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Quantitative Analysis of *n*-paraffins in Heavy Oil (Grade C) added to Media for Oil-decomposing Bacteria by a Simplified Internal Standard Method

By

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Oil pollution in coastal waters becomes serious year after year. And sometimes local heavy oil pollution, which is usually caused by accident, damages inshore fishery greatly. It is a well-known fact that the self-purification in the oil-polluted areas depends mainly on the bacterial activity. In the area polluted by the Mizushima Oil Refinery Accident[1], many oil-decomposing bacteria could be isolated from bottom sediments as well as from sea water. Among many components of oil, *n*-paraffins are a group of components most easily decomposed by bacteria[2,3] and the mechanism of bacterial decomposition has been clarified in detail[4,5], although this group is not the major component but the minor one in heavy oil (grade C). Gas chromatography is effectively applied to the quantitative analysis of *n*-paraffins, but it is necessary to separate *n*-paraffin fraction preliminarily from heavy oil and it consumes much labor. If the oil sample is analysed without the preliminary separation, the analysis becomes very complicated. In the field survey of examining oil pollution and self-purification after an oil accident, a simpler method on the determination of oil components is favorable, for it is necessary to analyse many samples derived from the same original oil within a short time with limited members. On the other hand, it is necessary to examine preliminarily the variation of the oil components caused by the treatment indispensable to the bacteriological work, i.e. by the sterilization in the presence of medium. Based on these facts, therefore, the ordinary method of internal standard method (i.e. standard adding method)[6] was simplified, and a concise method was carried out of quantitative analysis of *n*-paraffins of heavy oil (grade C) in media suitable to the laboratory work after the bacteriological field survey. By using this method, the components of heavy oil in media could be quantitatively analysed without the preliminary separation of *n*-paraffin fraction from the oil sample, and could yield the results of the same level of accuracy with those of the orthodox method.

The present report aimed, accordingly, at the following two points as a preliminary step to examine the activity of oil-decomposing bacteria separated from the oil-polluted area, and its results will be presented in the succeeding report: one is to show the base of the simplified method of quantitative analysis proposed here and employed in a series of these experiments, and the other is to show the outline of the variation of the oil components caused by autoclaving in the presence of the different media.

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Material and Method

The material of the present report was the desulfurized heavy oil (grade C) sampled from oil tank No.271 in the Mizushima Oil Refinery, which was the same heavy oil spilt from oil tank No.270 into Bisan Seto in the oil accident. Its commercial properties are shown in Table 1.

A 110—150 mg heavy oil was added to 50 m*l* of medium A or B[7] in a 500 m*l* culture flask in duplicate, and these flasks were stoppered with cotton. One in duplicate was used without treatment, but the other was autoclaved at 1.1 kg/cm^2 for 15 minutes and used in the present work. The hydrocarbons of heavy oil were separated, as shown in Fig. 1, by silicic acid column chromatography[8,9] from the *n*-hexane extractive fraction. The mg weights of *n*-hexane extractive fraction and of hydrocarbon fraction (F_2 and F_3 in Fig. 1, respectively) per 100 mg of heavy oil (F_1 in Fig. 1) were estimated.

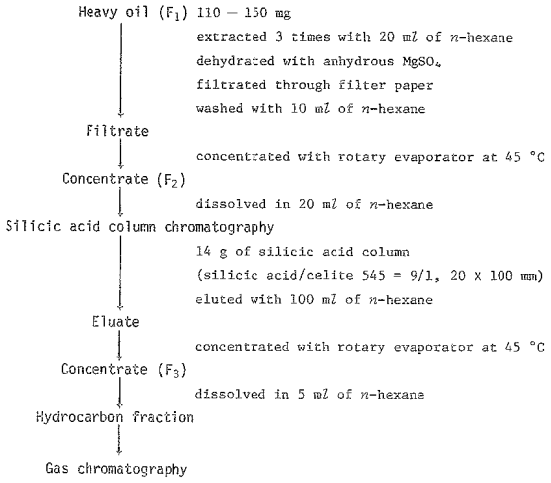

Fig. 1. Preliminary preparation of hydrocarbons from heavy oil for gas chromatography.

Table 1. Commercial properties of heavy oil (grade C) used in the present work.

Specific gravity (15/4 °C)	0.9214
Flash point (°C)	162
Kinematic viscosity (at 50°C, cm^2/sec)	78.61
Pour point (°C)	10
Sulfur content (wt %)	0.92
Carbon residue (wt %)	4.75

Note : Properties of heavy oil were analysed by the Mizushima Oil Refinery.

The components of *n*-paraffins in the above-mentioned hydrocarbon fraction were qualitatively and quantitatively analysed by a Hitachi 163 Gas Chromatograph under the conditions shown in Table 2.

Table 2. Analytical conditions of gas chromatography.

Apparatus	Hitachi Gas Chromatograph Model 163
Column	1 % Dexsil 300 GC on Chromosorb WHP (80-100 mesh), Stainless steel tubing (3 mm × 2 m)
Detector	Hydrogen flame ionization
Temperature	Column 80 °C to 320°C, Program rate 5 °C/min, Injection 350 °C
Carrier gas	Nitrogen
Flow rate	Carrier gas 40 m*l*/min, Hydrogen gas 35 m*l*/min, Air 550 m*l*/min
Sample size	1 or 2 μl

The components of *n*-paraffins in the hydrocarbon fraction were identified by comparing their retention time with that of the authentic *n*-paraffins (C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, C_{34}, C_{36}, C_{38} and C_{40} in chain length) added to the hydrocarbon fraction.

