Toxicity Testing of Synthetic and Biogenic Surfactants on Marine Microorganisms

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ABSTRACT

The toxicity of four synthetic surfactants, two commercial oil dispersants, and six biosurfactants have been examinated. The test systems were (a) bacterial growth inhibition, (b) microalgae growth inhibition, (c) microflagellate growth inhibition, (d) biodegradation, and (e) bioluminescence inhibition (Microtox test). The multiplication of bacteria was stimulated by surfactants, while that of microflagellates and microalgae was inhibited. This may be due to the metabolic usage of surfactants, especially biosurfactants, by bacteria. The bioluminescence was very sensitive to surfactants. No toxicity could be detected with glucose-lipid, produced by the marine bacterium *Alcaligenes sp.* MM1. Most biosurfactants were degraded faster and possessed higher EC_{50} values than synthetic dispersants.

INTRODUCTION

Surfactants have been used for 20 years for the abatement of marine oil spills. 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 (biogenic surfactants, biosurfactants) have been isolated and described (Cooper and Zajic, 1980; Gutnik and Minas, 1987; Lang and Wagner,

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TABLE I
Tested synthetic surfactants

Abbreviation	Chemical name	"Manufacturer"
E04,5	Nonylphenol-(ethylenoxide)$_{4,5}$-acetate	Hüls, Marl, FRG
E09	Nonylphenol-(ethylenoxide)$_9$-acetate	Hüls, Marl, FRG
TBS	Tetrapropylenbenzene-sulfonate	Merck, Darmstadt, FRG
CTAB	Cetyltrimethyl-ammoniumbromide	Merck, Darmstadt, FRG
DK50	Sucrose-stearate (30% monoester and 70% diester)	Chemische Fabrik, Grünau, FRG
DK160	Sucrose-stearate (70% monoester and 30% diester	Chemische Fabrik, Grünau, FRG
Pril	Commercial cleaning surfactant Pril	Böhme Chemie GmbH, Düsseldorf, FRG
Corexit	Commercial oil dispersant Corexit 9527	Esso, Hamburg, FRG
Finasol	Commercial oil dispersant Finasol OSR-5	Fina GmbH, Frankfurt, FRG

1987; Zajic and Panchal, 1976). After their discovery, the idea of a new generation of surfactants was borne. The 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 (Dörjes, 1984). These preliminary results induced further investigations about the toxicity of synthetic and biogenic surfactant with the use of several different test systems.

TESTED SURFACTANTS

The surfactants tested are listed in Tables I and II. 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).

INFLUENCE ON THE GROWTH OF MICROORGANISMS

The influence of surfactants on the growth of several strains of pro- and eucaryotic microorganisms was tested. An example is given in Fig. 1, but similar results were found with other surfactants, too. The curves indicate the relative (percentage) multiplication of the microalgae *Du-*

TABLE II
Tested biogenic surfactants

Abbreviation	Chemical name	"Manufacturer"
TL-2	Trehalose-dicorynomycolate (C_8, C_{10} fatty acids)	*Rhodococcus erythropolis* DSM 43215
TL-4	Trehalose-tetraester (C_8, C_{10}, C_{10}, fatty acids and succinate)	*Rhodococcus erythropolis* DSM 43215
RL	Rhamnose-lipid mixture	*Pseudomonas sp.* DSM 2874
SS	Sophorose-lipid (acidic form)	*Torulopsis bombicola* ATCC 22214
SL	Sophorose-lipid (lactonic form)	*Torulopsis bombicola* ATCC 22214
Sac	Saccharose-lipid	*Corynebacterium sp.* M 9b
GL	Glucose-lipid	*Alcaligenes sp.* MM1
Emu	Emulsan	*Acinetobacter calcoaceticus* ATCC 31012
LGP	Lipopolysaccharide	Marine bacterium SL-1 (strain classification in progress)

naliella tertiolecta (Chlorophyceae) and a mixed population of bacterivorous nanoflagellates, but the results of the two marine bacteria *Acinetobacter calcoaceticus* HO1-N and *Serratia marinorubra* DSM 30124 in the presence of an increasing amount of the biosurfactant RL are also shown.

