površinskim vodama i akseničnim kulturama aerobnih Gram-negativnih bakterija izoliranih iz mletačke lagune

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Udjel bakterija koje razgrađuju naftne ugljikovodike (n-parafine od C_{12} do C_{28}) određen je na tri postaje u sjevernom dijelu mletačke lagune. Koncentracije bakterija koje razgrađuju n-parafine u rasponu su od 8 do 70 na 100 mL u najmanje zagađenom dijelu (postaja najbliže otvorenom moru) do 33–345 na 100 mL u najzagađenijem dijelu (postaja pored Porto Marghera). Biološka potrošnja kisika porasla je dodatkom naftnih ugljikovodika na toj postaji, gdje je kisik potpuno potrošen za 7, odnosno 5 dana u studenom i lipnju. Dvadesetpet sojeva bakterija izolirano je s agara, kojemu su dodani naftni ugljikovodici kao jedini izvor ugljika i energije, samo dva su bila sojevi *Acintobacter* spp. Soj VE-3 rastao je u prisutnosti n-parafina. Rast je bio inducibilan, s generacijskim vremenom 2,77 sati i brzinom potrošnje kisika 53 mL h^{-1} po mg-stanica (suhe težine). Pet drugih sojeva uspijevalo je na međuproduktima oksidacije n-parafina.