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PARTICIPATION OF SULPHATE-REDUCING BACTERIA IN BIODEGRADATION OF ORGANIC MATTER IN SOILS CONTAMINATED WITH PETROLEUM PRODUCTS

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UDZIAŁ BAKTERII REDUKUJĄCYCH SIARCZANY W BIODEGRADACJI MATERII ORGANICZNEJ W GLEBACH SKAŻONYCH PRODUKTAMI ROPOPOCHODNYMI

W pracy badano udział bakterii redukujących siarczany (BRS) w biodegradacji materii organicznej. Mikroorganizmy izolowano z gleby poligonu wojskowego oraz z terenu rafinerii. Wyizolowane zespoły mikroorganizmów były zdolne do biodegradacji związków małocząsteczkowych będących produktami biodegradacji związków polimerowych występujących w środowisku. Stwierdzono, że udział BRS w biodegradacji związków organicznych w glebie był porównywalny z udziałem w biodegradacji materii organicznej w osadach morskich (40–55%).

Summary

Biodegradation of organic matter by sulphate-reducing bacteria (SRB) isolated from soil from military testing ground and petroleum plants were investigated. The isolated microorganisms utilized low molecular weight compounds and participation of SRB in biodegradation of these compounds was similar in marine sediments and in soil (40–55%).

INTRODUCTION

Sulphate-reducing bacteria (SRB) play a remarkable role in environments where oxygen is absent. They can utilize considerable amounts of organic compounds simultaneously using sulphates as the ultimate acceptors of hydrogen in the respiratory chain. These bacteria are encountered in anaerobic environments containing sulphate and organic compounds. They occur in soil, fresh water and marine sediments, hot springs and geothermal sites, crude oil and natural gas intakes [2]. However, the most characteristic environments where the SRB exist are marine sediments [5], where the concentration of sulphates is about 28 mM [10], oil fields and petroleum reservoirs [7] and environments polluted with petroleum products [11].

Since SRB are heterotrophs using sulphates, the presence of organic compounds and oxidized sulphur compounds is the main factor responsible for the rate of reduction of sulphates in both natural and anthropogenic environments. In anaerobic ecosystems, the process of mineralization of organic matter is usually more complex than in aerobic conditions, and requires the cooperation of different groups of microorganisms. Every group of microorganisms is responsible for a specific stage of oxidation of the substrates, and the ultimate products are metabolized by the next group of bacteria.

Organic substances occurring in water after death of plants are decomposed by extracellular enzymes, which are produced by microorganisms. As a result, proteins and their derivatives, saccharids, lipids, tannins, organic acids, as well as lignin, cellulose, hemicellulose, waxes and other substances appear in surface waters. Most of these compounds are assimilated and processed to obtain energy and cellular building agents. They are also used as secondary substrates for further decomposition and mineralization by microorganisms [6].

SRB take part in the terminal stage of organic matter mineralization, what results from the fact that they do not produce hydrolytic enzymes. SRB use intermediate products of biodegradation of polymeric compounds such as lactates, acetates, propionaians, butyrates, ethanols and alanines as the source of carbon (Fig. 1).

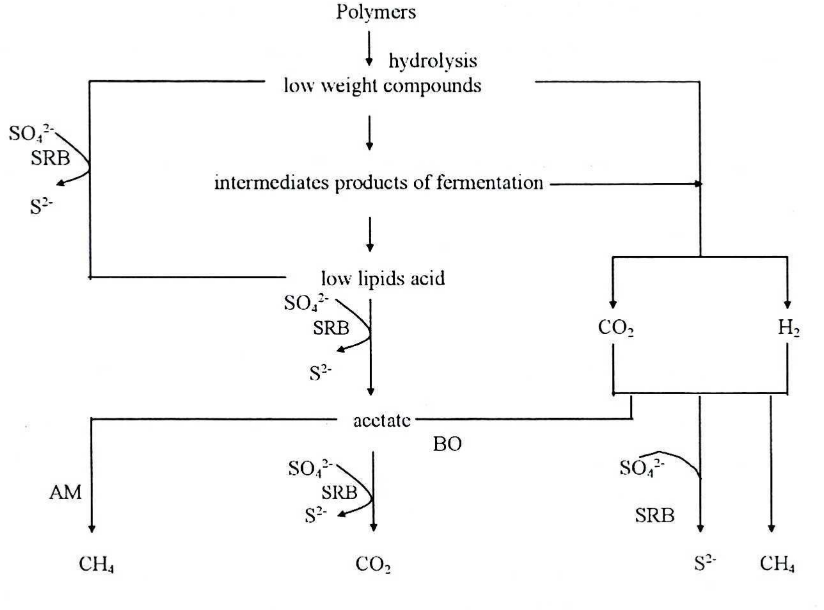

Fig. 1. Biodegradation of organic matter in anaerobic condition; SRB – sulphate reducing bacteria, AM – methanogenic archeon, BO – acetogenic bacteria

It is assumed that in near shore marine deposits the SRB take part in over 50% of organic matter [4]. SRB play a crucial role in the biogeochemical sulphur cycle, and are an important element of sulphur transformation of deposits of lakes, seas and oceans, as well as in beds of sedimentary rocks. In all cases these microorganisms occur only in anaerobic conditions, and their abundance is the largest in the surface parts of deposits (10^5–10^8 cells/cm^3). There are only a few papers focused on the role and participation of SRB in the biodegradation of organic compounds in soil, particularly in the environments polluted by oil-derived products.

