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RHAMNOLIPID-ENHANCED ELUTION OF ORGANIC COMPOUNDS FROM THE CREOSOTE-CONTAMINATED SOIL

Summary

Rhamnolipids are a group of biosurfactants produced by microorganisms, which are considered to be an eco-friendly enhancing agents in bioremediation of the soils. Here, using creosote-contaminated soil, we investigate the effectiveness of rhamnolipids aqueous solutions (at different concentrations) on removal of polar and non-polar contaminants from the soil matrix. The obtained results show that rhamnolipids at higher concentrations could be more effective as extraction agents compared to the organic solvent – dichloromethane. The highest overall concentration of creosote components (212.3 μg mL^{-1}) was detected in aqueous solution of rhamnolipids at concentration equal to 10 CMC. This was achieved mainly because of the high efficiency of this variant to elute both polar (e.g. phenols) and non-polar (e.g. PAHs) compounds. Therefore, aqueous solution of rhamnolipids at concentration of 10 CMC may be recommended for the remediation of areas contaminated by numerous structurally different (i.e. polar and non-polar) chemical compounds, as in the case of the studied creosote-polluted soil.
***Key words**: rhamnolipids, creosote, extraction, GC-MS, PAHs*

ELUCJA ZWIĄZKÓW ORGANICZNYCH Z GLEBY ZANIECZYSZCZONEJ KREOZYTEM WSPOMAGANA RAMNOLIPIDAMI

Streszczenie

Ramnolipidy są grupą biosurfaktantów (związków powierzchniowo czynnych pochodzenia mikrobiologicznego), które coraz powszechniej badane są w kontekście ich użyteczności w bioremediacji środowiska. W powyższym artykule zbadano wpływ wodnych roztworów ramnolipidów (w różnych stężeniach) na wypłukiwanie polarnych oraz niepolarnych związków organicznych z gleby skażonej kreozotem. Otrzymane rezultaty wskazują, że wodne roztwory ramnolipidów w wysokich stężeniach mogą być bardziej efektywnym rozwiązaniem niż użycie rozpuszczalnika organicznego – dichlorometanu. Analiza GC-MS wskazała, że najwyższe całkowite stężenie składników kreozotu (212.3 μg mL^{-1}) odnotowano po wypłukiwaniu gleby wodnym roztworem ramnolipidów w stężeniu 10 CMC. Wynika to z faktu wysokiej wydajności ekstrakcji zarówno związków polarnych (np. fenoli), jak i niepolarnych (np. WWA) zaobserwowanej dla wspomnianego wariantu ekstrahenta. Wobec tego wodne roztwory ramnolipidów w stężeniu 10 CMC powinny być rozważane jako potencjalnie przydatne odczynniki używane w trakcie prowadzenia remediacji gleb skażonych różnorodnymi (pod względem struktury i polarności) związkami chemicznymi, podobnie jak w wypadku badanej gleby skażonej kreozotem.
***Słowa kluczowe**: ramnolipidy, kreozot, ekstrakcja, GC-MS, WWA*

1. Introduction

The widespread pollution of terrestrial environment by organic contaminants originated from motor fuels and other commercially available mixtures poses a threat to plants and animals inhabiting upper layers of the soil. The contamination of soil is crucial for humans, since organic xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs), may not only reduce crop yields in agriculture, but also penetrate (or deposit on) crop plants tissues and then become dangerous to human health due to dietary intake [14]. Creosote is a complex organic mixture, which may be obtained from the distillation of coal tar and consists of thousands of organic compounds, especially PAHs and phenols [6]. For many years creosote has been widely used as wood preservative to protect railroad ties and other outdoor wooden elements.

