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Microbiotests with marine organisms for studying the toxic potential of biosurfactants and synthetic surfactants

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Abstract

During the last decade several surface active substances produced by microorganisms (biogenic surfactants, biosurfactants) have been described. Most of them are glycolipids composed of a hydrophilic sugar and one ore more lipophilic corynomycolic acids. A better biodegradability and lower toxicity of biosurfactants should be expected, because of their biogenic origin. However, data in this regard are missing.

This paper presents results of toxicity testing series, in which four synthetic surfactants, two commercial oil dispersants, and six biosurfactants have been examinated. The test systems were 1. bacterial growth inhibition. 2. microalgae growth inhibition. 3. microflagellate growth inhibition. 4. biodegradation rate, and 5. bioluminescence inhibition (Microtox test). The multiplication of bacteria was stimulated by surfactants, whilst that of microflagellates and microalgae was inhibited. This may be due to the metabolic usage of surfactants, especially biosurfactants, by the bacteria. The bioluminescence was very sensitive to surfactants. No toxicity could be detected with the glucoselipid GL, produced by the marine bacterium *Alcaligenes* sp. MM1. Most biosurfactants were degraded faster and possess higher EC_{50}-values than synthetic dispersants.

Introduction

Since more than 20 years surfactants are used for the abatement of marine oil pollutions. The aim is to break the oil slick into small droplets, to produce oil-in-water microemulsions, and to transfer the hydrocarbons into the water column. A disadvantage of the actually used surfactants is their own toxicity, which strongly limits their applicability. During the last decade several surface active substances produced by microorganisms have been isolated and described (LANG and WAGNER 1987). After their discovery the idea of a new generation of surfactants was borne. A first experimental investigation in this regard was done 1979: a tidal flat was experimentally oil polluted, and after treatment with the biogenic trehalose-dicorynomycolate (TL-2) it was less damaged than after treatment with the synthetic Finasol OSR-5 or without surfactants usage (DÖR-

JES 1984). This preliminary results induced further investigations about the toxicity of synthetic and biogenic surfactant with the use of several different test systems.

Material and methods

The following chemically synthesized surfactants were tested:

EO4,5 = nonylphenol- (ethylenoxide) $_{4,5}$-acetate (Hüls, FRG)
EO9 = nonylphenol- (ethylenoxide) $_{9}$-acetate (Hüls, FRG)
TBS = tetrapropylenbenzene-sulfonate (Merck, FRG)
CTAB = cetyltrimethyl-ammoniumbromide (Merck, FRG)
DK50 = sucrose-stearate, 30 % monoester and 70 % diester
DK160 = sucrose-stearate, 70 % monoester and 30 % diester (both DK-ester from Chemische Fabrik, Grünau, FRG)
Pril = a commercial cleaning surfactant (Böhme Chemie GmbH, FRG)
Corexit = the commercial oil dispergator Corexit 9527 (Esso, FRG)
Finasol = the commercial oil dispersant Finasol OSR-5 (Fina GmbH, FRG)

Tested biogenic surfactants and their producing strain:

TL-2 = trehalose-dicorynomycolate and TL-4 = trehalose-tetraester (C_8, C_{10}, C10 fatty acids and succinate), both from *Rhodococcus erythropolis* DSM 43215
RL = rhamnose-lipid mixture, *Pseudomonas* sp. DSM 2874
SS = sophorose-lipid (acidic form) and SL = sophorose-lipid (lactonic form), both from *Torulopsis bombicola* ATCC 22214
Suc = sucrose-lipid, *Corynebacterium* sp. M 9b
GL = glucose-lipid, *Alcaligenes* sp. MM1
Emu = Emulsan, *Acinetobacter calcoaceticus* ATCC 31012 (marine)
LGP = lipopolysaccharide, SL-1 (marine bacterium, not classified)

Emu was obtained from Prof. Dr. D.L. Gutnik, Tel Aviv (Israel). All other biosurfactants were isolated and purified by the Institute of Biochemistry and Biotechnology, Braunschweig, Germany.