The aim of this study was to assess the SRB participation in biodegradation of organic matter in soils contaminated with oil – derived products.

MATERIALS AND METHODS

Microorganisms

The microorganisms were multiplied from two study areas polluted by oil derived products: military testing ground (environment A) and a petroleum plant (environment B). Soils from military testing ground had been contaminated for about 30 years and from a petroleum plant about 10 years. Samples of soil were taken from the depth of 70 cm.

Media

Modified Postgate medium (Tab. 1).

Table 1. Composition of modified Postgate medium [g/dm^3]

S-SO_4	Ca	Fe	Al	Mg	Sr	Ba	Na	K	P	Mn	Ti
2.58	1.0	0.025	0.005	0.007	0.065	0.002	0.081	0.148	0.162	0.0002	0.002

This medium was supplemented with lactate (7.9 g/dm^3), ethanol (3 cm^3/dm^3), acetate (3.8 g/dm^3), casein (2.7 g/dm^3), lactose (3 g/dm^3) or phenol (1.5 g/dm^3) as the sole carbon source. Reazurin in the concentration of 0.001 g/dm^3 was added to all cultures as the indicator of redox conditions in the medium.

Culture conditions

Cultures were set up in 300 cm^3 bottles in anaerobic condition. The ratio of inoculum to medium was 1:10. The cultures were incubated in thermostatic room at 30°C or/and in a thermostat at 55°C.

Isolation of microorganisms

The soil microorganisms were multiplied using the "microcosms" method and enrichment cultures. The method of "microcosms" – soils from the study sites were placed in 100 cm^3 containers together with medium enriched with various carbon sources. The containers were tightly closed and incubated for 6 weeks at 30 or 55°C to select sulphidogenic communities of microorganisms capable of biodegradation of applied sources of carbon. Enrichment of cultures – soil was placed in 300 cm^3 glass bottles, which after the addition of appropriate medium were tightly closed. The ratio of soil to medium was 1:10.

Measurements

Sulphides in the cultures were determined using the iodometric method, sulphates – the hot barium method, COD – the dichromate method [3], pH – by a pH-meter.

Calculations

When calculating the results the following conversion factor was used for SRB – reduction of 1 mole SO_4 1 mole H_2S [9], $COD_{used}/SO_{4\ red}$ [mg/mg] = 0.67. Based on the maximal content of HS^- in the cultures and the total COD value and utilized COD value in cultures, the theoretical activity of SRB in the process of biodegradation of organic matter by SRB has been calculated.

All measurements were made three times, and in calculations the means were used.

RESULTS AND DISCUSSION

A total of 48 cultures were established of which each 24 were incubated at 30°C and/or 55°C, respectively. After 18 days blackening and the characteristic odor of hydrogen sulphide was noted only in 4 cultures incubated at 30°C on media with lactate, ethanol, casein and lactose.

During isolation only four active sulfidogenic bacterial communities were obtained, what is rather surprising. The number of all isolated communities is only 2% of the initial cultures. Mesophilic cultures prevail. The activity of SRB was not detected in cultures incubated at 55°C.

Lack of active termophilic SRB may be linked with the fact that the entire selection process took place at 30°C, what is optimal for the SRB. The number of so far isolated mesophilic communities or tribes of SRB is higher than that of thermopiles cultures. Among the applied carbon sources, a high activity of SRB communities was observed on media with ethanol, lactate, casein and/or lactose. No community was grown on a medium with acetate or phenol as the sole carbon source. More communities (3) were isolated from environment B than from environment A (Fig. 2). Most probably the activity and number of isolated communities depends on the age of pollution. This assumption, however, requires further studies.

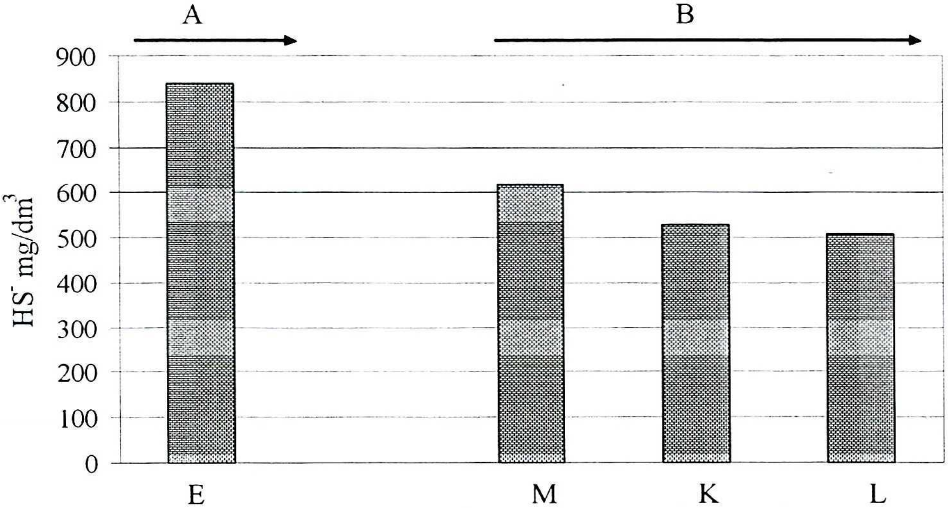

Fig. 2. Maximal content of sulphides obtained in anaerobic cultures of bacterial communities; the letters refer to the source of carbon in the medium: E – ethanol, M – lactate, K – casein and L – lactose

