

## Changes in the Structure of Macromolecules of Resin-Asphaltene Substances of Oil from the Ashalchinskoye Field in the Course of Biological Oxidation

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Received November 8, 2023; Accepted February 1, 2024

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### Abstract

The effect of biogenic oxidation of high-viscosity oil from the Ashalchinskoye field by native soil microflora (laboratory experiment) on the composition and structure of its resin-asphaltene components is studied. To conduct the experiment, a complex of physicochemical methods is used, such as elemental analysis, IR spectroscopy, selective chemical destruction of sulfide and ether bonds, and gas chromatography-mass spectrometry. It has been established that the biodegradation of resins and asphaltenes is accompanied by a change in the qualitative and molecular composition of compounds bound in their structure through sulfide and ether bridges. The share of aromatic hydrocarbons in the composition of compounds identified in the products of chemical degradation of biodegraded oil samples is lower, while the relative content of heteroorganic compounds is higher. The prevalence of saturated hydrocarbons, which are predominantly bound through oxygen in biodegraded asphaltenes and through sulfur in biodegraded resins, remains stable. In the course of biodegradation, the molecular composition of bound *n*-alkanes, *n*-alkylbenzenes, and naphthalenes changes most noticeably. At the same time, biomodified asphaltenes and resins have a similar distribution of *n*-alkanes and naphthalenes but differ in the composition of bound *n*-alkylbenzenes.

**Keywords:** Heavy oil; Soil microflora; Biodegradation; Resins; Asphaltenes; Structural group composition; Chemical degradation; Composition of bound fragments.

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## 1. Introduction

Biodegradation is a major factor in ecosystem self-remediation from oil and petroleum product pollution [1-3]. The efficiency of the processes of biodegradation of oil components largely depends on their structure and the activity of hydrocarbon-oxidizing microorganisms [4-5]. As a result of studies of the biotransformation of individual hydrocarbons, multicomponent mixtures of individual substances belonging to different classes, and samples of reservoir oils, it has been found out that the ability of oil components to biodegradation decreases in the series *n*-alkanes, branched alkanes (isoalkanes, isoprenoids), cycloalkanes, arenes, asphaltenes, and resins [6-8]. Most of the published works are devoted to the utilization of saturated and aromatic hydrocarbons. The study of the biodegradation of polar compounds, in particular resin-asphaltene substances (RAS) has been neglected, since it was believed that their complex macromolecules were resistant to microorganisms [9-10]. However, in recent years, there has been growing interest in such studies [11-19]. One of the reasons for this is the increase in the share of heavy oils, including biodegraded ones, in the total volume of hydrocarbons produced. The high content of high-molecular heteroatomic compounds – resins and asphaltenes – significantly complicates the processes of production, transportation, and processing of these oils due to the formation of asphalt, resin and paraffin deposits in the reservoir, in downhole and oil pumping equipment and deactivation of catalysts for oil refining processes [20-23]. The problem of restoration of ecosystems polluted by them is also acquiring a global scale. It is possible to reduce the concentration of oil pollution, in particular resins and asphaltenes due to the activation of the native microflora of contaminated objects. Some researchers reported that soil

microorganisms use asphaltenes and resins as a source of carbon and energy [13,24-32]. The recycling process is accompanied by noticeable changes in the structural and group characteristics of the mean molecules of resins and asphaltenes. Their linear and branched alkyl fragments, saturated cyclic structures, and aromatic rings containing heteroatoms undergo biodegradation.

Published data indicate that resins and asphaltenes are involved into the processes of oxidation by soil microorganisms and provide a general picture of changes in their structure. At the same time, it is known that some of the sulfur and oxygen atoms present in the composition of resins and asphaltenes are involved in the formation of sulfide and ether bridges through which individual structural fragments are bonded to each other or to the polycondensed core of RAS macromolecule [33-36]. Data on the effect of biodegradation by native soil microflora on the composition of structural fragments bound through sulfur and oxygen in complex high-molecular moieties of oil components of heavy oil were discussed in the work of Antipenko *et al.* [37]. It is shown that alkanes, tri- and tetracyclic aromatic hydrocarbons are present in bound form in the oils of biodegraded oil. O-bound *n*-alkanes predominate over O-bound isoprenanes and, in contrast to S-bound alkanes, are predominantly represented by low molecular weight homologues.

The study was conducted of the effect of aerobic bacteria *Nocardioides simplex*, which are widely present in soils and used in oil waste treatment plants, on the composition of compounds cross-linked by sulfide bridges in the structure of macromolecules formed by low molecular weight hydrocarbon fragments of high-sulfur crude oil [38]. A comparison of the desulfurization products of the original and biodegraded samples showed that the fragments represented by sulfur-bound *n*-alkanes are the first to undergo biodegradation. Information on the effect of biodegradation by native soil microflora on the composition of fragments bound via sulfur and oxygen in the macromolecules of asphaltenes and resins of heavy oil is not presented in the scientific literature. Obtaining such information will allow a more detailed assessment of the effect of this process on the composition and structure of resins and asphaltenes.

In this work, a comparative study of the composition and structure of asphaltenes and resins of heavy oil before and after its biodegradation by native soil microflora was carried out. The direction of change in the relative content of structural fragments of various types in the macromolecules of resins and asphaltenes during biooxidation has been revealed.

The aim of this work is to study the effect of biodegradation by native soil microorganisms on the composition and structure of asphaltenes and resins of heavy oils. This implies the finding of direction of changes in the relative content of structural fragments of various types in the macromolecules of resins and asphaltenes in the course of biooxidation. The task is also set to study the composition of sulfur- and ether-bound compounds in the composition of RAS macromolecules of initial and biodegraded oils. Experimental results will allow a more detailed assessment of the effect of the biodegradation process on the composition and structure of petroleum resins and asphaltenes. They may be also used to develop effective methods for oil waste disposal and new control methods for processing heavy hydrocarbon feedstocks.

## 2. Experimental

### 2.1. Materials

Solvents, i.e. 99.9 % *n*-hexane, benzene, chloroform, tetrahydrofuran, were purchased from JSC EKOS-1 (Russia), while methanol and ethanol were purchased from JSC "Vekton" (Russia). Sodium tetrahydroborate (98 %) and lithium aluminum hydride (97 %) were purchased from ABCR GmbH Co KG (Germany), while boron tribromide (99 %) was purchased from ABCR ORGANICS (Germany). Nickel chloride ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ) was purchased from LLC EKOTEK (Russia). ASC silica gel (0.25-0.50 mm) was purchased from HONG KONG CHEMICAL CORPR (Hong Kong), while silica gel L (100/160) for chromatography was supplied by LACHEMA (Czech Republic).

