

Tobin

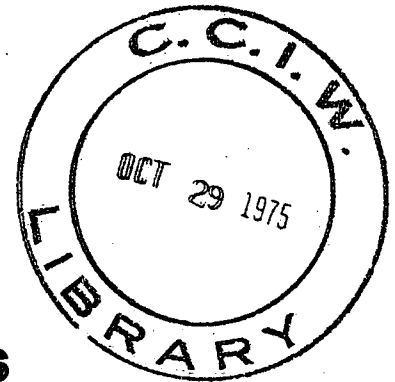


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**"TESTING THE BIODEGRADABILITY OF NONIONIC
SURFACTANTS BY THE O.E.C.D. DYNAMIC
SIMULATION TEST"**

Richard S. Tobin and D.H.J. Anthony

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SIMULATION TEST"

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Canada Centre for Inland Waters

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ABSTRACT

Biodegradability testing of nonionic surfactants was performed by the O.E.C.D. dynamic simulation (confirmatory) test. A bench-scale activated sludge plant was used with a continuous flow of synthetic sewage containing the surfactants to be tested. Details of operation and modifications to prevent excessive foaming are described. These techniques can be modified for testing the biodegradability and intermediate products of other classes of compounds.

Results showed the extent of primary biodegradation of the three nonionic surfactants tested followed the order: linear primary alcohol ethoxylate >> nonylphenol ethoxylate > branched alkylphenol ethoxylate.

INTRODUCTION

Nonionic surface-active agents (surfactants) presently constitute 25-30% of all surfactants in use. One report of the total quantities manufactured in Canada has been prepared (1) but, because of the proprietary nature of the products, the distribution of nonionic surfactant types in detergent formulations is difficult to ascertain. Results of an informal survey of manufacturers and distributors (Appendix A) indicates that all the most common household nonionic surfactants are the ethoxylate type, the most common being the linear primary alcohol ethoxylate. In spite of the extensive use of nonionic surfactants, little biodegradation testing has been performed in Canada, partially because of other detergent problems (viz. phosphate replacement, anionic surfactant determinations) and partially because of the lack of a standard, universally approved test method.

Parts of this study were performed as a contribution to "ring tests" by the Organization for Economic Cooperation and Development (O.E.C.D.) to develop suitable biological and analytical techniques for measuring the biodegradability of nonionic surfactants. The O.E.C.D.(2) has proposed a static culture "screening test" for anionic and nonionic surfactants, in addition to a dynamic simulation "confirmatory test" for surfactants which fail to satisfy the requirements of the screening test. A report on the operation of

the screening test has already appeared (3). The present report is designed to assist those involved in establishing and operating the dynamic simulation (confirmatory) test and presents some considerations for further studies.

MATERIALS AND METHODS

Surfactants

- Dobanol 25-9 Shell Research Laboratories, Egham, Surrey, England; Active ingredient 30% (w/w); average structure:
$$n-C_{12-15}H_{25-31}O(C_2H_4O)_9H.$$
- Nonylphenol EO₁₀ Chemische Werke Hüls, Aktiengesellschaft Marl-Kreis, Recklinghausen, Germany; Active Ingredient 99.9%; average structure:
$$n-C_9H_{19} \text{ (benzene ring) } O(C_2H_4O)_{10}H.$$
- Lissapol - NX Department of the Environment, Water Pollution Research Laboratories, Elder Way, Stevenage, Herts., Great Britain; Active ingredient 100%; average structure (branched alkyl phenol ethoxylate of unspecified alkyl group):
$$C_xH_y \text{ (benzene ring) } O(C_2H_4O)_9H.$$

TBS An anionic surfactant. "Hard", tetrapropylene-derived alkyl benzene sulphonate; American Soap and Glycerine Products, Inc., New York, New York, U.S.A.

Equipment

The bench-scale activated sludge plant (Fig. 1) is constructed of glass*according to dimensions specified by the OECD (2,4). The equipment consists of a 26 l storage vessel (A), a dosing pump (B) to deliver $24 \text{ l} \cdot \text{d}^{-1}$, a settling column (D), air lift pump (E) with a downspout (F) to recycle the activated sludge, and 26 l collection vessel (G) to collect the treated effluent. Air flow through a sintered glass aerator (H) is controlled by a meter (I). A stirrer (J) with a glass stirring rod was used to keep the sludge suspended in the aeration chamber(C).