The amount of each *n*-paraffin was determined by the below-mentioned simplified method and was compared with that estimated by the orthodox way of the standard adding method. In the latter case, the 15 standards of the authentic *n*-paraffins of even carbon numbers were used. The simplified method used in the present work is one of the modifications of the standard adding method, and needs only one standard substance (*n*-dodecane) and an equation showing the correction for the different sensitivity according to the length of carbon chain, and it is based on the following hypothesis: if it is possible to estimate the relative sensitivity of respective *n*-paraffins, there is no need to use many substances as standards to be added.

The following equations show the possibility of realizing the above-mentioned hypothesis:

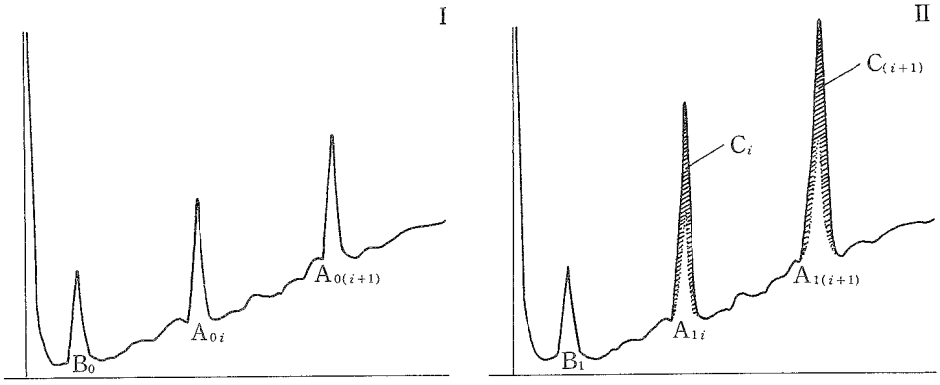

Fig. 2. Model of a pair of gas chromatograms of *n*-paraffins in hydrocarbon fraction of heavy oil through the standard adding method.

Note: I ..... Gas chromatogram of hydrocarbon fraction added with a standard substance (*n*-dodecane)
II..... Gas chromatogram of hydrocarbon fraction added with the authentic *n*-paraffins
B_0 ..... Peak area of standard substance (*n*-dodecane, e.g. 0.1 μg) in I
B_1 ..... Peak area of standard substance (*n*-dodecane, e.g. 0.1 μg) in II
A_{0i} .... Peak area of *n*-paraffin *i* (chain length) in I
A_{1i} .... Peak area of *n*-paraffin *i* (chain length) in II, $A_{0i} + C_i$
C_i ..... Increased peak area of *n*-paraffin *i* by adding the authentic substance (e.g. 0.1 μg) in II

Employing B_0 and B_1 in Fig. 2 as standard substances, weight of C_i, ΔW_{ai}, can be expressed by

$$\Delta W_{ai} = W_{0i}\left(\frac{A_{1i}}{A_{0i}} - 1\right) \quad \cdots\cdots\cdots\cdots (1),$$

where W_{0i}: Weight of A_{0i} in I of Fig. 2
ΔW_{ai}: Weight of C_i (the authentic *n*-paraffin *i*, e.g. 0.1 μg) in II of Fig. 2

W_{ai}: Weight of A_{1i} ($A_{0i}+C_i$) in II of Fig. 2, being equal to $W_{0i} + \Delta W_{ai}$
A_{0i}: A_{0i}/B_0
A_{1i}: A_{1i}/B_1.

And therefore, W_{0i} can be expressed by

$$W_{0i} = \frac{\Delta W_{ai}}{(\frac{A_{1i}}{A_{0i}} - 1)} \quad \text{.....(2).}$$

Based on this equation, the weight of each *n*-paraffin can be determined from peak areas of *n*-dodecane and other respective standard *n*-paraffins in a definite weight added to the hydrocarbon fraction. This is the standard adding method. For the purpose of finding out a method consuming less labor but yielding the results of the same level of accuracy, the possibility of substituting a series of correction coefficients or an equation showing them for adding standard substances was sought in the following way :

Employing the sensitivity of *n*-dodecane as the standard, the relative sensitivity of *n*-paraffin *i*, K_i, can be expressed by

$$K_i = \frac{A_{0i}}{W_{0i}} \times \frac{W_b}{B_0} \quad \text{.....(3),}$$

where W_b: A definite weight of the standard substance (*n*-dodecane, e.g. 0.1 μg) in I of Fig. 2.

By this equation, it is possible to estimate the relative sensitivity of each *n*-paraffin (the sensitivity of *n*-dodecane as the standard). The results of the preliminary experiment showed that the relative sensitivity changed with the chain length of *n*-paraffin keeping a clear linear equation, as shown in Fig. 5. Using the relative sensitivity estimated from this equation, weight of *n*-paraffin *i*, W_{0i}, can be expressed by

$$W_{0i} = W_b \times \frac{A_{0i}}{B_0} \times \frac{1}{k_i} \quad \text{.....(4).}$$

This equation indicates that μg weight of each *n*-paraffin per μl of hydrocarbon fraction prepared from heavy oil can be estimated from peak area of each of them and that of *n*-dodecane in a definite weight. And also the estimated value was used after it was converted into mg per 100 mg of hydrocarbon fraction. The validity of the method based on the equation (4) was tested by comparing the results of the present method with those of the standard adding method used in common which is based on the equation (2). And to examine the necessity of correction for the different sensitivity, the results of the former method before sensitivity correction was compared with those of the latter method.

