ELABORATION OF AN EXPERIMENTAL METHOD TO ASSESS BIODEGRADATION AGENTS : BIOREMEDIATION TRIALS ON OIL POLLUTED BEACH.

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ABSTRACT

Many new bioremediation processes are now proposed on the market for cleaning oil polluted shores.

However the efficiency of these processes when used in open field is still controversal ; it is very difficult to distinguish between the effect of the biodegradation itself and the effect of the mecanical washing due to the waves and tides action.

CEDRE is conducting trials on a sheltered beach in Britany (France), in order to define a methodology to control and to assess these biotreatments : trials have been already conducted over a 5 months period, on 6 parcels, beforehand polluted with light crude oil and then treated by 4 different bioprocesses.

Microbiological analysis, qualitative and quantitative chemical analysis (IR, GC-MS) have been performed to follow the evolution of the oil ; results are discussed here below.

1. INTRODUCTION

Among the many techniques proposed for restoring a polluted coastline, the processes involving oil biodegradation are currently being studied and improved.

The use of these techniques has evolved as a result of the Exxon Valdez accident in Alaska when "fertilizer type" products for biodegradation were massively employed.

** CEDRE : Center for Documentation, Research and Experimentation on Accidental Water Pollution*
*** SHOM : French Navy's Hydrographic Office*
**** MNHN : National Museum of Natural History of Paris*

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In order to clarify some questions concerning bioremediation agents, CEDRE was allowed to realize long term experiments on a beach belonging to the French Navy.

Conducted over a one year period in close collaboration with EPSHOM and MNHN, these tests aimed at assessing the fate of an oil treated by various biodegradation agents and processes over a period of one year.

2. EXPERIMENTAL OBJECTIVES

In addition to observing the biodegradation of a crude oil and to obtaining a generalized "first evaluation" concerning the various bioremediation agents tested, the experiments were also intended to define and improve an experimental testing method which would enable subsequent evaluations concerning the effects of various biodegradation processes and products.

3. TESTING

3.1. Testing Site

The test area is a beach located in the Brest Bay on the western coast of France (Brittany).

The beach is a pebbles spit sheltered behind a little rocky island, and is composed of a mixture of large grained sand (sea-shells debris) and shingles.

The slope of the beach is on the order of 10 %.

3.2. Experimental Set-up (fig. 1)

Six 3 m2 test plots were prepared in the intertidal zone at a height of 3.5 m above 0 (according to maritime charts, lowest sea level). Each test plot was enclosed by a breeze-block wall designed to protect the plot from extensive wave action. Two crates containing sand from the nearby beach area were placed within the breeze-block enclosures of each test plot.

The experimental plots were built about 1 month before the oil was applied.

3.3. Type of Pollutant

The testing was done using a light Arabian crude (BAL) topped at a temperature of 110°C.

3.4. Application of the Pollutant

The oil was applied (on the whole surface of each test plot) for a total concentration of 5 liters/m2, in two consecutive applications of 4 liters and 1 liter which were respectively accomplished 14 days and 10 days before the bioremediation agents were applied.

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3.5. Biodegradation Processes to be tested

Four different processes were evaluated.

3.5.1. ***Process Nr. 1*** involved the application of amicroflora adapted to the specific task of oildegradation. The freeze-dried bacteria enriched with additional nutritive elements were prepared several hours before application premixed with water with a specific additional ingredient (Tested in Test plot No. 1).

3.5.2. ***Process Nr. 2*** involved the use of an adapteddehydrated bacteria packed in small 50 gram net bags. The small bags were re-hydrated in sea water 20 minutes before being applied (Test plot No. 2).

3.5.3. ***Process Nr. 3*** involved an already known bioremediation formula of nutritive nitrogen and phosphorous which was previously used during the Exxon Valdez clean-up inAlaska (Reference : 1).
This product, defined as a "biodegradation starter" is designed to acts as a stimulus for indiginous bacteria (Test plots Numbers 3 and 5).

3.5.4. ***Process Nr. 4*** used a fine-grained, non water repellant chalk powder which is intended to favorize the action of indigenous bacteria by increasing the surface area for effective bacteria fixation (Test plot No. 6).

Test plot Nr. 4 was left un-treated in order to serve as a control plot.

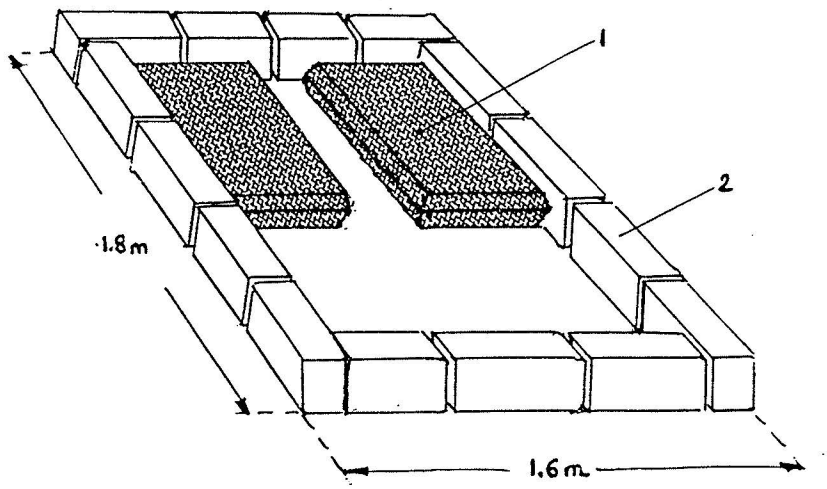

FIG. 1 - Experimental set up
1. Protection made with breeze-blocks
2. Crates containing sediment

