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Toxicity Test Accompanying Biodegradation Test of Anionic Surfactants

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In July 1974, the Minister of Agriculture in Israel, passed a bill prohibiting the manufacture, importation, distribution and marketing of "hard" anionic synthetic surfactants based on branched chains of alkyl benzene sulphonates. This prohibition does not include products for export, and solid synthetic soaps. By April 1975, the detergent industry had to change over completely to biodegradable anionic surfactnats in all washing and cleaning products in household and industrial use.

A year later, the Water Commissioner's Office commissioned a survey (NARKIS AND ZUR 1976) on the degree of obedience to this law, using the screening test of the OECD (OECD 1976).

Since at the beginning of the survey, many of the washing powders, bought in stores, proved to be nonbiodegradable, it was assumed that they represented an old stock, and the survey was continued on products received from the factory inventory. Even then, the screening test showed that many of the washing powders still contained anionic surfactants which were only slightly biodegraded. These are responsible for a high residual of 10-12 mg/l MBAS in effluent from biological treatment plant of sewage, in comparison to 25 mg/l MBAS before the changeover took place (NARKIS 1976).

In cases where the biodegradation test did not proceed smoothly a second surfactnat extraction was repeated.

During the incubation test samples of one of the prominent sources gave turbid and unpleasant odours, even though this source had declared its products to contain only soft anionic surfactants. This was repeated in a second sample of the same product from the same source, with the same results, thus showing a very low degree of biodegradation. In order to determine whether any constituent of the washing powder formulation which might be toxic to the micro-organisms was transferred to the ethanol extract, and interfered with the biodegradability test, a simple toxicity test was undertaken, in parallel with the screening test. This technique is recommended in order to determine whether toxic or inhibitory factors exist in problematic materials in the biodegradability test.

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EXPERIMENTAL

Materials: Household and cleaning products from factory inventory.
LAS - Linear Alkyl Benzene Sulphonate, soft standard anionic surfactant, with 100% of biodegradation after 5 days of incubation. Supplied by Zohar-Dalia.

ABS - Branched chain Alkyl Benzene Sulphonate, hard standard anionic surfactnat with 50.5% biodegradation after 19 days of incubation, supplied by Lankro.

The anionic synthetic surfactants were leached from the commercial samples by refluxing with ethanol. In samples containing soap concentration, equal to or higher than the anionic component, the soap was subsequently removed by acidification and hexane extraction. Perborates and percarbonates, where present, were destroyed by boiling a mixture of washing powder in distilled water for one hour, prior to the extraction with ethanol. There was no available chlorine in any of the products examined. The extent of the biodegradation of the separated anionic surfactant was tested according to the screening test of the OECD (OECD 1976). Each set tested for biodegradation included blank and reference standards of soft LAS and hard - ABS anionic surfactants. The reported biodegradation represent duplicate samples.

RESULTS AND DISCUSSION

Figs. 1 and 2 show the biodegradability screening test of the anionic surfactants from washing powders which were also tested by the toxicity test discussed later on. A soft biodegradable anionic surfactant is defined as one undergoing at least 80% removal of MBAS after 19 days of incubation. The biodegradation results of washing powders have shown that some of them still contain hard anionic surfactants, even though this is prohibited by law. The biodegradability test in many cases did not proceed smoothly. In some cases a lag period was required for acclimation. During this lag period there was only a slight decrease in MBAS concentration but by the end of the 19 day incubation period the MBAS decreased to correspond to more than 80% removal. In some extreme cases at the end of the lag period the MBAS concentration increased to nearly the original level or more and then the material was slightly biodegraded. This suggests that a part of the anionic surfactant was in a complex not detected by the MBAS test. At the end of the lag period, the anionic surfactnat was changed to a form that was both more readily degraded and detected by the MBAS test.

Similar behaviour was reported by BUZZELL & RYCKMAN (1968) who studied the biodegradation of pyridine by measuring COD, TOC and oxygen uptake. Pyridine is not detected by the COD test. The COD remained low until the end of the lag period. As oxygen uptake reached its maximum rate and the TOC value decreased, the

COD increased. These authors explained that this measurable COD was the result of microbial degradation, of pyridine giving intermediate products which were active in the COD test.

Toxicity test

In the screening tests giving the most difficulty, the solutions tested became increainsgly turbid, during the incubation period. This interference was accompanied by unpleasant odours and poor biodegradation of the MBAS tested. Since washing powders consist of mixtures of anionic surfactants and soap together with other organic and inorganic constituents it is reasonable to ask if any constituents of the washing powder formulation may have been transferred to the ethanol extract. It is possible that such a component is either toxic to the microorganisms or at such a high concentration that unaerobic conditions prevail, inhibiting the aerobic biodegradation.

