*J. Serb. Chem. Soc.* 66(2)95-99(2001) UDC 622.337:623.458.1
*JSCS – 2836* *Short communication*

SHORT COMMUNICATION

# Evidence of stability of sedimentary organic matter during bacterial desilicification of an oil shale

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(Received 11 October 2000)

Aleksinac oil shale organic matter appeared to remain unchanged, according to elemental, IR, P-GC and P-GC-MS analytical characterization, after exposure to *Bacillus circulans*-Jordan desilicification for 30 days. These experiments indicate that "siliceous bacteria" may have potential as an alternative, "biochemical agent" for the isolation of native kerogen, and justify further efforts toward continuedevaluation of this advantageous process.

*Keywords*: oil shale, Aleksinac shale, organic matter stability, bacterial desilicification, *Bacillus circulans*-Jordan, kerogen preparation.

## INTRODUCTION

Investigation of the chemical composition and structure of kerogen requires its isolation from sedimentary mineral matter. Due to large proportions of carbonates, silicates and pyrite in kerogen-containing sediment, the isolation of native kerogen is a difficult task. Chemical reagents, commonly used for this purpose, attack and compositionally alter the kerogen. Therefore, depyritization by the chemolithoautotrophic bacterium *Thiobacillus ferrooxidans*,[1,2] and desilicification by the chemoorganoheterotrophic bacterium *Bacillus circulans*[3,4] were proposed as alternative methods. These approaches avoid the use of chemical reagents, solving the problem of pyrite elimination. In addition, they directly address the particularly difficult problem of removing the silicates. Experimental evidence shows that kerogen remains unchanged during bacterial depyritization.[5] However, the question of kerogen stability during bacterial desilicification remains unanswered.

The latter problem is addressed in this paper, through the use of elemental, IR, P-GC and P-GC-MS analyses to examine the organic matter of Aleksinac oil shale prior to and after bacterial treatment.

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## EXPERIMENTAL

The Aleksinac oil shale sample (Serbia, Yugoslavia) was a composite of proportional quantities of the 365.1–390.1 m interval of core BS-14. The composite was homogenized and powdered to < 100 m. A dilute hydrochloric acid concentrate of the composite was used as the reference sample. Standard analytical methods were used for the characterization of the organic matter in the reference and bacterially treated samples,[6] as well as for the analysis of the ash, which served as a basis for evaluating the desilicification efficiency.

An activated culture of *Bacillus circulans*, Jordan, obtained from the Center of Microbiology, Sofia, Bulgaria, adapted to modified Ashby's medium, was used as the inoculum in the desilicification experiments, which were run on a rotary shaker for 30 days at 35 ºC, with reseeding every third day. After 30 days the substrate was finally separated by centrifugation and treated with dilute HCl, rinsed with distilled water and dried at 80 ºC.

Elemental organic analysis (C, H, N, S) was carried out by standard microanalytical methods. Infrared analyses were done using a Perkin-Elmer spectrometer, Fourier-Transform 1750X, range 4000–400 $cm^{-1}$ (KBr 1:100 pellet). Pyrolysis-gas chromatography-mass spectrometry (P-GC-MS) analysis was conducted using a high performance flow programmable thermal extraction-pyrolysis injector mounted on a Hewlett-Packard quadrupole mass spectrometer (volatile organics were purged at 320 ºC for 5 min; pyrolysis was carried out by heating the sample at 100 ºC/min to 600 ºC; the pyrolysis products were cryogenically focused before separation with a low bleed capillary column, and ionization and detection in the mass spectrometer).

## RESULTS AND DISCUSSION

To determine whether the composition and structure of the sedimentary organic matter had changed during bacterial desilicification with *Bacillus circulans*, the properties of the organic material in the reference sample were compared with the properties of the same material in the bacterially treated sample. The composition of the reference sample, *i.e.,* the HCl-concentrate of Aleksinac oil shale composite BS-14, is shown in Table I.