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4. ANALYTICAL METHODS

In addition to visual observations and photographies at the site, the methods employed in the evaluation included sampling and laboratory analyses :

- micro-biological analyses : comparison between the total microscopic bacteria and the microscopic bacteria specifically adapted to oil degradation (reference : 2 and 3).

- measurements of oil concentration in the sediment using spectrometry and infra-red analyses (reference : 4).

- qualitative analyses of the oil after its separation into various families (Cf. Annex 1) using gas chromatography methods on aliphatic and sulfur containing aromatic fractions (analyses made by EPSHOM) (reference : 5 and 6).

- qualitative analyses of the oil by gas chromatography coupled with mass spectrometer (analyses made by MNHN)

- measurments of the nitrogen (Kjeldhal) and phosphorous-concentrations in ambient sea-water.

The samples were taken from the test plots, and from surrounding beach areas to serve as control samples (non polluted area), at several meters from the test plots as well as at several tens of meters from the test plots.
Each time an "average sample" was prepared for a given test plot ; it was made by mixing ten random samples from the plot.

5. CHRONOLOGY

(Cf. Annex 2)

6. FIRST TEST RESULTS

At the present time, we have analyses available from samples taken during the first 4,5 months of the evaluation period.

6.1. Micro-biological analyses

Annex 3 presents the evolution of bacterial population (bacteria adapted to hydrocarbon and total bacteria) during the first 4.5 months of the evaluation period.

6.2. Oil concentration in sediment

Annexes 4 and 5 present the evolution of the oil concentration in the sediment measured by colorometric spectrometry and by infra-red spectrometry.

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6.3. Qualitative oil analyses using chromatography

Annexes 6 and 7 present several examples of chromatographic analyses on samples taken after two months for the aliphatic and aromatic oil fractions. Analyses have been done for test plot 4 (control plot) and for the more degraded oil in plots 2 and 3 (processes 2 and 3). Similar chromatographic analyses were made after 4.5 month, however they do not reveal a more advanced stage of oil degradation.

NOTA : only the mass spectrography analyses of the two first months have been yet performed on plots 2 and 4 and they confirm GC analyses).

6.4. Nitrogen and phosphorous concentrations

The nitrogen content of the surrounding sea water was determined to be 0.34 mg/l at the beginning of the test period and 0.15 mg/l after 2 months (total Kjeldalh nitrogen).

As far as the phosphorous content is concerned, a quantity of 0.09 mg/l (total phosphorous) was determined at the beginning of the test period as well as two months later.

7. DISCUSSION

7.1. Studies of the biodegradation processes

The microbiological data mainly show that specifically adapted bactera are much developped in the polluted plot than in the surrounding area (non polluted control).

The oil concentration in the sediment has rapidly decreased (about 80 - 90 % according to the I.R. measurements), and this can be explained by the tides action and wave washing effects.

The plots located at the two ends of the set-up are more exposed to the sea's washing action, especially plots 1, 5, and6. The waves' action was so intense that they were able to loosen and displace some of the breeze blocks. The oil concentrations decreased immediately at the beginning of the experimental period (between 8,000 - 11,000 ppm according to the I.R. measurements) and this concentration decreased increasingly more quickly in the more exposed test plots. For the plots 2 and 3 which were less exposed to the waves' action, the oil concentrations at the beginning of the test period were greater (between 13,000 - 18,000 ppm according to the I.R measurements), and the decrease in oil concentration required a longer period of time.

After 4.5 months, the difference in the oil concentration for the five treated test plots is less apparent (between 4,400 and 2,000 ppm according to the colorimetric analyses); at this point, only the oil strongly adsorbed on the sediment still remains.

Whether they were made on samples recovered after 2 or after 4.5 months, the chromatographic analyses have revealed only a small amount of biodegradation.

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Nevertheless, test plots 2, 3 and 5 have shown a significant degradation when compared to the control plot (despite the fact that their oil content is somewhat higher than in the other test plots).

The table 1 presents the percentage of oil which has been biodegraded (for the medium weight alcanes) in comparison with the original crude of oil and with the oil of the un-treated control plot (calculated according to the method of Bodennec & al. reference 7) :

(nC17/Pr)o - (nC17/Pr)/(nC17/Pr)o

where :

(nC17/Pr) is the ratio nC17/Pristane of the test plot.
(nC17/Pr)o is the ratio nC17/Pristane of the reference (oil initially applied , B.A.L. or the control plot, untreated).

