Comparison of two yeast biosurfactants for the removal of petroleum hydrocarbons

Marek ŠÍR[a], Zuzana HONZAJKOVÁ[a], Kristina TURNVALDOVÁ[b], Jiří MIKEŠ[b], Martin Kubal[a], Radek VURM[a], Juraj GRÍGEL[b], Miroslav MINAŘÍK[b]

[a]Institute of Chemical Technology Prague, Faculty of Environmental Technology, Technická 5, Praha 6, 166 28,
e-mail: sirm@vscht.cz
[b]EPS, s.r.o., V Pastouškách 205, 686 04 Kunovice,
e-mail: eps@epssro.cz

Summary

The aim of this paper was to produce, isolate and characterize two types of biosurfactant produced by yeasts Yarrowia lipolytica and Candida bombicola and quantify their potential for stimulated attenuation. Critical micelle concentration (CMC) and minimum surface tension (MST) were determined for both biosurfactants. CMC = 31 mg/L and MST = 40 mN/m (producer Yarrowia lipolytica), CMC = 83 mg/L and MST = 43 mN/m (producer Candida bombicola). Lower interfacial tension causes that non-polar carbon source can be easily utilized by microorganisms, thus dependence of interfacial tension between organic phase and surfactant solution was measured. Kerosene and crude oil were chosen as representatives of organic phase. Biosurfactant produced by Yarrowia lipolytica was found to be more effective for remediation application, because its solution decreased the interfacial tension by 80%.

Keywords: *biosurfactant, surface tension, critical micelle concentration, interfacial tension, petroleum hydrocarbons*

Introduction

Surfactants are substances that contain both hydrophilic and hydrophobic part. Surfactants can reduce surface tension of aqueous solutions, reduce interfacial tension between immiscible liquids, stabilize emulsions, and finally increase the solubility of hydrofobic organic substances slightly soluble (or insoluble) in aqueous medium.

Synthetically produced surfactants are classified according to their hydrophilic groups. Biosurfactants are classified according chemical composition and microbial origin. Hydrophilic part is generally composed of mono-, di- or polysacharides, amino acids or peptide anions or cations. The hydrophobic part consists of saturated, unsaturated or fatty acids. The main groups of biosurfactants are glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids and polymeric surfactants.

Some representatives of biosurfactants and their producers are as follows: glycolipids are rhamnolipids (*Pseudomonas aeruginosa*), trehalolipids (*Rhodoccocus erythropolis*) and sophorolipids (*Candida bombicola*, *Candida apicola*), lipopeptides such viscosin (*Pseudomonas fluorescens*) and carbohydrate-protein-lipid complexes such emulsan (*Acinetobacter* sp.)[1].

An important issue in the production process is determining the optimal conditions for culture medium. There are several methods for determining of maximum production of biosurfactant. Methods were used for monitoring of the growth phase based on the relationship between growth, utilization of substrate and the production of biosurfactant. Microorganisms produce surfactants partly as a primary part of their metabolism. However, increased production occurs in the stationary phase of growth due to substrate limitation. Especially important is limitation of nitrogen and microelements[2].

We can include carbon source, nitrogen source and the presence of biogenic elements among the factors affecting the production of biosurfactants. The production is also affected by environmental factor limiting growth of cells and their activity such as temperature, pH value, stirring and oxygen availability. Water soluble compounds (glucose, glycerol, mannitol, ethanol) are usually used as a carbon source. Water insoluble compounds (oils, n-alcanes, paraffin) are usually used as a second carbon source, because they form a phase boundary. The utilization of two carbon sources leads to higher yields of biosurfactants[3].

Oil spreading technique is a method for determining the presence of biosurfactant in solution. Principle is as follows: 30 mL of distilled water is poured in Petri dish, 15 µL of oil is dripped in the middle of dish, than 5 mL of sample is dripped on the oil surface. The presence of surfactant clears the zone whose diameter is measured[4].

Extraction is the most suitable method for isolation of biosurfactant from culture medium. Suitable solvents are ethyl acetate, mixture of ethyl acetate and isopropanol[5] or mixture of chloroform and methanol[6]. In some cases it is possible to isolate biosurfactant using acid precipitation by addition of hydrochloric acid[7].

Biosurfactants can be applied for remediation. In this case rhamnolipid surfactats are usually used. Rhamnolipids are biosurfactants produced by bacteria, which are described generally more than biosurfactants produced by yeasts. Rhamnolipids were tested for solubilization of non-polar model organic contaminants (toluene, ethylbenzene and butylbenzene)[8] or for solubilization of petroleum hydrocarbons[9]. These tests dealt with solubilization of organic substances, but did not deal with interfacial tension measurement between these phases.

