Analysis of light hydrocarbons in marine sediments by headspace technique: Optimization using design of experiments

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Abstract:

Headspace technique is a common method to analyze light hydrocarbons in deep marine sediments. The use of an automatic headspace sampler instead of a manual injection is the guarantee for a better repeatability and furthermore it is less time consuming. But to increase the sensitivity and therefore, to achieve a better detection limit, it is necessary to optimize the automatic headspace sampler parameters. The theory of design of experiments was applied here by studying them. As a response, methane, which is an important gas in marine sediments, was chosen for its short analysis time. Regarding the parameters for automatic headspace sampler, eight variables were selected and then, a screening of them was carried out with a fractional factorial design to determine the influential factors. Finally, optimization was conducted with four factors: Sample Loop Fill time (t_{slf}), Oven temperature (T°_o), Vial Pressurization time (t_{vp}) and Vial pressure (P_v). They were modeled with a Doehlert experimental design. Then, the model was validated by a conventional statistical test (analysis of variance) and the optimum has been found and checked by three experiments. Results on light hydrocarbons measured in sediments from the Congo–Angola Basin are given, as an example.

Keywords: Sediments; Gas; Headspace technique; Design of experiments

1. Introduction

Gas data are very important to understand fluid circulation along mid-oceanic ridges [1,2,3] and on continental margins [4,5]. In marine sediments, the light hydrocarbon signature enables the identification and study of different processes involved in the production and control of either gas generation or consumption. For example, methane discharges from sediments may be linked to the decomposition of natural gas hydrate [6], which is known to be a potential reserve of energy on continental margins. In the other hand, the C1/C2 ratio and carbon isotopic measurements are generally used to know the origin of hydrocarbons and discriminate between biogenic and thermogenic gas [7,8].

Generally two methods are used to analyze light hydrocarbons in marine sediments and seawater: the "purge and trap" technique [9,10] and the "headspace" technique [11,12]. In the "purge and trap" technique, dissolved gases may be completely extracted from the sediments or seawater and concentrated at – 80°C inside two 1/8 inches O.D. stainless steel traps containing activated alumina for trapping the ethane and propane and activated charcoal for the methane. In a second step, by increasing the temperature, light hydrocarbons are desorbed from the traps and injected into a gas chromatograph for quantitative analysis.

Headspace method consists on putting a seawater volume or a fraction of sediment in a sealed vial and keeping it in equilibrium with a gaseous phase (Fig. 1). Using a gas-tight syringe or a headspace sampler, an aliquot of this gas phase is injected into a gas chromatographic system for separation and quantification of each component. The sensitivity can be improved by modifying the aqueous sample matrix.

The purpose of this study is to optimize the "headspace" method in order to quantify light hydrocarbons in marine sediments using an automatic headspace sampler.

2. Material and methods

2.1. Apparatus

A HP 7694 Headspace Sampler has been used with an automated method and a six port valve; it is able to run up to 44 samples consecutively without operator's attention (Fig. 2). The vial containing sediment or seawater, is automatically heated during a period of time defined by the operator (Vial Equilibration Time). Meanwhile, that vial can be stirred (shaking step) as well. Next, the sampling step is performed by perforation of the vial with a needle. After the pressurization of vial (Vial pressure), the opening of the vent valve leads to the filling of 1ml sample loop by the gas (sample loop fill time). Both pressured and vent valves are closed during the time assigned to the loop equilibration. Finally, the sample is injected into the gas chromatograph: switching of the six-port valve to make the loop, still filled with sample, in connection with the carrier gas flow (helium).

A heated transfer line ensures the connection between the HP 7694 headspace and the HP 5890 II gas chromatograph. This latter is equipped with two detectors in series: a thermal conductibility detector for the detection of permanent gases and a flame ionization detector for hydrocarbons. All data acquisition have been done with chemstation software. The analytical column is a megapore Poraplot Q from Chrompack (50m length, 0.53mm i.d.). For calibration, standards CH4 (10 ± 0.2 ppmv, 102 ± 2 ppmv and 10800 ± 200 ; confidence level 95%) and standard C2-C3(100 ± 5ppmv ethane and propane in helium) have been used at appropriate pressure and temperature.