	Reference	nC17/Pr	nC18/Ph	Pr/Ph	% biodegradation	
					B.A.L.	Plot 4
	B.A.L.	6.67	3.13	0.56-	-	
Process 1	Plot 1	4.17	2.22	O.56	34.9	0
Process 2	Plot 2	2.63	1.37	0.54	59.1	36.8
Process 3	Plot 3	2.78	1.47	0.56	57.4	33.3
Control	Plot 4	4.17	2.33	0.55	35.4	-
Process 3	Plot 5	3.57	2.04	0.55	45.1	14.3
Process 4	Plot 6	4.17	2.38	0.56	34.9	0

Table 1 : Evaluation of the percentage of Biodegradation

The chromatographic analyses of the sulfur containing aromaticfractions confirm the prior results. For all the test plots, we observe an almost total disappearance of the benzothiophenes (which might also be partially due to the phenomenon of evaporation); however, the disappearance of the dibenzothiophenes and their methyl derivatives for test plots 2 is attributed to the result of biodegradation.

These findings have led us to the conclusion that processes 2 and 3 (tested in plots 2, 3, and 5) have had a favorable effect on the oil biodegradation. Nevertheless, when taking into account the original composition of the oil, and according to previous work which has been done to assess the relative length of time necessary for the biodegradation of various oil fractions (reference 8), we can estimate that the total of degradation (when compared to the whole composition of the oil), is on the order of only 10 - 15% of the total original oil for test plots 1, 4, 6 and about 20 % for the test plots 2 and 3. Thiscorresponds to the first stage of biodegradation as defined byOudot-Dutrieux (reference 9).

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It is interesting to note that after 4.5 months in situ, the biodegradation which has occurred is on the same order as what was obtained after only a few days under optimal laboratory conditions (nutritive minerals, temperature, bacteria, etc.), (reference 8). Available nitrogen is apparently a limiting factor : a quick calculation using as reference an optimum ratio C/N = 10), shows that the nitrogen concentrations in sea water (0.15-0.3 mg/l) enable, in the best case situation, (where the total amount of available nitrogen is consumed), a biodegradation of 10 to 20 ppm of oil in the sediment per month, (which is much less than the oil concentrations actually observed in the test plots).

7.2. Experimental Methods

Although the beach area selected for the test site was relatively protected, there was still a fairly strong agitation (waves, etc.) which caused much sediment movement along the shore.

Under such conditions, the use of crates to hold the polluted sediment was seen to be a good solution for such long term testing.

For any further test, the method can be improved especially by half buried the crate of sediment in the beach rather than simply set them on the beach surface ; in this manner, the polluted sediment will be under more realistic conditions in terms of the agitation, wave action, percolation, etc.

The variations in the oil concentrations in the sediment from one sampling site to another (due to the amount of pollution and the sediment granulometry) give proof of the sediment heterogeneity within each crate.

In terms of comparing the various bioremediation agents, it will be better to use a sifted sand (to provide a more uniform sand grain size), and to thoroughly mix the oil with the sediment (in a mixing machine).

Observations have shown that the test site, although relatively small (~ 60 m), does not present the same oceanic conditions ; it was especially noticeable that the beach's extremities were more exposed to the waves'agitation than the center.

In order to overcome these local variations, it will be preferable to test each individual product in at least two (and better yet, three) test plots located at various sites along the beach.

8. CONCLUSIONS

Under the given oceanic-temperate test conditions, this long term study has clearly shown that the oil biodegradation in surface sediment along the coast takes place very slowly and that the oil's disappearance is principally due to wave washing action and the effects of the tides.

Then these tests, which were initially planned to cover a period of six months, will be extended for a total of one year.

The main factor limiting the biodegradation of oil is the availability of nitrogen; this emphasizes the need for the development of bioremediation products able to progressively provide the correct amount of necessary nutrients.

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The tested products were not proven to be of any particular effectiveness. Only the Processes 2 and 3 were able to show a slight increase in the oil's biodegradation and this observation should be confirmed in the test follow-up.

The experimental methods (especially the use of crates for holding polluted beach sand) will enable us to perform a long term study of the oil's evolution in the absence of perturbing external elements such as waves, storms, sediment movement, etc.

