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Can microorganisms play a beneficial role in oil spill clean-up?

Czy mikroorganizmy mogą odgrywać korzystną role w usuwaniu wycieków ropy?

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Keywords: *oil spill, hydrocarbons, metabolism, enzymes, bacteria, consortium, bioremediation*

Abstract
Flourishing petroleum industry is the main cause of environmental pollution. According to the estimates, annually from 3 up to 6 million metric tons of crude oil are released into the world's water reservoirs due to oil seepage and spills. Supertanker accidents and oil rig disasters are the cause of major oil spills in the ocean, the oil slick while floating on the water surface can spread out by wind and currents to disrupt the ecosystem at long distances from the source of the spill. To limit the scale of environmental damage some oil spill clean-up techniques have been implemented, like for example skimming – physical separation of oil from water, use of sorbents such as, for example, volcanic ash and shavings of polyester-derived plastic to absorb oil hydrocarbons or application of surfactants to disperse oil plume in a water column and thus make it available for bacterial degradation. Recently, more attention is being paid to bioremediation process employing indigenous and exogenous hydrocarbon degrading bacteria to remove spilled oil fractions. This approach seems most promising and beneficial as ecosystem clean-up and recultivation method, since based on activity of naturally occurring microorganisms it is safer, more sustainable and providing diminished human impact on environment in comparison with other techniques.

Słowa kluczowe: *wycieki ropne, węglowodory, metabolizm, enzymy, bakterie, konsorcja bakteryjne, bioremediacja*

Streszczenie
Rozwijający się przemysł naftowy jest główną przyczyną zanieczyszczenia środowiska. Według szacunków do światowych zbiorników wodnych trafia od 3 do 6 milionów ton ropy naftowej rocznie w wyniku wycieków. Wypadki supertankowców i katastrofy na platformach wiertniczych są przyczyną dużych wycieków ropy do oceanów. Plamy ropy unoszące się na powierzchni wody mogą rozprzestrzeniać się przez wiatr i prądy zakłócając ekosystem na duże odległości od źródła wycieku. Aby ograniczyć skalę szkód środowiskowych wdrożono techniki usuwania wycieków ropy, takie jak np. skimming – fizyczne oddzielenie ropy od wody, stosowanie sorbentów, takich jak np. popioły lub pyły wulkaniczne, lub tworzyw sztucznych na bazie poliestrów w celu sorpcji smug ropnych na powierzchni wody, a tym samym umożliwiania ich degradacji bakteryjnej. Ostatnio coraz więcej uwagi poświęca się procesowi bioremediacji z wykorzystaniem rodzimych i egzogennych bakterii rozkładających węglowodory do usuwania rozlanych frakcji ropy naftowej. Podejście to wydaje się najbardziej obiecujące i korzystne jako metody oczyszczania i rekultywacji ekosystemów ponieważ oparte są na aktywności naturalnie występujących mikroorganizmów. Techniki te są bezpieczniejsze i bardziej ekologiczne zapewniając mniejszy wpływ człowieka na środowisko w porównaniu z innymi technikami.

Development of modern civilization requires continuous supply of power fuels, among others petroleum, which plays exceptional and irreplaceable role, so far. The ongoing pursuit of new vast oil and gas deposits led to the general acknowledgement that abundant deep sea resources provide easily accessible and cost effective global energy resources when compared with the inland ones. The first offshore drilling in shallow waters of California (USA) started as early as in 1897, 38 years after the first American commercial inland oil exploration [1,2]. Nowadays, application of advanced engineering technology enables drilling in deep-water (<200 m), some attempts are also made to start the crude oil output in ultra-deep water (<1000 m). It is being anticipated, in the Gulf of Mexico gas and oil reserves can be explored from the depth of 3000 m in the near future [1]. Worldwide offshore exploitation of chemical raw materials, such as fossil fuels, becomes routine in the mining industry, however, it may pose a direct threat to the marine ecosystem at any production stages. Over the course of last 60 years many oil spill disasters of long lasting detrimental influence on marine and coastal environment took place. They include oil tankers accidents, such as among others, Sinclair Petrolore (1960), Torrey Canyon (1967), Urquiola (1976), Olympic Bravery (1976), Amoco Cadiz (1978), Atlantic Empress (1979), Castillo deBellver (1983), Exxon Valdez (1989), Prestige (2002), Hebei Spirit (2007), Sanchi (2018) as well as oil spills from rigs and wells. These are disasters of Ixtoc I (Mexico 1979), Gulf War (1991) and Deepwater Horizon (DWH) (Gulf of Mexico 2010) [3], the largest oil spill accident of the US petroleum history. In the aftermath of oil rig explosion on April 20th 2010, 4,1 – 4,9 million of barrels of oil were spilled into the Gulf of Mexico (1 oil barrel = 42 US gallons = 158,987 liters) over 87 days, causing contamination of 11000 km2 of ocean area and 2000 km of the coastline [4,5]. Following the DWH incident some post-disaster research of contaminated area and recovery programs were launched to monitor chemical transformations of released oil and