TABLE I. Composition and intensity ratios of certain functional group bands in the IR-spectra of the reference and bacterially treated samples

| | Reference sample | Bacterially treated sample |
|---|---|---|
| Atomic ratios | | |
| H/C | 1.79 | 1.75 |
| $O+S_{org}/C$ | 0.18 | 0.26 |
| Ash/% | 53.8 | 43.4 |
| Content of ash components, relative to the reference sample/% | | |
| $Al_2O_3$ | 7.8 | 6.2 |
| $SiO_2$ | 38.6 | 29.0 |
| $Fe_2O_3$ | 4.8 | 3.6 |
| IR-band intensity ratios of selected functional groups | | |
| CH/>C=O | 2.19 | 2.25 |
| CH/OH | 3.34 | 3.60 |
| >C=O/OH | 1.53 | 1.60 |

Desilicification experiments, which consisted of leaching for 30-days with *Bacillus circulans*-Jordan in a modified Ashby's medium, were repeated four times. In all of these experiments, a progressive decrease in the pH was observed, indicating that organic acids, responsible for the desilicification,[7] were constantly being produced. In spite of this, the leaching effect, expressed *via* the sum of $SiO_2$, $Al_2O_3$ and $Fe_2O_3$ relative to the contents of the same oxides in the reference sample, was not 100 % efficient. This suggests that the leaching effect depends on the type of mineral components in the shale sample, as well as on the relatively low desilicification efficiency of this particular *Bacillus circulans* strain. It is important to note, however, that the aim of this paper was not to study the desilicification process itself. Rather, we wish to examine the stability of the sedimentary organic matter towards the *Bacillus circulans* strain, and thus show whether further efforts aimed at enhancing this proposed desilicification process are justified. We considered a comparison of the composition and structure of the organic matter prior to and after a 30-day exposure to *Bacillus circulans* to be a sound basis for checking the kerogen stability during *Bacillus circulans* desilicification. The product resulting from the most efficient desilicification experiment (elimination of 24.3 % of the $SiO_2 + Al_2O_3 + Fe_2O_3$ relative to reference sample) was used for further examination.

As the desilicification efficiency of the used *Bacillus circulans*-Jordan strain was still rather low ( 25 %), and our main motive in demineralizing the sediment was to remove the mineral matter interfering with the organic matter analysis, the relatively high remaining mineral matter content implied that elemental analysis may not be the most efficient method for checking the stability of sedimentary organic matter during bacterial desilicification. This fact must be considered when evaluating the elemental analysis results.

Atomic H/C ratios of the organic matter prior to and after 30-day exposure to *Bacillus circulans* (Table I) differed only slightly, suggesting that the composition of the organic matter during bacterial desilicification remained stable with respect to this ratio. In contrast, the $O+S_{org}/C$ ratio of the organic matter from the bacterially treated sample was somewhat higher than that of the initial sample. These results suggest that further investigation of the real source of the observed difference is needed, as are further checks on the reliability of elemental analysis of samples containing high concentrations of mineral components.[8]

The stability of the sedimentary organic matter during bacterial treatment was also monitored by infrared spectroscopy. The spectra of the substrates are complex, and direct correlation is inconclusive. In this work, the ratios of quantitative estimates of the absorption bands of certain functional groups, representative of the structures of specific sedimentary organic matter, were taken as the basis for the evaluation of structural changes caused by bacterial desilicification (Table I). In summary, the IR data do not suggest dramatic structural changes resulting from the 30-day bacterial treatment.

P-GC and P-GC-MS analyses were also completed for our sample set. The total ion chromatogram of the bacterially treated sub-sample is not significantly different from that of the untreated sample with respect to the straight-chain hydrocarbon components (*n*-alkanes). Moreover, the pyrogram of the bacterially treated sample shows a bimodal distribution in the aliphatic hydrocarbon homologous series (*n*-$C_7$–$C_{35}$), as does the pyrogram of the reference sample. In addition, P-GC-MS analyses for the same sample pair showed no substantial changes resulting from bacterial treatment