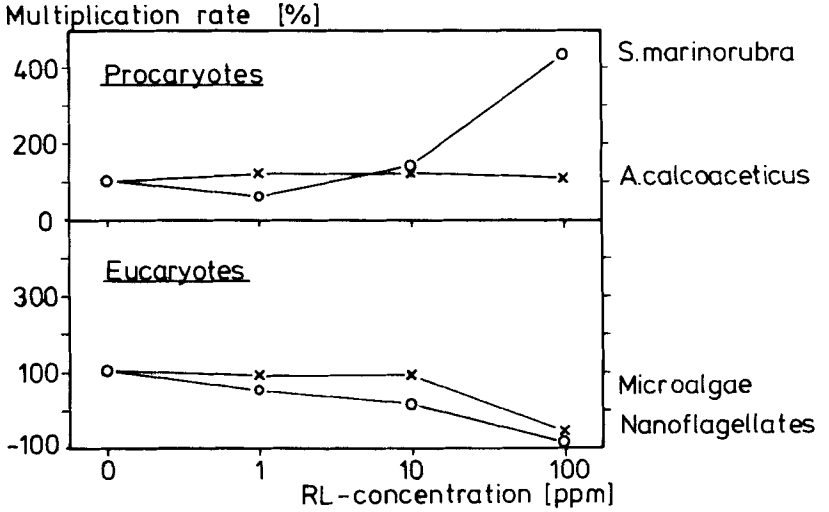

Fig. 1. Influence of RL on multiplication of marine microorganisms.

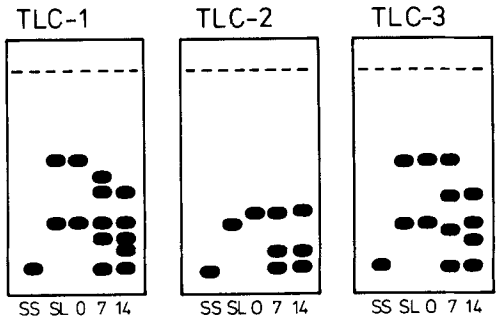

Fig. 2. TLC of SL-degradation cultures after 0, 7, and 14 days, SS and SL are references; TLC-1: anisaldehyde (hydrocarbons), TLC-2: Bromcresol green (acidic groups), TLC-3: dichlorfluoresceine (lipids).

While the growth of the tested eucaryotic organisms decreased or was inhibited, the multiplication of bacteria remained nearly uneffected or was stimulated. These findings documentate a generally greater sensibility of marine eucaryotes than marine bacteria to surfactants. Similar results are known (Bringmann and Kuhn, 1980) for several other xenobiotics. The missing sensibility of bacteria could be the result of the biodegradability of surfactants (see below).

BIODEGRADATION OF SURFACTANTS

The marine biodegradation of surfactants was measured with the biochemical oxygen demand (BOD) method in closed bottles, which were supplemented with 1.0 mg surfactant per L freshly collected seawater. The average daily BOD of each substance was documentated for getting comparable data. Moreover, a qualitative verification of the attack of the biosurfactant SL was done by thin layer chromatography (TLC) analysis (Fig. 2). The developing system used for this TLC was $CHCl_3$/CH_3OH/acetic acid = 65/15/2 (v/v/v).

The TLC chromatograms show that over the duration of the experiment (14 days) SL changes its reaction against detecting reagents getting more acidic groups—similar to the reference substance SS, and becomes more and more hydrophilic.

BIOLUMINESCENCE INHIBITION (MICROTOX)

The bioluminescence inhibition test is a generally accepted toxicity test, although it does not indicate a definite toxic reaction (Krebs, 1983).