One of the ways to clean up contaminated soil consists in the use of physicochemical methods, such as leaching of the soil with the appropriate extracting agent. The use of organic solvents, such as dichloromethane or chloroform is efficient, but their high density and significant toxicity negates their application for remediation purposes. Surfactants are surface active chemical compounds, which increase the availability of hydrophobic compounds by their solubilization or by creating micro- and macroemulsions [8]. Thus water solutions of surfactants may be used to leach the soil and remove hydrophobic compounds. Since most of the synthetic surfactans are toxic to microorganisms, plants and animals, environmentalists are interested in the use of biosurfactants i.e. surfactants of microbial origin, which may be less toxic to soil organisms and easy biodegradable.

One of the examples of biosurfactans are rhamnolipids, which are studied by many authors due to their possible positive effect on bioremediation of hydrocarbon-contaminated areas [13]. It was confirmed that the removal efficiency of total petroleum hydrocarbon (TPH) from soil

was much higher in the presence of biosurfactants than in the presence of synthetic surfactants [7]. The previous literature reports also indicated a positive effect of rhamnolipids on the solubilization of petroleum hydrocarbons such as PAHs [10]. Other studies performed by Chrzanowski et al. [4] indicated that the presence of rhamnolipids may accumulate toxic organic compounds inside micelles and simultaneously decrease the negative effect of the contaminants on the environment. However, it is unknown, which group of chemical compounds could be readily extracted from the soil using rhamnolipids. To our best knowledge, there are no reports investigating the influence of rhamnolipids on the extraction efficiency and susceptibility of both polar and non-polar organic compounds to be removed from the creosote-contaminated soil.

The aim of the study was to investigate the leaching capability of water extracts of rhamnolipids to remove the organic compounds from the creosote-contaminated soil of Solec Kujawski (Poland). During the experiments rhamnolipids were used at different concentrations (ranging from 150 to 1500 mg L^{-1} of water, where 150 mg L^{-1} correspond to 1 CMC).

2. Materials and methods

2.1. Soil and rhamnolipids

Soil used in the experiment originated from the area of Solec Kujawski (Poland), (53°04'37.3"N 18°14'44.8"E) - the place permanently contaminated by creosote, used as wood preservation. More than 100-year activity of the company resulted in a significant contamination of the soil environment by creosote components. Additionally, commercial solution of high-purity rhamnolipids from AGAE Technologies (USA) was used in the research.

2.2. Experimental variants and extracts preparation

Depending on the variant, soil extracts were prepared as follows: 200 g of contaminated soil was supplemented by: (i) 1-L of tap water (hereafter named as 0 CMC); (ii) 1-L of tap water + rhamnolipids at concentration of 1 CMC (150 mg L^{-1}); (iii) 1-L of tap water + rhamnolipids at concentration of 5 CMC (750 mg L^{-1}); (iv) 1-L of tap water + rhamnolipids at concentration of 10 CMC (1500 mg L^{-1}). Next all soil systems were mixed using a mechanical stirrer (IKA T18 basic) for 60 min at 14 000 rpm and then filtered. Additional experimental variant was prepared to check the leaching efficiency with the use of organic solvent – dichloromethane (CH_2Cl_2).

2.3. Preparation of samples for GC-analysis

In order to determine the content of organic compounds in soil eluted by dichloromethane, the studied soil samples were prepared for GC-analysis in a following way: prior to extraction a 25 g portion of a field moist soil sample was dried by mixing with 10 g of anhydrous magnesium sulphate. The dried sample was then placed into a extraction cell of a Soxhlet device and subjected to extraction with dichloromethane for six hours. In the next step, an aliquot of the extract was dried with anhydrous magnesium sulphate, spiked with an aliquot of internal standards solution in acetone (d10-acenaphthene, d10-anthracene and d12-chrysene), diluted to suitable concentration and subjected to GC-analysis.

