Correlation between biosurfactant synthesis and microbial degradation of crude oil hydrocarbons

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The production of biosurfactans in media with tetradecane, hexadecane and docosane as the sole carbon source by five bacteria strains was observed by increasing the emulsification properties that could be used for the preliminary screening of the active strains of secreted biosurfactans. The emulsification properties in growth medium – the decay constant (K_d) and the extent of dispersity of microemulsion (OD_{max}) – correlated with the biodegradation level of crude oil hydrocarbons.

Key words: biodegradation, biosurfactants, emulsification properties, hydrocarbons

INTRODUCTION

Bioremediation of waste sites with crude oil and its processing products is one of the important ecological problemes today. The list of microorganisms that grow in oil-containing medium cannot be completed, because new populations and individuals with the new ways of metabolism and enzime specificity are formed.

The extent and rate of crude oil biodegradation depend on the oil low aqueous solubility and strong adsorptive capacity to a soil [1, 3, 8, 9, 10]. One way to enhance the solubility of hydrophobic organic compounds found in the environment as pollutants is to apply mobilizing agents such as surfactants. Under their influence, a droplet of crude oil can be solubilized in water, thus increasing the contact area between the substrate and the microorganisms and improving the aeration.

Chemical synthesized surfactants added to oil-contaminated soil become pollutants of the environment by themselves and may be highly toxic to the microorganisms. The biosurfactants are under increasing consideration, because of their broad range of potential applications, including a role in the growth of microorganisms on water-insoluble hydrophobic materials such as hydrocarbons [2, 6, 7, 9].

We focused our attention on the relation among the biodegradation of crude oil by bacteria, their secreted biosurfactants and quantitative emulsification properties. On the other hand, the emulsification properties of biosurfactants synthesized by microorganisms could be used for the preliminary selection of active strains.

MATERIALS AND METHODS

Organism and culture conditions

Five bacteria strains (*Bacillus* sp. 5.1, *Bacillus* sp. 1.2, *Micrococcus* sp. 1a, *Micrococcus* sp. 1d and *Arthrobacter* sp. 2a) were isolated from oil-polluted soil in our department on the basis of their ability to grow on various crude oil hydrocarbons as the only source of carbon and energy. The bacteria strains were maintained on nutrient agar slants and subcultured every two weeks. Yeast extract-peptone-dextrose agar containing 1% yeast extract, 2% peptone, 2% glucose, 2% agar was used as a multipurpose growth medium.

The studies were carried out in bath culture for production of biosurfactants. The bacteria were grown in synthetic medium containing the following constituents (gl^{-1}): NH_4NO_3 1.0; $MgCl_2$ 0.1; KH_2PO_4 3.0; K_2HPO_4 7.0; $CaCO_3$ 1.0. The pH of the growth media was adjusted to 7.0, and the media were sterilized 0.1 MPa for 30 min. The cell suspension was 1×10^{-6} cell/ml. Cultures were grown at 35 °C without shaking 7 days. The normal acyclic hydrocarbons such as tetradecane, hexadecane and docosane were supplied at concentrations of 8 gl^{-1} and crude oil 20 gl^{-1}.

Analytical methods

To recover the residual hydrocarbons after bacteria degradation, the cultures from the mediam were extracted with chloroform. The extracts were dried over Na_2SO_4 and concentrated to constant weight. Hyd-

rocarbon degradation was determined as a percent decrease of dry weight as compared to the control sample.

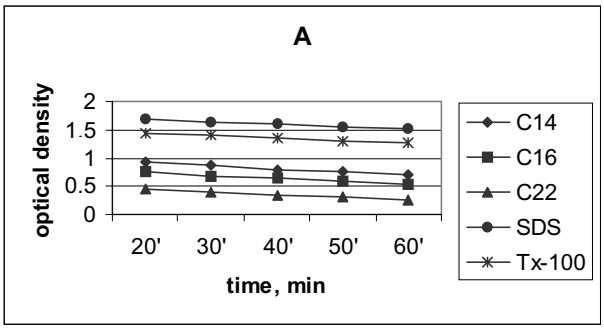
To determine the degradation of saturated fraction of crude oil (paraffin) by bacteria, the residual crude oil, as well as controls, were fractionated by liquid-column chromatography [4]. The undegraded fraction was assayed by weighing.