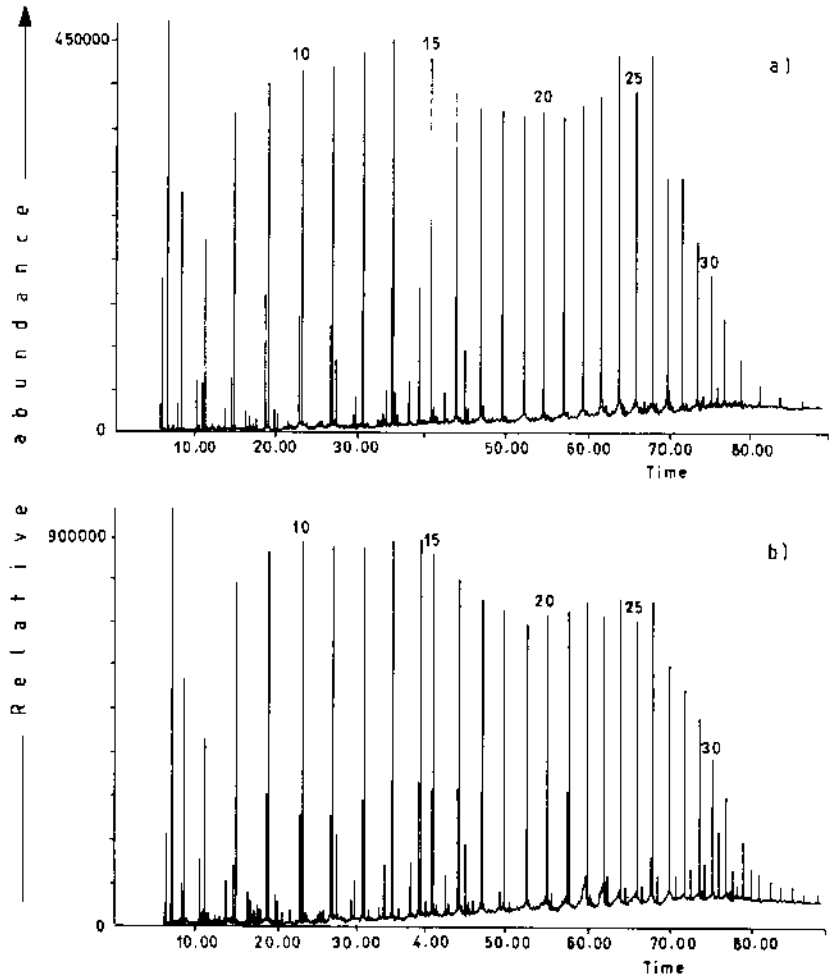


Fig. 1. P-GC-MS (*m*/*z* 191.18 and 217.19) mass chromatograms of the reference and bacterially treated samples.

(Fig. 1). The pyrolyzates were very similar, and only minor variations were observed in the relative peak intensities. All major components were present in both pyrolyzates. In conclusion, our observations suggest only minor modifications of the kerogen due to bacterial treatment.

Further studies on bacterial preparation of silicate-free organic matter concentrates from oil shales, combined with improvements in the efficiency of the process, should lead to more reliable and accurate compositional conclusions concerning organic matter in general and kerogen concentrates in particular.

И З В О Д

## ДОКАЗ О ПОСТОЈАНОСТИ СЕДИМЕНТНЕ ОРГАНСКЕ СУПСТАНЦЕ ПРИ БАКТЕРИЈСКОЈ ДЕСИЛИЦИФИКАЦИЈИ ЈЕДНОГ УЗОРКА БИТУМИНОЗНОГ ШКРИЉЦА

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На основу елементарне, инфрацрвене и пиролитичко-гаснохроматографско-масеноспектрометријске анализе пре и после 30-дневне обраде помоћу *Bacillus circulans*-Jordan, указано је на релативно добру постојаност органске супстанце алексиначког битуминозног шкриљца при бактеријској десилицификацији. Чињеница да се седиментна органска супстанца при овој бактеријској обради није битније мењала оправдава даље напоре ка побољшању ефикасности бактеријског десилицификационог процеса.

(Примљено 11. октобра 2000)

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