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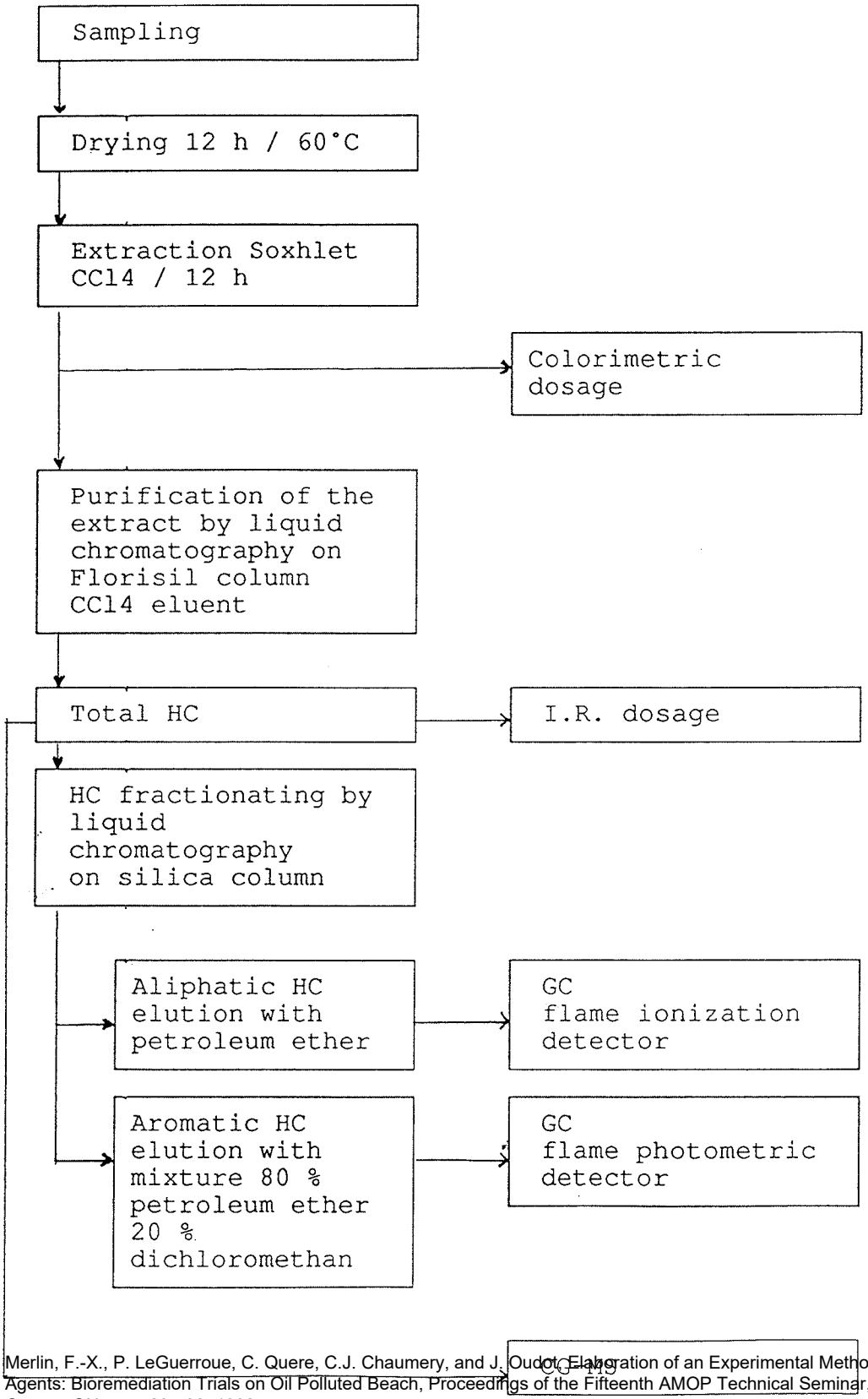
ANNEX 1

PURIFICATION, FRACTIONATION AND ANALYSES OF OILY SEDIMENTS SAMPLES



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ANNEX 2

CHRONOLOGY

Time (days)	ANALYSES						
	Bacterio	Quantitative			Quali-tative		
		Colorimetry	I.R.	Gravimetry	GC	GC-MS	N&P
- 42	Preparation of test plots on the beach						
- 14	X						
	First application of oil on test plots						
- 10	Second application of oil on test plots						
- 3		X	X				X
0	X						
	First application of biodegraders (plots : 1, 2, 3, 5, 6)						
4		X	X				
14	X						
	Second application of biodegraders (plots : 1, 2, 5, 6)						
34	X	x	x				
	Third application of biodegraders (plots : 1, 2, 5)						
49	X						
62	X	X	X	X	x	x (plots 2,4)	X
140	X	X	X	X	X	X	
futur (#240)	X	X	X	X	X	X	X
(#365)	X	X	X	X	X	X	