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[*)] **Oliwia Zegrocka-Stendel,** dr nauk chemicznych, Warszawski Uniwersytet Medyczny, Wydział Nauk o Zdrowiu gas hydrocarbon components, chemical dispersants as well as to keep track of ecosystem changes, particularly within microbial community [6,7,8]. The degradation fate of crude oil depends on the spill magnitude and type of area the spillage accident took place, on water or land sites. Oil released on land infiltrates vertically into porous soil that hampers evaporation of more volatile hydrocarbon components and UV-induced decomposition, thus resulting in a long term soil contamination. Oil spilled at seawater when floating spreads out to cover large water area, the heavier oil fractions may be adsorbed on the particulates naturally present in the water column and descent to the sea floor to remain in the sediments. This process depends chiefly on the oil density and viscosity, water temperature and wind strength, usually 1 g of petroleum covers 1-10 m2 of the water area [9]. Crude oil spread on water surface is subject to weathering processes such as evaporation, photodegradation, dispersion and emulsification. It is assumed, during first few days following the spill, up to 25-35% of initial amount of oil hydrocarbons is lost due to evaporation and photodegradation [1,9]. These processes may be regarded advantageous contributing to remediation of polluted sites but they can also be environmentally harmful, like for example UV radiation, which may produce toxic acidic and phenolic compounds due to oxidation of some oil components. The statistical data revealed 0.08-0.4% of the petroleum world production ends up in the oceans remaining the main cause of pollution of extensive water area with profound impact on seawater ecosystem as well as coastal and estuarine wildlife [1,11].

Crude oil is a complex mixture of hydrocarbons, which are nonpolar hydrophobic molecules of diverse structure and molecular weight. As far as the molecular structure is concerned major oil hydrocarbons usually are divided into four classes including:

- aliphatics (termed also alkanes or paraffines) consisting of straight or branched hydrocarbon chains (methane, ethane, butane, hexanes, heptanes, eicosanes, etc.),
- cycloalkanes (cyclopentanes, cyclohexanes),
- aromatics (benzene, naphthalene, toluene, xylene, phenanthrene, etc.) embracing also polycyclic aromatic hydrocarbons (PAHs),
- asphaltenes and resins – the mixture of nonaromatic, aromatic, heteroaromatic and heteropolyaromatic hydrocarbons of high molecular weight [9].

As far as the resin fraction is concerned, on account of its complexity, this group is not defined in terms of the chemical constituents [12].

The physical parameters of oil components, such as viscosity, density, water miscibility and state of matter are molecular structure and size-dependent. At ambient temperature and pressure hydrocarbons containing up to four carbon atoms exist in the gas phase, molecules consisting of five up to fifteen carbon atoms are in the liquid state and these containing eighteen or more carbon atoms are solids. The aforementioned physical properties of oil-derived hydrocarbons directly influence the rate of biodegradability, usually the simpler the molecular structure and better water solubility the higher rate of biodegradation by microorganisms. Unbranched n-alkanes consisting of 10 to 24 carbon atoms (C10-C24) are the fraction of the highest metabolic rate by marine microbiota. The C5-C10 homologues exert cytotoxic effect due to the high bioavailability, resulting from better water solubility, and the ability to disrupt the lipid membrane of microorganisms. Cyclo – and branched alkanes are fairly resistant to microbial metabolism and the susceptibility to enzymatic degradation decreases proportionally to the increase of branch number in a molecule. The low-molecular-weight aromatic hydrocarbons, such as benzene or substituted benzene derivatives are of similar toxicity to marine microorganisms as the C5-C10 alkanes, but they are also relatively easily degraded by certain microbial communities [13]. The higher molecular weight and structural complexity the lower biodegradation rate, it explains why hydrocarbons consisting of five or more aromatic rings remain in the ecosystem for a long period of time as well as the 'heaviest' asphaltenes and resins, which are consider recalcitrant fraction among oil components. As far as the latter are concerned, they might be subject to microbial degradation due to so called co-oxidation process, which regards the oxidation of non-growth hydrocarbons, such as asphaltenes and resins, in the presence of growth substrates, like n-alkanes C12-C18 [14]. Except for molecular structural complexity there are some other factors influencing biodegradation rate of crude-petroleum hydrocarbons, such as concentration of hydrocarbon components, emulsion formation and temperature. High concentration of hydrocarbons is associated with the formation of oil slicks in water, which limit oxygen and nutrients accessibility, necessary for bacterial growth and proliferation. It has been observed that high carbon/nitrogen and carbon/phosphorous ratio and lower availability of inorganic nutrients decrease microbial degradation rate of hydrocarbons in estuarine water, seawater and marine sediments as well as in freshwater of lakes. As a proof of this notion may serve the use of urea-phosphate and ammonium-phosphate fertilizers in contaminated land area, which elicited substantial increase of crude oil degradation by soil microorganisms [15, 16]. Most oil components are water insoluble but due to wind and wave-evoked agitation, oil-in-water emulsion is formed, dispersion of oil in the water column mitigates microbial degradation of hydrocarbons due to increased surface area of spilled oil and thus enhanced accessibility for enzymatic transformations. The excess amount of oil spilled may lead to the formation of water-in-oil emulsion, so called mousse, which represents unfavorably low surface to volume ratio that inhibits microbial biodegradation. The mousse, stabilized by high ratio of asphaltenes and resins which enhance oil viscosity, may persist for a long period of time and can travel with waves towards coastline to degrade coastal ecosystem [11, 12] .