Experimental

Production of two types of biosurfactants followed these steps: Cultivation was carried out in liquid medium in Erlenmeyer flask. Flask was placed on a laboratory shaker and shaken at 120 rpm. Basic conditions are defined by the pH and temperature values of the solution. Generally, acidification of the environment occurs for yeast cultivation. Decrease of pH from 6.5 to 3 during 48 hours is typical phenomenon. Adjustment of pH by buffer solution was not applied. Yeasts are facultative anaerobic microorganisms, thus cultivation medium was aerated to prevent fermentation process. Cultivation was carried out at room temperature 20 – 25 °C. Yeast biosurfactants are usually consisted of a hydrophilic carbohydrate part and a hydrophobic chain of fatty acids. Production medium contained two different source of organic carbon – glucose and vegetable oil. Other components of the solution were yeast extract, peptone and minerals.

Isolation and purification of biosurfactant was carried out as follows: crude production medium (volume V = 500 ml) was exposed to ultrasound in an ultrasonic bath PS04000A (Notus-POWERSONIC Ltd.) for 15 minutes. Subsequently, this solution was transferred into a separating funnel and extracted three times with ethyl acetate in a volume ratio of 3:1. Ethyl acetate was evaporated in a rotary vacuum evaporator RVO 64 and crude product was washed with hexane five times.

Surface tension and interfacial tension were measured using the school K6 tensiometer (Kruss GmbH). Du Noüy ring method with a platinum ring was used. Volume of V = 20 ml of biosurfactant solution was used to measure the surface tension. A two-phase mixture was prepared to measure the interfacial tension. Volume of V = 20 ml of non-polar substance was poured on the surface of biosurfactant solution. Interfacial tension between these two phases was determined for different concentration of biosurfactant. Kerosene and crude oil were chosen as representatives of non-polar substances.

The critical micelle concentration (CMC) was determined by the method of surface tension measurement. The principle is that surface tension of solution decrease with increasing concentration of surfactant. Surfactant micelles affect this dependence, thus turning point on the curve corresponds to the critical micelle concentration. Minimum surface tension (MST) was determined from the dependence of surface tension on the concentration of biosurfactant.

Results and discussion

Biosurfactant of *Yarrowia lipolytica* is a white crystalline powder. Biosurfactant produced by *Candida bombicola* is a yellow-brown viscous liquid. Surfactants are completely soluble in water in concentration range from 0 – 500 mg/L. Dissolution can be accelerated by ultrasound in an ultrasonic bath.

Basic parameters of surfactants were determined. The parameters were critical micelle concentration (CMC) and minimum surface tension (MST) (Table 1). We can compare these values with values in other studies. The same type of biosurfactant had the CMC = 500 mg/L and MST = 50 mN/m[10] and the same yeast produced the biosurfactant with the CMC = 12 mg/L and MST = 40 mN/m in the previous study[11]. In the case of surfactant produced by *Candida bombicola* we can also compare its basic parameters with other. Daverey reported that CMC = 34 mg/L and MST = 59 mN/m[12]. In another study, authors presented lower values, while biosurfactant was produced under the same measured conditions. Specific values were CMC = 27 mg/L and MST = 34 mN/m[5].

Differences between the observed values can be explained by the different culture conditions, mainly by the differences in carbon source. It can be also related with the morphology of the yeasts[13]. Follow-up research on this topic should be conducted.

Table 1: Characterization of studied biosurfactants.

Producer	Critical micelle concentration (mg/L)	Minimum surface tension (mN/m)	Maximum decrease of surface tension (%)
Yarrowia lipolytica	31	40	44
Candida bombicola	83	43	40

Remediation potential of biosurfactant is associated with its ability to reduce the interfacial tension between the organic and aqueous phases. Lower interfacial tension promotes formation of emulsions, thus non-polar carbon source can be utilized by microorganisms. Dependence of interfacial tension between organic phase and water solution on the concentration of biosurfactant was determined (detailed trend in ***Figure 1*** and ***Figure 2***). Kerosene and crude oil were chosen as representatives of petroleum products. Dependence of interfacial tension between phases was as follows in the case of surfactant produced by *Yarrowia lipolytica*: interfacial tension decreased by 80% (kerosene/surfactant solution) and by 63 % (crude oil/surfactant solution). Dependence of interfacial tension between phases was as follows in the case of surfactant produced by *Candida bombicola*: interfacial tension decreased by 28 % (kerosene/surfactant solution) and by 25 % (crude oil/surfactant solution). Decrease in the interfacial tension was not affected by critical micelle concentration of biosurfactant. Biosurfactant produced by *Yarrowia lipolytica* was more effective in the reduction of interfacial tension.