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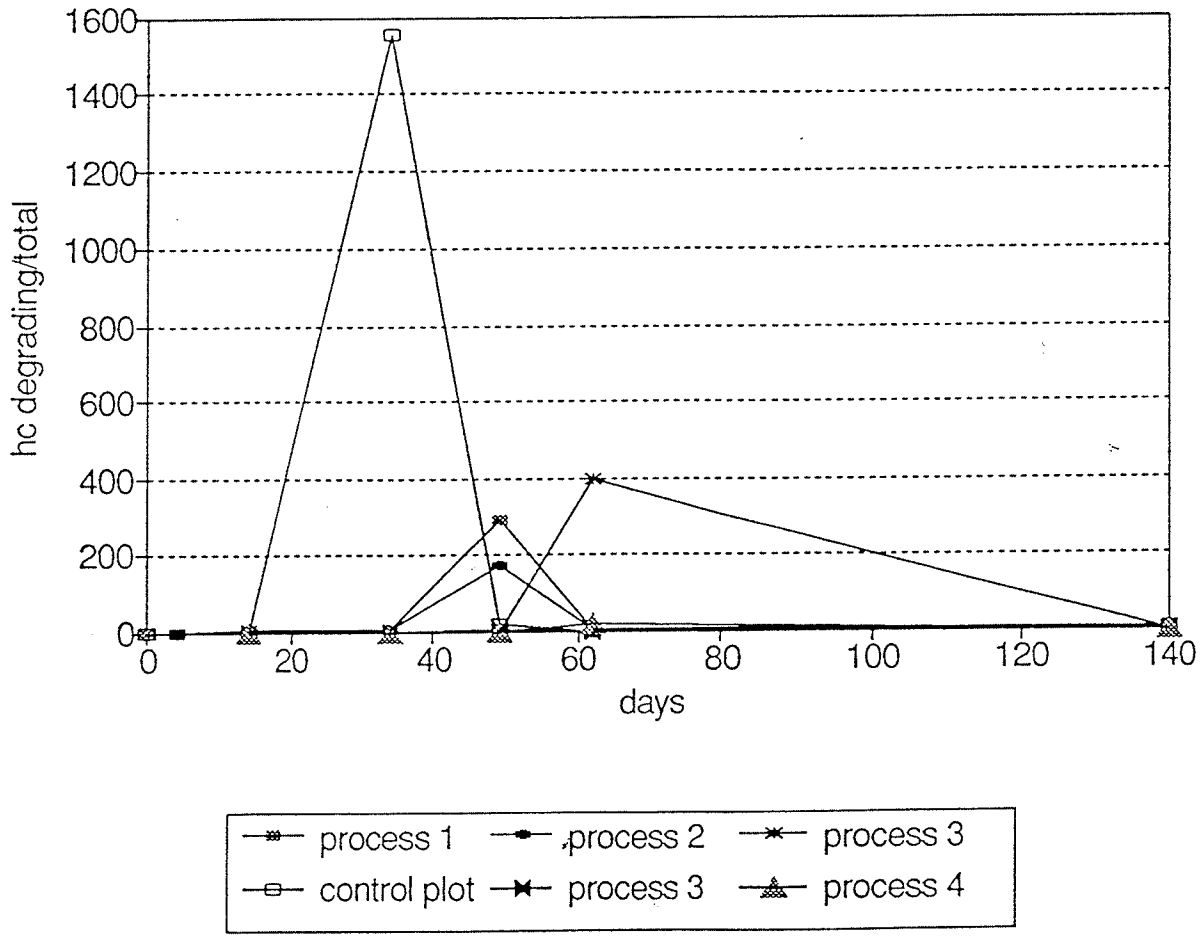
ANNEX 3

RATIO HC degrading microbial population/total population

process days	n 1	n 2	n 3	control	n 3	n 4	control unpollute area 1	control unpollute area 2
0	0,13			0,56			0,41	0,00
4	3,11	0,04					0,02	
14	4,74	0,00	0,00	0,10	0,01	0,00	0,04	0,00
34	6,00	6,25	0,16	1555,56	0,09	0,40	0,04	0,00
49	291,67	175,00	0,00	22,73	9,33	1,56		
62	4,74	4,74	400,00	0,00	1,80	23,75	1,80	0,03
140	0,42	4,74	1,00	9,47	0,06	0,08	0,20	0,02



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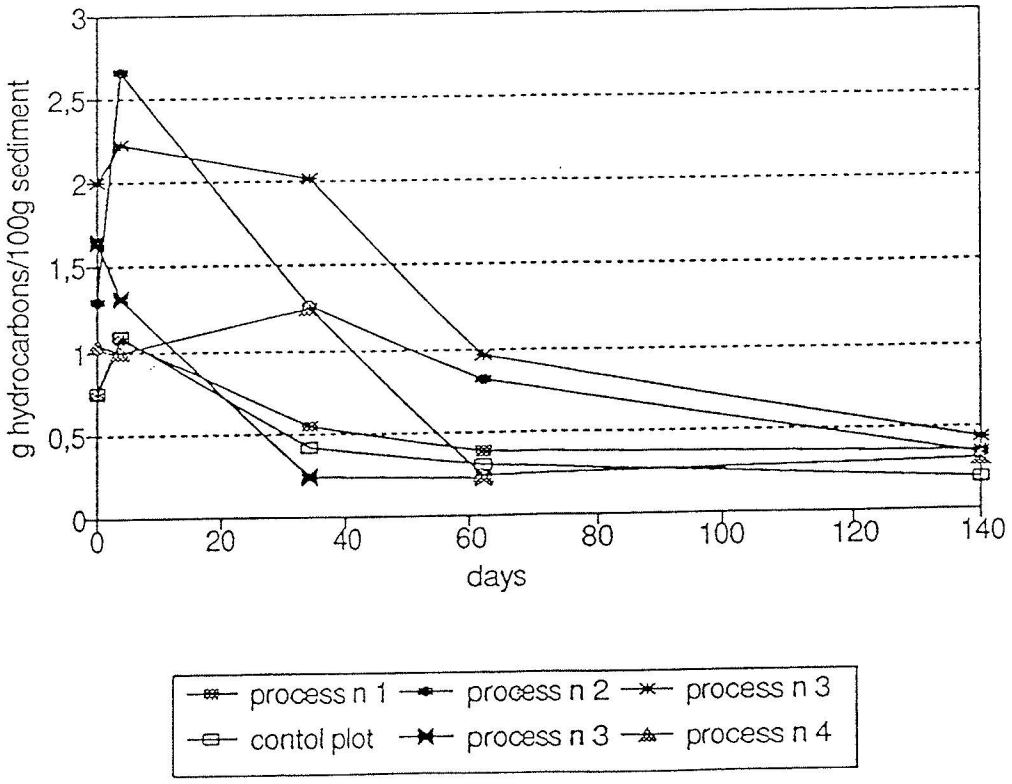
ANNEX 4