### 2.2. Samples

The objects of study are resins and asphaltenes isolated from heavy oil sampled at the Ashalchinskoye field (Republic of Tatarstan, Russia) before and after its biodegradation by

native soil microflora under laboratory conditions. This oil belonging to the class of bituminous oils ( $\rho$  978 kg/m<sup>3</sup> at 20°C) is characterized by a high content of asphaltenes (6.4 wt%) and resins (26.2 wt%). The choice of oil is also conditioned by the fact that today at the Ashalchinskoye oilfield the liquid hydrocarbons are produced with a flow rate of about 1000 tons/day [39]. The reserves of the Ashalchinskoye oilfield are estimated at 10.248 million tons.

### 2.3. Experiment techniques

A sample of biodegraded oil was manufactured at the Laboratory of Colloidal Chemistry of the Institute of Petroleum Chemistry of the Siberian Branch of the Russian Academy of Sciences according to the method described in detail by Filatov *et al.* [26].

The separation of asphaltenes and resins from the original (A and R) and biodegraded (AB and RB) oils was carried out according to the standard procedure [40].

To cleave sulfide and ether bridges in samples A, R, AB, and RB, nickel boride and boron tribromide were used, the use of which enables the breakage of carbon–sulfur bonds in sulfides and carbon–oxygen bonds in ethers and/or esters under mild conditions [33,41]. The destruction of sulfide and ether bonds took place at a temperature of 60°C according to the methods described by Peng *et al.* [33]. Desulfurization was carried out in a tetrahydrofuran and methanol medium, while the breaking of C–O bonds was performed in a chloroform medium. The products of chemical degradation of asphaltenes and resins soluble in n-hexane were separated on silica gel L (100/160) into fractions of non-polar and polar compounds, using for desorption mixtures of n-hexane and benzene (7:3 by vol.) and benzene and ethanol (1:1 by vol.) respectively. Non-polar alkyl bromides were reduced with lithium aluminum hydride. The products of reduction of alkyl bromides and the nonpolar fraction of desulfurization products were analyzed by the method of gas chromatography-mass spectrometry.

### 2.4. Analysis methods

For the comparative characterization of A, R, AB, and RB samples, the methods of elemental composition determination, IR-Fourier spectroscopy, and selective chemical destruction were used. The content of C, H, S, N was determined using an automatic elemental 'Vario EL Cube' analyzer (Table 1). The absolute error of the analysis for each determined element did not exceed  $\pm 0.1\%$ . IR spectra were recorded in the range 4000–400 cm<sup>-1</sup> using a Nicolet 5700 FTIR spectrometer. In the case of resins, films obtained from a CHCl<sub>3</sub> solution were analyzed, while in the case of asphaltenes, tablets with KBr were analyzed.

Table 1. Characteristics of the objects under study.

Sample	Content in oil, wt %	Content, wt %					H/C <sub>at</sub>
		C	H	N	S	O	
R	18.39	78.87	8.65	1.52	5.35	5.61	1.32
RB	2.59	73.11	6.79	0.89	4.88	14.33	1.11
A	6.40	75.04	6.19	1.59	9.35	7.38	0.99
AB	2.86	72.99	6.82	0.93	4.92	14.34	1.12

To estimate the conventional content of structural fragments in the RAS macromolecules of the original and biodegraded oil, the IR spectra were processed using the OMNIC 7.3 software. The values of optical densities obtained were used to calculate the  $C_1 = D_{1600}/D_{720}$ ,  $C_2 = D_{720} + D_{1380}/D_{1600}$ ,  $C_3 = D_{1380}/D_{1465}$ ,  $C_4 = D_{1710}/D_{1465}$ , and  $C_5 = D_{1030}/D_{1465}$  spectral coefficients, which are the ratios of the optical densities of the absorption bands at frequencies 1710, 1600, 1465, 1380, 1030, and 720 cm<sup>-1</sup>. They represent the relative content of aromatic fragments ( $C_1$ ) in the RAS macrostructure, the share of aliphatic fragments ( $C_2$ ) and the degree of their branching ( $C_3$ ), as well as the share of fragments containing carbonyl ( $C_4$ ) and sulfoxide ( $C_5$ ) functional groups, respectively [42].

GC-MS analysis of liquid products of chemical degradation was performed using a Thermo Scientific DFS magnetic chromatography-mass spectrometer. The compounds were separated

using a quartz capillary column 30 m long and 0.25 mm in inner diameter with a stationary 0.25  $\mu\text{m}$  thick DB-5MS phase. The chromatographer operated with helium as a carrier gas at a constant flow rate of 0.8 mL/min. The thermostat program was as follows: initial temperature 80°C (3 min), rise to 300°C (4°C/min), hold up time at final temperature 30 min. Operating mode of the mass spectrometer was as follows: ionization method was an electron impact at energy of ionizing electrons 70 eV and the temperature of the ionization chamber was 250°C. Mass spectra were scanned every second in the mass range from 50 to 500 amu. Mass spectral data were processed using the Xcalibur program. Literature data and the computer library of mass spectra of the National Institute of Standards and Technology were used to identify individual compounds.

### 3. Results and discussion

#### 3.1. General characteristics of resins-asphaltene components of the original and biodegraded oils

As follows from the data given in Table 1, the total biodegradation of asphaltenes and resins under the experimental conditions was 55.3% and 85.9%, respectively. The RAS of the original and biodegraded oils differ in the H/C atomic ratio and the content of heteroatoms.

A decrease in the H/C value from 1.33 to 1.11 is observed for resins, while for asphaltenes its increase is from 0.99 to 1.12. These features of the biodegradation of resins and asphaltenes can be associated with different structures of their macromolecules. The decrease in the saturation of resin molecules is most likely due to the destruction of acyclic fragments in their structure, while the increase in the saturation of asphaltene molecules is due to the destruction of naphthenic cycles [27].

As for a change in the heteroatomic composition, it is most pronounced for oxygen compounds. In resins and asphaltenes of biodegraded oil, the oxygen content is much higher (14.34 and 14.33%) than in resins and asphaltenes of the original oil (5.61 and 7.38%). Such a significant increase in the total number of oxygen atoms directly indicates the microbiological oxidation of RAS macromolecules.

The observed decrease in the number of sulfur and nitrogen atoms in the macromolecular structure of resins and asphaltenes may be due both to the biodegradation of aliphatic fragments containing these heteroatoms and to the oxidation of heteroatoms in the cyclic aromatic fragments of RAS macromolecules, which leads to the process of their decomposition [43-44]. More noticeable differences in the sulfur content for A and AB samples compared to R and RB are most likely due to the fact that A macromolecules differ from R macromolecules in the presence of a larger amount of bioavailable sulfur-containing fragments.

The data on the elemental composition of the RAS of the original and biodegraded oils are consistent with the results of their analysis by IR spectroscopy.