Results and Discussion

Yields of *n*-hexane extractive fraction and hydrocarbon fracton from heavy oil

The amounts of *n*-hexane extractive fraction and hydrocarbon fraction obtained from heavy oil in media A and B, are shown in Table 3. As shown in this table, the amounts of hydrocarbon fraction (F_3/F_1 and F_3/F_2) and *n*-hexane extractive fraction (F_2/F_1) of all the samples were estimated with accuracy. The results of two-way layout analysis of variance of these values (between media A...and B...and between treatments...autoclaved and unautoclaved...), are shown in Tables 4, 5 and 6.

Table 3. Yields of *n*-hexane extractive fraction and hydrocarbon fraction from heavy oil contained in the different media.

Sample No.	Treatment	Heavy oil (F_1 mg)	*n*-Hexane extractive fraction (F_2 mg)	Hydrocarbon fraction (F_3 mg)	F_2/F_1 (mg/100 mg of F_1)	F_3/F_1 (mg/100 mg of F_1)	F_3/F_2 (mg/100 mg of F_2)
1	Original heavy oil	129.3	113.9	99.2	88.1	76.7	87.1
2	〃	156.6	132.9	110.5	84.9	70.6	83.1
3	〃	115.9	98.4	90.6	84.9	78.2	92.1
4	〃	126.7	105.8	97.5	83.5	77.0	92.2
	Average				85.35±1.69	75.63±3.00	88.63±3.80
5	Heavy oil in unautoclaved medium A	124.7	108.2	95.5	86.8	76.6	88.3
6	〃	110.8	100.0	88.7	96.3	80.0	88.7
7	〃	132.3	119.5	105.1	90.3	79.4	87.9
8	〃	127.9	112.6	96.9	88.0	75.8	86.1
	Average				88.85±1.75	77.95±2.06	87.75±1.15
9	Heavy oil in autoclaved medium A	131.5	107.8	95.6	82.0	72.7	88.7
10	〃	155.2	128.2	113.1	82.6	72.9	88.2
11	〃	113.2	92.1	82.5	81.4	72.9	89.6
12	〃	132.6	110.4	97.3	83.2	73.4	88.1
	Average				82.30±0.77	72.98±0.30	88.65±0.69
13	Heavy oil in unautoclaved medium B	143.8	124.7	110.1	86.7	76.6	88.3
14	〃	122.9	107.4	94.9	87.4	77.2	88.4
15	〃	150.0	134.1	118.1	89.4	78.7	88.1
16	〃	128.9	116.8	104.9	90.6	81.4	89.8
	Average				88.53±1.80	78.48±2.14	88.65±0.78
17	Heavy oil in autoclaved medium B	113.9	98.3	87.2	86.3	76.6	88.7
18	〃	140.5	119.2	105.9	84.8	75.4	88.8
19	〃	120.8	106.9	98.3	88.5	81.4	92.0
20	〃	114.3	96.4	84.3	84.3	73.8	87.4
	Average				85.98±1.88	76.80±3.27	89.23±1.96

Table 4. Two-way layout analysis of variance of the yields of *n*-hexane extractive fraction (F_2 / F_1) from heavy oil contained in media.

Factor	Sum of squares	Degree of freedom	Mean square	F_0
Between media (M)	11.22	1	11.22	4.31
Between treatments (T)	82.81	1	82.81	31.78**
M×T	16.00	1	16.00	6.14*
Residual	31.27	12	2.61	
Sum	141.30	15		

Note : The original data are shown in Table 3.
F_0 = estimated value of SNEDECORE's F, F_{12}^{1} (0.05) = 4.75, F_{12}^{1} (0.01) = 9.33
* significant at 0.05 level
** significant at 0.01 level

Table 5. Two-way layout analysis of variance of the yields of hydrocarbon fraction (F_3 / F_1) from heavy oil contained in media.

Factor	Sum of squares	Degree of freedom	Mean square	F_0
Between media (M)	18.92	1	18.92	3.85
Between treatments (T)	44.22	1	44.22	9.01*
M×T	10.89	1	10.89	2.22
Residual	58.93	12	4.91	
Sum	132.96	15		

Note : The original data are shown in Table 3.
F_0 = estimated value of SNEDECORE's F, F_{12}^{1} (0.05) = 4.75
* significant at 0.05 level

Table 6. Two-way layout analysis of variance of the yields of hydrocarbon fraction (F_3 / F_2) from heavy oil contained in media.

Factor	Sum of squares	Degree of freedom	Mean square	F_0
Between media (M)	2.18	1	2.18	1.40
Between treatments (T)	2.18	1	2.18	1.40
M×T	0.11	1	0.11	0.07
Residual	18.66	12	1.55	
Sum	23.13	15		

Note : The original data are shown in Table 3.
F_0 = estimated value of SNEDECORE's F

Tables 4 and 5 showed that significant between-treatment difference but insignificant between-medium difference were found in both F_2/F_1 and F_3/F_1. As shown in Table 6, no significant between-treatment and between-medium differences could be found in F_3/F_2, because autoclaving caused a decrease in both of F_2 and F_3.