In order to determine the content of organic compounds in soil extracts obtained by the elution of the studied soil with appropriate solution of rhamnolipids, the samples were prepared for GC-analysis as follows: prior to extraction 200 ml of each sample was passed through a paper filter to remove any suspended matter. Then the pH of the samples was measured, and an aliquot of 50 ml was transferred into separatory funnel. Prior to LLE 15 g of NaCl was dissolved in the samples for salting-out of the analytes and to promote the phase separation. Next the sample was acidified to the pH 2 by addition of a 2 ml aliquot of 2,5M HCl and subjected to 4-step extraction with 5 ml aliquots of dichloromethane; the extracts obtained during each step were combined in a 50 ml volumetric flask. The pH of the sample was adjusted back to pH 7 by addition of a 2 ml aliquot of 2,5M NaOH and subjected to 1-step extraction with 5 ml aliquot of dichloromethane; the extract was combined with the extracts obtained during previous step. The sample was then alkalised to the pH 13 by addition of a 2 ml aliquot of 2.5M NaOH and subjected to 4-step extraction with 5 ml aliquots of dichloromethane; the extracts were combined with the extracts obtained during previous steps. The volume of the extract was made to 50 ml by addition of dichloromethane. 1 ml aliquot of the extract was transferred into GC vial and dried with anhydrous magnesium sulphate, spiked with an aliquot of internal standards solution and subjected to GC-analysis.

2.4. GC-analysis

For analysis of the soil contaminants comprehensive two-dimensional gas chromatography was employed. The GCxGC-TOF-MS system consisted of Agilent 7890 chromatograph equipped with cryogenic modulator and LECO Pegasus 4D TOF-MS spectrometer. The operating conditions were as follows: column setup – 1st dimension SGE BPX5, 30 m, 250 μm, 0.25 μm; 2nd dimension Restek RXI-17, 0.95 m (10 cm in the modulator, 74 cm in the secondary oven, 21 cm in the transfer line), 100 μm, 0.1 μm; PTV injection – splitless, initial temperature 50°C held for 6 s, than ramped to 300°C at 12°C/s and held for 5 min; separation conditions – carrier gas (helium) flow constant at 1 ml/min, main oven program: 45°C held for 0.3 min, than ramped to 280°C at 6°C/min and held for 20 min, secondary oven program: 55°C held for 1 min, than ramped to 305°C at 6°C/min, the modulator temperature was set 15°C above the temperature of the secondary oven; detection conditions - ion source temperature 250°C, ion source voltage 70 eV, solvent delay 290 s, acquisition range 33-300 m/z, acquisition rate 150 spectra/s.

For quantitative analysis of the samples, a set of model analytes, representing the main groups of creosote constituents, was selected, based on a literature review. These included *n*-alkanes, monoaromatic and polyaromatic hydrocarbons, phenols, as well as nitrogen-, sulphur and oxygen-heterocyclic aromatics. For these compounds 5-point calibration was performed against internal standards added both to the standard solutions and the sample extracts. The peaks of the other analytes were identified based on the library search and were semi-quantified based on the calibration curves obtained for the standard compounds of a similar structure. For this purpose the area of the peaks was calculated based on the deconvoluted TIC.