It follows from the analysis of the IR spectra that the structure of all samples under study contains aliphatic (2925–2854 and 1461–1377  $\text{cm}^{-1}$ ), aromatic (3060, 1602, 870–746  $\text{cm}^{-1}$ ) fragments, and fragments containing a sulfoxide group (1036–1032  $\text{cm}^{-1}$ ) (Fig. 1).

The presence of oxygen-containing fragments in structure of R of the original oil can be inferred by the absorption of the carbonyl group of carboxylic acids and/or esters at 1700  $\text{cm}^{-1}$  and by the band at 3232  $\text{cm}^{-1}$  attributed to hydroxyl functions. The appearance of a band in the 3500–3200  $\text{cm}^{-1}$  region is assigned to the absorption of hydroxyl groups, which suggests the presence of oxygen-containing fragments in the structure of A of the original oil.

The absorption bands characteristic of nitrogen-containing fragments do not appear in their IR spectra due to the low concentration of nitrogen in the resins and asphaltenes of the original oil. In addition, the high-intensity absorption bands of fragments containing oxygen functions do not allow them to appear.

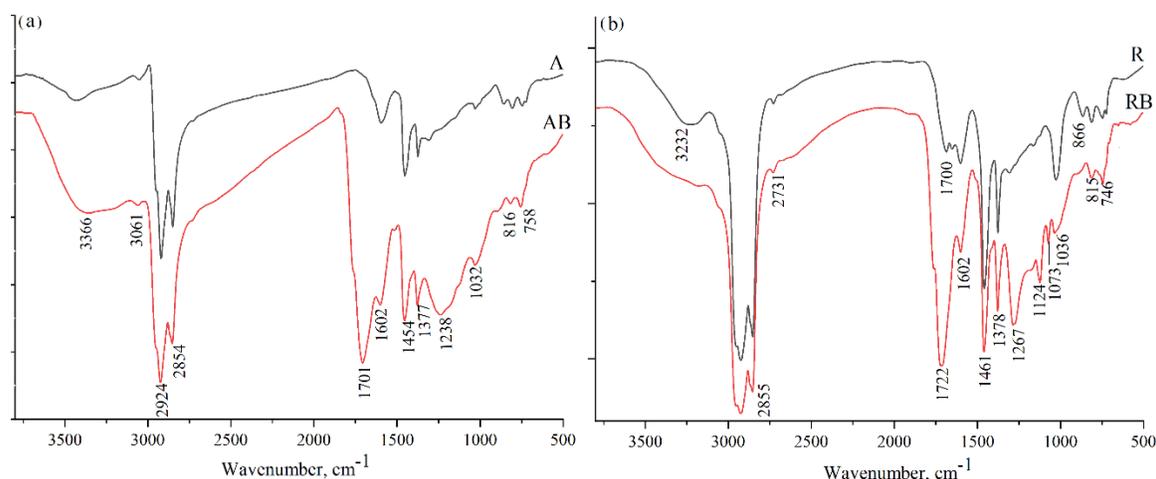


Fig. 1. IR spectra of the original (A) and biodegraded (AB) asphaltenes (a) and the original (R) and biodegraded (RB) resins of oil (b).

A comparative analysis of the data given in Table 2 showed that biodegradation has a significant effect on the quantitative distribution and composition of structural fragments in RAS macromolecules.

Table 2. Characterization of asphaltenes and resins according to data of FTIR analysis.

Samples	Spectral indices*				
	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>5</sub>
A	1.907	2.184	0.767	-	0.332
AB	2.516	1.405	0.886	1.416	0.628
R	2.044	2.484	0.615	-	0.370
RB	2.218	2.153	0.689	1.147	0.336

$$*C_1 = D_{1600}/D_{720}; C_2 = D_{720}+D_{1380}/D_{1600}; C_3 = D_{1380}/D_{1465}; C_4 = D_{1710}/D_{1465}; C_5 = D_{1030}/D_{1465}$$

Thus, in AB and RB, the conditional content of aromatic fragments (C<sub>1</sub>) is higher, while the conditional content of aliphatic fragments (C<sub>2</sub>) is lower than that in A and R. At the same time, the share of terminal methyl groups (C<sub>3</sub>) in the alkyl substituents of AB and RB increases, that is, their degree of branching increases. RAS of biodegraded oil are more oxidized. The share of structural fragments containing carbonyl (C<sub>4</sub>) and sulfoxide (C<sub>5</sub>) functional groups noticeably increases in the composition of AB, while in the composition of RB the share of carbonyl-containing fragments increases. A clear difference in the relative content of sulfoxide groups in the RB and AB samples may be due to structural features of A and R and, as a result, with different bioavailability of C–S bonds in their macromolecules.

The higher relative content of S=O groups in the AB structure is most likely due to the biological oxidation of the C–S bonds of aliphatic sulfides, structural fragments of most geomacromolecules [34,45], which results in the formation sulfoxides and sulfones [38]. The fact of the formation of sulfones is confirmed by the appearance of a band at 1124 cm<sup>-1</sup> in the IR spectrum of SB.

The composition of carbonyl-containing structures changes most significantly. In the IR spectrum of AB, an absorption band of the carbonyl group of aldehydes and/or ketones (1701 cm<sup>-1</sup>) appears, while an absorption band of the carbonyl group of aliphatic acids (1722 cm<sup>-1</sup>) appears in the IR spectrum of RB. In the spectra of both biodegraded samples, the intensity of the C–O–C 'ether band' (1300–1100 cm<sup>-1</sup>) increases and the absorption band of hydroxyl groups in the region 3400 cm<sup>-1</sup> becomes broader.

A wider set of oxygen-containing fragments in the structure of the RAS of biodegraded oil and a high value of their C<sub>4</sub> parameter suggest the oxidative nature of the processes occurring during the biodegradation of resin and asphaltene macromolecules.

### 3.2. Chemical destruction of sulfide and ether bonds in asphaltene molecules of original and biodegraded oils

Using the method of selective chemical destruction, it was found that the structure of asphaltenes of both original and biodegraded oil contains fragments linked through sulfide and ether bridges. It follows from a comparison of the results of the quantitative distribution of the products of destruction of C-S and C-O bridge bonds that, as a result of oxidative transformation, the yield in liquid products of chemolysis of asphaltene molecules increases from 33.3 to 56.2% in the case of the destruction of the C-S bond. In the case of the destruction of the C-O bond it increases from 8.9 to 32.1%. The predominance of soluble desulfurization products in the composition of products of AB chemolysis may indicate that the share of bioavailable oxygen-bound fragments in structure A is higher than that of bioavailable sulfur-bound fragments. According to literature data, among the ester-bound structural fragments of asphaltenes, the fragments connected with the core of their macromolecules through the functional group of esters are more susceptible to biodegradation [14].