Temperature significantly alters hydrocarbon biodegradation, first of all, due to the impact on distribution and population dynamics of hydrocarbon-utilizing bacteria as well as the rate of enzymatic activity which reaches its optimum at the range of 30-40oC. Temperature change influences physico-chemical properties of hydrocarbons, which in turn determine the uptake and metabolic rate of oil components. Temperature decline is the cause of the decreased volatility, increased water solubility and increased oil viscosity of the short-chain toxic alkanes. All these factors contribute to inhibition of the hydrocarbon microbial biodegradation, although there are some reports about crude oil degradation by some species of marine bacteria at 3oC and at soil and estuarian sediments at – 1,1oC or lower temperature [17].

Oil hydrocarbons metabolizing species of microalgae, fungi and bacteria are ubiquitously present in terrestrial and aquatic environments. According to the scientific reports, in non-contaminated marine environments hydrocarbon degrading bacteria account for about 0,1% of marine microbiota and they can proliferate up to 100% in crude oil polluted areas [9]. Among indigenous microbial community Alcanivorax dieselolei, Acinetobacter sp., Pseudidiomarina maritima, Marinobacter hydrocarbonoclasticus, and Vibrio hepatarius genera isolated from oil contaminated beach sands of Florida are worth mentioning, as well as another group embracing Achromobacter, Acinetobacter, Alkanindiges, Alteromonas, Arthrobacter, Burkholderia, Dietzia, Enterobacter, Kocuria, Marinobacter, Mycobacterium, Pandoraea, Pseudomonas, Staphylococcus, Streptobacillus, Streptococcus, and Rhodococcus genera isolated from oil contaminated soil, due to crucial role they play in oil hydrocarbon degradation [13,18,19].

