The Egyptian Journal of Hospital Medicine Vol., 7 : 53 – 57 June 2002
I.S.S.N: 12084

Toxicity of Biosurfactants and Synthetic Surfactants on Marine Organisms

Prepared By:
Eman I. Siam
Assistant Professor in Marine Environmental Protection Programme
Arab Academy for Science and Technology and Maritime Transport

Abstract

This paper presents results of toxicity testing series, in which four synthetic surfactants, two commercial oil dispersants, and six bio surfactants have been examined.

The test systems were bacterial growth inhibition, microalgae growth inhibition, and microflagellate growth inhibition and biodegradation rate. 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. No toxicity could be detected with the glucose lipid GL, produced by the marine bacterium Alcaligenes sp. MMI. Most biosurfactants were degraded faster than synthetic dispersants.

Introduction

Surfactants are used since 50 years for the combating of marine oil pollution, surfactants are mixtures which include surface active agents to reduce the interfacial tension between oil and sea-water, this makes it possible for an oil slick to break into very fine droplets (less than 100 microns in diameter) which are rapidly distributed throughout the water column because of natural water movement.

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 WAGNEN 1987). After their discovery the idea of a new generation of surfactants was born.

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 (Dörjes 1984). These preliminary results induced further investigations about the toxicity of synthetic and biogenic surfactant with the use of several different best systems.

Material and Methods

Tested biogenic surfactants and their producing strain

TL – 2 = trehalose – dicorynomycolate, and

TL – 4 = trehalose – tetraester (C_8, C_{10} fatty acids and succinate), both from Rhodococcus erythropolis.

RL = rhamnose – lipid mixture , Pseudomonas sp

SS= sophorose – lipid (acidic form)

SL= sophorose – lipid (lactonic form)

SUC= sucrose – lipid, corynebacterium sp.

GL= glucose – lipid, Alcaligenes sp.

Ema = Emalsan, Acinetobacter Calcoaceticus (marine)

Refree : Prof ; *Dr. Abla M . Helmy Afify.*

LGP = Lipopolysaccharide
SL-1 marine bacterium (not classified)
All biosurfactants were isolated and purified by the high institute of Public Health, Alex-Egypt.

The following chemically synthesized surfactants were tested:
$EO_{4,5}$ =nonylphenol – (ethylenoxide) 4, 5-acetate
EO_9 = nonylphenol – (ethylenoxide) 9-acetate
TBS = tetraprophylenbenzene - sulfonate
CTAB =cetyltrimethyl – ammonium bromide
DK_{50} = sucrose – stearate, 30% monoester and 70% diester
DK_{160} = sucrose – stearate, 70% monoester and 30% diester
Both DK-ester
Pril = a commercial cleaning surfactant
Corexit=the commercial oil dispergator corexit
Finasol=the commercial oil dispergent Finasol

Test Systems

1. The growth inhibition of bacter - iaserratia marinorubra, photobacterium phospherium, Acinetobacter calcoacet - icus, mixed marine population of microflagelates (mixed marine popul - ation), and microalgae (Dunaliella tertiolecta, scripsiella trochoidea) by surfactants was calculated by incubating the organisms in a sufficient medium (bacteria and flagellates on pepton-broth, algae on mineral-broth in light) supplemented with 0-1000 ppm surfactant. The multiplication was studied by cell counting and the logarithmic growth documented.

From these results the surfactant concentration was calculated, at which 50% of growth was inhibited (EC_{50} – value).

2. The biodegradability of surfacta -nts by marine bacteria (serratia marinor -ubra, mixed marine population) was studied by measuring the biological oxygen demand in closed bottles and calculating an average daily oxidation rate.