HYDROCARBON CONCENTRATIONS VERSUS TIME

(Spectrocolorimetry method)

process days	n 1	n 2	n 3	control	n 3	n 4	control unpolluted area 1	control unpolluted area 2
0	0,74	1,30	2,00	0,76		1,05		
4	1,07	2,66	2,22	1,08	1,65	0,99	0,030	
34	0,54	1,26	2,02	0,42	1,31	1,25	0,020	0,020
62	0,38	0,81	0,96	0,30	0,24	0,25	0,009	0,002
140	0,35	0,34	0,44	0,2	0,22	0,3	0,007	0,018



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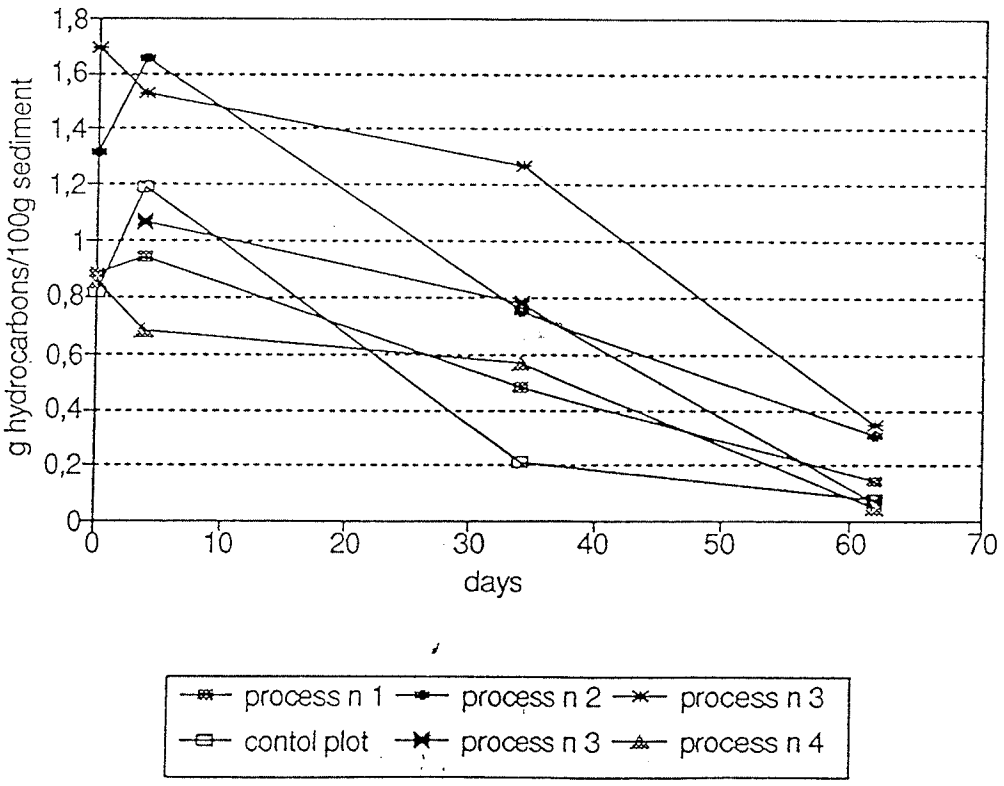
ANNEX 5

HYDROCARBON CONCENTRATIONS VERSUS TIME

(Infrared measurment method)

process days	n 1	n 2	n 3	control	n 3	n 4	control unpolluted area 1	control unpollute area 2
0	0,88	1,32	1,69	0,82		0,86		
4	0,94	1,65	1,53	1,19	1,07	0,68	0,005	
34	0,48	0,75	1,27	0,21	0,78	0,57	0,02	0,002
62	0,14	0,31	0,35	0,08	0,07	0,05	0,002	0,02