Synthetic Sewage

Each litre of synthetic sewage was made up with tap water and contained 160 mg peptone, 110 mg beef extract, 30 mg urea, 7 mg NaCl, 4 mg $\text{CaCl}_2 \cdot 2 \text{H}_2\text{O}$, 2 mg $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, 5 mg Dow Corning Antifoam B (Fisher Scientific Co., 184 Railside Road, Don Mills, Ontario, Canada), 5 mg (of active ingredient) of the non-ionic surfactant being tested, 3 mg "Hard" standard TBS.

* Glassblowing by Rudy Palme, Hamilton, Ontario. Approximate cost of glass and glassblowing: \$150.

Operation of Activated Sludge Plant

Innoculation was effected by 1.5 ml of unfiltered secondary effluent from a domestic sewage treatment plant. The sludge plant was maintained at $20 \pm 0.1^{\circ}\text{C}$ in an environmental chamber. The nutrient flow rate was 1 litre per hour with solution prepared fresh each day. The dissolved oxygen content was at least 2 mg/litre. The running-in period was one week.

Analysis of Samples

Microscopic examination, DOC and loss of dry weight upon ignition were performed at least once per week. Samples for anionic surfactant analysis were taken twice per week and analyzed by the Methylene Blue Test (5). Samples for nonionic surfactant analysis were taken three times per week and analyzed by the Wickbold method. This technique consists of gas stripping the surfactant from the sample into ethyl acetate. After evaporation of solvent, the residue is dissolved and the surfactant precipitated with modified Dragendorff reagent ($\text{KBiI}_4 + \text{BaCl}_2 + \text{glacial acetic acid}$). The precipitate is collected by filtration, washed, redissolved and the Bi^{3+} titrated with pyrrolidinedithiocarbamate using either a Radiometer or Orion platinum-calomel combination electrode. Titration was performed semi-automatically with a Radiometer Model PHM26-TT11-SBR2C-ABU11 recording titrator.

Endpoints were read directly from the ABU-11 autoburette or, in the case of less pronounced titration curves, calculated graphically. The precision obtained with either method of endpoint determination, using DOBANOL 25-9 as a reference substance in replicate determinations, was not significantly different, nor was the accuracy. The precision experienced in the determination of the external reference, CuSO_4 , was typically of the order Coefficient of Variability (CV) = 0.2% (N = 7) while the precision in determination of unextracted surfactant was CV = 1.2% (N = 7). This precision, however, demands the special care necessary in analytical operations with surfactant solutions (e.g. ref. 6, page 41). Biodegradation results were calculated as prescribed by the OECD (2).

RESULTS

Operation of the Bench-Scale Activated Sludge Plant

The dynamic simulation (confirmatory) test adopted by the O.E.C.D. in its recommendations for surfactant biodegradability testing is designed to simulate a conventional activated sludge waste treatment unit. A synthetic sewage mixture containing nonionic surfactants at 10 ppm (or the level of 5 ppm used in these studies to more closely simulate domestic sewage treatment operations) is pumped at the rate of $1 \text{ l} \cdot \text{h}^{-1}$ into a 3 l

aeration chamber (Fig. 1, C) where it is degraded by polyvalent aerobic microorganisms (inoculum from an unfiltered secondary effluent source). At a flow rate of $1 \text{ l} \cdot \text{h}^{-1}$ and 3 l capacity, the mean retention time for the synthetic sewage is 3 h. A flow of synthetic sewage to the settling chamber (Fig. 1, D) is maintained by gravity and the sludge is settled and recycled to the aeration chamber by an air lift (Fig. 1, E). The clarified effluent drains into the collection vessel (Fig 1, F) where the 24 h composite samples are taken as required, preserved with 50 ppm HgCl_2 and stored in the dark at $4-5^\circ$ until analyzed.

Periodic microscopic examination is required to determine the quantity of protozoans as they may, in sufficient quantities, interfere with the sludge performance. The dry weight content of the material in the aeration chamber should be performed twice weekly. If it is more than $2.5 \text{ g} \cdot \text{l}^{-1}$, the excess activated sludge must be discarded.