Qualitative analysis of *n*-paraffins in heavy oil

Gas chromatograms of hydrocarbon fraction of heavy oil and of the sample containing the authentic *n*-paraffins (C_{12}, C_{14}, C_{16}, C_{18}, C_{20}, C_{22}, C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, C_{34}, C_{36}, C_{38} and C_{40} in chain length), are shown in Fig. 3. As shown in Fig. 4, the wide range of chain length of *n*-paraffins resulted in a slightly curved relation between chain length and retention time of the authentic *n*-paraffins added to the hydrocarbon fraction. The retention time of long-chain *n*-paraffins, especially that of longer *n*-paraffins than C_{28}, was slightly shorter than that expected from the supposed linear relation. Based on the retention time shown in Fig. 4, *n*-paraffins in hydrocarbon fraction of heavy oil were identified to be in the range from C_{13} to C_{40} or thereabout in chain length.

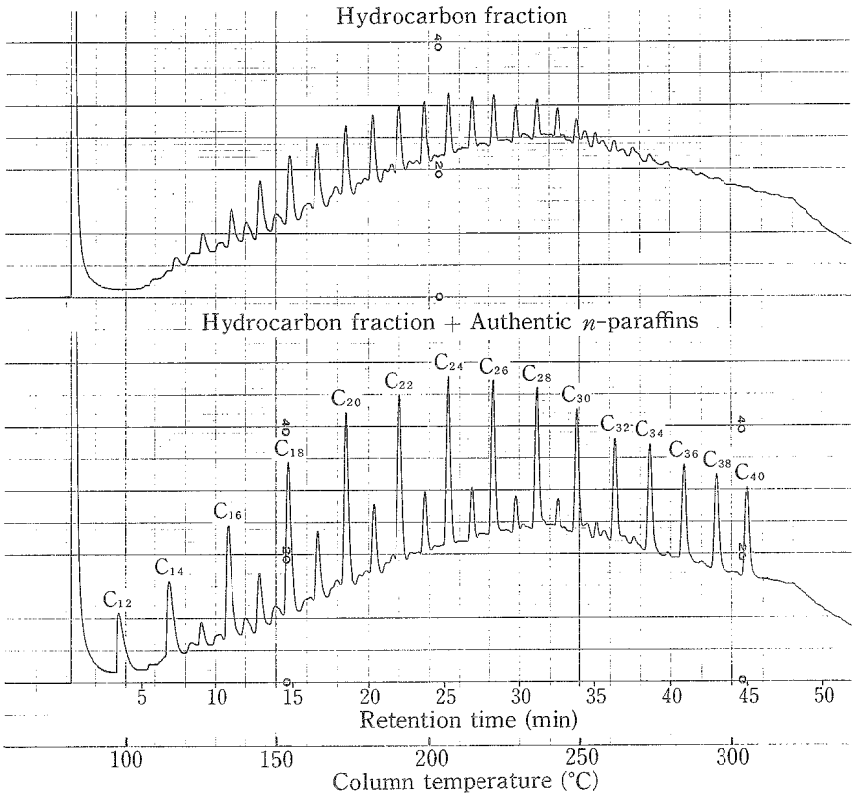

Fig. 3. Gas chromatograms of hydrocarbon fraction in heavy oil with and without the authentic *n*-paraffins.

Note : C_{12}–C_{40} indicate chain length of *n*-paraffins added.

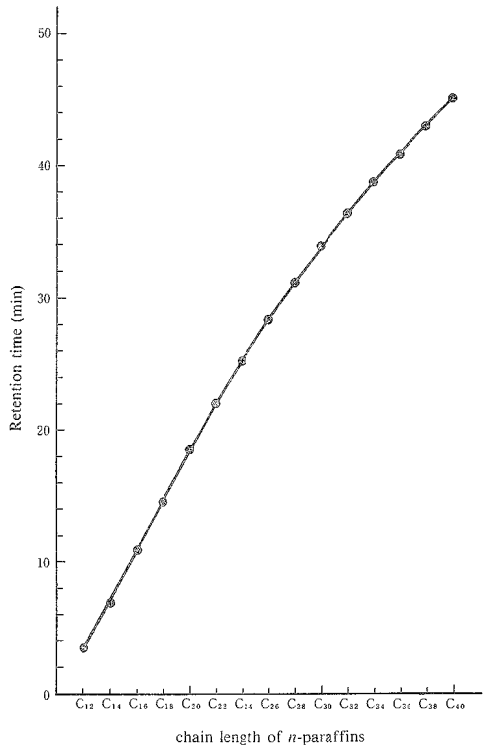

Fig. 4. Relation between chain length and retention time of the authentic *n*-paraffins added to hydrocarbon fraction of heavy oil.

Note : The original data are shown in Fig. 3.

Examination on the accuracy of the method used in the present work

Preceding to the application of the simplified method, it is necessary to estimate the correction coefficients for different sensitivity according to the length of carbon chain as shown in Fig. 2 and in the equation (3). The same hydrocarbon fraction from heavy oil was analysed by the simplified method and by the standard adding method. And the results are shown in Table 7. The correction coefficient of sensitivity for each *n*-paraffin was estimated from these records, and it was found out that there existed a clear linear relation between chain length and relative sensitivity as shown in Fig. 5, which was expressed by $K_i = 1.273 - 0.008\ i$.