Preparation of samples

As previously mentioned, methane, suitable here for its short analysis time and principal component in sediments, was selected. To simplify the preparation of samples, a standard seawater (salinity 35°/∘∘ or chlorinity 19.374) was used for all experiments.

Regarding experimental design, similar samples (equal methane concentration) were needed. For this reason, two methods were tested to solve this sensitive point:

Procedure 1:
An aliquot of 250 ml of standard seawater is placed into a glass bottle (volume 500ml) with a headspace made with a calibrated methane/helium mixing. The glass bottle was shaked by hand vigorously. After 12 hours at ambient temperature, an aliquot of liquid phase is transferred in five headspace vials (10 ml in each vial). After clamping with silicone septa and aluminum seal, methane in the gas phase was analyzed by the Headspace sampler coupled with a HP 5890 gas chromatograph.

Procedure 2:
An aliquot of 250 ml of standard seawater is flushed by methane until saturation. Then, a fraction of this solution is transferred in five vials (500 µl in each vial) and completed by 10 ml of degassed standard seawater before sealing. The methane in the gas phase in those five vials was analyzed by headspace sampler coupled with a Gas chromatograph HP 5890. The results obtained with the second procedure show a better repeatability (%RSD: 3 % and 6 % for procedure 1), moreover being also easier, it was used.

3. Results and discussion

3.1. Screening design

According to the number of factors, in order to limit the number of runs and to take into account the major effects, a screening study is necessary. Consequently, a first step of screening was conducted using a fractional factorial design. The fractional factorial design with eight factors (table 1) was a 2^{8-4} design involving a minimum of sixteen experiments. Resolution IV has been chosen to get the main effects free of two factors interactions with the following independent generators: I=2345=1346=1237=1248. The factors selected were: P_v Vial Pressure, S_h Shaking, $T°_o$ Oven temperature, t_{ve} Vial Equilibration time, t_{vp} Vial Pressurization time, t_{slf} Sample Loop Fill time, t_{le} Loop Equilibration time, T_l Loop temperature. Experimental domain for each variable was defined with an HP 7694 operating and service manual and preliminaries runs. All the calculations were carried out using NEMRODW software [13]. Table 2 gives the screening design with methane response (quantity of methane in nmol injected by headspace sampler and detected by flame ionization detector). The design was duplicated and three center point experiments corresponding to a central value only for the quantitative factors were carried out. Qualitative factor (shaking) was set to "Low", which correspond to an intermediate level between "Off" and "High". The objective was to check whether there was a non-linear relationship between the variables and the response.

We can observe in table 2 that the mean value $\bar{y}$ is different from the mean value of centre points $\bar{y_m}$. That means that the model is not linear and underlines at least one or several quadratic effect. Fig. 3 shows the different effects of variables P_v to T_l on methane response. Influence of t_{ve} and t_{le} is not significant. Main effects are Pv and t_{vp}. Excepted for S_h et t_{le}, the variables have a negative effect: the response decreased as the factor changes from its low level to its high level.

3.2. Optimization

choice of factors

With the fractionnal factorial design $2^{(8-4)}$, the main effects have been estimated and their influence evaluated. For an optimisation purpose, criteria of choice were as follows:
- Variables which had no significant effect were discarded: t_{ve} (Vial Equilibration time) and t_{le} (Loop Equilibration time). t_{ve} is fixed to 30 mn and t_{le} to 1 mn compatible with recommended time by literature [14,15].

- Variables which had the more significant effect were selected: t_{slf} (Sample Loop Fill time), P_v (Vial pressure) and t_{slf} (Sample Loop Fill time) had a total contribution of 67%.
- The variable "Shaking" is qualitative and its effect was significant: Three choices are possible: no (-1), Low, High (+1). Its influence is positive, therefore its level should be set to +1. Its level has been voluntarily set to −1 (Low) to avoid contamination of septum that were sometimes observed in "High " shaking mode.
-The variable T_l (Loop temperature) was disregard because it is generally recommended to work at a temperature equal or higher than the oven temperature. It was fixed to 80°C.
-Finally the $T°_o$ (Oven temperature) was selected: its effect is significant. This parameter is also of particular interest as different values are proposed in the literature [4,12,15].

