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The emulsifying activity of several hydrocarbon-degrading microorganisms

Abstract: It is known that biosurfactants are capable of increasing the bioavailability of hydrocarbons in oil and petroleum products, as well as modifying the external surfaces of bacteria by hydrophobizing providing direct contact with hydrocarbon molecules. In this study the index of emulsification index was studied for four hydrocarbon-degrading strains of microorganisms such as *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Tessaracoccus flavescens*, *Acinetobacter calcoaceticus*. Diesel fuel and hexadecane were used as hydrophobic substrates.

It is shown that for all members of natural hydrocarbon-degrading microorganisms are characterized by high rates of emulsification index. The highest index of emulsification has shown for *Acinetobacter calcoaceticus* - 80-87%. Microorganisms having an emulsification index bigger than 50% are considered promising producers of surfactants. Since the microbial cell oxidizes hydrocarbons by adsorbing on their surface, as a result, the oil-oxidizing activity of the crops depends on its ability to utilize the hydrocarbon substrate. This could indicate a destructive activity of microorganisms.

Keywords: hydrocarbon-degrading microorganisms, emulsifying activity, hydrophobic substrates.

Introduction. Contamination of soil by petroleum hydrocarbons and their derivatives is a serious environmental problem all over the world.

Bioremediation is effective and environmental friendly but it often takes time and is not cost-effective on treating large volumes of polluted materials.

The primary mechanism for elimination of hydrocarbons from contaminated sites is biodegradation by the natural populations of microorganisms [1]. The enhancement of natural biological degradation processes in what is termed "bioremediation" can be a preferred cost-effective method of removing contaminant hydrocarbons from oil-contaminated environments [2]. Bioremediation, which is the use of microorganism's consortia or microbial processes to degrade and detoxify environmental contaminants, is also amongst these new technologies, which derives its scientific justification from the emerging concept of Green Chemistry and Green Engineering [3]. The success of bioremediation of oil spill not only depends on the ability of the strains but also on the physical, chemical and biological conditions of the contaminated environment [8].

One of the main problems that limit the effectiveness of this process is very low solubility and high hydrophobicity of oil pollutants. These compounds strongly bind to soil particles, and thereby they are poorly available for bacterial cells [5].

A large number of bacteria, yeast and fungi are able to metabolize one or more compounds in crude oil. A few species of microorganisms, in particular bacteria, are able, by de novo synthesis, to produce surface active compounds, so called biosurfactants. Crude oil emulsifying microorganisms are able to transform crude oil into an oil-in-water emulsion, often by a combination of production of biosurfactants and generation of surface active degradation products through their metabolism of crude oil components [6].

Biosurfactant reduces surface tension or interfacial tension of an interface, depending whether it is a water/air or water/oil interface. In water/oil interface, biosurfactant molecule generates a new surface area by forming a surfactant oriented monolayer around the hydrocarbon particle with hydrophobic tail of the surfactant pointing out to the liquid phase [6]. This leads to increase in surface area of hydrocarbon substrate and facilitates emulsification. The entire phenomena enhances the bioavailability of contaminants for microbial degradation through better solubilization of hydrocarbons in water or water in hydrocarbons [7]. Due to the lower toxicity and biodegradable nature in comparison to their synthetic counterparts, biosurfactants are considered to be more suitable

for environmental applications such as hydrocarbon remediation. The biosurfactant molecules enhance the solubility of these sparsely soluble hydrophobic pollutants through emulsification, thereby leading their better bioavailability for the existing micro-flora [8]

The aims of the study were to characterize emulsifying activity of natural hydrocarbon oxidizing microorganisms.

Materials and methods

Microbial strains: The bacterial cultures were selected from samples of sludge. Day cultures of microorganisms were transferred from agar medium on a standard medium.

Hydrophobic substrates: Diesel fuel and hexadecane were used as hydrophobic substrates. Diesel fuels consist primarily of C9-C20 hydrocarbons.

Hexadecane (also called cetane) is an alkane hydrocarbon with the chemical formula C₁₆H₃₄. Hexadecane consists of a chain of 16 carbon atoms, with three hydrogen atoms bonded to the two end carbon atoms, and two hydrogens bonded to each of the 14 other carbon atoms. Cetane is often used as a shorthand for cetane number, a measure of the detonation of diesel fuel. Cetane ignites very easily under compression; for this reason, it is assigned a cetane number of 100, and serves as a reference for other fuel mixtures. It has one of the lowest octane ratings, at < -30.