Table 7. Relative sensitivity (k_i) for each n-paraffin obtained through a pair of gas chromatograms of hydrocarbon fraction in heavy oil and its correction coefficient ($1/k_i$).

	Chain length of n-paraffins							
	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}
B_0 (mm²)	56.68							
B_1 (mm²)	48.00							
A_{0i} (mm²)		7.56	23.04	41.76	40.00	37.36	29.68	23.52
A_{1i} (mm²)		60.96	74.40	91.08	92.48	82.80	77.84	70.56
W_{0i} ($\mu g/\mu l$)		0.0117	0.0355	0.0635	0.0578	0.0629	0.0477	0.0394
k_i	1.0000	1.1400	1.1450	1.1603	1.2210	1.0591	1.0978	1.0559
$1/k_i$	1.0000	0.8772	0.8734	0.8618	0.8190	0.9442	0.9109	0.9471

	Chain length of n-paraffins						
	C_{28}	C_{30}	C_{32}	C_{34}	C_{36}	C_{38}	C_{40}
B_0 (mm²)							
B_1 (mm²)							
A_{0i} (mm²)	19.32	12.88	5.46	4.68	1.00	1.50	0.50
A_{1i} (mm²)	64.96	56.84	47.60	52.80	50.56	49.92	46.72
W_{0i} ($\mu g/\mu l$)	0.0337	0.0237	0.0108	0.0081	0.0017	0.0026	0.0009
k_i	1.0115	0.9588	0.8919	1.0194	1.0378	1.0179	0.9802
$1/k_i$	0.9886	1.0430	1.1212	0.9810	0.9636	0.9824	1.0202

Note : B_0, B_1, A_{0i}, A_{1i}, W_{0i}, K_i and $1/K_i$ are shown in Fig. 2 and the equations (1)-(4).

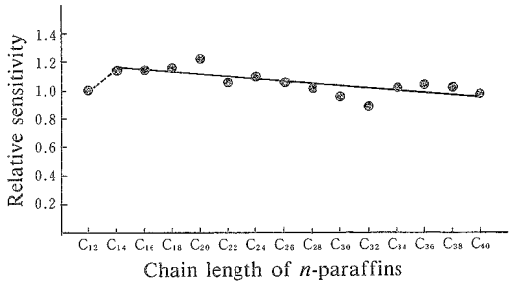

Fig. 5. Correlation between chain length of n-paraffins in hydrocarbon fraction of heavy oil and relative sensitivity of ones in a pair of gas chromatogrms.

Note : The original data are shown in Table 7.
The correlation was expressed by the linear regression equation,
$k_i = 1.273 - 0.008\ i$, which was significant at 0.01 level ($F_0 = 16.02 > F_{12}^{1}(0.01) = 9.33$).
k_i.....relative sensitivity of n-paraffin i to sensitivity of n-dodecane
i.....chain length of n-paraffin

These results opened the possibility of the present method to be applicable to the quantitative analysis of *n*-paraffins in heavy oil with less labor but with the same level of accuracy. Before applying the present method practically, however, it is necessary to test its validity through comparing the results estimated by this method with those estimated by the orthodox method i.e. the standard adding method.

For this purpose, the amounts of *n*-paraffins in the same heavy oil sample were estimated again by the orthodox method based on the equation (2) and by the present method based on the equation (4). In the latter case, the correction coefficients were estimated from the above-mentioned regression equation. And the results are shown in Table 8. The results of the present method before correction for different sensitivity were added to this table, for the purpose of testing whether it would be necessary to make correction or not, because all the correction coefficients took the values nearly equal to 1.0.

Table 8. The comparison of the amounts of *n*-paraffins obtained by the simplified method with those by the standard adding method from the same heavy oil sample.

Method	Chain length of *n*-paraffins						
	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	C_{26}
Standard adding method	0.065	0.196	0.350	0.319	0.347	0.263	0.217
Simplified method	0.066	0.204	0.374	0.364	0.346	0.278	0.224
before sensitivity correction	0.076	0.230	0.406	0.411	0.339	0.277	0.233

Method	Chain length of *n*-paraffins						
	C_{28}	C_{30}	C_{32}	C_{34}	C_{36}	C_{38}	C_{40}
Standard adding method	0.186	0.131	0.060	0.045	0.009	0.014	0.005
Simplified method	0.187	0.126	0.057	0.048	0.010	0.016	0.005
before sensitivity correction	0.176	0.125	0.056	0.041	0.007	0.011	0.004

Note : Amounts of *n*-paraffins were expressed with mg per 100 mg of hydrocarbon fraction.

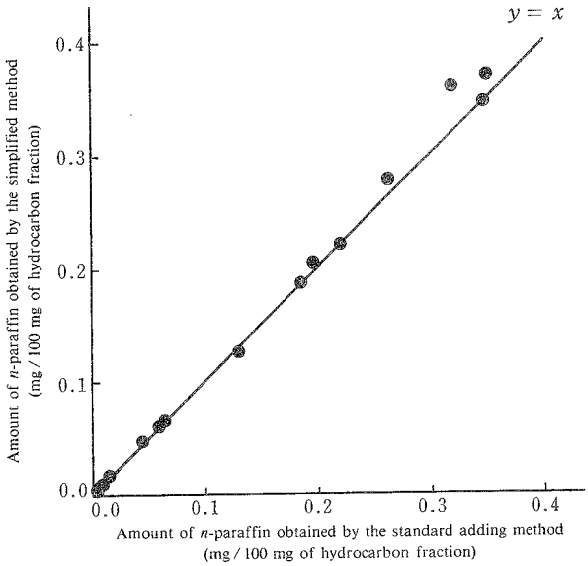

Fig. 6. Correlation between amount of *n*-paraffin obtained by the standard adding method and that by the simplified method from the same heavy oil sample.