As follows from the data in Table 3, C-O and C-S bound fragments in molecules A and AB are represented by a complex mixture of saturated and aromatic hydrocarbons (HC) and heteroorganic compounds (HOCs). In molecules A, among both types of 'bound' compounds, alkanes of normal and branched structure, *n*-alkylcyclopentanes, *n*-alkylcyclohexanes, cheilanthanes, hopanes, *n*-alkylbenzenes, *n*-alkyltoluenes, phenylalkanes with different positions of the phenyl substituent, naphthalenes, phenanthrenes, and dibenzothiophenes have been identified. Ethyl esters of *n*-alkanoic acids have been additionally identified in the composition of ester-bound fragments and steranes, while *n*-alkanoic acids have been additionally identified in the composition of sulfur-bound fragments. The composition of the products of rupture of sulfide and ether bridges in AB molecules differs from the composition of similar products in A molecules (Table 3). Hence, *n*-alkylcycloalkanes and cheilanthanes are absent in the composition of S- and O-linked fragments of AB, but additionally *n*-alk-1-enes with an even number of carbon atoms have been revealed.

Table 3. Composition of liquid products of chemical destruction of ether (C-O) and sulfide (C-S) bonds in asphaltene molecules of original and biodegraded oils.

Compound, m/z	Asphaltenes			
	A		AB	
	C-O	C-S	C-O	C-S
Aliphatic hydrocarbons				
<i>n</i> -alk-1-enes, 55	not identified	not identified	C <sub>14</sub> , C <sub>16</sub> , C <sub>18</sub>	C <sub>14</sub> , C <sub>16</sub> , C <sub>18</sub> , C <sub>20</sub>
<i>n</i> -alkanes, 57, 71	C <sub>13</sub> -C <sub>27</sub>	C <sub>15</sub> -C <sub>31</sub>	C <sub>14</sub> -C <sub>32</sub>	C <sub>14</sub> -C <sub>31</sub>
methylalkanes, 57, 71	C <sub>13</sub> -C <sub>24</sub>	C <sub>16</sub> -C <sub>27</sub>	C <sub>13</sub> -C <sub>26</sub>	C <sub>15</sub> -C <sub>31</sub>
isoprenoid alkanes	C <sub>18</sub> -C <sub>20</sub>	C <sub>18</sub> -C <sub>20</sub>	C <sub>18</sub> -C <sub>20</sub>	C <sub>18</sub> -C <sub>20</sub>
<i>n</i> -alkylcyclopentanes, 68, 69	C <sub>14</sub> -C <sub>24</sub>	C <sub>15</sub> -C <sub>28</sub>	not identified.	not identified
<i>n</i> -alkylcyclohexanes, 82, 83	C <sub>12</sub> -C <sub>23</sub>	C <sub>15</sub> -C <sub>29</sub>	not identified	not identified
cheilanthanes, 191	C <sub>20</sub> -C <sub>26</sub>	C <sub>23</sub> -C <sub>25</sub>	not identified	not identified
hopanes, 191	C <sub>27</sub> -C <sub>32</sub>	C <sub>27</sub> , C <sub>29</sub> -C <sub>33</sub>	C <sub>27</sub> -C <sub>32</sub>	C <sub>27</sub> , C <sub>29</sub> -C <sub>33</sub>
steranes, 217	not identified	C <sub>21</sub> -C <sub>22</sub> , C <sub>27</sub> -C <sub>30</sub>	not identified	C <sub>27</sub> -C <sub>29</sub>
Aromatic hydrocarbons				
<i>n</i> -alkylbenzenes, 92	C <sub>11</sub> -C <sub>24</sub>	C <sub>15</sub> -C <sub>29</sub>	C <sub>14</sub> -C <sub>31</sub>	C <sub>15</sub> -C <sub>28</sub>
<i>n</i> -alkyltoluenes, 105	C <sub>13</sub> -C <sub>18</sub>	C <sub>15</sub> -C <sub>28</sub>	C <sub>14</sub> -C <sub>21</sub>	C <sub>16</sub> -C <sub>29</sub>
phenylalkanes, 91	C <sub>17</sub> -C <sub>19</sub>	C <sub>17</sub> -C <sub>19</sub>	C <sub>17</sub> -C <sub>19</sub>	C <sub>17</sub> -C <sub>19</sub>
naphthalenes, 128 +14	C <sub>0</sub> -C <sub>3</sub>	C <sub>1</sub> -C <sub>4</sub>	C <sub>1</sub> -C <sub>3</sub>	C <sub>3</sub> -C <sub>4</sub>
phenanthrenes, 178+14	C <sub>0</sub> -C <sub>3</sub>	C <sub>0</sub> -C <sub>4</sub>	C <sub>0</sub> -C <sub>3</sub>	C <sub>0</sub> -C <sub>2</sub>
Heteroorganic compounds				
dibenzothiophene, 184+14	C <sub>0</sub> -C <sub>1</sub>	C <sub>0</sub> -C <sub>2</sub>	C <sub>0</sub> -C <sub>1</sub>	C <sub>0</sub> -C <sub>2</sub>
<i>n</i> -alkanoic acids, 60	not identified	C <sub>15</sub> -C <sub>19</sub>	not identified	C <sub>15</sub> -C <sub>29</sub>
ethyl esters of <i>n</i> -alkanoic acids, 88	C <sub>18</sub> , C <sub>20</sub>	not identified	C <sub>14</sub> -C <sub>26</sub>	not identified

In the composition of 'sulfur-bound' compounds, low molecular weight C<sub>21</sub>-C<sub>22</sub> steranes disappear but high molecular weight *n*-alkanoic acids appear. The composition of 'ester-bound' fragments is distinguished by a wider range of ethyl esters of *n*-alkanoic acids. Ethyl esters of *n*-alkanoic acids and *n*-alk-1-enes with an even number of carbon atoms are most likely products of the geochemical transformation of the original organic matter, which were captured by kerogen macromolecules and then inherited by asphaltenes formed from it [46-48]. In the course of biodegradation, the complex structure of A is destroyed and the compounds clogged in it are released. The formation of C<sub>20</sub>-C<sub>29</sub> fatty acids can be considered as a result of bacterial oxidation of structural fragments of A macromolecules represented by sulfur-linked *n*-alkanes [35,38].

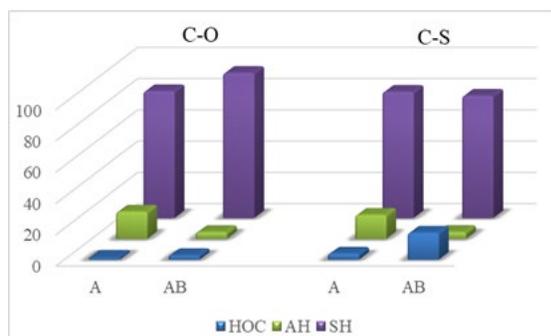


Fig. 2. Distribution of saturated (SH) and aromatic (AH) hydrocarbons and heteroorganic compounds (HOC) in the products of chemical degradation of ether (C-O) and sulfide (C-S) bridges of asphaltenes in the original (A) and biodegraded (AB) oils.