choice of model and matrix

Because of the none-linearity of the model, a polynomial to degree two with a Doehlert matrix [16] was postulated to describe the phenomenon evolution.
With four factors X1, X2, X3 and X4 the quadratic model is expressed such as:

$$\hat{y} = b_0 + b_1 * X_1 + b_2 * X_2 + b_3 * X_3 + b_4 * X_4 + b_{11} * X_1^2 + b_{22} * X_2^2 + b_{33} * X_3^2 + b_{44} * X_4^2$$

$$+ b_{12} * (X_1*X_2) + b_{13} * (X_1*X_3) + b_{23} * (X_2*X_3) + b_{14} * (X_1*X_4) + b_{24} * (X_2*X_4) + b_{34} *$$

$$(X_3*X_4)$$

With a Doehlert design, often used in analytical chemistry [17], the study can be immediately conducted with a quadratic model without any step of a factorial design. Another advantage is its potential for sequentially [18], where experiments can be re-used when the boundaries have not been well chosen at first. It is also possible to re-use the experiments and add other factors: these additional experiments must be set to level 0 (coded variable) in the original design. With the Doehlert matrix, the number of level is not the same for all variables. This property has been used to assign the factors as shown in table 3. The Doehlert matrix is given in table 4: six centre points have been done in order to have the best estimation of predicted value by the model on the whole experimental domain by decreasing the variance function of the calculated response. A part of the design was duplicated to have an estimation of experimental error not only at center but also in different point of the explored domain.

Calculation of model

Coefficients of the model were calculated using software Nemrodw (table 5) without taking account experiment 12 due to high residual. The adjusted R^2 was well within acceptable limits of $R^2 \geq 0.8$ [19] which revealed that the experimental data well fitted the second-order polynomial equation. According the student test (t exp), we can observe that the choice of a quadratic model was justified: the coefficients b11 ($X1^2$) and b33 ($X3^2$) are statistically significant at 95% confidence level. To estimate the quality of the model and validate it, analysis of the variance and the residual values (difference between the calculated and the experimental result) were examined. According to the residual (Figure 4), the choice of model was appropriate: a systematic behavior was not observed in the plot, for example, an increase in residual suggesting the necessity to transform the response. The $\chi 2$ test shows that data are normally distributed and that is also confirmed by normal probability plot. In fact, from this latter, we can observe points correctly aligned.
A complete analysis of variance (Table 6) consists in two Fisher tests. A first test compares regression variance with residual variance named here ANOVA 1 and a second test compares lack of fit variance with pure error variance named here ANOVA2 when there are replicates among the experiments. In our case, with a risk of 5%, the probability for ANOVA 1 is smaller than 5% and while it is bigger than 5% pour ANOVA 2. These two conditions are sufficient to consider that the model is valid.

Optimum research

The theoretical optimum was found using tool "desirability" available with the software.

This tool involves transformation of each predicted response, ŷ, to a dimensionless partial desirability function, d_i, which includes the researcher's priorities and desires when building the optimization procedure. One or two-sided functions are used, depending on whether the response has to be maximized or minimized, or has an allotted target value. If the response i has or is wanted to be maximized the quantity d_i is defined as:

$$d_i = \left(\frac{\hat{y}-A}{B-A}\right)^{wi}, \quad A \leq y \leq B \quad (3)$$

$d_i = 1, \quad \hat{y} > B$
$d_i = 0, \quad \hat{y} < A$

Likewise, d_i can be defined if the response has to be minimized or if there is a target value for the response. In Eq. (3), A and B are the lowest and the highest values, respectively, obtained for the response i, and wi is the weight. d_i ranges between 0, corresponding to a completely undesired response, and 1, corresponding to a fully desired response. In both cases, d_i will vary non-linearly while approaching the desired value. But with a weight of 1, d_i varies linearly. In this work we chose to maximize the response and to use a weight equal to 1.

Table 7 gives the parameters for which the response is optimal and fig. 5 shows the contour plot around optimal response. The optimal response calculated is 12.1 ± 0.4 (confidence level 95%), so that means an interval [11.7; 12.5]. Three check points have been done in optimal conditions and they gave following values: 11.65; 12.89 and 12.52 from which a mean value of 12.35 is calculated. This mean value being in the interval [11.7; 12.5], the modeling around the optimum is suitable.