Note : The correlation was expressed by the linear regression equation, $y = -0.004 + 1.017x$, which was significant at 0.01 level ($(F_0 = 593.3 > F_{12}^{1}(0.01) = 9.33)$).
x ..... amount of *n*-paraffin obtained by the standard adding method
y ..... amount of *n*-paraffin obtained by the simplified method

Table 9. Statistical tests for the validity of the simplified method for quantitative analysis of *n*-paraffins in heavy oil.

	a_0	a_1	F_0	n_2	t_0	t_1	F_e
Simplified method	−0.004	1.017	593.30**	12	−0.26	0.40	0.11
before sensitivity correction	−0.008	1.132	446.48**	12	2.00	2.46*	5.04*

Note : The linear regression equation, $y = a_0 + a_1x$
x ..... estimated value by the standard adding method
y ..... estimated value by the simplified mehtod or that before sensitivity correction
F_0 ..... SNEDECORE's F for a_1 with 1 and n_2 degrees of freedom
t_0 ..... STUDENT's t under the null hypothesis of a_0 being equal to 0.0 with n_2 degrees of freedom
t_1 ..... STUDENT's t under the null hypothesis of a_1 being equal to 1.0 with n_2 degrees of freedom
F_e ..... SNEDECORE's F of the comparison between the estimated linear regresson equation and the expected one, with 2 and n_2 degrees of freedom
* significant at 0.05 level
** significant at 0.01 level

As shown in Fig. 6, the present method (y) could yield the results very closely similar to the orthodox method (x). The validity of y to be identical with x was verified by estimating the observed regression equation of y on x ($y = a_0 + a_1x$) and by testing whether a_0 would be equal to 0.0 or not and whether a_1 would be equal to 1.0 or not. The results of these tests shown in Table 9 revealed the following facts: Regression analysis y on x yielded an extremely large F_0. This fact means that the observed values are distributed in a very narrow belt around the estimated regression equation, and therefore, a very small difference of a_0 from 0.0 is regarded as significant difference. The same is true to the difference of a_1 from 1.0. In spite of these facts, all the t_0, t_1 and F_e of the present method were insignificant, indicating that the results of this method could be regarded as identical with those of the orthodox method. This fact means that the present method is applicable to the quantitative analysis of *n*-paraffins in this heavy oil. The significant values of t_1 and F_0 for the estimated regression equation before sensitivity correction indicate that it is necessary to make correction for different sensitivity, in spite of the fact that the correction coefficients took the values similar to 1.0, as shown in Table 7.

Variation of *n*-paraffin components caused by the procedure indispensable to the bacteriological work

One of the principal aims of the present report is to clarify the variation of the estimated components of *n*-paraffins in heavy oil cuased by the procedure indispensable to the bacteriological work. If heavy oil used in this work had been low in viscosity, it would have been possible to apply a filter sterilization. In the present case, however, it was difficult to do so. And it is probable that the components are, more or less, varied during sterilization, for heavy oil is sterilized usually by autoclaving in the presence of medium, which is the mixture of various compounds. The survey was mainly conducted in the coastal waters, for the oil accident occurred there. And therefore, the two media A and B, which are suitable for studying oil-decomposing bacteria in the eutrophicated waters, were adopted[7]. For the purpose of clarifying the variation of the *n*-paraffin components, the 20 samples (four samples in the two media under the two sterilization conditions...autoclaved or not..., and four samples of the original heavy oil) were quantitatively analysed by the method proposed here.

The results shown in Table 10 revealed the following facts :

1. The contents of *n*-paraffins of all the samples showed a dull bell-shaped change with the increase in carbon chain length, indicating that the major components were C_{18} to C_{22}.
2. The difference in media used here did not cause any difference in the components of *n*-paraffins after autoclaving.
3. The influence of autoclaving was found in the decrease of the contents of the short-chain *n*-paraffins...in the range from C_{13} to C_{20} or thereabout, especially in the minor components, C_{13}, C_{14}, and C_{15}.
4. The *n*-paraffins of the original heavy oil and those contained in the unautoclaved media, consisted of C_{13} to C_{40} in chain length.
5. Those of heavy oil in the autolaved media consisted of C_{14} to C_{40}, showing the possibility of C_{13} having been by autoclaving.

Table 10. Variation of *n*-paraffin components in heavy oil caused by autoclaving in the presence of different media.

