

Analysis of Surface Tension During Biodegradation of Hydrocarbons

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Abstract

Biodegradation of a model mixture of hydrocarbon (dodecane and hexadecane, 1:1 w/w), as well as hydrocarbon emulsified by synthetic and natural emulsifiers, and their mixtures were analyzed in the presence of *Pseudomonas spp.* and *Bacillus spp.* bacterial strains. Changes in surface tension were measured by the static and dynamic methods during biodegradation processes.

During the biodegradation processes of hydrocarbon and emulsified hydrocarbon, various changes in surface tension were observed. In some systems, the initial decrease of surface tension after the addition of an emulsifier was greater after a few days of biodegradation. Decrease in the surface tension does not seem to be related to the degree of hydrocarbon biodegradation, both for the hydrocarbon and emulsified hydrocarbon systems.

Keywords: biodegradation, biosurfactant, emulsifiers, hydrocarbons, surface tension

Introduction

Pollution of the environment, despite significant increase of ecological consciousness, is still a big problem. In recent years biodegradation has become one of the methods used in restoration of oil-polluted sites. Many different microbial species such as bacteria, yeast and moulds are involved in a biodegradation process [1, 2, 3, 4]. Bacterial strains use chemical compounds as a carbon source for amino acid synthesis.

The surfactants have applications in the biodegradation processes of organic compounds, especially crude oil substances. Leakage of crude oil from tankers in harbours still remains a major environmental problem. With the newest generation of emulsifiers it is possible to maintain the oil near the surface, thus making it more accessible to microorganisms which can then degrade the oil. Some bacterial strains can produce active agents, which are able

to emulsify oil in water assisting biodegradation of compounds with limited water solubility [1,5,6,7,8]. Biosurfactants could be a useful instrument in the bioremediation and control of crude oil pollution.

The biological role of biosurfactants has not been fully explored. One of its functions could be participation in the increasing degradation of compounds with limited water solubility. A larger degree of biodegradation may follow the following mechanisms:

- Natural surfactants increase the solubility of hydrocarbon, which in turn increases its bioavailability to microorganisms [9].
- Interaction between biosurfactant and degrading cells leads to an increase in the hydrophobic surface [10]. Maier and Soberon-Chavez proposed an interaction between rhamnolipides and surface cells of *Pseudomonas*, causing the secretion of lipopolysaccharides (LPS). This leads to an increase in the hydrophobic surface [11].

A larger quantity of emulsifiers is required to increase the solubility of hydrocarbon in water more than

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to change surface properties of cells. Biosurfactants may also be a source of carbon for microorganisms. However, an increase in concentration does not always improve biodegradation. This could mean that an effect of surface cell hydrophobicity is probably more important than a concentration of biosurfactant.

The aim of this research was to analyze changes in surface tension during biodegradation of hydrocarbon and emulsified hydrocarbon, as well as to find the correlation between changes in the surface tension and the degree of hydrocarbon biodegradation. Synthetic emulsifiers, biosurfactant and a mixture of natural and synthetic emulsifiers were used to emulsify the model mixture of hydrocarbon (dodecane-hexadecane). Biodegradation tests were done in the presence of *Pseudomonas spp.* and *Bacillus spp.* bacterial strains.

Experimental Procedures

Biodegradation tests were done with the model mixture of hydrocarbon: dodecane and hexadecane (1:1, w/w) as well as an emulsified mixture of hydrocarbon. The following bacterial strains were used (biochemical profile): *Pseudomonas aeruginosa*: Pa 10 (20573067072), Pa TK (20573067073), *Pseudomonas putida* (42072067073), as well as *Bacillus subtilis* (1706165255761100). Biochemical tests ID 32 GN and API 50 CHB (prod. bio-Merieux France) were used for *Pseudomonas spp.* and *Bacillus spp.* identification. Bacterial strains were isolated from bioprepate, from food industry sewage and from soil contaminated by crude oil. The time of experiments was 7 days. Samples were incubated in Erlenmeyer flasks (250mL) at 20-30°C in a shaker. Bacterial suspension (42 mL) was added to a solution of 100 cm³ water with 1.5 g of the model mixture of hydrocarbons. The number of bacteria was 10⁵-10⁶ CFU/mL (Colony Forming Unit/mL).