The results of GC-MS analysis indicate that, under experimental conditions, the biodegradation of asphaltene components is accompanied by a change in the qualitative and molecular composition of compounds bound in their structure through sulfide and ether bridges.

It follows from the data of quantitative distribution of the identified compounds that the biodegradation process leads to a noticeable decrease in the share of aromatic hydrocarbons (AH) in bound fragments of both types and an increase in the relative content of HOS, which is more noticeable for sulfur-bound structures (Fig. 2).

The predominance of saturated hydrocarbons in the composition of the identified compounds remains stable. In the biodegraded sample these compounds are predominantly represented by compounds bound through oxygen. The increased content of ester-bound saturated hydrocarbons in the AB structure may be due both to the microbial oxidation of C-S bonds to sulfoxides and sulfones in A macromolecules, and to the bio-oxidation of S-bound hydrocarbons to carboxyl-containing structures. This assumption is in good agreement with the results of IR spectrometry (Table 2) and GC-MS analysis (Table 3), according to which the share of fragments containing the sulfoxide group increases sharply in the composition of AB, while the set of *n*-alkanoic acids in the composition of the products of their desulfurization increases significantly.

In this work, we paid attention to the characterization of sulfur-bound and ether-bound alkanes and aromatic hydrocarbons, for which the most noticeable changes in the group and molecular composition are observed during biodegradation.

According to the data given in Table 3, S- and O-bound alkanes identified in the products of selective chemical destruction of A and AB molecules are represented by linear, branched, and isoprenoid structures. It follows from the comparison of the corresponding mass chromatograms (Fig. 3) that in the course of biodegradation the content of both types of bound *n*-alkanes decreases against the background of an increase in the concentration of branched structures (Fig. 3b and 3d). In the case of ether-bound alkanes, signs of pristane and phytane biodegradation are pronounced, which suggests the participation of isoprenoids in the process of microbial oxidation [7].

As can be seen from Fig. 3, an increase in the share of macromolecular compounds is observed for asphaltenes of biodegraded oil after the destruction of ether (Fig. 3b) and sulfide bridges (Fig. 3d) in the composition of bound *n*-alkanes. An increase in the relative content of high-molecular-weight homologues can be due both to the higher resistance of high-molecular-weight linear alkanes to biodegradation [19,49,50] and to their formation during the metabolism of the bacterial mass [51-52]. Among both types of bound *n*-alkanes, the relative content

of homologues with an even number of carbon atoms increases, which indicates the microbial oxidation of asphaltenes [38,52].

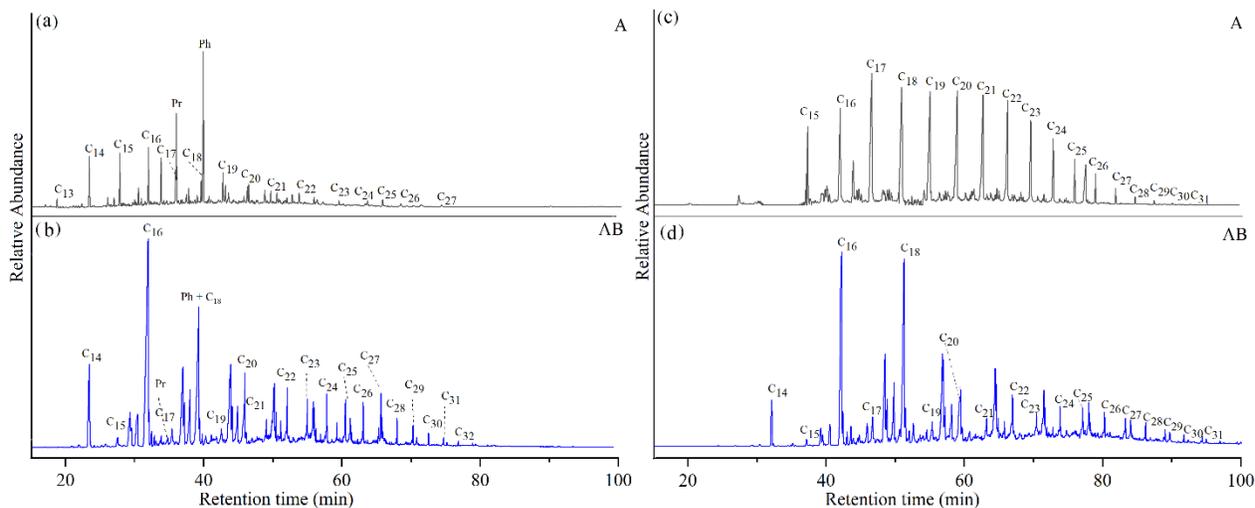


Fig. 3. Mass chromatogram of the products of cleavage of C-O bonds in A (a) and AB (b) molecules and C-S bonds in A (c) and AB (d) molecules at  $m/z$  71.

S- and O-bound AHs identified in the products of selective chemical degradation of A and AB molecules are represented by *n*-alkylbenzenes, *n*-alkyltoluenes, phenylalkanes, naphthalenes, and phenanthrenes (Table 3). In the course of biodegradation, the most noticeable changes occur in the composition of bound monoalkylbenzenes (Fig. 4) and compounds of the naphthalene series (Fig. 5), which are the least biodegradable aromatic structures [7]. In this case, the nature of changes in the composition of *n*-alkylbenzenes is similar to changes in the composition of bound *n*-alkanes. Among *n*-alkylbenzenes, the relative content of low-molecular homologues also decreases, while the share of high-molecular compounds increases. This occurs in the range from C<sub>23</sub> to C<sub>31</sub> for ether-bound *n*-alkylbenzenes (Fig. 4b) and in the range from C<sub>19</sub> to C<sub>28</sub> for sulfur-bound *n*-alkylbenzenes (Fig. 4d).