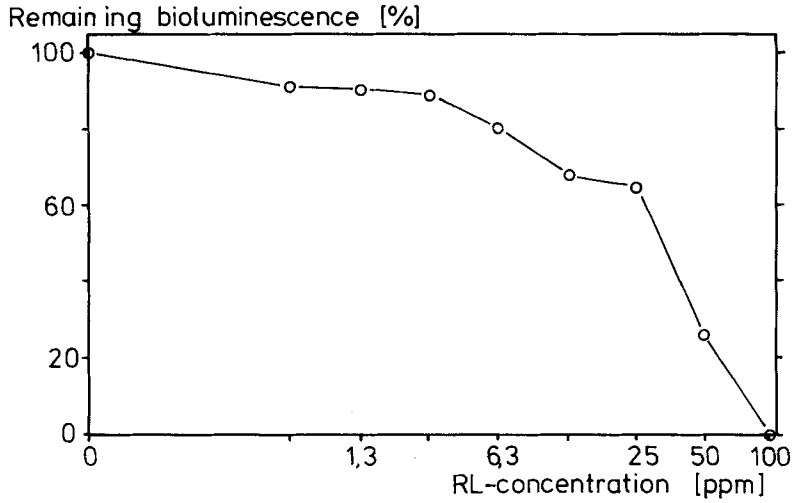

Fig. 3. Influence of RL on bioluminescence.

Figure 3 shows a representative curve of decreasing bioluminescence with increasing concentration of the biosurfactant RL. Data from all tested surfactants were collected and the EC_{50} (concentration of surfactant, which inhibites 50% luminescence) was documentated for comparison.

COMPARISON OF TOXICITY TAKING ALL SYSTEMS INTO ACCOUNT

Each test system was used to calculate toxicity data. The growth inhibition experiments gave the EC_{50} value of a surfactant concentration, which inhibits 50% growth rate. The lowest data were obtained from the bioluminescence test; thus it was the most sensitive test. The data concerned 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 (Fig. 4), as previously described (Wilson, 1974).

The generally higher toxicity of synthetic products is significant. Only DK surfactants break this rule. Moreover, the well-described relationship (James, 1965; Pelczar *et al.*, 1988) between toxicity and ionogenic structure of the surfactants—this means that cationics are more toxic than anionics, and nonionics are the least toxic ones—be-

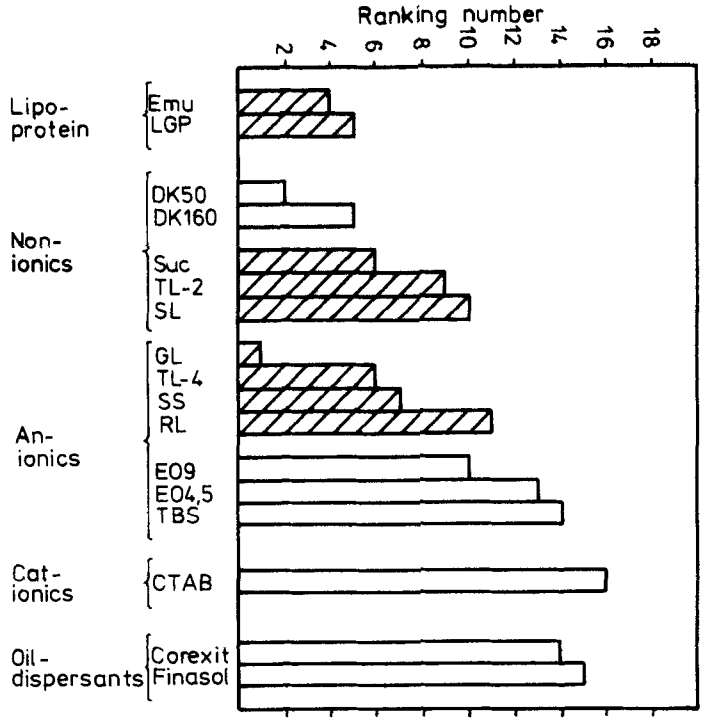

Fig. 4. Average ranking number of surfactants taking five toxicity tests into account; shaded columns are biosurfactants, unshaded columns are synthetic surfactants.

comes obvious, but only in the case of synthetic surfactants. Although biosurfactants miss the conformity with this rule, perhaps because their hydrophilic sugar residue 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 biosurfactants tested miss such an inert compound and should be totally mineralizable. The good oxidation of DK surfactants is in agreement with the following interpretation: DK surfactants are synthetic glycolipids and of homological structure as the biogenic glycolipids.

Finally, the low toxicity of GL is noteworthy. This "marine" surfactant missed nearly any response in growth inhibition tests and exhibited the fastest biodegradation of all tested substances. Nevertheless, we think it is too early to make its marine origin responsible for its lack of 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 can be stated.

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