Optimal conditions for light hydrocarbons other than methane

The solubility of ethane and propane (for example solubility at 20 °C: 34 ml/l for methane, 45 for ethane and 32 for propane) and their behavior are similar when the salinity increase [20]. Accordingly, optimal conditions will be also applied to quantify other light hydrocarbons in sediments.

4. Applications

Using a Headspace Sampler-Gas chromatograph system, sediments from Congo-Angola Basin and Nigerian basin were analyzed. These sediment samples were collected by using standard gravity coring techniques during the French Zairov-Leg 2 and Neris II cruise on the R/V L'Atalante in 2001 and 2004. Upon retrieval, the sediment contained in a plastic liner was removed from the core barrel and sectioned at specific depths. Then an aliquot of 10 ml of sediment was sampled with a syringe and placed into a sealed vial of 20 ml in capacity. A solution of NaCl-HgCl2 was added to inhibit microbial activity and modify the matrix. All samples were analyzed on shore by the previously described method.

Fig. 6 shows the distribution of methane concentration versus depth in a core from the Congo-Angola basin. Geological settings of this zone have been described in detail by Ondreas *et al.* [21] and Gay *et al.* [22]. This area is marked by the presence of a pockmark (800-m wide, 15-20 m deep) named Regab, located in a deep abyssal setting at a water depth of 3160m. In this core, pieces of gas hydrate [6] were discovered after opening the core on board. This profile shows a methane anomaly corresponding to the depth where gas hydrate specimens were discovered (photo 1) and it indicates some decomposition of gas hydrate with release of methane. Ethane was also measured. The high C1/C2 ratio (≅2000) related to the isotopic ratio of carbon and Deuterium of CH4 in the gas hydrate (δ^{13}C of −69.3 ‰ (PDB) and a δD of −199 ‰ (V-SMOW)) indicates microbial CO2 reduction origin of CH4 in the sediments of this area. It could be noted also that the existence of these hydrocarbon emissions and its accumulation is also very important for biological and chemosynthetic

communities [23].

Conclusion

In the present paper, the main objective was to optimize the automatic headspace sampler parameters in order to quantify light hydrocarbons present as traces in marine sediments and in seawater. A methodological approach was investigated by means of the theory of design of experiments. This study was conducted in two steps: the first step was to detect influential factors with a screening design (fractional factorial design 2^{8-4}) and the second step was the modeling and find an optimal response with a Doehlert design. With the screening design, it was possible to compare the different effects on the methane response, to identify the major effects (vial pressure and vial pressurization time) and to set their sign. With a Doehlert design, the optimum was determined by response surface methodology. This whole strategy enabled to improve the limit detection (ratio: 2) according the defaults parameters of Headspace sampler. Application was made on sediments issued from a mud volcanoe from the Congo-Angola basin to study the hydrocarbon emission of this area.

Acknowledgements

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Tables

Natural variable	Coded variable	Level (-1)	Level (+1)
Vial pressure (bar)	P_v	0.5	1.5
Shaking	S_h	Off	High
Oven temperature (°C)	$T°_o$	40	80
Vial equilibration time (mn)	t_{ve}	0	30
Vial pressurization time (mn)	t_{vp}	0.01	1
Sample loop fill time (mn)	t_{slf}	0.02	1
Loop equilibration time (mn)	t_{le}	0.01	1
Loop temperature (°C)	T_l	60	100