Next, the isolated microorganisms communities were passaged on a Postgate medium with different carbon sources. During incubation the concentration of sulphides was measured in the cultures (Table 2).

Table 2. Maximal content of HS^- [mg/dm^3] after incubation on a Postgate medium with different sources of carbon

Environment of isolation →	Military testing ground	Soil from petroleum plant		
Source of carbon	ethanol	lactate	casein	lactose
ethanol	637	461	391	478
lactate	272	376	366	383
phenol	314	461	392	384
acetate	242	150	188	208
lactose	348	257	286	383
casein	365	435	453	351

The most active bacterial community was isolated from soil from the military testing ground and multiplied on medium with ethanol as the sole carbon source. The highest concentration of sulphides noted in this culture reached 637 mg/dm^3.

The domination of a particular group of microorganisms in a given environment depends mainly on the COD/SO_4 ratio. If in the medium 1 g of sulphates is correlated with 0.67 g or less COD, then according to the stoichiometry of the reduction process, the bulk of organic compounds can be mineralized to CO_2 and H_2S [1, 2, 7]. When the value of this ratio is higher, more organic compounds are decomposed during metanogenesis. At $ChZT/SO_4 > 10$ sulphates are not produced [8]. It seems that the coexistence of the two groups of microorganisms is possible at $ChZT/SO_4$ in the range from 1.7 to 2.7 mg, whereas below 1.7 mg the SRB prevail [1].

At the known content of SO_4 in the cultures (2580 mg/dm^3) and value of COD (3000 mg/dm^3), the ratio COD/SO_4 can be calculated; the grown cultures showed the value of 1.16. Thus, conditions favorable for the selection of SRB have been created in the cultures.

In all cultures of sulphidogenic consortia, apart from the active reduction of sulphates, the distinct utilization of organic compounds has also been detected. The highest percentage content of SRB in the biodegradation of organic compounds has been noted in cultures of microorganisms isolated from the area of the petroleum plant and multiplied on a medium with lactate. The SRB content was *ca.* 60%. In the remaining cultures the mean values were similar and reached *ca.* 30% (Fig. 3).

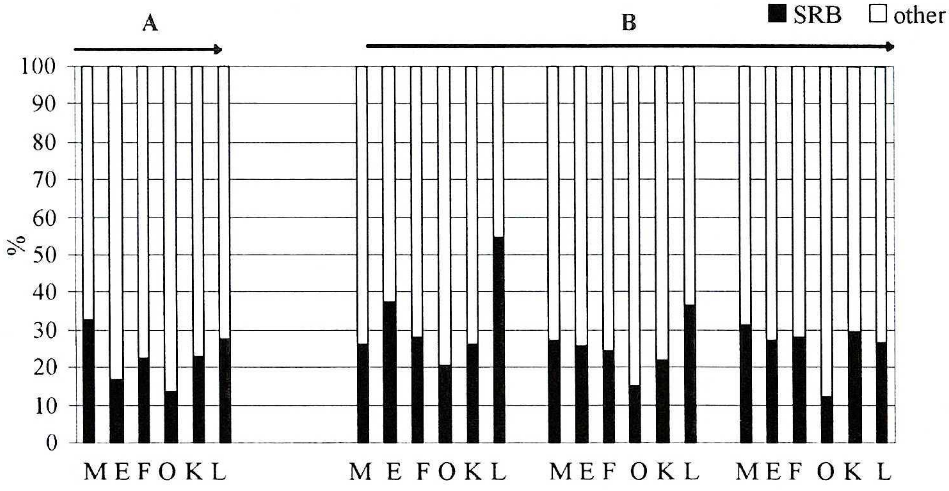

Fig. 3. Reduction of COD by bacterial community; the letters refer to the source of carbon in the medium: M – lactate, E – ethanol, F – phenol, O – acetate, K – casein, L – lactose and environments A – military testing ground, B – soil from petroleum plant

This paper presents preliminary studies focused on the activity of anaerobic communities of microorganisms in the biodegradation of organic matter. This topic is of great importance from both the theoretical as well as practical point of view.

CONCLUSIONS

- Sulphate-reducing bacteria take part in the ultimate stage of organic matter decomposition in conditions rich in oxygenated sulphur compounds and organic matter.
- The content of sulphate-reducing bacteria in the biodegradation of organic matter in soils is similar to that marine deposit, what points to the important role of these bacteria also in soils contaminated by petroleum.

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