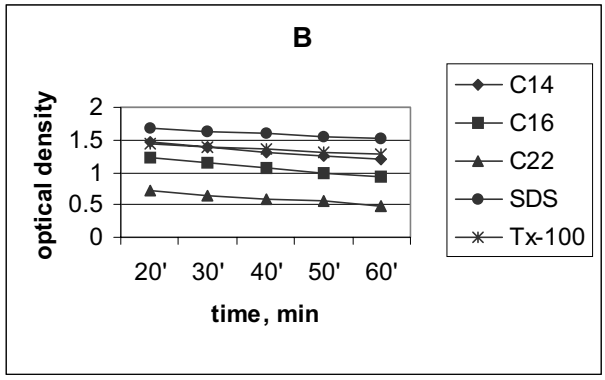
Emulsification activity after removing the residual hydrocarbons was measured by vortexing 4 ml of medium with 1 ml of hexadecane for 2 min [5]. Microemulsion of oil in water (O/W) was allowed to sediment for 10 min, and then its optical density (turbidity) was measured at 540 nm. All assays were carried out in triplicate.

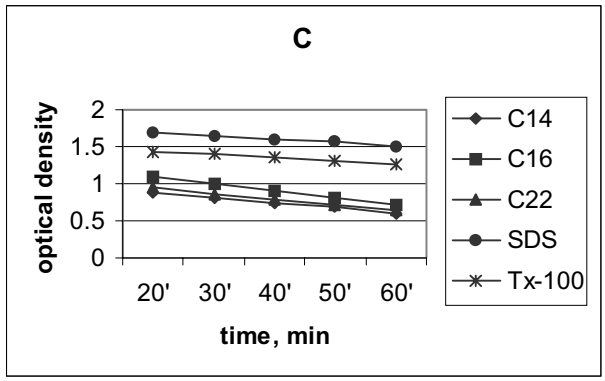
RESULTS AND DISCUSSION

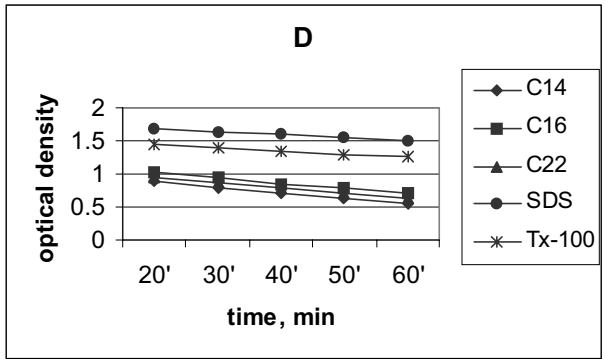
The optical methods are widely used to study the dispersed particles in solutions. The intensity of incident light passing through the emulsion decreases as a result of two different processes: absorption and scattering. The portion of the light passed through the emulsion (omission) depends on what part of light beam is absorbed and scattered by a dispersed substance. The absorption in the range of visible light (400–800 nm) is extremely unimportant, as the particles, in most cases, are colorless and transparenty. So the omission in this range of light depends upon the value of scattering on the basis of light reflection from the surface of particles and its diffraction. Light scattering increases with particle concentration in a microemulsion. The omission or optical density was measured as an intensity of light transmitted through the microemulsion. In this way we measured emulsification activities of the growth medium of different bacteria. The data (Fig. 1) show that the bacteria produced different amounts of extracellular biosurfactants when they grew with the oil hydrocarbon sources. The stability of the obtained microemulsion *versus* time was given by straight lines in coordinates OD-t in accordance with the equation:

$$OD = \mathbf{a} + K_d \times t,$$









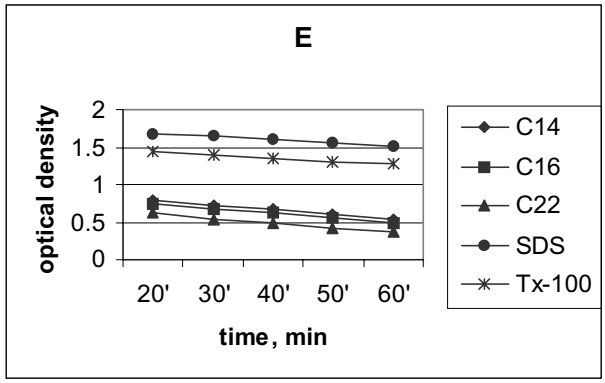


Fig. 1. Stability of hexadecane microemulsion in the presence of biosurfactant from cell-free culture growth medium with time (A – *Micrococcus* sp. 1a; B – *Arthrobacter* sp. 2a; C – *Micrococcus sp*. 1 d; D – *Bacillus sp.* 1.2; E – *Bacillus* sp. 5.1 *versus* SDS and Triton X-100)

where OD is optical density; t is the time (h); **a** and K_d are the parameters defined the microemulsion.

The point of intersection of the line with the ordinate axis gives the value of **a**. According to the equation, when t = 0, **a** = OD_{max}, the meaning of OD_{max} is the theoretically possible extent of dispersity for a given microemulsion. The value of OD_{max} could be taken as a quantitative characteristic of preliminary selection of the microorganisms that synthesize extracellular biosurfactants. However, the increasing dispersion does not always correlate with an increasing biodegradation. An important factor of the microorganism growth on water-insoluble hydrocarbons is the decay constant (K_d) of emulsion, though. A plot of OD *versus* t gives a slope that is equal to the decay constant. We can take the decay constant as a qualitative characteristic of a given microemulsion, because it depends on the chemical nature of produced biosurfactants responsible for stability of the system. The lower K_d shows the better stability of a microemulsion. The decay constants of the systems studied are presented in Table 1. The emulsification properties of the systems are compared with commercial lauryl sulfate (SDS) and Triton X-I00 used at their critical micellar concentration (I cmc).

The cultures *Micrococcus* sp. 1a, *Micrococcus* sp. 1d and *Arthrobacter* sp. 2a. demonstrated the ability to utilize different hydrocarbons as a sole carbon source for their growth to different extent (Fig. 2).

All test cultures grew on tetradecane. The percentage of tetradecane degradation was higher by a

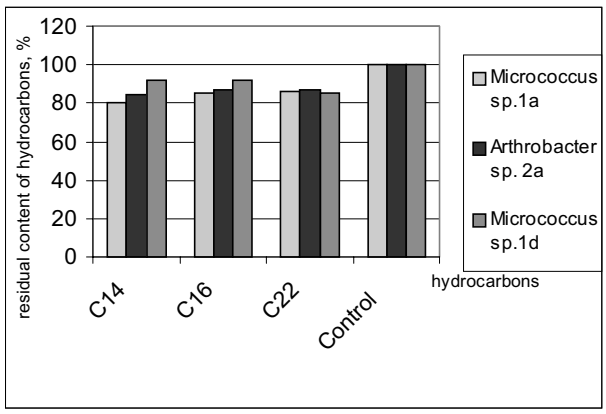


Fig. 2. Residual content of tetradecane (C14), hexadecane (C16) and docosane (C 22) after biodegradation for 7 days by bacteria

culture of *Micrococcus* sp. 1a. It was about 19.6%. The *Micrococcus* sp. 1d demonstrated a lower degradation percentage of tetradecane – 7.7% after 7 days of incubation. A comparison of tetradecane degradation by *Micrococcus* sp. 1a and bacterium emulsification properties in the growth medium showed the lower K_d value (0.3) and the higher degradation percentage (19.6%). In the cases of *Micrococcus* sp. 1d and *Arthrobacter* sp. 2a cultures, when the growth source was tetradecane, the surfactants synthesized by the cultures formed emulsions with the same K_d (0.4). The microemulsion decay constants were higher than in the case described above (*Micrococcus* sp. 1a), so the percentage of biodegradation was lower. When the K_d of microemulsions was the same, the biodegradation percentage of tetradecane depended on the OD_{max} – a quantitative characteristic of a microemulsion. The OD_{max} and biodegradation level were higher for *Arthrobacter* sp. 2a (respectively 1.52 and 15.2%), as compared to *Micrococcus* sp. 1d (0.90 and 7.9%). Similar data were obtained for the same cultures when the growth source of carbon was hexadecane and docosane.