Table 1: Description of experimental variables in the screening design

N°Exp	P_v	S_h	$T°_o$	t_{ve}	t_{vp}	t_{slf}	t_{le}	T_l	Y1
1	0.5	off	40	0	0.01	0.02	0.01	60	18,57
2	0.5	off	40	0	0.01	0.02	0.01	60	20,01
3	1.5	off	40	0	0.01	1	1	100	18,93
4	1.5	off	40	0	0.01	1	1	100	19,41
5	0.5	High	40	0	1	0.02	1	100	17,48
6	0.5	High	40	0	1	0.02	1	100	17,02
7	1.5	High	40	0	1	1	0.01	60	9,56
8	1.5	High	40	0	1	1	0.01	60	10,18
9	0.5	off	80	0	1	1	1	60	15,15
10	0.5	off	80	0	1	1	1	60	15,43
11	1.5	off	80	0	1	0.02	0.01	100	12,45
12	1.5	off	80	0	1	0.02	0.01	100	11,27
13	0.5	High	80	0	0.01	1	0.01	100	16,59
14	0.5	High	80	0	0.01	1	0.01	100	16,99
15	1.5	High	80	0	0.01	0.02	1	60	19,76
16	1.5	High	80	0	0.01	0.02	1	60	19,06
17	0.5	off	40	30	1	1	0.01	100	13,28
18	0.5	off	40	30	1	1	0.01	100	14,28
19	1.5	off	40	30	1	0.02	1	60	11,20
20	1.5	off	40	30	1	0.02	1	60	11,93
21	0.5	High	40	30	0.01	1	1	60	23,18
22	0.5	High	40	30	0.01	1	1	60	21,48
23	1.5	High	40	30	0.01	0.02	0.01	100	18,97
24	1.5	High	40	30	0.01	0.02	0.01	100	19,91
25	0.5	off	80	30	0.01	0.02	1	100	16,34
26	0.5	off	80	30	0.01	0.02	1	100	17,65
27	1.5	off	80	30	0.01	1	0.01	60	16,46
28	1.5	off	80	30	0.01	1	0.01	60	17,28
29	0.5	High	80	30	1	0.02	0.01	60	18,88
30	0.5	High	80	30	1	0.02	0.01	60	20,24
31	1.5	High	80	30	1	1	1	100	7,48
32	1.5	High	80	30	1	1	1	100	7,13
								$\overline{y}$	16,05
Centre	1	Low	60	15	0.5	0.51	0.5	80	11,64
Centre	1	Low	60	15	0.5	0.51	0.5	80	11,11
Centre	1	Low	60	15	0.5	0.51	0.5	80	11,58
								$\overline{y_m}$	11,44

Table 2: Screening design with methane response Y1 (nmol)

	Variable	Unit	Center	Step
Sample Loop Fill time	X1: tslf	mn	0.510	0.490
Oven temperature	X2: T°o	°C	60	20
Vial Pressurization time	X3: tvp	mn	0.505	0.495
Vial pressure	X4: Pv	bar	1.00	0.50

Table 3: Specification of variables and the experimental domain in Doehlert design (step is in natural variable and correspond to a range from level 0 to +1 in coded variable)

N°Exp	X1	X2	X3	X4	Y (methane)
1	1.0000	0.0000	-0.0101	0.0000	9.06
2	-1.0000	0.0000	-0.0101	0.0000	10.02
3	-1.0000	0.0000	-0.0101	0.0000	11.24
4	0.4898	0.8500	-0.0101	0.0000	7.46
5	-0.5102	-0.8500	-0.0101	0.0000	10.05
6	0.4898	-0.8500	-0.0101	0.0000	8.16

7	-0.5102	0.8500	-0.0101	0.0000	6.81
8	0.4898	0.3000	0.8182	0.0000	6.82
9	0.4898	0.3000	0.8182	0.0000	6.44
10	-0.5102	-0.3000	-0.8182	0.0000	12.36
11	0.4898	-0.3000	-0.8182	0.0000	10.05
12	0.0000	0.6000	-0.8182	0.0000	8.09
13	-0.5102	0.3000	0.8182	0.0000	7.02
14	0.0000	-0.6000	0.8182	0.0000	8.33
15	0.0000	-0.6000	0.8182	0.0000	8.88
16	0.4898	0.3000	0.2121	0.8000	5.05
17	-0.5102	-0.3000	-0.2121	-0.8000	10.55
18	0.4898	-0.3000	-0.2121	-0.8000	10.21
19	0.4898	-0.3000	-0.2121	-0.8000	9.39
20	0.0000	0.6000	-0.2121	-0.8000	9.57
21	0.0000	0.0000	0.6162	-0.8000	8.90
22	-0.5102	0.3000	0.2121	0.8000	5.03
23	-0.5102	0.3000	0.2121	0.8000	4.48
24	0.0000	-0.6000	0.2121	0.8000	7.26
25	0.0000	0.0000	-0.6162	0.8000	7.93
26	0.0000	0.0000	-0.0101	0.0000	7.57
27	0.0000	0.0000	-0.0101	0.0000	7.41
28	0.0000	0.0000	-0.0101	0.0000	7.36
29	0.0000	0.0000	-0.0101	0.0000	8.36
30	0.0000	0.0000	-0.0101	0.0000	7.87
31	0.0000	0.0000	-0.0101	0.0000	7.43

X1: Sample Loop Fill time (t_{slf}),   X2: Oven temperature ($T°_o$),
X3: Vial Pressurization time(t_{vp}),   X4: Vial pressure (P_v).