Oxyethylated alcohol (L-10 prod. Sigma Aldrich Chemie GmbH), alkylpolyglycosides (Lutensol GD 70 prod. BASF AG Ludwigshafen), a mixture of oxyethylated alcohol and ester (AT 7 prod. ECO Atlantol Belgium) and the biosurfactant JBR 425 (prod. Jeneil Biosurfactant Company USA, mixture of rhamnolipides R1 and R3) and a mixture of synthetic emulsifiers and the biosurfactant were used as emulsifiers. The concentration of emulsifiers in tested systems was 8.5 · 10⁻³% below critical micellar concentration (CMC). CMC of emulsifiers were the following: AT 7 – 0.03%, L-10 – 0.02%, Lutensol GD 70 – 0.051%. In relation to the hydrocarbon the concentration of emulsifiers was 0.8%.

During hydrocarbon and emulsified hydrocarbon biodegradation surface tensions were estimated using the dynamic and static methods. For the static method a Kruss K 12 tensometer was used. For the dynamic method surface tensions were measured using the maximal vesicles pressure method. The surface tension was measured with a SITA apparatus. In this method non-equilibrium values of surface tension (lifetime of bubbles was 40 s) were

estimated. The weight of hydrocarbon before and after biodegradation was measured to calculate the degree of hydrocarbon biodegradation, using an extraction of hydrocarbon by diethyl ether. The values of surface tension are an average of 5 measurements with a precision of ± 0.09. In hydrocarbon biodegradation the following concentration of emulsifiers were used: 4.2 · 10⁻³%, 2.1 · 10⁻³%, 4.2 · 10⁻³%, 8.5 · 10⁻³% and 13 · 10⁻³%.

Results and Discussion

Results of the surface tension, measured by the static method, after seven days of biodegradation indicate that both bacterial strains (*Pseudomonas aeruginosa* Pa 10 and *Bacillus subtilis*) used in experiments, reduced the surface tension of water (Figs. 1 and 2). This effect is advantageous for the *Pseudomonas aeruginosa* Pa 10 strain. After seven days of biodegradation the surface tension of water was reduced 18.5%. The *Pseudomonas aeruginosa* strain is able to produce rhamnolipides biosurfactants. These biosurfactants reduce the surface tension of water [12,13]. Fewer changes in the surface tension were observed for the *Bacillus subtilis* strain (6%). This strain is able to produce surfactin as biosurfactant, but only when it grows in a water-soluble carbon source [14]. Analysis of changes in the surface tension during biodegradation of AT 7 alone showed that the biggest increase in surface tension was during day 2. These values in comparison to initial values were lower by 8.6% and 3.5% lower for *Pseudomonas aeruginosa* Pa 10 and Ba-

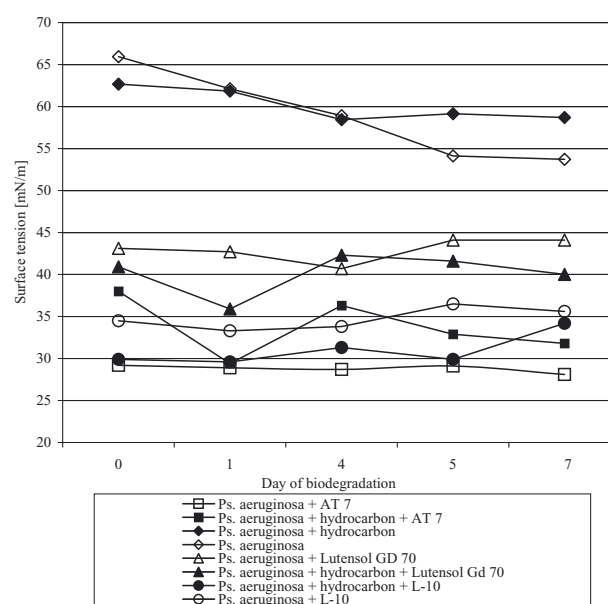


Fig. 1. Changes in surface tension (estimated by the static method) of water, water solution of hydrocarbon and emulsified hydrocarbon by AT 7, Lutensol GD 70 and L-10 in systems with the *Pseudomonas aeruginosa* Pa 10 strain during seven days of biodegradation.

Bacillus subtilis, respectively. A gradual increase in surface tension was later observed. The reason for this could be the slow biodegradation of emulsifiers. The analysis of surface tension did not indicate the biodegradation of the Lutensol GD 70 emulsifier by *Pseudomonas aeruginosa Pa 10* strain. These values were only 2.2% higher than initial values after day 7. In the case of the *Bacillus subtilis* strain, the drop was observed on day 2 (11.8%) and later oscillations were noticed. For both bacterial strains oscillation in the surface tension were observed when L-10 emulsifier was used.

