5th International Conference SCIENCE AND HIGHER EDUCATION IN FUNCTION OF SUSTAINABLE DEVELOPMENT



4th and 5th of October 2012, Uzice, Serbia

PROCEEDINGS

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About SED

The First Scientific Conference "Economy development and effectively business management - challenges of sustainable regional development" - was held upon the initiative of management of High Business Technical School of Uzice on the occasion of 30 years of working of High Business Technical School of Uzice. It was held on 20th and 21st of January, 2006, in Uzice, Republic of Serbia. Eighty papers were presented on the Conference. Number of authors and coauthors were 140.

The Second Scientific Conference "Higher Education and effectively business management- challenges of sustainable regional development" was held on 21st and 22nd of December, 2007, in Uzice, Republic of Serbia. More than 90 papers were presented. Number of authors and coauthors was 150.

After that, management of Business Technical College decided to organize, for the first time, an International Conference.

The First International Conference

The First International Conference "Science and Higher Education in Function of Sustainable Development" SED 2008 was held on 17-18. September, year 2008, in Užice, Serbia. Ninety papers were presented on the Conference. Number of authors and coauthors was 150.

The Second International Conference

The Second International Conference "Science and Higher Education in Function of Sustainable Development" SED 2009 was held on 14-15. September, year 2009, in Užice, Serbia. More than 80 papers were presented. Number of authors and co-authors exceeded 100.

The Third International Conference

The Third International Conference "Science and Higher Education in Function of Sustainable Development" SED 2010 was held on 7th and 8th of October, 2010, in Uzice, Serbia. One hundred and twenty one (121) paper were presented, while one hundred and ninety five (195) authors and co-authors took part in the Conference.

The Fourth International Conference

The Fourth International Conference "Science and Higher Education in Function of Sustainable Development" SED 2011 was held on 7th and 8th of October, 2011, in Uzice, Serbia. One hundred and fourty five (145) papers were presented, while two hundred and fourty three (243) authors and co-authors took part in the Conference.

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THE ROLE OF *n*-ALKANES IN THE INVESTIGATION OF PETROLEUM POLLUTANT FATE IN THE ENVIRONMENT

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Summary: In most cases, <u>n</u>-alkanes are the most abundant organic compounds in the petroleum. It is well known that parameters calculated on the basis of <u>n</u>-alkane abundances and distributions in saturated hydrocarbon fractions are very useful tool for estimation of source and geological history of crude oils in organic geochemical studies. However, these parameters are also unavoidable in the environmental chemistry. Firstly, they can be used for the identification of oil type pollutant in the environment and for evaluation of its biodegradation level in soil, sediments and groundwaters and surface waters. In bioremediation studies changes of <u>n</u>-alkanes during experiments have shown microbial efficacy and help in define the best conditions. This review is focused on these hydrocarbons and their role in the investigation of petroleum pollutant fate in the environment and bioremediation experiments.

Keywords: n-alkanes, petroleum-type pollutants, identification, biodegradation, bioremediation

1. INTRODUCTION

Studies on the environmental fate of petroleum-type pollutants remain to be an actual scientific interdisciplinary problem [as an example: 1-3]. It can be monitored the most accurately by determining its quantity and studying its composition in the polluted samples from the same or close locality, and during different periods of time. The role of n-alkanes, as the most abundant organic compounds in most petroleums, in these studies is very important.

For the purpose of identification of oil type pollutants in recent sediments it is necessary to possess reliable for a precise distinction between the anthropogenic and native compounds, including exact knowledge on the type and composition of autochthonous organic matter. Organic geochemical investigations have shown that the main constituents of recent sediments' organic matter are fulvic acids, humic acids and humin, i.e. compounds insoluble in commonly used solvents. The soluble organic matter, bitumen, is present only in minor amounts [as an example: 4,5]. However, since the range of native bitumen content is not exactly defined, neither in recent nor in other sedimentary formations, the quantification of hydrocarbons is not sufficient for detecting pollution in recent sediments. Especially if minor amounts are present, both a natural, as well as an anthropogenic (oil derived) origin of the soluble organic matter can be anticipated. In our recent paper the aim was to determine the origin of soluble organic matter, i.e. to identify oil-type pollution, on the basis of *n*-alkane abundance and distribution patterns in the alkane fractions (determined by gas chromatograph, GC).

Bearing in mind the fact that oil type n-alkanes in crude oil reservoir rocks are extremely susceptible to microbiological degradation [6,7], we have performed further investigations of the fate of these pollutants in the environment, this time assuming that their abundance could lead to conclusions about their biodegradation in soils and alluvial formation and about microbial efficacy and the best conditions during bioremediation experiments.

2. THE ROLE OF *n*-ALKANES

2.1. *n*-Alkanes as a tool for identification of oil type pollution

An example of typical petroleum *n*-alkane distribution (in the form of a gas chromatogram of sample II representing a petroleum-type pollutant found in underground water from the area of Pančevo Oil Refinery), and several examples of distributions characteristic of organic substance derived from recent sediments, typical for all other samples (B2, B4, B6, B8, B10, B11, D1 and D2) originating from different localities of the River Danube alluvial formations, are shown in Figure 1 [8]. *n*-Alkane fraction in sample I1 was characterized by uniform distribution of odd and even homologues and a maximum at a lower member (n-C₁₉). Carbon preference index (CPI) a ratio of the sum of odd-numbered hydrocarbons to the sum of even-numbered hydrocarbons was around 1. On the other hand, the distribution of *n*-alkanes in nonpolluted sediments was characterized by domination of odd homologues (CPI considerably above 1) and a maximum at some of the higher homologue members (n-C₂₉ or n-C₃₁). The soluble sedimentary organic substance, called bitumen, was generally found in very small amounts. Its higher content in sediments may therefore indicate the presence of anthropogenic contaminants. The conclusion is that *n*-alkanes may successfully be used for differentiating the petroleum-type pollutants, as anthropogenic organic substances, from native organic matter in recent sedimentary formations.