Table 4: Doehlert matrix (four factors) and methane response measured (nmol)

Standard error of response	0.469
R^2	0.966
R^2A	0.934
R^2 pred	0.846
PRESS	14.950
Degrees of freedom	15

Name	Coefficient	Standard error	exp.t	Signif. %
b0	7.644	0.192	39.88	< 0.01 ***
b1	-0.702	0.189	-3.72	0.205 **
b2	-1.337	0.228	-5.86	< 0.01 ***
b3	-2.196	0.237	-9.25	< 0.01 ***
b4	-2.219	0.188	-11.81	< 0.01 ***
b11	2.201	0.339	6.49	< 0.01 ***
b22	-0.144	0.393	-0.37	72.0
b33	1.775	0.377	4.71	0.0278 ***
b44	-0.240	0.315	-0.76	45.9
b12	1.491	0.552	2.70	1.64 *
b13	0.508	0.568	0.90	38.5
b23	-1.674	0.655	-2.55	2.20 *
b14	-0.039	0.549	-0.07	94.4
b24	-1.602	0.581	-2.76	1.47 *
b34	-0.504	0.606	-0.83	41.9

Table 5: Statistical data and coefficients of response model: y (methane) =f(X1, X2,X3,X4)

Source of variation	Sum of squared	Degrees of freedom	Mean square	Ratio	Signif.	
Regression	93.9660	14	6.7119 (Vreg)	30.4574	< 0.01 ***	ANOVA 1
Residual	3.3055	15	0.2204 (Vres)			
Lack of fit	1.1081	5	0.2216 (Vlof)	1.0085	46.1	ANOVA2
Pure Error	2.1974	10	0.2197 (Vpe)			
Total	97.2715	29				

Table 6: Multiple regression analysis: ANOVA 1 and 2 (95% confidence level)

Variable	Value	Factor	Value
X1	-0.777660	tslf	0.129
X2	-0.315307	T°o	54
X3	-0.539346	tvp	0.238
X4	-0.167682	Pv	0.92

Table 7: Values of factors X1, X2, X3, X4 corresponding to optimal response calculated by the tool desirability (NEMRODW software)