The values of surface tension after day 7 of hydrocarbon biodegradation were similar to initial surface tension. It is interesting that the increases in surface tension in the initial days of hydrocarbon biodegradation accompanied the drop in surface tension in the system with bacteria and emulsifier. The addition of the AT 7 emulsifier generated a reduction in surface tension of 16.3% and 11.3% for the *Pseudomonas aeruginosa Pa 10* strain and the *Bacillus subtilis* strain, respectively, after day 7 of biodegradation. In the case of Lutensol GD 70 emulsifier no significant changes in tension were observed for the *Pseudomonas aeruginosa Pa 10* strain after day 7. However, for the *Bacillus subtilis* strain the surface tension was less after day 7 (9.3%). The increase in surface tension was measured for day 7 of the biodegradation for both bacterial strains where L-10 was used in the system.

When surface tensions were measured by the dynamic method, no significant changes in surface tension were found during the biodegradation process in the system with hydrocarbon as well as in the system with the *Pseu-*

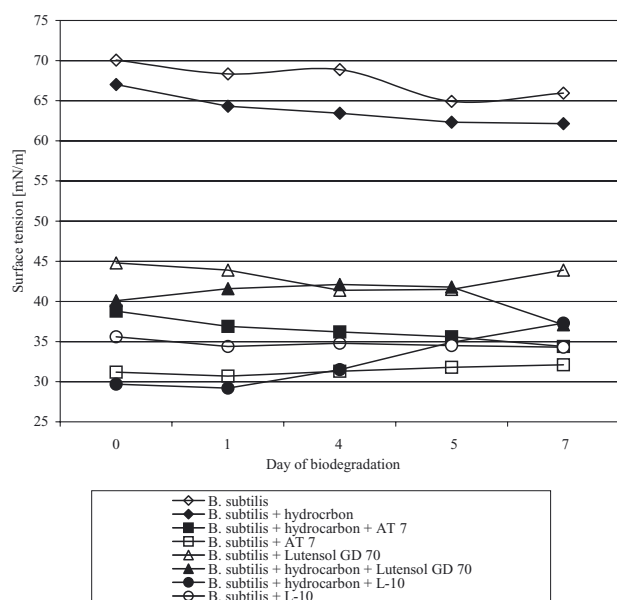


Fig. 2. Changes in surface tension (estimated by the static method) of water, water solution of hydrocarbon and emulsified hydrocarbon by AT 7, Lutensol GD 70 and L-10 in the systems with the *Bacillus subtilis* strain during seven days of biodegradation.

domonas aeruginosa Pa 10 strain alone (Table 1). For systems where microorganisms were with emulsifiers (AT 7, L-10), a decrease in surface tension was observed after day 4 of biodegradation. In the days following, oscillations were observed. In this case, emulsified hydrocarbon fluctuations in surface tension were observed for all emulsifiers. Surface tension results obtained by the static and dynamic methods, for biological systems, indicate that values from the dynamic method are always higher (Table 1). The dynamic method only allows the estimation of non-equilibrium values of surface tension (lifetime of bubbles was 40s). Therefore, in some examined systems we could not observe the same trend.

During all experiments the quantities of bacterial strains (CFU) were analyzed. Analysis of the changes in surface tension, measured by the dynamic method, and the activity of microorganisms displays no simple relationship between these parameters.

Changes in surface tension, measured by the static method, were compared with the degree of biodegradation after 7 days (Table 2). The highest level of biodegradation for the *Pseudomonas aeruginosa Pa 10* strain was observed for the system with AT 7 emulsifier (42.9%) and without emulsifier (44.6%). The greater drop of the surface tension for the system emulsified with AT 7 accompanied, an insignificant, smaller degree of hydrocarbon biodegradation in comparison to the system without emulsifier. For the *Bacillus subtilis* strain, the smallest surface tension was observed after day 7 in the system with AT 7 emulsifier where hydrocarbon biodegradation was the highest (48.4%).

When the hydrocarbon systems were emulsified with the biosurfactant (rhamnolipides) a large decrease in surface tension (estimated by the dynamic method) was observed in all systems with the *Pseudomonas aeruginosa Pa TK* strain (Fig. 3). The addition to the system with rhamnolipides of synthetic emulsifier permits an extra reduction in surface tension of 4-5 mN/m. After day 7 of biodegradation, a considerable change in surface tension was observed only for the system with L-10 emulsifier when the lowest degree of hydrocarbon biodegradation was obtained.

Lower values in surface tension after the addition of synthetic emulsifier did not result in a greater biodegradation of hydrocarbon in comparison to the system with rhamnolipides. This shows a limited relationship between a decrease in surface tension and biodegradation.

Considerable differences in the degree of hydrocarbon biodegradation for systems with similar initial values in surface tension could be explained by different interactions of individual surfactants with bacterial cells.