Sample No.	Chain length of *n*-paraffins																
	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}
1	0.016	0.075	0.160	0.263	0.357	0.437	0.376	0.405	0.399	0.390	0.347	0.344	0.255	0.241	0.164	0.197	0.177
2	0.014	0.072	0.160	0.223	0.322	0.400	0.354	0.382	0.382	0.349	0.323	0.285	0.239	0.217	0.170	0.177	0.161
3	0.016	0.066	0.120	0.202	0.300	0.367	0.336	0.367	0.346	0.328	0.277	0.267	0.239	0.221	0.162	0.177	0.152
4	0.016	0.089	0.161	0.204	0.295	0.378	0.353	0.375	0.363	0.326	0.297	0.293	0.244	0.234	0.175	0.175	0.154
Average	0.016	0.076	0.150	0.223	0.319	0.396	0.355	0.382	0.373	0.348	0.311	0.297	0.244	0.228	0.168	0.182	0.161
5	0.009	0.095	0.175	0.262	0.311	0.397	0.364	0.384	0.381	0.345	0.328	0.285	0.252	0.217	0.179	0.189	0.162
6	0.008	0.092	0.181	0.306	0.341	0.433	0.392	0.417	0.420	0.380	0.347	0.313	0.264	0.249	0.201	0.220	0.171
7	0.012	0.071	0.161	0.257	0.308	0.377	0.345	0.363	0.364	0.332	0.300	0.281	0.224	0.214	0.163	0.208	0.163
8	0.008	0.064	0.181	0.252	0.316	0.385	0.350	0.393	0.380	0.339	0.313	0.301	0.253	0.240	0.169	0.195	0.171
Average	0.009	0.081	0.175	0.269	0.319	0.398	0.363	0.389	0.386	0.349	0.322	0.295	0.248	0.230	0.178	0.203	0.167
9	—	0.014	0.086	0.187	0.276	0.363	0.354	0.391	0.403	0.350	0.350	0.330	0.244	0.252	0.207	0.206	0.183
10	—	0.025	0.102	0.181	0.302	0.411	0.371	0.426	0.413	0.374	0.361	0.343	0.270	0.261	0.199	0.216	0.184
11	—	0.008	0.074	0.184	0.290	0.362	0.347	0.370	0.375	0.369	0.341	0.310	0.250	0.241	0.167	0.204	0.188
12	—	0.013	0.079	0.154	0.241	0.331	0.326	0.346	0.363	0.341	0.333	0.270	0.244	0.217	0.171	0.204	0.163
Average	—	0.015	0.085	0.177	0.277	0.368	0.350	0.383	0.389	0.359	0.346	0.313	0.252	0.243	0.186	0.208	0.180
13	0.019	0.087	0.189	0.253	0.340	0.416	0.378	0.402	0.401	0.342	0.325	0.285	0.261	0.239	0.190	0.203	0.184
14	0.004	0.082	0.184	0.234	0.284	0.420	0.379	0.407	0.408	0.368	0.332	0.299	0.245	0.233	0.178	0.197	0.171
15	0.011	0.090	0.189	0.250	0.339	0.426	0.393	0.416	0.426	0.365	0.349	0.319	0.281	0.264	0.204	0.216	0.196
16	0.007	0.074	0.168	0.256	0.329	0.439	0.371	0.398	0.405	0.370	0.352	0.318	0.267	0.248	0.172	0.186	0.180
Average	0.010	0.083	0.183	0.248	0.323	0.425	0.380	0.406	0.410	0.361	0.340	0.305	0.264	0.246	0.186	0.201	0.183
17	—	0.005	0.077	0.171	0.268	0.378	0.382	0.423	0.434	0.368	0.387	0.351	0.263	0.250	0.203	0.229	0.191
18	—	0.004	0.062	0.168	0.264	0.361	0.339	0.352	0.369	0.336	0.334	0.303	0.246	0.238	0.179	0.186	0.173
19	—	0.014	0.081	0.179	0.281	0.387	0.363	0.401	0.412	0.358	0.346	0.296	0.278	0.262	0.213	0.216	0.183
20	—	0.006	0.059	0.154	0.241	0.347	0.330	0.371	0.382	0.349	0.347	0.299	0.268	0.237	0.172	0.201	0.179
Average	—	0.007	0.070	0.168	0.264	0.368	0.354	0.387	0.390	0.353	0.354	0.312	0.264	0.247	0.192	0.208	0.182

Table 10. — (Cont'd)