Figures
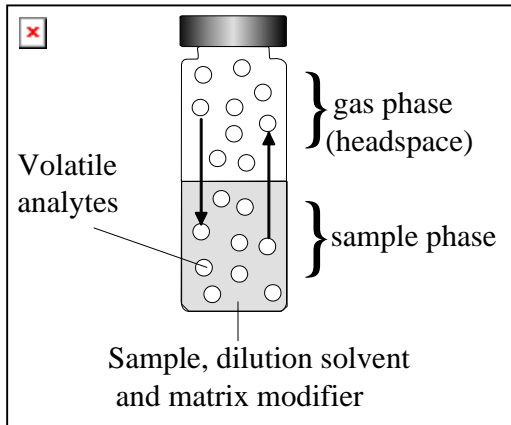
Fig. 1: The two phases of a Headspace vial
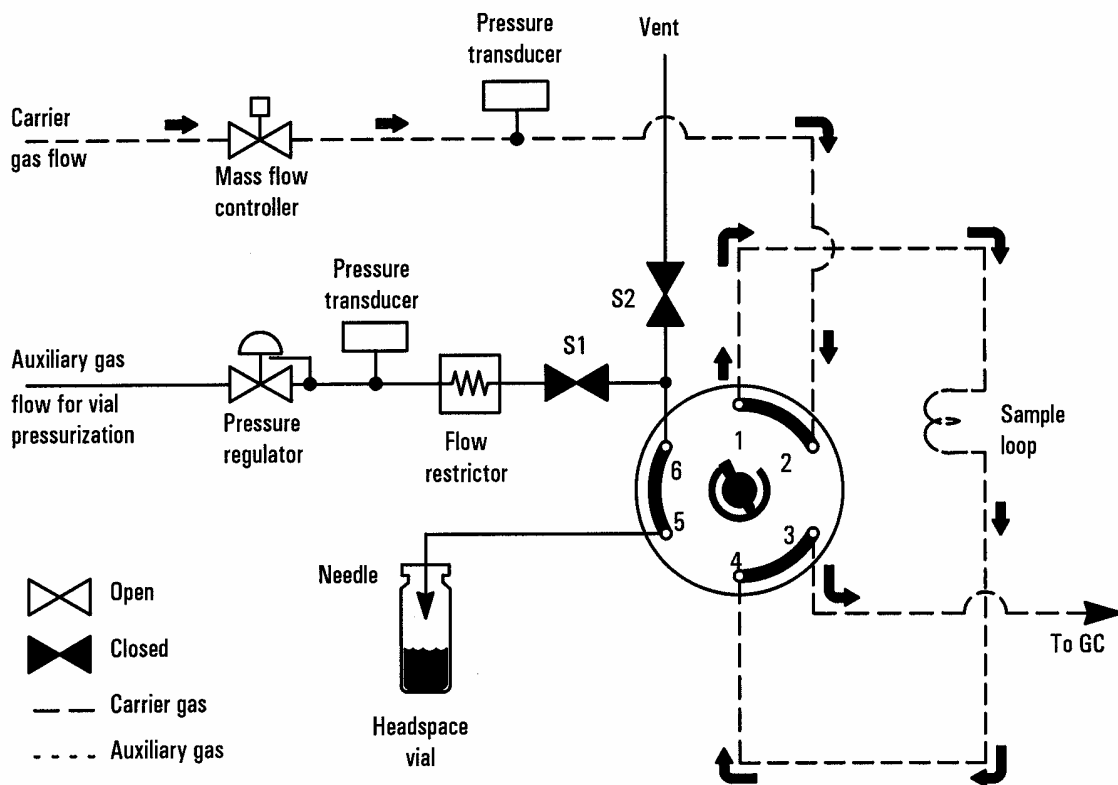
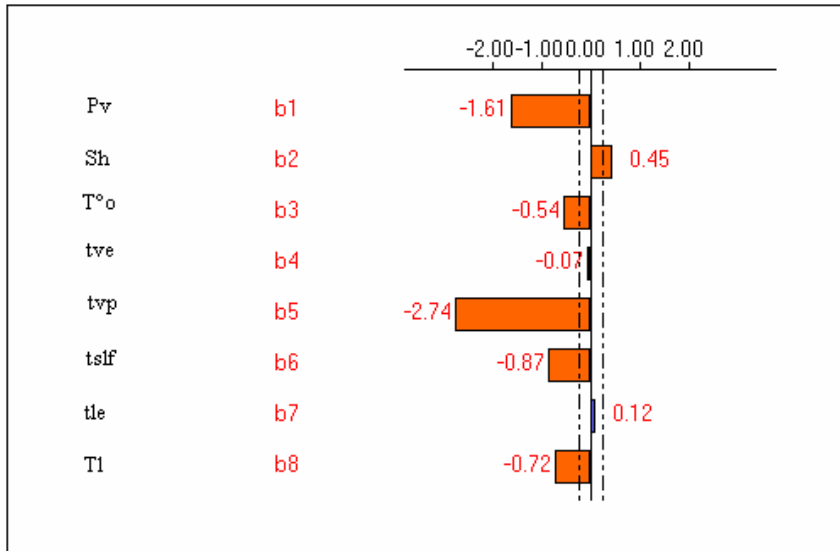Fig. 2: Configuration of Headspace Sampler (injection step into gas chromatograph)