Emulsification properties were investigated for *Bacillus* sp. 5.1 and *Bacillus* sp. 1.2, when a carbon substrate was tetradecane, hexadecane and docosane. The emulsifying activity of *Bacillus* sp. 5.1 growth medium was better than of *Bacillus* sp. 1.2, with a lower K_d and nearly the same OD_{max}. The biodegradation ability of *Bacillus* sp. 5.1 and *Bacillus* sp. 1.2 was examined by their growth ability on a saturated hydrocarbon fraction of fuel oil as the sole carbon source. *Bacillus* sp.

Table. **Emulsification activity of biosurfactans**

Cultures	Carbon Source	OD (0.5 h)	OD (1 h)	K_d	OD_{max}
Micrococcus sp. 1a	$C_{14}H_{30}$	0.86	0.71	0.30	0.95
	$C_{16}H_{34}$	0.69	0.53	0.32	0.80
	$C_{22}H_{46}$	0.39	0.26	0.26	0.50
Arthrobacter sp. 2a	$C_{14}H_{30}$	1.40	1.18	0.40	1.52
	$C_{16}H_{34}$	1.15	0.95	0.40	1.26
	$C_{22}H_{46}$	0.66	0.52	0.36	0.75
Micrococcus sp. 1d	$C_{14}H_{30}$	0.80	0.60	0.40	0.94
	$C_{16}H_{34}$	0.94	0.74	0.40	1.12
	$C_{22}H_{46}$	0.85	0.68	0.34	1.00
Bacillus sp. 5.1	$C_{14}H_{30}$	0.71	0.56	0.32	0.82
	$C_{16}H_{34}$	0.75	0.51	0.30	0.80
	$C_{22}H_{46}$	0.53	0.39	0.26	0.65
Bacillus sp. 1.2	$C_{14}H_{30}$	0.80	0.58	0.44	0.98
	$C_{16}H_{34}$	0.94	0.71	0.40	1.05
	$C_{22}H_{46}$	0.85	0.63	0.40	1.00
Control	SDS	1.64	1.52	0.24	1.72
	Triton X-100	1.40	1.28	0.24	1.48

5. 1 utilized 56% of saturated hydrocarbon fraction, while *Bacillus* sp. 2.1 utilized only 6.0%.

The emulsifying properties of the five investigated bacteria (their K_d and OD_{max}) correlated with the biodegradation extent (level): the lower K_d, the higher OD_{max}, *i.e.* the higher the biodegradation level.

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KORELIACIJA TARP MIKROBŲ PAVIRŠIAUS AKTYVIŲ MEDŽIAGŲ SINTEZĖS IR NAFTOS ANGLIAVANDENILIŲ DEGRADACIJOS

S a n t r a u k a

Viena pagrindinių priežasčių, kodėl naftos produktai ilgai išlieka gamtoje kaip teršalai, yra jų mažas tirpumas vandenyje. Mikroorganizmų gebėjimas išskirti emulguojančias medžiagas yra vienas iš požymių, rodantis, kad organizmas asimiliuoja angliavandenilius. Angliavandenilių buvimas terpėje paprastai gali padidinti išskiriamų emulsiklių kiekį, dėl kurių padidėja ir pačių angliavandenilių tirpumas terpėje. Darbe tirti penki bakterijų izoliatai, išskirti iš nafta užteršto grunto. Jie buvo auginti terpėse su tetradekanu, heksadekanu ir dokozanu kaip vieninteliais anglies šaltiniais, lygiagrečiai įvertinant į terpę išskiriamų paviršiaus aktyvių medžiagų kiekius bei jų sudaromų dispersinių sistemų stabilumą. Rezultatai parodė, kad tirtų angliavandenilių biodegradacijos lygis susijęs su susidariusių emulsijų dispersijos laipsniu ir skilimo konstanta: kuo didesnė biodegradacija, tuo mažesnė dispersinės sistemos skilimo konstanta ir didesnis susidariusios emulsijos dispersijos laipsnis.