Effects of the emulsifier concentrations (AT 7, Lutensol GD 70, L-10 and JBR 425) on the biodegradation of hydrocarbon in the presence of *Bacillus subtilis* were analyzed (Table 3). The obtained results indicated that the various concentration emulsifiers was the best for hydrocarbon biodegradation. All tested concentrations of emulsifiers were below CMC, where micellarization has

Table 1. Results of surface tension (σ) estimated by the dynamic (dyn.) and static (stat.) method during the biodegradation process by *Pseudomonas aeruginosa Pa 10* strain.

Day of process	Surface tension σ [mN/m]															
	Bacterial suspension								Bacteria + hydrocarbon							
	-		AT 7		L-10		Lutensol		-		AT 7		L-10		Lutensol	
	σ dyn.	σ stat.	σ dyn.	σ stat.	σ dyn.	σ stat.	σ dyn.	σ stat.	σ dyn.	σ stat.	σ dyn.	σ stat.	σ dyn.	σ stat.	σ dyn.	σ stat.
0	69.7	65.9	56.5	29.2	39.7	34.5	50.7	50.7	71.1	62.7	45.9	38.0	35.0	29.9	51.1	40.9
2	69.3	60.1	48.4	26.7	39.0	35.2	50.3	50.3	72.0	56.5	47.1	30.3	43.0	29.8	50.1	45.0
4	67.8	58.9	41.6	28.7	38.7	33.8	50.9	50.9	69.9	58.4	47.9	36.3	36.7	31.3	52.4	42.3
6	71.9	53.7	49.0	29.7	41.8	35.8	58.8	58.8	70.2	53.8	52.1	36.2	45.9	37.0	55.6	42.4
8	69.1	*	44.3	*	43.5	*	57.8	*	69.9	*	51.3	*	47.3	*	50.3	*
10	68.8	*	49.4	*	40.0	*	58.8	*	70.2	*	47.6	*	47.1	*	47.2	*
12	72.0	*	48.2	*	41.5	*	52.0	*	72.0	*	58.3	*	46.0	*	48.4	*
14	73.4	*	52.7	*	41.7	*	53.4	*	72.0	*	54.5	*	48.5	*	49.6	*

*not measured

Table 2. Changes of surface tension (estimated by the static method) and degree of hydrocarbon and emulsified hydrocarbon biodegradation after 7 days with *Pseudomonas aeruginosa Pa 10* and *Bacillus subtilis*.

System	<i>Ps. aeruginosa Pa 10</i>			<i>B. subtilis</i>		
	Initial surface tension	Surface tension after 7 days	Degree of hydrocarbon biodegradation	Initial surface tension	Surface tension after 7 days	Degree of hydrocarbon biodegradation
	mN/m	mN/m	%	mN/m	mN/m	%
Bacteria + hydrocarbon	62.7	58.7	44.6	67.0	62.2	34.9
Bacteria + hydrocarbon + AT 7	37.9	31.8	42.9	38.8	34.4	48.4
Bacteria + hydrocarbon + L-10	29.9	34.2	36.2	29.7	37.1	37.4
Bacteria + hydrocarbon + Lutensol	40.9	40.0	40.8	40.1	37.3	23.6

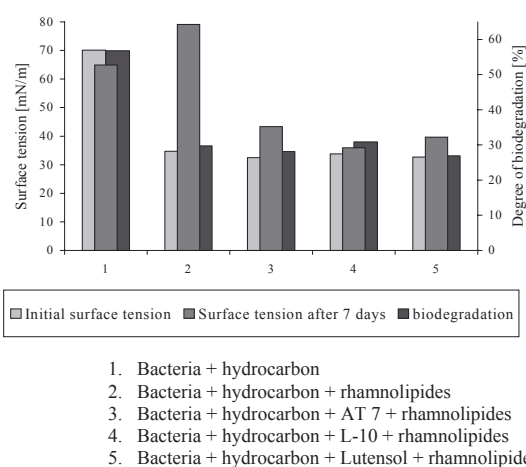


Fig. 3. Changes of surface tension (estimated by dynamic method) and degree of emulsified hydrocarbon biodegradation (synthetic and rhamnolipides) after 7 days of process for the *Pseudomonas aeruginosa Pa TK* strain.

not occurred but only solubilizations of hydrocarbon in the water phase.

Conclusions

During the biodegradation processes of hydrocarbon and emulsified hydrocarbon, various changes in surface tension were observed. In some systems the initial decrease of surface tension after the addition of an emulsifier was greater after a few days of biodegradation. The decrease in surface tension does not seem to be related to the degree of hydrocarbon biodegradation, both for the hydrocarbon and emulsified hydrocarbon systems.