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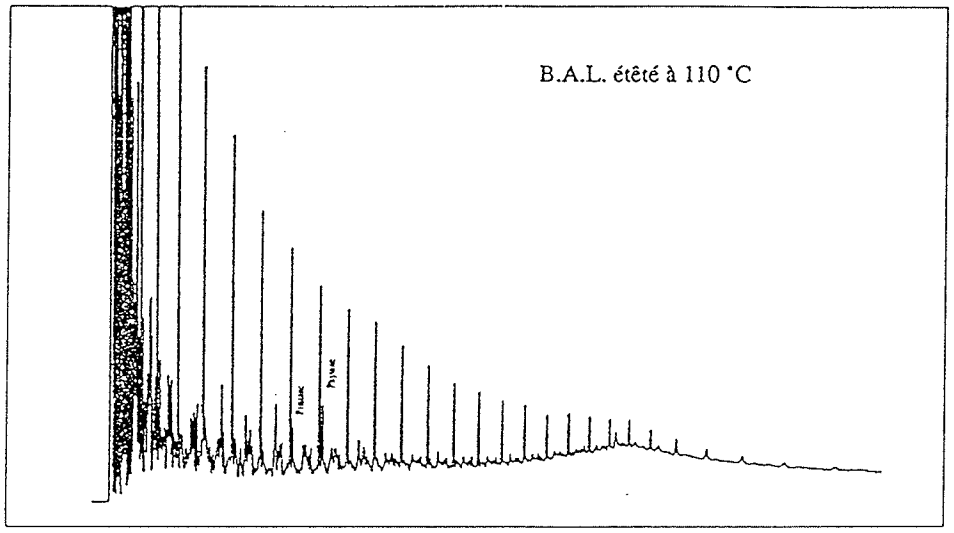
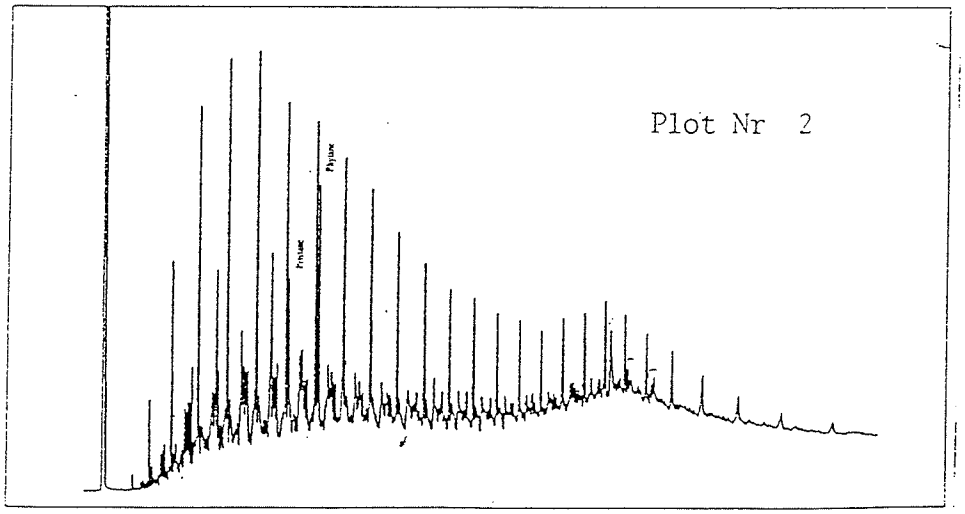
ANNEX 6

GC of aliphatic fractions





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ANNEX 6

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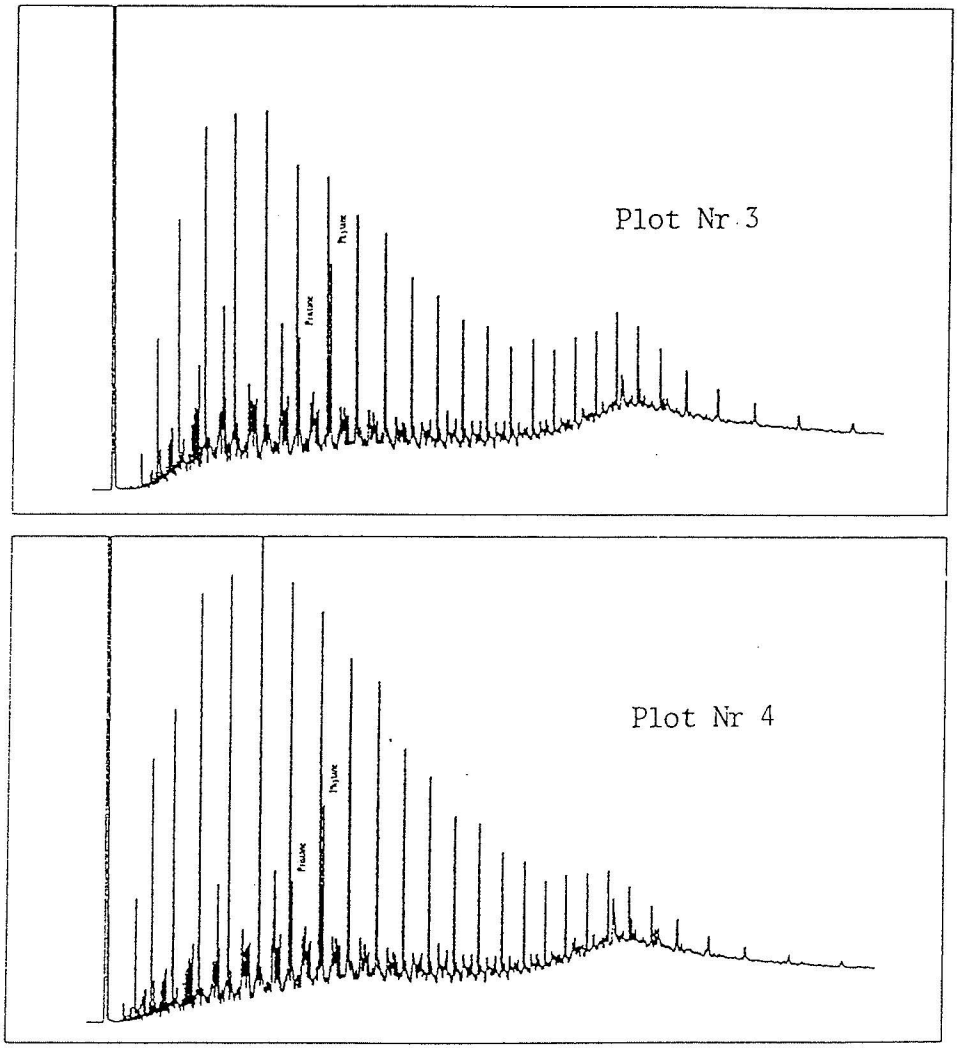