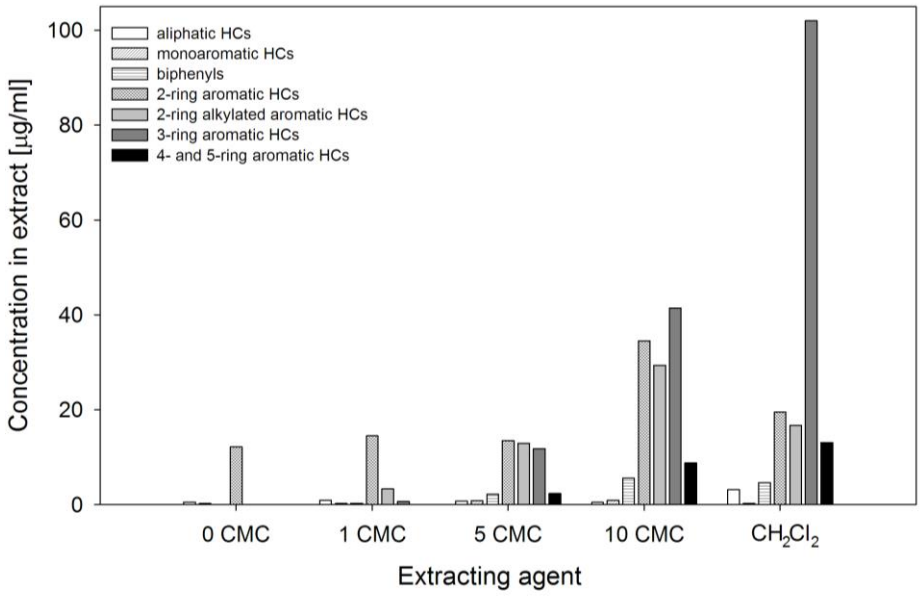
2.5. Statistical analysis

All analyses were replicated three times. Analysis of variance (ANOVA) was employed to statistically verify the obtained results. The calculations were performed using Statistica 6.0 software.

3. Results and discussion

Based on the GC analyses, more than one hundred creosote components were identified and classified into structurally similar groups. As can be seen in Fig. 1, the ability of rhamnolipids solutions to extract non-polar compounds (hydrocarbons) from the soil, depended on the applied biosurfactant concentration. We observed, that the higher concentration was, the more hydrocarbons were leached from the soil matrix. Satisfactory results were obtained for the highest used concentration of rhamnolipids equal to 10 CMC. It was observed, that for some hydrocarbons the extraction efficiency was even higher than in the case of dichloromethane (with exception of 3-, 4- and 5- ring aromatic hydrocarbons) – less polar solvent compared to aqueous solutions of rhamnolipids.

On the other hand, as can be seen in Fig. 2, the capability of rhamnolipids solutions to leach more polar compounds – especially N-heterocyclic aromatics and monoaromatic phenols – was higher in the case of lower concentrations of rhamnolipids (the best results were obtained for pure tap water).



Source: Own work / Źródło: opracowanie własne

Fig. 1. Concentrations of non-polar compounds (hydrocarbons) in extracts. CMC – critical micelle concentration, HCs – hydrocarbons, CH_2CL_2 – dichloromethane

Rys. 1. Stężenia niepolarnych związków (węglowodorów) w ekstraktach. CMC – krytyczne stężenie micelizacji, HCs – węglowodory, CH_2CL_2 – dichlorometan

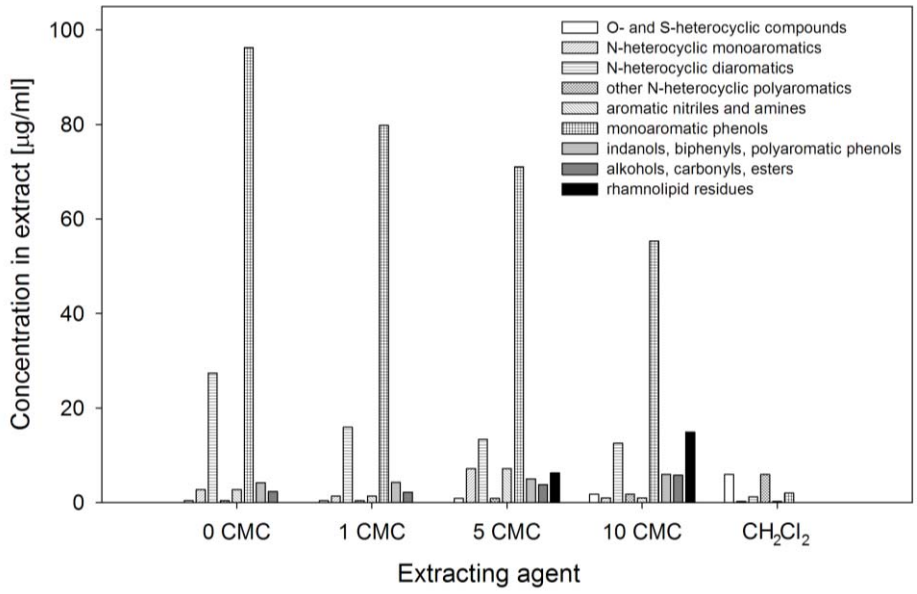


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Fig. 2. Concentrations of other, more polar compounds in extracts. CMC – critical micelle concentration, CH_2CL_2 – dichloromethane