A running-in period of one week or more was required before sufficient sludge was formed for proper operation of the settling chamber. Additions of 2 ml aliquots of 5% ferric chloride were made to the contents of the aeration chamber to augment sludge settling. During the running-in period, the surfactant being studied is present in the synthetic sewage to allow microbial acclimatization to that compound. It was noted early in the testing procedure that foaming of the surfactants in the aeration

chamber would be a problem. Because surfactant is concentrated in foam, excessive foaming in the aeration chamber would tend to remove significant quantities of surfactant from contact with the bulk of the activated sludge. Biodegradation results might then reflect degree of foaming in the aeration chamber to a great extent. Since the degree of foaming was found to be attributed to the method of aeration (i.e. sparging through sintered glass) the following modifications were made to the operation of the aeration chamber: (1) the inclusion of Dow Corning Antifoam B (silicone oil emulsion) in the synthetic sewage, (2) the use of a stirring motor and propeller to keep the sludge in suspension in the aeration chamber, allowing the air flow to the sintered glass bubbler (Fig. 1, H) to be decreased and (3) the addition of a downspout (Fig. 1, F) to the air lift return to eliminate splashing. These three measures greatly decreased foaming and enabled a more accurate determination of biodegradability. It was, however, still necessary to scrub and rinse down the side of the aeration vessel at least once every day to remove sludge residue and adhering surfactant from the wall above the liquid level. Modifications (2) and (3) were not used in the studies presented here, but were evaluated and adopted in subsequent experiments.

Biodegradability of Dobanol 25-9

A three week biodegradability test was performed with the linear primary alcohol ethoxylate, Dobanol 25-9. The dissolved

organic carbon (DOC) analyses (Table 1) indicated an active sludge which degraded the organic material of the synthetic sewage an average of $75.7 \pm 4.1\%$ (mean \pm standard error) throughout the test period. Degradation of the "hard" anionic surfactant, TBS, was greater than expected (Table 2) with a mean degradation of $67.7 \pm 3.7\%$. The high degree of activity may be partially due to the high dissolved oxygen concentration (about 5.4 ppm) because of the dual role of the aerator in aeration and keeping the sludge from settling. The later modification of adding a stirrer (Fig. 1, J) allowed a decreased air flow to be used (and dissolved O_2 to decrease to a value closer to the minimum of 2 ppm specified by the O.E.C.D.) and lower degradation of the TBS standard resulted.

The test material, Dobanol 25-9 was degraded an average of $95.9 \pm 0.2\%$ (Table 3) in the three week test. The uniformity of degradation of this compound was in contrast with results obtained with the anionic surfactant (Fig. 2) and the other nonionic surfactants studied (see below) and suggests that greatest reproducibility will be observed in studies of those surfactants which are most easily degraded.

Biodegradability of Alkylphenol Ethoxylates

To compare the behaviour of other types of nonionic surfactants with Dobanol 25-9 in the dynamic simulation test, one week experiments were performed on nonylphenol EO₁₀

and Lissapol-NX. The degradation of TBS was $66.0 \pm 3.2\%$ (Table 4) in the nonylphenol EO_{10} experiment, in good agreement with the Dobanol 25-9 trials. The nonylphenol EO_{10} degradation was erratic with an average of $45.7 \pm 8.0\%$ degradation.

In the test of the branched alkylphenol ethoxylate, Lissapol-NX, TBS degraded $66.3 \pm 1.8\%$ (Table 5) and Lissapol-NX degraded $36.7 \pm 1.9\%$. In this test flocculation of "micellar-like" material occurred in the aeration and settling chambers, suggesting that large quantities (5 ppm in influent) of very "hard" nonionic surfactants may significantly impair the activated sludge process.

DISCUSSION

The dynamic simulation test has been chosen as the "confirmatory" test for surfactant biodegradability by the O.E.C.D. "Group of Experts on the Biodegradability of Nonionic Surfactants" because of the similarity of operation to actual common waste-treatment facilities. The test requires a reasonable amount of expense to set up (glassblowing, pumps, flowmeters), considerable daily attention throughout the test period and can only test one surfactant in a minimum of eight weeks of operation (i.e. an experiment consisting of a one week running-in period and three weeks test period, performed in duplicate). In spite of the limitations, results appear to be reasonably consistent over the test period and the apparatus can be used for testing compounds

other than surfactants (unpublished results). At present, the correlation of results obtained in this simulation and actual sewage treatment facilities has not been precisely determined.

The results presented here on the biodegradability of nonionic surfactants agree with published data (for a review, see ref. 6). Primary biodegradation of the linear alcohol ethoxylate was almost complete in the test period while the alkylphenol ethoxylate and branched alkylphenol ethoxylate were more refractory. The order of primary biodegradability, linear alcohol ethoxylate >> alkylphenol ethoxylate > branched alkylphenol ethoxylate confirms published data (3,6) and to some extent, validates this approach.