Rate of decrease in the concentration of contaminant at the site could be determined in the following study.

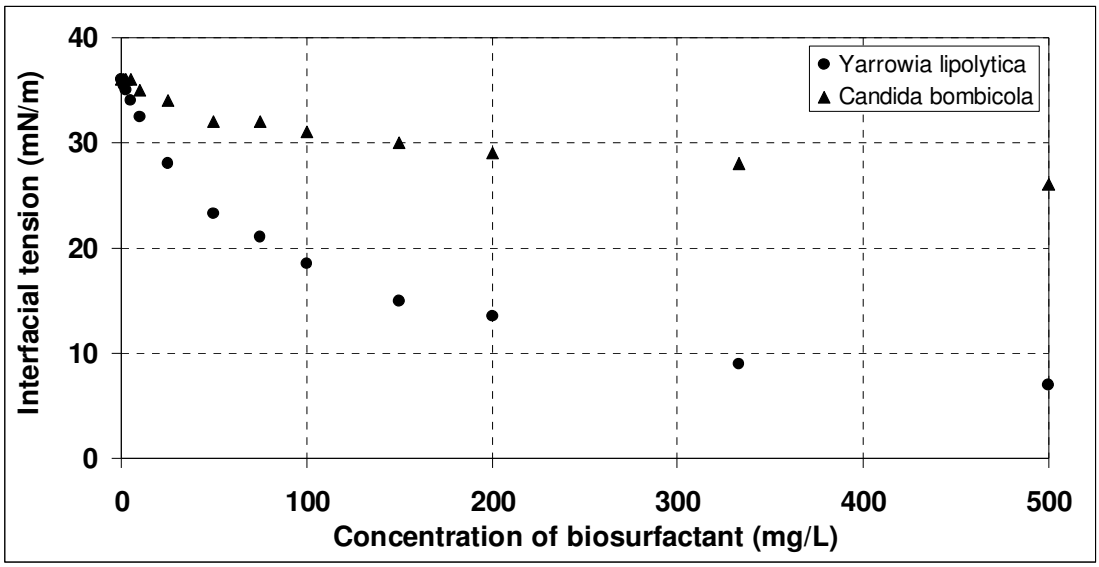

Figure 1: Dependence of interfacial tension between kerosene and biosurfactant solution.

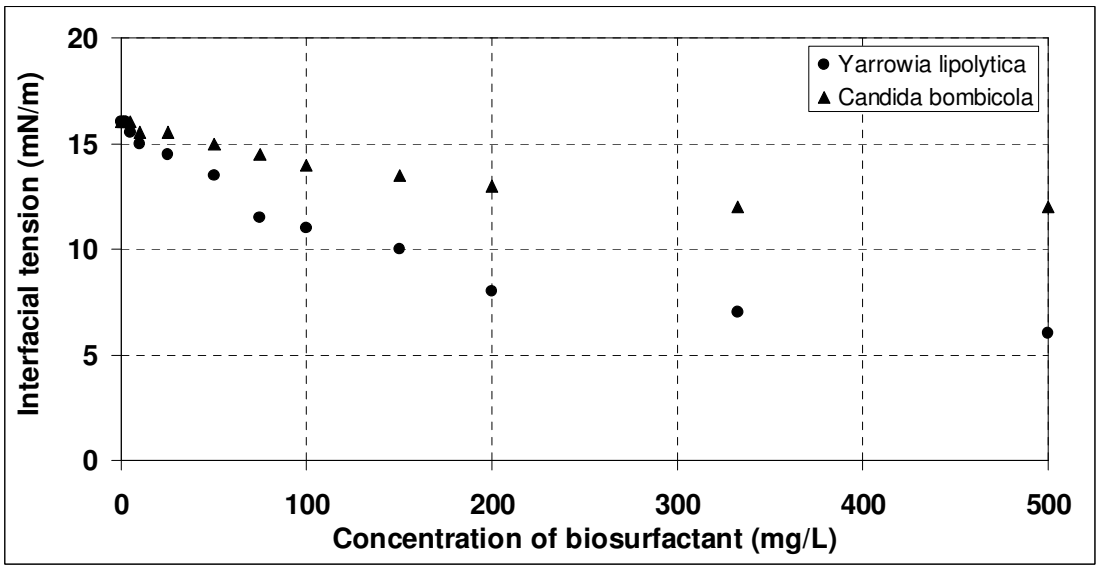

Figure 2: Dependence of interfacial tension between crude oil and biosurfactant solution.