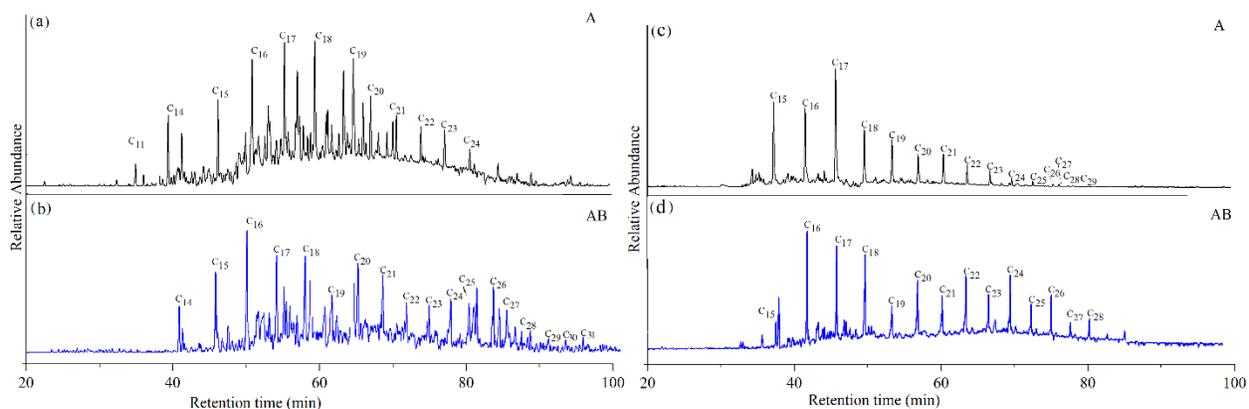


Fig. 4. Mass chromatogram of the products of cleavage of C-O bonds in A (a) and AB (b) molecules and C-S bonds in A (c) and AB (d) molecules at  $m/z$  92.

The ether-bound naphthalene is absent in the composition of the products of AB chemolysis, ether- and sulfur-bound methylnaphthalenes are not identified, the contents of C<sub>2</sub>-alkylnaphthalenes bound through oxygen and C<sub>3</sub>-alkylnaphthalenes bound through sulfur are noticeably reduced, but the relative contents of ether-bound C<sub>3</sub>-alkylnaphthalenes and sulfur-bound C<sub>4</sub>-alkylnaphthalenes increase (Fig. 5b and 5d).

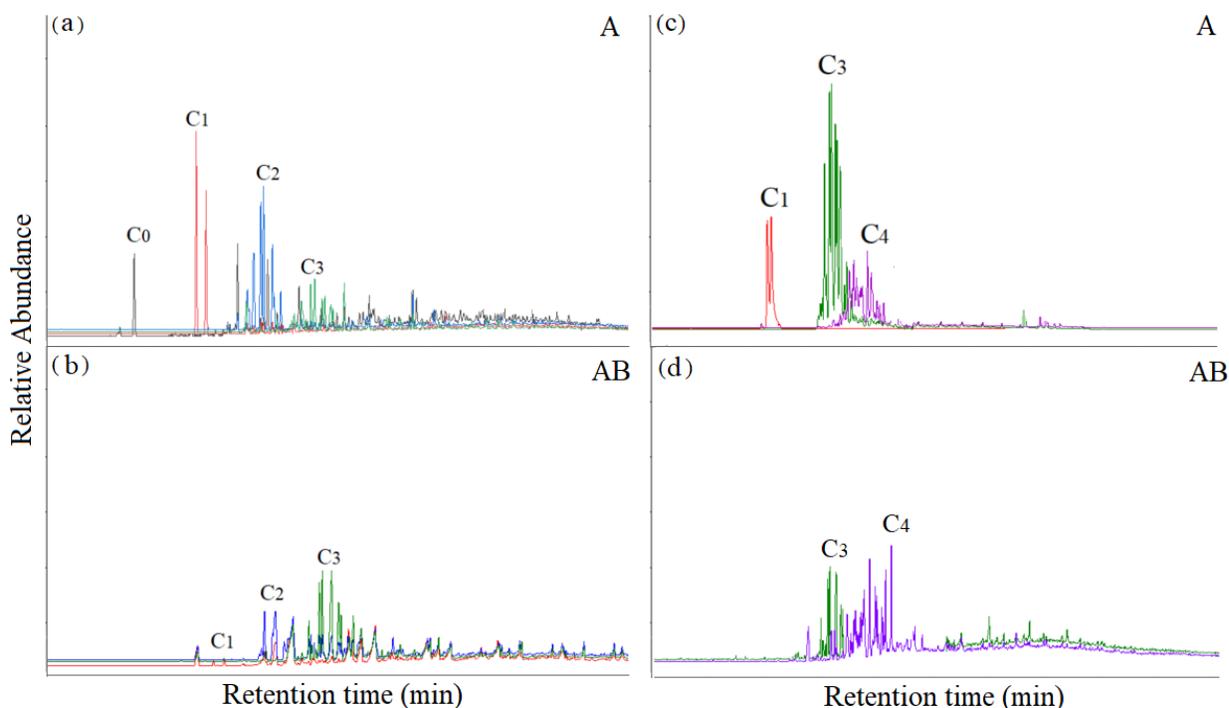


Fig. 5. Mass chromatogram of the products of cleavage of C-O bonds in A (a) and AB (b) molecules and C-S bonds in A (c) and AB (d) molecules at  $m/z$  128(C<sub>0</sub>)+142(C<sub>1</sub>)+ 156(C<sub>2</sub>)+170(C<sub>3</sub>)+184(C<sub>4</sub>).

These features of the composition of naphthalenes bound in the structure of A and AB can be due both to different bioavailability and resistance of the identified compounds to biodegradation. According to data reported by Peters *et al.*, the resistance of alkyl naphthalenes to biodegradation increases with an increase in the number of alkyl substituents [7]. It is also known that tri- and tetrasubstituted alkyl homologues of naphthalene are more resistant to microbiological oxidation than unsubstituted and mono- and dialkyl-substituted structures [53-54]. It should be noted that the asphaltenes of the original oil differ in the composition of O- and S-bonded naphthalenes. Thus, among the sulfur-bound hydrocarbons of the naphthalene series, unsubstituted naphthalene and C<sub>2</sub>-alkyl naphthalenes have been not found (Fig. 5a and 5c). The absence of these non-biodegradable homologues may indicate that heavy oil from the Ashhalchinskoye oilfield was partially biodegraded in the reservoir.

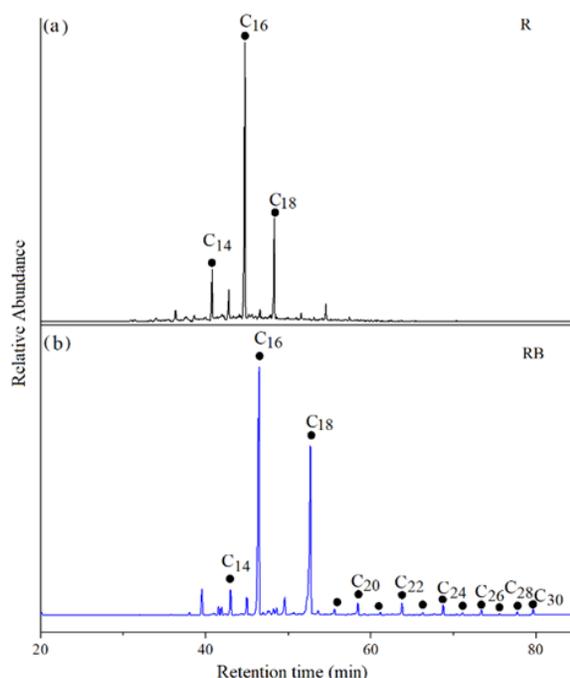
### 3.3. Chemical destruction of sulfide and ether bonds in molecules of resins of original and biodegraded Ashalchinskaya oils

In contrast to asphaltenes, the fragments bound through sulfur in the structure of the resin components of Ashalchinskaya oil are more accessible for biological oxidation by native soil microflora due to the structure of resin macromolecules. This follows from the fact that the yield in products of desulfurization of resins of biodegraded oil is lower (64.40%), but the yield in products of destruction of ester bonds is higher (89.30%) than the yield of the similar products of degradation of resins of the original oil (80.34 and 42.60%, respectively).