Emulsification index: Emulsification index (E24) In this study, diesel fuel and hexadecane were added to the cell free supernatant in a ratio of 1:1 and vortexed vigorously for two minutes. After 24 hours of incubation at room temperature, the height of the emulsified layer was measured. The emulsification index (E24) was estimated as: $E24 (\%) = (HEL/HS) \times 100$ Where, E24 is the emulsification activity after 24 hours, HEL is the height of the emulsified layer, and HS is the height of the total liquid column [2].

Results and Discussion. In this study, as natural strains used strains isolated from sludge. Natural strains of the following microorganisms are - *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Tessaracoccus flavescens*, *Acinetobacter calcoaceticus*.

All selected natural strains were able to grow in synthetic medium with addition of diesel fuel and crude oil.

Emulsification activity is one of the criteria to determine the potential of biosurfactants. When evaluating the surface-active properties of microorganisms, an emulsifying activity indicator is used which is based on the property of surfactants to form an emulsion of a culture fluid of microorganisms with a hydrocarbon. Experiments were conducted to determine the ability of natural to form stable emulsions with an oil phase (emulsifying activity). Emulsifying activities (E24) determine the productivity of bioemulsifier and given as a percentage of the height of the emulsified layer divided by the total height of the liquid column.

Emulsification index of 30% or more was considered as significantly positive emulsification activity.

Our study revealed that all natural hydrocarbon-degrading microorganisms showed high rates of emulsification index.

The potential of emulsifying activity in the culture liquid as the hydrophobic substrate of hexadecane (% E24) of 80-87% was obtained from *Acinetobacter calcoaceticus*. In the supernatant, the index of the emulsification index was 45-50% (Fig.1, 2).

It is shown, that as a hydrophobic substrate used a diesel fuel, the emulsion index values for *Acinetobacter calcoaceticus* were 50-77% in the culture liquid and 40-50% in the supernatant.

When *Bacillus subtilis* used a diesel fuel as a hydrophobic substrate, the index of the emulsification index was 20-30%. In the supernatant, the index of the emulsification index was 4-5%.

It is shown, that as a hydrophobic substrate used a hexadecane, the emulsion index values for *Bacillus subtilis* were 30-40% in the culture liquid and 6-9% in the supernatant.

In this study, as a hydrophobic substrate used a hexadecane, the emulsion index values for *Tessaracoccus flavescens* were 40-42% in the culture liquid and 11-13% in the supernatant.

The potential of emulsifying activity in the culture liquid as the hydrophobic substrate of diesel fuel (% E24) of 30-35% was obtained from *Tessaracoccus flavescens*. In the supernatant, the index of the emulsification index was 10-14%. (Fig.1, 2).

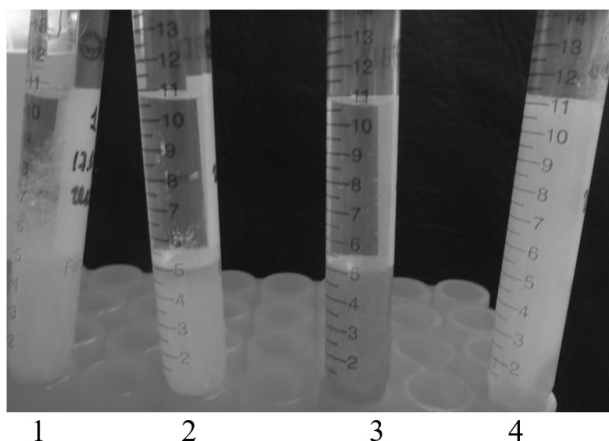


FIGURE 1 – Emulsifying activity of hydrocarbon degrading microorganisms in a culture liquid using hexadecane as a hydrophobic substrate

1 - *Achromobacter xylosoxidans*, 2 - *Bacillus subtilis*, 3-*Tessaracoccus flavescens*, 4 -*Acinetobacter calcoaceticus*

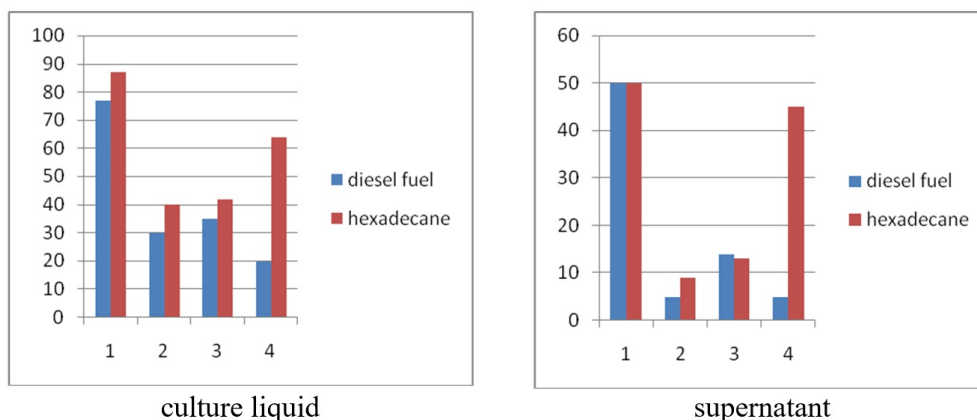


FIGURE 2 – The rates of emulsification index of hydrocarbon degrading microorganisms