A preliminary toxicity test was carried out on samples which did not biodegrade after 10 days of incubation in the screening test Tinokleen proved to be netirely nonbiodegradable (Fig. 1), with residual of 11.5 mg/1 MBAS (0% removal). At this stage 10.8 mg/1 of LAS was added to a combined sample taken from the original duplicate samples, giving a total MBAS concentration of 22.3 mg/1. This mixture was incubated on a shaker for an additional 9 days together with new reference standard solutions of LAS in nutrient broth and seeding. The extent of biodegradation is given in Table 1, showing that substantially all of the added LAS decomposed in both the test sample and the reference standard solutions. The preliminary test showed that in this case neither a toxic nor any inhibition effect exist and the material which does not decompose is in fact nonbiodegradable. On the other hand, a soft surfactant, such as LAS, decomposed easily under these conditions.

TABLE 1

Preliminary toxicity test by addition of a soft LAS to Tinokleen solution after 10 days of incubation in the OECD screening test.

Surfactant tested	Screening test	Preliminary toxicity test		
	MBAS conc. after 10 days mg/1	LAS added mg/1	Initial total MBAS conc. mg/1	Residual MBAS conc. after 9 days mg/1
LAS Standard	-	19	19	0.7
LAS Standard	-	11.8	11.8	0.5
Tinokleen	11.5	10.8	22.3	13.3

Due to the possibility of toxicological effects it was decided to carry out a simple toxicity test in addition to the screening test of the OECD.

The same solutions prepared for the screening test containing nutrients, seeding and tested surfactant, were also used for the toxicity tests. After determination of the initial surfactant concentration a weighed sample of the soft surfactant LAS was added to a volume of 250 ml, taken from the screening test solution. After the LAS was dissolved by mixing for 1 hour, the total MBAS concentration of the test surfactant and the LAS were determined. It was assumed that a presence of toxic component would inhibit the biological decomposition of the soft standard, which has previously been shown to completely biodegrade after 5 days incubation. These biodegradation tests were carried out for 10 days at $25^0 \pm 1^oC$, on a shaker in a dark room. A summary of the toxicological results is given in Table 2. This table also presents results of the screening tests after 10 and 19 days.

In samples tested the residual total MBAS concentration after 10 days was lower than the initial MBAS concentration before the addition of the soft LAS.

The complete decomposition of the LAS demonstrates the absecne of a toxic substance, and proved that the surfactant tested present in the same erlenmeyer tested is a nonbiodegradable material. The anionic surfactnats which do not degrade in the screening tests are nonbiodegradable in the described test as well, while the added soft LAS degrades. A synergistic effect has been noted on addition of the biodegradable LAS on the surfactant tested. This surfactant decomposed at a faster rate in the presence of LAS. In some cases a period of 10 days incubation resulted in decomposition greater than after a full 19 days incubation by the OECD screening test.

In conclusion it is proposed to carry out a toxicity test in parallel to the OECD test, by addition of 10 mg/l LAS soft surfactant to a part of a solution of the surfactnat tested, nut rients and seeding, prepared for the screening test. The residual MBAS concentration is then tested after 10 days of incubation ruling out the possibility of toxic or inhibiting effects.

An additional advantage of this proposed procedure is that a shorter incubation time is required in the presence of LAS than is required by the screening test. In this way modifying the OECD test by addition of soft LAS can both shorten the period of incubation and give information on possible toxic effects.

TABLE 2

Parallel toxicity test and OECD screening test carried out by addition of soft LAS to a part of the surfactant, nutrients and seeding solutions

Cleaning product tested	OECD SCREENING TEST					TOXICITY TEST				
	0	10 days		19 days				10 days of incubation		
	Initial conc. mg/1	Residual mg/1	Degradation % Ave.	Residual mg/1	Degradation % Ave.	LAS added mg/1	Total initial MBAS mg/1	Residual MBAS mg/1	% degradation, Ave. LAS	% degradation, Ave. Surfactant tested
LAS Standard	12.8 12.8	0.0 0.0	100	0.0 0.0	100					
ABS Standard	14.1 14.1	8.5 8.5	39.3	6.9 7.0	50.5	26.1 26.1	40.2 40.2	7.5 7.5	100	46.8
Bioness Automati	9.4 9.4	7.3 7.3	20.2	2.3 2.1	77.1	18 18	27.4 27.4	1.4 1.4	100	85.1
Textile Shampoo	11.0 11.0	8.8 8.2	22.7	0.9 0.85	92.0	30.7 30.7	41.7 41.7	0.4 0.4	100	96.3
Tasbin	9.0 9.0	5.8 6.3	32.8	1.6 1.3	83.8	16.8 16.8	25.8 25.8	2.9 2.9	100	67.7
Tinokleen yad	15.5 15.5	11.7 11.4	25.5	9.2 9.1	40.9	17.7 17.7	33.2 33.2	16.2 16.2	97.2	0
Gal	13.7 13.7	12.6 12.9	6.9	12.5 12.7	8.0	12.9 12.9	26.6 26.6	11.3 11.3	100	17.5
Kvis-yad	10.7 10.7	6.9 6.2	38.8	1.3 1.0	89.2	14.9 14.9	25.6 25.6	0.8 0.8	100	92.5
Bioness ragil	12.5 12.5	9.1 9.3	28.1	0.6 0.6	95.2	18.7 18.7	31.2 31.2	1.0 1.0	100	92.0
Bioplus	11.0	10.7	2.7	1.0	90.9	10.2	21.2	0.6	100	94.5
Galit	17.1 17.1	15.0 15.1	11.9	2.6 2.6	84.8	32.9 32.9	50 50	12.5 12.5	100	26.9