Test systems:

1. The growth inhibition of bacteria (*Serratia marinorubra, Photobacterium phosphoreum, Acinetobacter calcoaceticus,* mixed marine population), of microflagellates (mixed marine population), and microalgae (*Dunaliella tertiolecta, Scripsiella trochoidea*) by surfactants was calculated by incubating the organisms in a sufficient medium (bacteria and flagellates: pepton-broth, algae: mineral-broth and light) supplemented with 0 - 1000 ppm surfactant. The multiplication was studied by cell countings and the logarithmic growth documentated. From this results the surfactant concentration was calculated, at which 50 % of growth was inhibited (EC_{50}-value).

2. The biodegradability of surfactants by marine bacteria (*Serratia marinorubra,* mixed marine population) was studied by measuring the biological oxygen demand in closed bottles and calculating an average daily oxydation rate.

3. The bioluminescence inhibition test with *Photobacterium phosphoreum* was carried out according to the method described by KREBS 1983.

Results and discussion

The tested surfactants showed different results in the used test systems. The growth of eucaryotic test organisms (microalgae, flagellates) was slowed down or was inhibited by surfactants, while the multiplication of bacteria remained nearly uneffected or was stimulated. This findings document a generally greater sensibility of marine eucaryotes than marine bacteria against surfactants. Similar results are known (BRINGMANN and KÜHN 1980) for several other xenobiotics. Moreover, most synthetic surfactants possessed a lower EC_{50} than biosurfactants. The missing bacterial growth inhibition could be the result of the biodegradability of surfactants, which was investigated in the degradation experiments. The pure culture of *Serratia marinorubra* showed smaller degradation rates compared with a mixed population of marine bacteria. This may be due to the greater range of available enzyms of a whole population compared to a single strain. Most biosurfactants were degraded faster than synthetic surfactants.

The biosurfactants showed their general smaller toxicity compared to synthetic surfactants also in the bioluminescence inhibition tests.

Each test system was used to calculate a toxicity data. The growth inhibition experiments gave EC_{50}-value of a surfactant concentration, which inhibites 50 % growth rate. The lowest data were obtained from the bioluminescence test, thus it was the most sensitive test. The data were concerned for rankings, in which a high toxicity (high ranking number) stands for a low EC-value in growth or bioluminescence inhibition and slow biodegradation rate. Taking all rankings into account was possible by the calculation of the average ranking number (Table 1) as previously described (WILSON 1974).

Table 1. Ranking of surfactants concerning several microbioassays; a high number stands for great toxicity and a low number for a small toxicity.

Surfactant	Origin	Ionic state	Ranking number
Emu	b		4
LGP	b		5
DK50	s	n	2
DK160	s	n	5
Suc	b	n	6
TL-2	b	n	9
SL	b	n	10
GL	b	a	1
TL-4	b	a	6
SS	b	a	7
RL	b	a	11
EO9	s	a	9
EO4,5	s	a	13
TBS	s	a	14
CTAB	s	c	16
Corexit	s		14
Finasol	s		15

a: anionic, b: biogenic, c: cationic, n: nonionic, s: synthetic

The generally higher toxicity of synthetic products is significant. Only DK-surfactants break this rule. Moreover, the well described relationship (JAMES 1965, PELZAR et al. 1988) between toxicity and ionogenic structure of the surfactants - this means, that the cationic surfactants are more toxic than the anionic, and the nonionic are the least toxic ones - becomes obviously, but only in the case of synthetic surfactants. Although biosurfactants miss the conformity with this rule; maybe, because their hydrophilic sugarresidue possess enough ionic strength to mediate glycolipids an ionic-like character.

The better degradability of biosurfactants may be due to their specific molecular structure. While the synthetic EO-surfactants contain the hardly attackable aromatic benzene ring (SWISHER 1970), the tested biosurfactants miss such an inert compound and should be totally mineralizable. The good oxidation of DK-surfactants is in agreement with this interpretation: DK-surfactants are synthetic glyco-lipids and of homological structure as the biogenic glyco-lipids.

Finally, the small toxicity of GL is noteworthy. This "marine" surfactant missed nearly any response in growth inhibition tests and exhibites the fastest biodegradation of all tested substances. Nevertheless, it is too early to make its marine origin responsible for its missing toxicity against marine test organisms. GL has just been discovered (SCHMIDT et al. 1990) and further investigation should take place, before a special qualification of GL for an application in the marine environment could be stated.

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