3. The bioluminescence inhibition test with photobactererium phospher - eum 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. These findings document are generally of greater sensibility for marine eucaryotes than marine bacteria against surfactants. Similar results are known (BRINCMANN and KÜHN 1990) for several other xenobiotics. Moreover, most synthetic surfactants possessed a lower EC_{50} than biosur-factants. The missing bacterial growth inhibition could be the result of the bio-degradability of surfactants, which was investigated in the degradation experi-ments. The pure culture of *Serratia marinorubra* showed smaller degrada - tion rates compared with a mixed population of marine bacteria. This may be due to the greater range of available enzymes 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 inhibits 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 bio-luminescence 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 relation - ship (JAMES 1985, 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 1570), the tested biosurf - actants miss such an inert compound and should be totally mineralizable. The good oxidation of DKsurfactants 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 CL is noteworthy. This “marine” surfactant missed nearly any response in growth inhibition tests and exhibits 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 been discovered recently (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.

References

1. ***BRINGMANN, C. and K. KÜHN, 1990***. Comparison of the toxicity thresholds of water pollutants to bacteria, algae, and protozoa in the cell multiplication test. Water Res. 14, 231-241.
2. ***DÖRJES, J., 1984***. Experimentelle Untersuchungen zur Wirkung von Rohöl und Rohöl/Tensid-Gemischen im Ökosystem Wattenmeer. XVI. Zusammenfassung und SchluBfolge -rungen. Senkenbergiana marit. 16, 267-271.
3. ***JAMES, A.M., 1985***. Surface-active agents in microbiology. 3-21. In: Surface activity and the microbial cell, S.C.I. Monograph 19, Staples Printers Ltd., Kent, GB.
4. ***LANG, S. and F. WAGNER, 1987***. Structure and properties of biosurf -actants. 21-45. In: Biosurfactants a biotechnology. Ed: N. KOSARIC. Marcel Dekker Inc. N.Y.
5. ***KREBS, F., 1983***. Toxizitätstest mit tiefgefrorenen Leuchtbakterien- Gewässerschutz Wasser Abwasser 63, 173-230.
6. ***PELZAR, M.J., E.C.S. CHAN and N.R. KRIEC, 1988***. Microbiology. McGraw-Hill Book Co., Singapore.
7. ***SCHMIDT, M., A. PASSERI, D. SCHULZ, S. LANG, F. WAGNER, K. POREMBA, W. GUNKEL and V. WRAY, 1990***. Formation of biosurfactants by oil degrading marine microorganisms. 811-814. In: Dechema Biotechnol. Conferences, Vol. 3, VCH Verlagsgesellschaft, Weinheim, FRG.
8. ***SWISHER, R.D., 1970***. Surfactant biodegradation. Surfactant Science Series. Vol. 3, Marcel Dekker Inc., N.Y.
9. ***WILSON, K.B., 1974***. Toxicity testing for ranking oils and oil dispersants. 11-22. In: Ecological aspects of toxicity testing of oils and dispersants. Ed.: L.R. BEYON and E.B. COWELL, Applied Science Publishers LTD, London, G.B.

التأثير السام لمنظفات الطبيعية والصناعية على الكائنات البحرية

د / إيمان إبراهيم صيام

أستاذ مساعد بقسم حماية البيئة البحرية بالأكاديمية العربية للعلوم والتكنولوجيا

تتناول هذه الدراسة نتائج تجارب عملية تم فيها اختيار درجة سمية بعض المركبات المصنعة والطبيعية مثل المنظفات الصناعية ومشتتات الزيت على نمو ونشاط بعض أنواع من الكائنات البحرية الدقيقة مثل البكتريا والطحالب الدقيقة والسوطيات الدقيقة لمقارنة تأثير استخدام هذه المركبات على الكائنات البحرية .

وقد استنتج من التجارب أن معظم المنظفات الحيوية تتحلل في البيئة البحرية بسرعة اكثر من المنظفات المصنعة ولوحظ أيضا تثبيط نشاط الطحالب والسوطيات الدقيقة في حين نشطت البكتريا وازداد معدل انقسامها في وجود هذه المركبات في البيئة البحرية