According to the GC-MS analysis, among both types of 'bound' compounds, *n*-alkanes, branched alkanes, including isoprenoids (pristane, phytane), *n*-alkylcyclopentanes, *n*-alkylcyclohexanes, hopanes, *n*-alkylbenzenes, *n*-alkyltoluenes, phenylalkanes with various positions of the phenyl substituent, naphthalenes, phenanthrenes and dibenzothiophenes are present in the structure of resins (Table 4).

Table 4. Composition of liquid products of chemical destruction of ether (C-O) and sulfide (C-S) bonds in asphaltene molecules of original and biodegraded oils.

Compound, <i>m/z</i>	Resins			
	original		biodegraded	
	C-O	C-S	C-O	C-S
Aliphatic hydrocarbons				
<i>n</i> -alk-1-enes, 55	not identified	not identified	C <sub>14</sub> , C <sub>16</sub> , C <sub>18</sub> , C <sub>20</sub>	C <sub>14</sub> , C <sub>16</sub> , C <sub>18</sub> , C <sub>20</sub>
<i>n</i> -alkanes, 57, 71	C <sub>12</sub> -C <sub>29</sub>	C <sub>14</sub> -C <sub>30</sub>	C <sub>14</sub> -C <sub>34</sub>	C <sub>14</sub> -C <sub>31</sub>
methylalkanes, 57, 71	C <sub>13</sub> -C <sub>26</sub>	C <sub>16</sub> -C <sub>30</sub>	C <sub>16</sub> -C <sub>28</sub>	C <sub>16</sub> -C <sub>30</sub>
isoprenoid alkanes	C <sub>18</sub> -C <sub>20</sub>	C <sub>18</sub> -C <sub>20</sub>	C <sub>18</sub> -C <sub>20</sub>	C <sub>18</sub> -C <sub>20</sub>
<i>n</i> -alkylcyclopentanes, 68, 69	C <sub>13</sub> -C <sub>23</sub>	C <sub>12</sub> -C <sub>24</sub>	not identified	not identified
<i>n</i> -alkylcyclohexanes, 82, 83	C <sub>13</sub> -C <sub>26</sub>	C <sub>12</sub> -C <sub>24</sub>	not identified	not identified
hopanes, 191	C <sub>27</sub> , C <sub>29</sub> -C <sub>32</sub>	C <sub>27</sub> , C <sub>29</sub> -C <sub>35</sub>	C <sub>27</sub> , C <sub>29</sub> -C <sub>32</sub>	C <sub>27</sub> , C <sub>29</sub> -C <sub>35</sub>
Aromatic hydrocarbons				
<i>n</i> -alkylbenzenes, 92	C <sub>12</sub> -C <sub>26</sub>	C <sub>16</sub> -C <sub>28</sub>	C <sub>16</sub> -C <sub>27</sub>	C <sub>15</sub> -C <sub>23</sub>
<i>n</i> -alkyltoluenes, 105	C <sub>12</sub> -C <sub>24</sub>	C <sub>16</sub> -C <sub>26</sub>	C <sub>16</sub> -C <sub>25</sub>	C <sub>17</sub> -C <sub>22</sub>
phenylalkanes, 91	C <sub>17</sub> -C <sub>19</sub>	C <sub>17</sub> -C <sub>19</sub>	C <sub>17</sub> -C <sub>19</sub>	C <sub>17</sub> -C <sub>19</sub>
naphthalenes, 128 +14	C <sub>0</sub> -C <sub>3</sub>	C <sub>0</sub> -C <sub>3</sub>	C <sub>1</sub> -C <sub>4</sub>	C <sub>2</sub> -C <sub>4</sub>
phenanthrenes, 178+14	C <sub>0</sub> -C <sub>3</sub>	C <sub>0</sub> -C <sub>4</sub>	C <sub>0</sub> -C <sub>3</sub>	C <sub>0</sub> -C <sub>4</sub>
Heteroorganic compounds				
dibenzothiophenes, 184+14	C <sub>0</sub> -C <sub>1</sub>	C <sub>0</sub> -C <sub>3</sub>	C <sub>0</sub> -C <sub>1</sub>	C <sub>0</sub> -C <sub>3</sub>
bicyclic sulphides, 183	C <sub>13</sub> -C <sub>22</sub>	not identified	not identified	not identified
<i>n</i> -alkanoic acids, 60	not identified	C <sub>16</sub> , C <sub>18</sub>	not identified	C <sub>16</sub> -C <sub>31</sub>



Palmitic and stearic acids have been additionally identified among the 'sulfur-bound' fragments and bicyclic sulfides among the ester-bound fragments. Changes in the qualitative composition of 'sulfur-bound' and 'ether-bound' structural fragments of resins in the process of oil biodegradation are symmetrically to changes in the composition and distribution of bound fragments in the structure of asphaltenes. Thus, the composition of the products of RB chemolysis, as well as the composition of products of AB chemolysis is characterized by the absence of monocyclic alkanes, the appearance of *n*-alkenes (Table 4) and a significant increase in the set of 'sulfur-bound' *n*-alkanoic acids (Fig. 6).

Fig. 6. Mass chromatogram of the products of the cleavage of C-S bonds in the molecules of the initial R (a) and biodegraded RB (b) resins at *m/z* 60.

The distinctive features of RB include the absence of bicyclic sulfides in the products of the cleavage of ether bonds, which may be due to their biological oxidation to sulfoxides and sulfones. The formation of the latter is confirmed by the appearance of an absorption band of the sulfo group in the IR spectrum of RB (Fig. 1).

Among the compounds identified in the products of RB chemolysis, saturated hydrocarbons also predominate, the share of AH is noticeably lower, while the share of HOC is higher (Fig. 7).

However, unlike in AB (Fig. 2), in the resins of biodegraded oil the relative content of saturated hydrocarbons bound through oxygen is lower than the share of sulfur-bound compounds, while the relative content of AH and HOC, on the contrary, is higher.