Figure 1. Distributions of *n*-alkanes and $\delta^{13}C_{PDB}$ values of individual *n*-alkanes of recent sediments' bitumen fractions and one oil type pollutant from Pančevo Oil Refinery locality; Pr: Pristane; Phyt: Phytane [8].

An alternative method to solve the problem may be $\delta^{13}C$ analysis. Being a mature organic substance in the geosphere, crude oil contains the greatest amount of heavier carbon isotope, ¹³C. Consequently, the ratio of ¹²C and ¹³C carbon isotopes $\delta^{13}C_{PDB}$ in petroleum is less negative compared to organic matter in recent sediments. For example, the sample I1 presented in Figure 1 showed less negative $\delta^{13}C_{PDB}$ with C₂₅, C₂₇, C₂₉ and C₃₁ *n*-alkanes compared to all other samples. As mentioned above, this particular sample was characterized by a typical petroleum *n*-alkane distribution, in contrast to all other samples demonstrating distributions typical for recent sediments. Hence, it is suggested that environmental native and anthropogenic organic substances may be differentiated based on comparison of their carbon isotopes ratios – in other words, $\delta^{13}C_{PDB}$ may be used as a tool for revealing petroleum-type pollutants in the environment.

2. 2. *n*-Alkanes as a tool for estimation of the biodegradation of oil-type pollutants in alluvial sediments

The process of biodegradation of petroleum-type pollutants in underground waters from Danube alluvial sediments (the locality of Pančevo Oil Refinery) was followed through a period from November 1997 to February 2000 [9,10].

Saturated hydrocarbon fractions from 5 investigated oil samples were isolated again by column chromatography. Distributions of *n*-alkanes and and isoprenoids C_{19} , pristane (Pr) and C_{20} , phytane (Phyt) in these fractions were defined by GC (Figure 2).

In the period from November 1997, when the first sample was taken, to February 2000, when the fifth sample was taken, important changes of the chemical composition were obvious (especialy in well I). Relative contribution of *n*-alkanes as compared to pristane and phytane in sample I*a* indicated changes defined as "initial petroleum biodegradation". Gas chromatogram of sample I*b* showed that in the period from November 1997 to May next year the amount of *n*-alkanes relative to isoprenoids was reduced, a phenomenon typical for biodegradation intensity in geochemical literature defined as "very slight" or "minimal biodegradation". Later, in September 1998 (sample Ic), the amount of *n*-alkanes was still smaller. Finally, during next one year, *n*-alkanes

were almost completely degraded (sample Id). In a relative short period of time, from September 1999 to February 2000, the alkane fraction of the petroleum-type pollutant suffered an unexpected change (sample Ie, Figure 1). Namely, in this fraction of the pollutant new even carbon-number C_{16} to C_{30} *n*-alkanes were observed. It was supposed that these even carbon-number *n*-alkanes were biosynthesized by some microorganisms (*Desulfovibrio desulfuricans, Corynebacterium sp., Escherichia coli, Rhizopus stolonifer* or *Penicillium sp.* [9,10].



Figure 2: GC analyses of alkanes from oil polluted alluvial ground waters (Pančevo locality, I and II wells): November 1997 (a), May 1998 (b), September 1998 (c), September 1999 (d) and in February 2000 (samples e) [9,10].

2. 3. *n*-Alkanes as a tool for estimation of the bioremediation efficiency

The bioremediation potential of zymogenous microorganisms isolated from soil was investigated under controlled laboratory conditions using a mixture of paraffinic types of oils as a substrate [11]. The ability and efficiency of these microorganisms in crude oil bioremediation was assessed by comparing the composition of samples which were exposed to the microorganisms with a control sample which was prepared and treated in the same way, but containing no microorganisms. Biodegradation was stopped by sterilization at 120 °C for 25 min, after which samples were taken on days 15, 30, 45, 60, and 75 days, while the control experiment was sampled only after 75 days and hydrocarbons were analyzed by the GC techniques.

The GC of the hydrocarbon fraction from the control and treated samples are shown in Figure 3. The dominant compounds in the hydrocarbon fraction of the control sample were n-alkanes and the isoprenoids pristane and phytane. These preliminary analyses showed a gradual decrease in the amount of n-alkanes and isoprenoids during 45 days. After 60 days of the experiment, n-alkanes and isoprenoids could not be observed in chromatograms indicating their possible complete degradation. At the end of the study, chromatograms were dominated by sterane biomarkers as the most abundant compounds in the fraction of hydrocarbons. The dominant compounds in the hydrocarbon fraction of the control sample were n-alkanes and the isoprenoids pristane and phytane.

According to GC analysis by the end of the study, after 75 days of exposure to the microorganisms, the *n*-alkanes and isoprenoids had been completely degraded.



Figure 3. GC Chromatograms of the hydrocarbon fractions isolated from the extracts of the control sample and from the samples during the biodegradation experiment after 15, 30, 45, 60, and 75 days; Pr: Pristane; Phyt: Phytane; Sq: squalane [11].

3. CONCLUSION

Abundance and distribution of *n*-alkanes in saturated hydrocarbon fractions are very useful tool in identification oil pollution in environment and in studying the fate of petroleum type pollutants in the environment and procedures for its removal in process of bioremediation.

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