Rys. 2. Stężenia pozostałych, bardziej polarnych związków w ekstraktach. CMC – krytyczne stężenie micelizacji, CH_2CL_2 – dichlorometan

Compared to the water, the extraction efficiency with the use of rhamnolipids at the highest concentration (10 CMC) was lower by 43% and 54% with respect to monoaromatic phenols and N-heterocyclic aromatics respectively. The extraction efficiency of dichloromethane to remove polar compounds from the soil was significantly lower – even compared to rhamnolipids at the highest used concentration (10 CMC).

Taking into account the overall leaching capability of the used extracting agents, the highest concentration of all extracted polar and non-polar compounds (212.3 μg mL^{-1}, ignoring rhamnolipds residues) was reached for the most concentrated rhamnolipids solution (10 CMC). In the case of other variants, the overall concentration in extract reached 152; 128.9; and 151.2 μg mL^{-1}, for 5 CMC, 1 CMC and pure tap water (0 CMC) respectively. The application of dichloromethane allowed for extraction of 171.9 μg mL^{-1} of all analyzed creosote components. Therefore, the solution of rhamnolipids at concentration of 10 CMC was the most efficient extraction agent for the cleaning up of the studied creosote-contaminated soil.

GC-MS results indicated that the main components of the studied creosote were PAHs, N-heterocyclic diaromatics and monoaromatic phenols. This observation corresponds well with the results obtained by Hartnik et al. [6], who showed a high content of PAHs in creosote-contaminated soil, but also demonstrated a dominance of polar compounds (such as phenols and heterocyclic compounds) in groundwater downstream from the contaminated area. The use of surfactants to remove hydrophobic organic compounds from the contaminated soil was widely studied by authors over the years. In general, our results are in agreement with other studies showing positive influence of surfactant (either synthetic surfactant or biosurfactant) addition on hydrocarbons removal from the experimentally contaminated soil [1, 3, 9]. Study performed by Lai et al. [7] and Scheibenbogen et al. [11] indicated that the leaching capability of rhamnolipids could be even higher than synthetic surfactants such as Tween 80 or Triton X-100.

Regarding the altered creosote-contaminated soil, most of the studies investigate the influence of synthetic surfactants (such as SDS or Triton X-100) addition on leaching of PAHs from the soil. Study performed by Deschênes et al. [5] showed that mobilization of PAHs in the presence of synthetic surfactants depends on the structure of analyzed PAH. On the other hand, Carriere and Mesania [2] indicated positive effect of surfactant addition on desorption of PAHs (compared to variants with buffered water solution only). However, our results demonstrate that the influence of surfactant on removal of more polar and hydrophilic compounds should also be considered, since some of them (especially monoaromatic phenols) are often dominant chemical groups in the composition of creosote. Although rhamnolipids and other biosurfactants are generally considered as "green surfactants", their eco-friendliness has still to be confirmed [12]. However, in the case of physiochemical remediation methods, not only the toxicity of the used extracting agent should be taken into account, but also its ability to recycle in the cleaning system.

4. Conclusions

Our study showed that the use of rhamnolipids aqueous solutions significantly promoted the elution of the hydrophobic compounds (especially PAHs) from the creosote-contaminated soil, but simultaneously reduced the elution of polar compounds such as phenols. However, among all the used variants, aqueous solution of rhamnolipids at concentration of 10 CMC was the most effective with respect to overall efficiency of extraction (considering both polar and non-polar components). Therefore, highly concentrated aqueous solutions of rhamnolipids may be recommended for the remediation of areas contaminated by both polar and non-polar organic compounds, as in the case of the studied creosote-polluted soil.

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