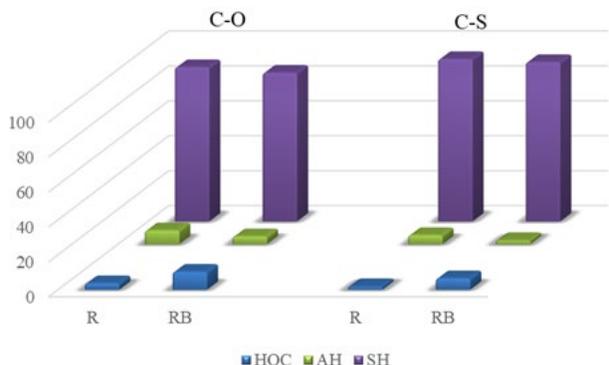


Fig. 7. Distribution of saturated (SH) and aromatic (AH) hydrocarbons and heteroorganic compounds (HOC) in the products of chemical degradation of ether (C-O) and sulfide (C-S) bridges of resins of the original (R) and biodegraded (RB) oils.

The effect of biodegradation on the composition of bound fragments, as in the case of asphaltenes, is most pronounced for sulfur-bound and ether-bound alkanes, monoalkylbenzenes, and naphthalenes. The changes observed for saturated hydrocarbons are similar to their changes in the structure of asphaltenes (Fig. 8). Among alkanes, the relative content of compounds of normal structure decreases and the share of branched structures increases. The share of high-molecular compounds and the share of homologues with an even number of carbon atoms in the chain increase in the composition of *n*-alkanes (Fig. 8b and 8d).

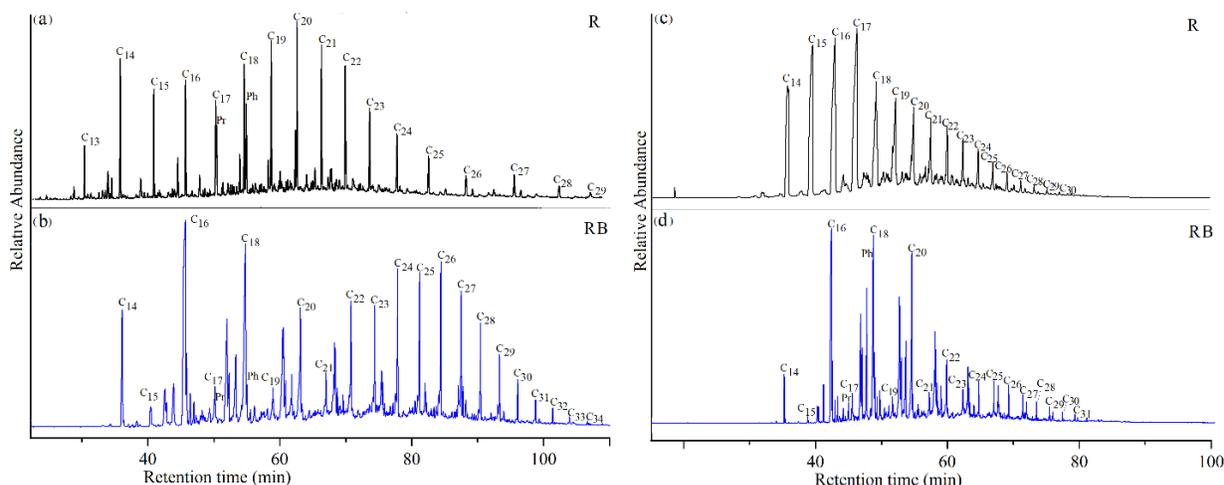


Fig. 8. Mass chromatogram of the products of cleavage of C-O bonds in R (a) and RB (b) molecules and C-S bonds in R (c) and RB (d) molecules at  $m/z$  71.

The nature of the change in the mass chromatograms of *n*-alkylbenzenes bound in resin macromolecules through oxygen and sulfur indicates that monoalkylbenzenes of resins, unlike those of asphaltenes, are subject to biooxidation in the entire range of the number of carbon atoms. Changes in the composition of bound naphthalenes in the structure of resins are similar to their changes in the structure of asphaltenes. The products of RB chemolysis contain no unsubstituted naphthalene and the relative content of C<sub>1</sub> and C<sub>2</sub> homologues is lower, while the share of tri- and tetraalkyl-substituted naphthalenes is higher. That is, in the case of resins, naphthalenes as the least stable compounds are also the first to undergo biodegradation.

#### 4. Conclusions

The direction of change in the relative content and composition of structural fragments of various types in the macromolecules of resins and asphaltenes of heavy oil from the Ashalchinskoye oilfield during its biooxidation by the native soil microflora has been revealed. It has been established that in the course of biodegradation the share of alkyl fragments decreases in the structure of resin-asphaltene substances, while the relative content of aromatic fragments and oxygen- and sulfur-containing structures increases. The biodegradation

of resins and asphaltenes is accompanied by a change in the qualitative and molecular composition of compounds bound in their structure through sulfide and ether bridges. The share of aromatic hydrocarbons decreases, while the relative content of heteroorganic compounds increases in compounds identified in the products of chemical degradation of biodegraded samples. The prevalence of saturated hydrocarbons, which are predominantly bound through oxygen in biodegraded asphaltenes and through sulfur in biodegraded resins, remains stable. In the course of biodegradation, the most noticeable are the changes in the molecular composition of bound *n*-alkanes, *n*-alkylbenzenes and naphthalenes.

Asphaltenes and resins of biodegraded oil are similar in the nature of changes observed for both types of bound alkanes and naphthalenes, but differ in the nature of changes in bound *n*-alkylbenzenes. In the composition of ether- and sulfur-bound alkanes of biomodified asphaltenes and resins, the content of linear hydrocarbons decreases, while the share of branched structures, macromolecular compounds, and homologues with an even number of carbon atoms increases. Among naphthalenes, the relative content of the least resistant to microbial oxidation C<sub>0</sub>, C<sub>1</sub>, and C<sub>2</sub> homologues decreases, while the share of C<sub>3</sub> and C<sub>4</sub> homologues increases. The nature of changes in the composition of *n*-alkylbenzenes of asphaltenes is similar to changes in the composition of bound *n*-alkanes. Biooxidation affects mainly low molecular weight *n*-alkylbenzenes, which results in an increase in the relative content of high molecular weight compounds. Monoalkylbenzenes of resins, unlike those of asphaltenes, are subject to biooxidation over the entire range of the number of alkyl carbon atoms. The revealed differences in the composition and structure of biodegraded asphaltenes and resins are most likely associated with structural features of the RAS macromolecules of the original oil and, as a result, with different degrees of bioavailability of the bound compounds.

### Acknowledgment

We thank Dr. Filatov DA. for conducting a laboratory experiment on oil biodegradation. This work was supported by the Ministry of Science and Higher Education of the Russian Federation (Registration Number 121031200185-6).

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