Fig. 3: Signification of the studied factors: graphic of effects P_v to T_l on methane response
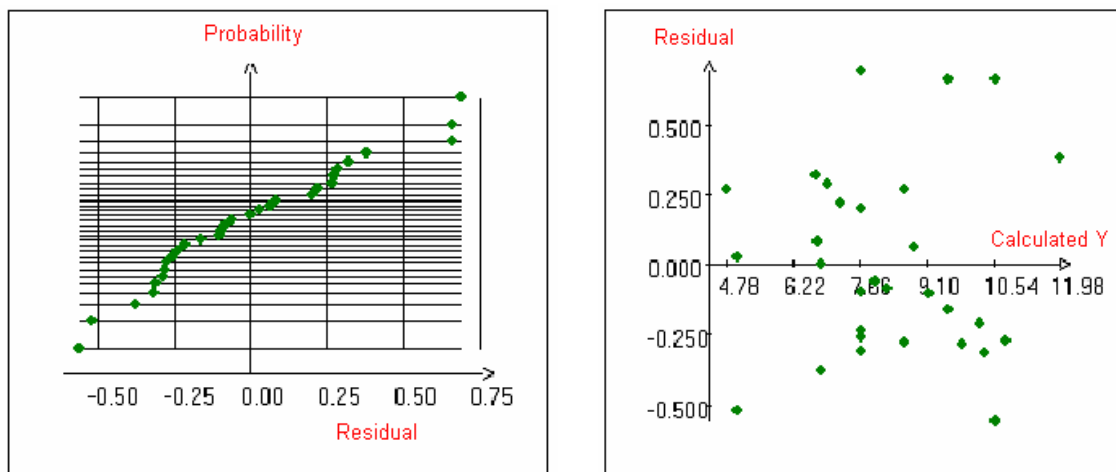

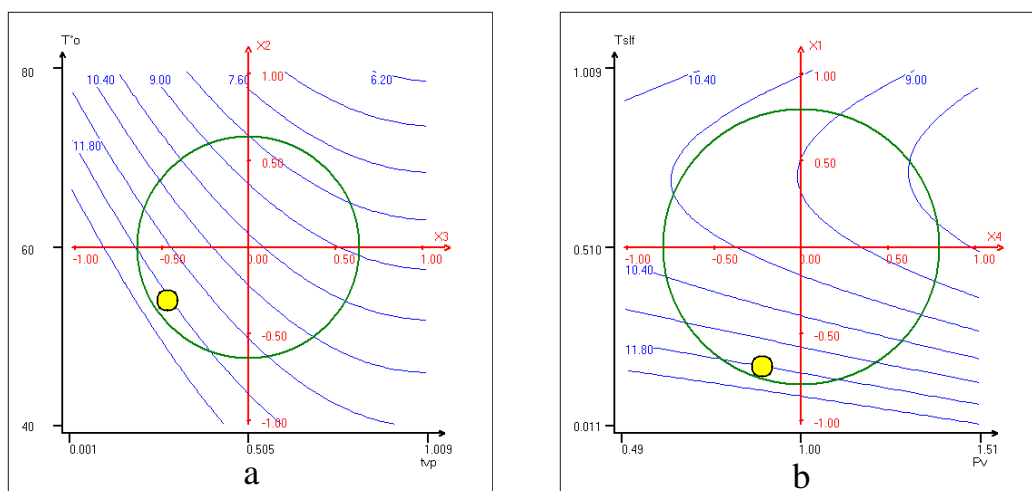Fig. 4: Overview of residual: Normal probability and residual plot

a

b

Fig. 5 : Contour plots with optimum ○ : values of fixed factors correspond to optimum response (plot a :tslf= 0.129 mn and Pv= 0.92bar   plot b: T°O= 54°C and tvp= 0.238mn)
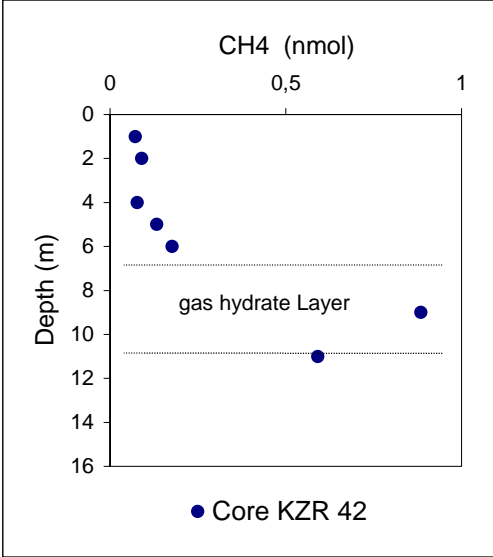

Fig. 6 : Methane versus depth in a sediment core from a gas hydrate pockmark in the Congo-Angola Basin

Photo 1 : Photography of solid sample of gas hydrate 10 cm diameter