1 - *Achromobacter xylosoxidans*, 2 - *Bacillus subtilis*, 3-*Tessaracoccus flavescens*, 4 -*Acinetobacter calcoaceticus*

The lowest values of the emulsification index of the culture liquid when used as a hydrophobic substrate for diesel fuel are shown for bacteria of the species *Achromobacter xylosoxidans*. The emulsification index was 10-20%. In the supernatant, the emulsification index values were 3-5%.

However, when using hexadecane as the hydrophobic substrate, the emulsification index values were 60-64% (Fig. 1, 2). In the supernatant, the index of the emulsification index was 40-45%.

The ability of biosurfactants to create emulsions of hydrocarbon compounds, and thereby increase their bioavailability, often used as a basis for determining their effectiveness in environmental bioremediation of hydrophobic contaminants. The biosurfactant produced showed a high hydrocarbon emulsification index.

According to the literature, it is known that cultures with an emulsification index of more than 30% are considered promising producers of surfactants.

All bacterial cultures had a high destructive activity, and this is due primarily to the oxidation of hydrocarbons. The availability of the hydrocarbon substrate is possibly provided by the synthesis of biosurfactants. Microorganisms isolated in this study could be a valuable source of novel environmentally friendly biosurfactants for the future replacement of synthetic surfactants.

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Эмульгирующая активность ряда углеводородокисляющих микроорганизмов

Аннотация: Известно, что биосурфактанты способны повышать биологическую доступность углеводородов нефти и нефтепродуктов, а также модифицировать внешние поверхности бактерий путём гидрофобизации обеспечивая прямой контакт с молекулами углеводородов.

В работе был исследован индекс эмульгирования углеводородокисляющих микроорганизмов видов *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Tessaracoccus flavescens*, *Acinetobacter calcoaceticus*. В качестве гидрофобных субстратов использовали дизельное топливо и гексадекан.

Показано, что для всех представителей природных углеводородокисляющих микроорганизмов характерны высокие показатели индекса эмульгирования. Наибольший индекс эмульгирования показан для *Acinetobacter calcoaceticus* – 80-87%. Микроорганизмы, имеющие индекс эмульгирования больше 50%, считаются перспективными продуцентами поверхностно-активных веществ. Поскольку микробная клетка окисляет углеводороды, адсорбируясь на их поверхности, вследствие этого нефтеокисляющая активность культур зависит от ее способности утилизировать углеводородный субстрат. Следовательно приведенные данные могут свидетельствовать о высокой деструктивной активности микроорганизмов.

Ключевые слова: углеводородокисляющие микроорганизмы, эмульгирующая активность, гидрофобные субстраты.

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Кейбір көмірсутектотықтырушы микроағзалардың эмульгирлеуші белсенділігі

Биосурфактантардың мұнай және мұнай өнімдердің көмірсутектірінің биологиялық жеткіліктігін ұлғайту қабілеті белгілі. Сонымен бірге көмірсутекті молекулалармен тікелей байланыста болатын гидрофобизациялау арқылы бактериялардың сыртқы беттерін өзгертуі. *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Tessaracoccus flavescens*, *Acinetobacter calcoaceticus* табиғи көмірсутектотықтырушы микроағзалардың өкілдерінің эмульгирлеу индексіні зерттелді. Гидрофобтық субстраттар ретінде гексадекан және дизельды отын пайдаланды. Алынған табиғи көмірсутектотықтырушы микроағзалар өкілдерінің эмульгирлеу индексінің жоғарғы көрсеткіштеріне тән болғаны көрсетілді. Эмульгирлеудің ең жоғарғы индексі *Acinetobacter calcoaceticus* үшін көрсетіледі - 80-87%.

Эмульгаторлық индексі 50% -дан асатын микроағзалар беттік-белсенді заттардың перспективті өндірушілері болып табылады. Микробтық жасуша көмірсутегі субстратының пайдалану қабілетіне байланысты оның ерітіндісінің мұнай-тотықтырғыш белсенділігіне байланысты көмірсутектерді бетіне сіңіріп тотықтырады. Бұл микроағзалардың деструктивті белсенділігінің жоғары болуына байланысты.

Түйін сөздер: көмірсутектотықтырушы микроағзалар, эмульгирлеуші белсенділік, гидрофобтық субстраттар.

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