Surface tension results obtained by the static and dynamic methods for biological systems, indicate that values from the dynamic method are always higher but in some examined systems we could not observe the same trend.

Table 3. Influence of emulsifier concentration: AT 7, L-10, Lutensol GD 70, on hydrocarbon biodegradation by the *B. subtilis* strain and number of bacteria after 7 days.

Kind of emulsifiers	Degree of biodegradation in following emulsifiers concentration					
	0.0%	0.00042%	0.0021%	0.0042%	0.0085%	0.012%
	%	%	%	%	%	%
AT 7	34.88	25.0	21.75	35.94	48.44	27.34
L-10		26.97	29.90	24.47	37.44	26.21
Lutensol GD 70		30.96	36.57	27.27	23.60	24.25
JBR 425		19.68	35.12	17.96	29.75	25.22

The analyzed process of hydrocarbon biodegradation in the presence of synthetic and natural emulsifiers is a complex, biological system, where many factors have to be considered. The values of surface tension depend on various factors, e.g. the type and concentration of emulsifier and bioemulsifier, the quantity and type of bacterial strains and the features of hydrocarbons and emulsifier biodegradation products. Their resultant influences on water could be expressed as changes in surface tension. The obtained results show that the changes in hydrocarbon and emulsifier concentration do not influence the majority of changes in surface tension. Therefore, the measurements of surface tension do not allow the prediction of the degree of hydrocarbon biodegradation and to find a correlation between these parameters. None of methods used assists in finding such a relationship.

Also, a decrease in surface tension does not depend on an increase in the activity of microorganisms during hydrocarbon biodegradation.

Acknowledgements

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References

- ATLAS R.M. Microbial degradation of petroleum hydrocarbons; an environmental perspective. *Microbiol. Rev.* **45**, 180, **1981**.
- SPENCER J., RAGOUT DE SPENCER A., LALUCE C. Non-conventional yeast. *Appl. Microbiol. Biotechnol.* **58**, 147, **2002**.
- IJAH U.J.J. Studies on relative capabilities of bacterial and yeast isolates from tropical soil in degrading crude oil. *Waste Manage.* **18**, 293, **1998**.
- OKERENTUGBA P., EZERONYE O., Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *Afr. J. Biotechnol.* **2** (9), 288, **2003**.
- BARTHA R., ATLAS R.M. The microbiology of aquatic oil spills. *Advan. Appl. Microbiol.* **22**, 225, **1977**.
- DESAI J.D., BANAT I.M. Microbial production of surfactants and their commercial potential. *Microbiol. Mol. Biol. Rev.* **61**, 47, **1997**.
- DESAI J.D., DESAI J.A. Production of biosurfactants. In: *Biosurfactants*, editor. N. Kosaric; p. Marcel Dekker: New York, pp 65-97, **1993**.
- FOGHT J.M., GUTNICK D.L., WESTLAKE D.W.S. Effect of emulsan on biodegradation of crude oil by pure and mixed bacterial cultures. *Appl. Environ. Microbiol.* **55** (1), 36, **1989**.
- ZHANG Y., MILLER R.M. Enhanced octadecane dispersion and biodegradation by a *Pseudomonas* rhamnolipid surfactant (biosurfactant). *App. Environ. Microbiol.* **58**, 3276, **1992**.
- ZHANG Y., MILLER R.M. Effect of a *Pseudomonas* rhamnolipid biosurfactant on cell hydrophobicity and biodegradation of octadecane. *Appl. Environ. Microbiol.* **60**, 2101, **1994**.
- MAIER R.M., SOBERON-CHAVEZ G. *Pseudomonas aeruginosa* rhamnolipids: biosynthesis and potential applications. *Appl. Microbiol. Biotechnol.* **54**, 625, **2000**.
- BENINCASA M., ABALOS A., OLIVEIRA I., MANRESA A. Chemical structure, surfaceproperties and biological activities of the biosurfactant produced by *Pseudomonas aeruginosa* LBI from soapstock. *Anton. Leeuw.* **85**(1), 1, **2004**.
- SIM L., WARD O.P., LI Z-Y. Production and characterization of a biosurfactant isolated from *Pseudomonas aeruginosa* UW-1. *J.Ind.Microbiol.Biotechnol.* **19**, 232, **1997**.
- COOPER D.G., MACDONALD C.R., DUFF S.J.B., KOSARIC N. Enhanced production of surfactin from *Bacillus subtilis* by continuous product removal and metal cation additions. *Appl. Environ. Microbiol.*, **42**, 408, **1981**.