Conclusions

Two biosurfactants produced by yeasts *Yarrowia lipolytica* and *Candida bombicola* were isolated and characterized. Critical micelle concentration (CMC) and minimum surface tension (MST) were determined for biosurfactants. CMC = 31 mg/L and MST = 40 mN/m (producer *Yarrowia lipolytica*), CMC = 83 mg/L and MST = 43 mN/m (producer *Candida bombicola*).

Interfacial tension between organic phase and surfactant solution was determined to estimate the possibilities of contaminant removal in the environment. Lower interfacial tension promotes formation of emulsions, thus non-polar carbon source can be easily utilized by microorganisms. Kerosene and crude oil were chosen as representatives of petroleum contaminants. Interfacial tension between kerosene and

surfactant solution decreased by 80 % in the case of biosurfactant produced by *Yarrowia lipolytica*. This surfactant was found to be more effective for remediation application than surfactant produced by *Candida bombicola*.

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Porovnání dvou typů kvasinkových biosurfaktantů pro odstraňování ropných uhlovodíků

Marek Šír[a], Zuzana HONZAJKOVÁ[a], Kristina TURNVALDOVÁ[b], Jiří MIKEŠ[b], Martin KUBAL[a], Radek VURM[a], Juraj GRÍGEL[b], Miroslav MINAŘÍK[b]

[a]VŠCHT Praha, Fakulta technologie ochrany prostředí, Technická 5, Praha 6, 166 28, e-mail: sirm@vscht.cz

[b]EPS, s.r.o., V Pastouškách 205, 686 04 Kunovice, e-mail: eps@epssro.cz

Souhrn

Biosurfaktanty zahrnují širokou skupinu látek převážně z řad glykolipidů, lipopeptidů, fosfolipidů, mastných kyselin a dalších látek lipidové povahy produkovaných řadou mikroorganismů. Mezi konkrétní zástupce biosurfaktantů a jejich nejvýznamnější producenty lze zařadit např. rhamnolipidy (*Pseudomonas aeruginosa*), trehalolipidy (*Arthrobacter paraffineus*, *Mycobacterium sp.*) sophorolipidy (*Candida apicola*, *Candida bombicola*), polyollipidy (*Rhodoturula glutinus*) nebo glykolipidy (*Arthrobacter sp.*).

Biosurfaktanty produkované kvasinkami se obvykle skládají z hydrofilní sacharidové části a hydrofobního řetězce mastné kyseliny. Jejich složení jim dává schopnost ovlivňovat povrchové napětí, mezifázové napětí na rozhraní organické fáze/roztoku surfaktantu a podporuje vznik emulzí. Některé typy kvasinek produkují také surfaktanty s nízkým podílem proteinů. V produkčním médiu jsou obvykle obsaženy dva zdroje uhlíku: zdroj v kultivačním roztoku (např. glukóza) a ve fázi (např. rostlinný olej).

Cílem studie bylo vyprodukovat, izolovat a charakterizovat dva typy biosurfaktantů produkovaných kvasinkami *Yarrowia lipolytica* a *Candida bombicola* a kvantifikovat jejich potenciál pro stimulovanou přirozenou atenuaci. Byly určeny jejich základní parametry (kritická micelární koncentrace a minimální povrchové napětí) a stanovena závislost mezifázového napětí v systému organická fáze/vodný roztok surfaktantu. Jako zástupci organických látek byly vybrány letecký petrolej a surová ropa.

V příspěvku nebyla zkoumána přímo solubilizace kontaminantů pomocí biosurfaktantu, ale potenciál pro odstranění kontaminantu v přirozeném prostředí. Schopnost biosurfaktantů snižovat mezifázové napětí v systému vodný roztok/nepolární látka přímo souvisí s dostupností nepolárního substrátu pro mikroorganismy a s rychlostí rozkladu dané látky.

Testy bylo zjištěno, že pro sanační aplikaci má vyšší potenciál biosurfaktant produkovaný kvasinkou *Yarrowia lipolytica*.

Klíčová slova: *biosurfaktant, povrchové napětí, kritická micelární koncentrace, mezifázové napětí, ropné uhlovodíky*