Sample No.	Chain length of n-paraffins														Total amounts of n-paraffins
	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}	C_{35}	C_{36}	C_{37}	C_{38}	C_{39}	C_{40}	C_{41}	C_{42}	C_{43}	
1	0.105	0.095	0.081	0.070	0.048	0.037	0.033	0.020	0.012	0.021	0.014	*	*	*	5.139
2	0.120	0.085	0.069	0.059	0.056	0.031	0.025	0.016	0.016	0.018	0.010	*	*	*	4.735
3	0.124	0.068	0.056	0.047	0.044	0.022	0.009	0.017	0.014	0.007	0.005	*	*	*	4.356
4	0.124	0.083	0.061	0.051	0.048	0.023	0.011	0.013	0.015	0.010	0.008	*	*	*	4.579
Average	0.118	0.083	0.067	0.057	0.049	0.028	0.020	0.017	0.014	0.014	0.009	*	*	*	4.705
5	0.126	0.097	0.072	0.066	0.051	0.039	0.036	0.029	0.021	0.015	0.014	*	*	*	4.901
6	0.143	0.095	0.075	0.064	0.065	0.040	0.028	0.024	0.018	0.019	0.005	*	*	*	5.311
7	0.114	0.086	0.075	0.054	0.042	0.030	0.023	0.019	0.011	0.016	0.013	*	*	*	4.626
8	0.117	0.084	0.072	0.054	0.038	0.031	0.022	0.015	0.008	0.011	0.008	*	*	*	4.770
Average	0.125	0.091	0.074	0.060	0.049	0.035	0.027	0.022	0.015	0.015	0.010	*	*	*	4.904
9	0.127	0.091	0.077	0.051	0.046	0.029	0.026	0.017	0.005	0.008	0.005	*	*	*	4.683
10	0.126	0.098	0.081	0.060	0.044	0.031	0.030	0.016	0.013	0.018	0.001	*	*	*	4.957
11	0.139	0.088	0.077	0.064	0.047	0.031	0.023	0.002	0.003	0.003	0.001	*	*	*	4.561
12	0.121	0.094	0.065	0.052	0.025	0.029	0.022	0.007	0.016	0.004	0.001	*	*	*	4.232
Average	0.128	0.093	0.075	0.057	0.041	0.030	0.025	0.011	0.009	0.008	0.002	*	*	*	4.610
13	0.129	0.096	0.080	0.069	0.052	0.041	0.025	0.024	0.022	0.020	0.009	*	*	*	5.081
14	0.121	0.086	0.078	0.058	0.049	0.030	0.030	0.007	0.010	0.013	0.008	*	*	*	4.915
15	0.156	0.103	0.077	0.070	0.055	0.037	0.037	0.018	0.011	0.005	0.009	*	*	*	5.312
16	0.119	0.086	0.066	0.055	0.050	0.026	0.026	0.018	0.009	0.016	0.001	*	*	*	5.012
Average	0.131	0.093	0.075	0.063	0.052	0.034	0.030	0.017	0.013	0.014	0.007	*	*	*	5.083
17	0.116	0.087	0.080	0.057	0.055	0.028	0.031	0.018	0.010	0.017	0.013	*	*	*	4.892
18	0.114	0.077	0.062	0.056	0.043	0.021	0.020	0.010	0.011	0.009	0.007	*	*	*	4.344
19	0.130	0.088	0.073	0.061	0.056	0.028	0.014	0.015	0.012	0.015	0.016	*	*	*	4.778
20	0.132	0.089	0.071	0.052	0.048	0.033	0.025	0.019	0.010	0.009	0.004	*	*	*	4.432
Average	0.123	0.085	0.072	0.057	0.051	0.028	0.023	0.016	0.011	0.013	0.010	*	*	*	4.609

Note : Amounts of n-paraffins in heavy oil were expressed with mg per 100 mg of hydrocarbon fraction. Samples are all the same as ones shown in Table 3.
* trace

The autoclaving caused the decrease in the contents of the short-chain *n*-paraffins, but the presence of media and the difference of media did not cause any notable difference in the contents regardless of the chain length.

Based on these results, it may be concluded thus :

1. There is no need to pay any attention to the variation due to the difference in media.
2. When the contents of short-chain *n*-paraffins are exclusively discussed, there is the need taking into consideration of their decrease due to autoclaving, but in other cases, there is no need to pay any attention to the variation of the componenets caused by autoclaving.

Summary

In the bacteriological work, especially in the field survey of examining oil pollution and self-purification after an oil accident, a simpler method on the determination of oil components is favorable, for it is necessary to analyse many samples derived from the same original oil within a short time with limited members. The present report, therefore, showed a concise method which does not need any preliminary separation of *n*-paraffin fraction from heavy oil (grade C), and is applicable to the quantitative analysis of *n*-paraffin components of heavy oil in media for oil-decomposing bacteria. This method is based on the standard adding method, in which many authentic *n*-paraffins are needed as the standard substances. But instead of many standards, the present method needs only one standard substance (*n*-dodecane) and the correction coefficient for the sensitivity of each *n*-paraffin (the sensitivity of *n*-dodecane as the standard) or an equation showing a relation between chain length and relative sensitivity. The accuracy of this method was statistically tested by comparing it with the results of the orthodox method. The components of *n*-paraffins in the 20 samples (four samples in the two media under the two sterilization conditions... autoclaved or not, and four samples of the original heavy oil) were quantitatively analysed by the present method, for the purpose of clarifying the variation of *n*-paraffin components caused by the procedure indispensable to the bacteriological work...by autoclaving treatment in the presence of the different media. The results obtained were summarized as follows :

1. The simplified method could yield the results capable of being regarded to be identical with those of the orthodox method. And therefore, it may be said that the present method is applicable to the quantitative analysis of the *n*-paraffin components of heavy oil (grade C) in media without labors of adding many standard substances and of separating *n*-paraffin fraction preliminarily from heavy oil.
2. The contents of *n*-paraffins of all the samples showed a dull bell-shaped change with the increase in carbon chain length, indicating that the major components were C_{18} to C_{22}.
3. The difference in media used here did not cause any difference in the components of *n*-paraffins after autoclaving.
4. The *n*-paraffins of the original heavy oil and those contained in the unautoclaved media, consisted of C_{13} to C_{40} in chain length, while those in the autoclaved media consisted of C_{14} to C_{40}, showing the possibility of C_{13} having been evaporated by autoclaving.
5. Based on these results, it may be said that there is no need to pay any attention to the variation due to the difference in media, and there is no need of taking into consideration of the degradation of the long-chain components caused by autoclaving, although attention

should be paid to the decrease in the short-chain minor components due to autoclaving only when the contents are exclusively discussed.

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