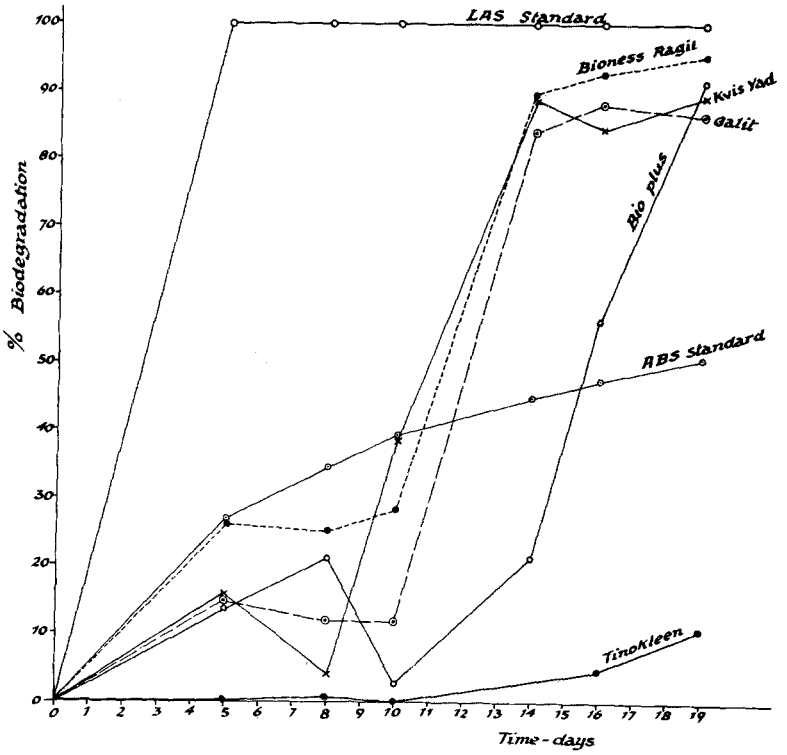

Fig. 1. Biodegradability OECD screening test of anionic surfactants leached from washing powders.

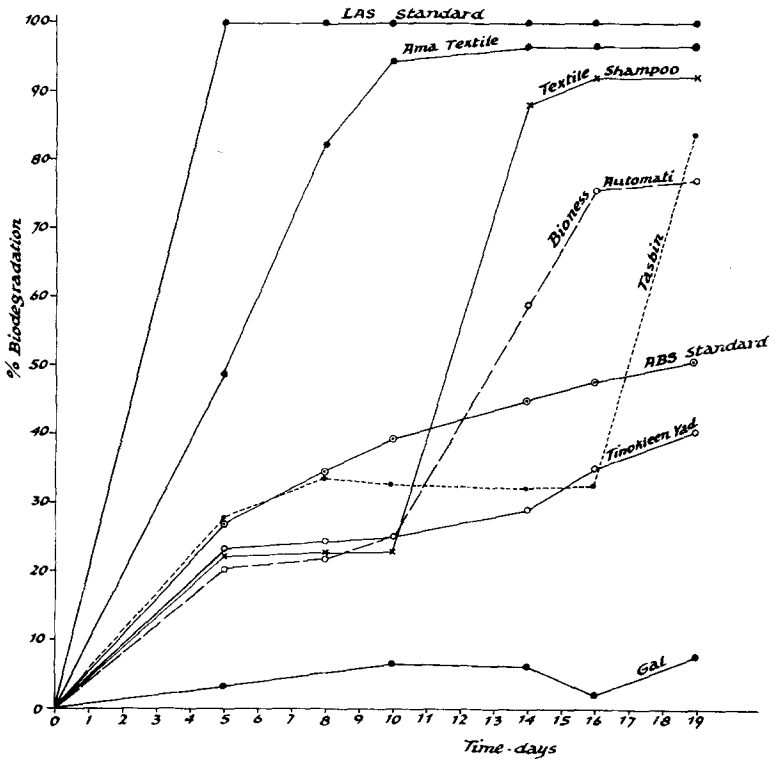

Fig. 2. Biodegradability OECD screening test of anionic surfactants leached from washing powders.

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