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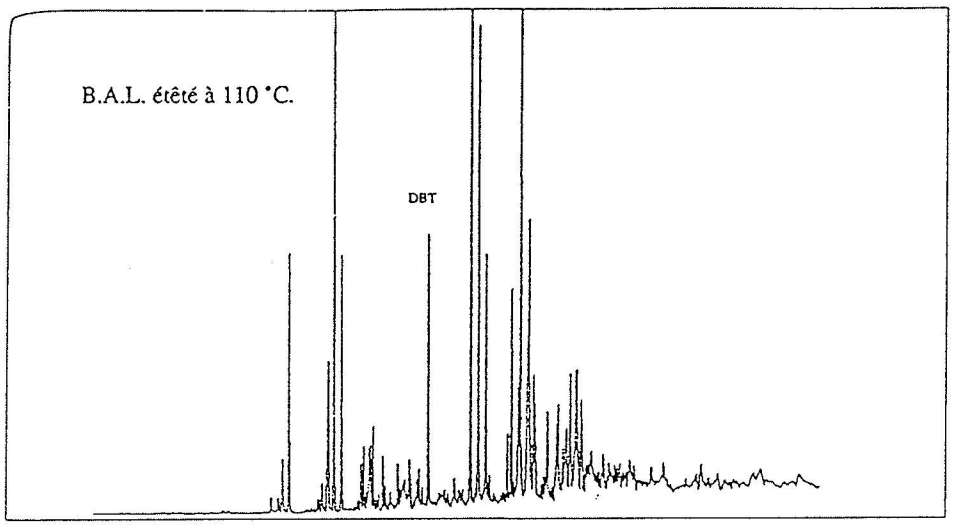
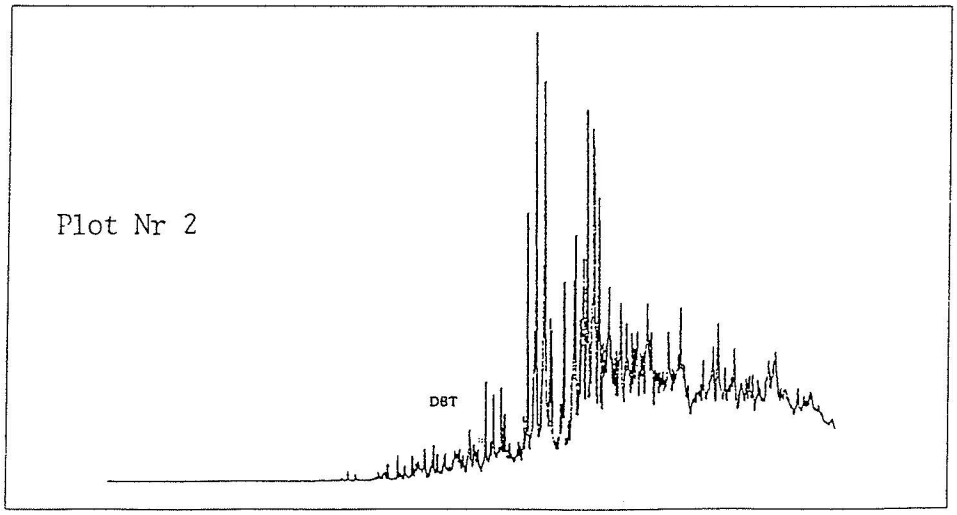
ANNEX 7
GC of sulfur containing aromatic fractions





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