Flurry of research has been carried out on isolation, culturing, gene expression and protein expression profile analyses as well as population heterogeneity to elucidate adaptation processes of oil-degrading bacterial species under changing environmental conditions in a time course after oil spill [20-25]. Bacterial species using hydrocarbons as a source of energy and carbon are endowed with various specific enzymes, which are employed in oxidative, usually aerobic but also anaerobic, degradation of crude oil hydrocarbon fractions. In the degradation of alkanes: 1-monooxygenase, alcohol dehydrogenase, cyclohexanol dehydrogenase, methane monooxygenase, cyclohexanone 1,2-monooxygenase, cytochrome P-450 are involved. Naphthalene 1,2-dioxygenase, ferredoxin reductase component, cis-2,3-dihydrobiphenyl-2,3-dioldehydrogenase and salicylaldehyde dehydrogenase are associated with naphthalene derivatives degradation. Benzene, toluene, ethylbenzene dioxygenases catalyze oxidative transformations of aromatics and bacterial laccase – multicopper oxidase participates in oxidative degradation of phenolic compounds, aromatic amines and polycyclic aromatic hydrocarbons (PAH) [18, 26-28]. Particular bacterial species are capable of degrading only certain petroleum hydrocarbon components due to the presence of specific oxygenases. None of the studied bacterial genera and species have been proved to be able to metabolize entirely all hydrocarbon fractions. Some bacterial species, such as Dietzia sp., Pseudomonas sp., Oleispira antarctica, Rhodococcus ruber, Alcanivorax sp. can degrade only aliphatics, Achromobacter xylosoxidans, Mycobacteirum cosmeticum, Pseudomonas aeruginosa, Cycloclasticus, Sphingomonas, Bacillus Licheniformis etc. are capable to metabolize only aromatics and Pseudomonas sp., Bacillus sp., Citrobacter sp. can degrade asphaltenes and resins merely [18,29]. Bacteria are known for their unique phenotypic plasticity that means the capability to alter their metabolic activity in response to environmental changes, such as limitation or abundance of oxygen, nutrients or toxic substances. Oil spills on one hand impose disastrous consequences to the environment on the other hand deliver substrates serving as carbon and energy source for hydrocarbon degrading bacteria, promoting they proliferation and growth. There is also a concern, that at spill sites the exciding rate of proliferation of hydrocarbon degrading bacteria may occur, they become dominant species changing the bacterial community composition and diversity of polluted water and sediments. Based on this observations some attempts have been made to utilize hydrocarbon degrading bacteria in bioremediation of crude petroleum contaminated sites [29,30]. To achieve pollutants removal more effective, instead of individual bacterial strains, consortium of different bacterial species have been used in the studies. Bacterial isolates consisting of Ochrobactrum sp., Stenotrophomonas maltophilia and Pseudomonas aeruginosa were proved to decompose 83,49% of crude oil fractions, the co-culture of indigenous soil bacteria and exogenous Bacillus subtilis accelerated oil fractions degradation at yield 85,01% in soil, according to the reports [31,32]. Another example of artificial consortium consisting of Aeromonas hydrophila, Alcaligenes xylosoxidans, Gordonia sp., Pseudomonas fluorescens, Pseudomonas putida, Rhodococcus equi., S. maltophilia, Xanthomonas sp. yielded 89% of hydrocarbon degradation in contaminated soil [33]. Neither the surface area that bacterial community were seeded nor duration of the experiments were strictly reported, nevertheless the outcomes are very encouraging for further research and implementation.

The application of bacterial community in a clean-up and bioremediation technique at the oil spill sites is being still developed under various research programs. Bioremediation employing natural microorganisms is less expensive, less intrusive, enables complete remediation of toxic pollutants, facilitates faster ecological balance restoration when compared with techniques used so far, such as excavation methods or chemical dispersants usage. Unfortunately, there are some environmental constrains which influence rate of microbial hydrocarbon metabolism that limit current successful application of bioremediation. Bacteria are single cell organisms and their activity as well as community dynamics are influenced by such parameters as temperature, nutrients availability, pH, salinity (in seawater) and time necessary for protein synthesis. For example, temperature dependence was corroborated in comparative study under laboratory and field conditions in which temperature was the only variable. The rate of hydrocarbon degradation by the same bacterial community was much faster in a laboratory site, where temperature was maintained at 20oC than in outdoor conditions [30].

Research findings proved the rate of hydrocarbon biodegradation relates to bacterial community structure which shows major dynamics, resulting from bacterial adaptation to changing environmental conditions that cannot be easily assessed [34,35]. Introduction of PCR gene amplification and the next-generation sequencing (NGS) techniques, enables precise analysis of 16S ribosomal RNA sequence (16S rRNA gene), which is the marker commonly used for the taxonomic identification of bacterial strains and thus can be a convenient tool in microbial community composition estimates [36,37]. The samples of various ecotopes, such as water column, sediments and soil of contaminated sites are collected to perform the assessment of indigenous bacterial community composition and to perform comparative studies with microbial communities subjected to bioaugmentation with exogenous hydrocarbon-degrading bacteria of Rhodococcus erythropolis strains, Arthrobacter oxydans ITRH49, Pseudomonas sp. ITRI73 and Pseudomonas sp. MixRI75 for instance [38,39]. Some experimental results show, the inoculation of exogenous hydrocarbon-degrading bacteria into natural microbiome increases the efficiency of oil hydrocarbon mineralization but within short period of time (28 days) and kinetics of this process declines during a long time period (109 days) [40]. Some attempts were performed, within the bioremediation strategy, to employ genetically modified microorganisms, like for example Pseudomonas putida PaW85 and Pseudomonas fluorescens HK44 strains, to clean up oil contaminated areas [41]. However, these genetically engineered organisms have not been introduce into natural environment so far, due to the concern of possible regulatory obstacles as well as the unpredictable effects of the interactions with natural microbiome.

Microbial-assisted bioremediation still remains at the development stage and seems to be a promising, environmentally beneficial solution to oil contamination problem in the coming future. There is a belief, employment of modern technologies based on bioengineering and bioinformatics analyses will contribute to elucidation of numerous factors influencing microbial diversity and microbial community profiling and thus enable implementation of bioremediation into of oil spill sites successfully. ■

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