It must be stressed that data obtained by this confirmatory test and using the O.E.C.D.-recommended Wickbold analysis for nonionic surfactants yield data on primary degradation only. That is, the method detects only molecules which have surface-active properties. Molecules which have, for example, been cleaved at the alkyl-polyethoxylate ether bond are not extracted by gas stripping and thus are not detected. As a result, no information is provided on ultimate biodegradability (mineralization to $\text{CO}_2 + \text{H}_2\text{O}$) nor is any information provided on the amount or types of products formed. These points are important because of recent evidence (7,8) that linear alcohol ethoxylates (Dobanol 25-9) undergo primary degradation with rapid removal of the alkyl moiety but polyethoxylates are liberated and these undergo slow and incomplete

degradation. In one of the studies, the dynamic simulation apparatus was used for biological degradation and samples analyzed by a HBr-ether cleavage and gas chromatographic technique (8) to determine the degradation products of the surfactant. It is thus possible to use the dynamic simulation test with any number of general or specific analytical techniques for a more complete assessment of the primary and ultimate biodegradability as well as a characterization of the products formed by incomplete degradation.

The main class of nonionic surfactants in household use in Canada are the linear primary alcohol ethoxylates (Appendix A) because of emphasis on (primary) biodegradability. None of the less biodegradable alkylphenol ethoxylates were noted in this survey although they are one of the predominant types in industrial and institutional applications where the same criteria are not applied. In view of the extensive use of nonionic surfactants in Canada (1) and U.S.A. (9) and lack of knowledge of their impact on the environment due to uncertainties of the extent of ultimate biodegradability and nature of the products formed, further testing is important. While the O.E.C.D. dynamic simulation test (and their recommended analytical technique) effectively measures parameters closely related to foaming potential, complete biodegradability testing should incorporate other techniques which measure the extent of ultimate degradation and the nature of any products formed.

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TABLE I. Reduction of dissolved organic carbon in the dynamic-simulation test of Dobanol 25-9 degradation. Samples were centrifuged at 45,000 xg for 20 minutes and analyzed on a Beckman Model 915 Total Carbon Analyzer with an IR-215B analyzer.

No.	Day of Test	DOC mg/l		Decrease (%)
		IN	OUT	
1	7	75	22	71
2	14	83	23	72
3	19	135	22	84

Average Decrease = $75.7 \pm 4.1\%$

TABLE 2. Biodegradation of TBS in the dynamic simulation test of Dobanol 25-9 degradation.

No.	Day of Test	Concentration (ppm)		Degradation (%)
		IN	OUT	
1	2	3.0	0.67	78
2	5	2.9	0.78	73
3	9	2.8	1.30	54
4	12	2.7	0.70	74
5	16	2.5	0.95	62
6	19	2.6	0.90	65

Average Degradation = $67.7 \pm 3.7\%$

TABLE 3. Biodegradation of Dobanol 25-9 in the dynamic simulation test

No.	Day of Test	Concentration (ppm)		Degradation (%)
		IN	OUT	
1	1	5.4	0.29	94.6
2	3	5.5	0.20	96.4
3	6	5.6	0.27	95.2
4	8	5.5	0.23	95.8
5	10	5.9	0.21	96.4
6	13	6.4	0.23	96.4
7	15	6.6	0.25	96.2
8	18	5.8	0.21	96.3
9	21	6.0	0.27	95.5

Average Degradation = $95.9 \pm 0.2\%$

TABLE 4. Determination of the biodegradability of Nonylphenol EO₁₀ in the dynamic simulation test. Analyses were performed on samples taken during a one-week period.

Sample	Day	TBS			Nonylphenol EO ₁₀		
		IN (ppm)	OUT (ppm)	Degradation (%)	IN (ppm)	OUT (ppm)	Degradation (%)
1	1	2.7	0.76	72	5.6	2.2	61
2	4	2.8	0.98	65	5.2	3.4	34
3	6	2.7	1.05	61	5.0	2.9	42

Average = $66.0 \pm 3.2\%$

Average = $45.7 \pm 8.0\%$

TABLE 5. Determination of the biodegradability of Lissapol-NX in the dynamic simulation test. Analyses were performed on samples taken during a one-week period.

Sample	Day	TBS			Lissapol-NX		
		IN (ppm)	OUT (ppm)	Degradation (%)	IN (ppm)	OUT (ppm)	Degradation (%)
1	1	2.7	1.0	63	4.9	3.0	39
2	4	2.8	0.94	67	4.8	3.0	38
3	6	2.8	0.86	69	4.2	2.8	33

Average = $66.3 \pm 1.8\%$

Average = $36.7 \pm 1.9\%$

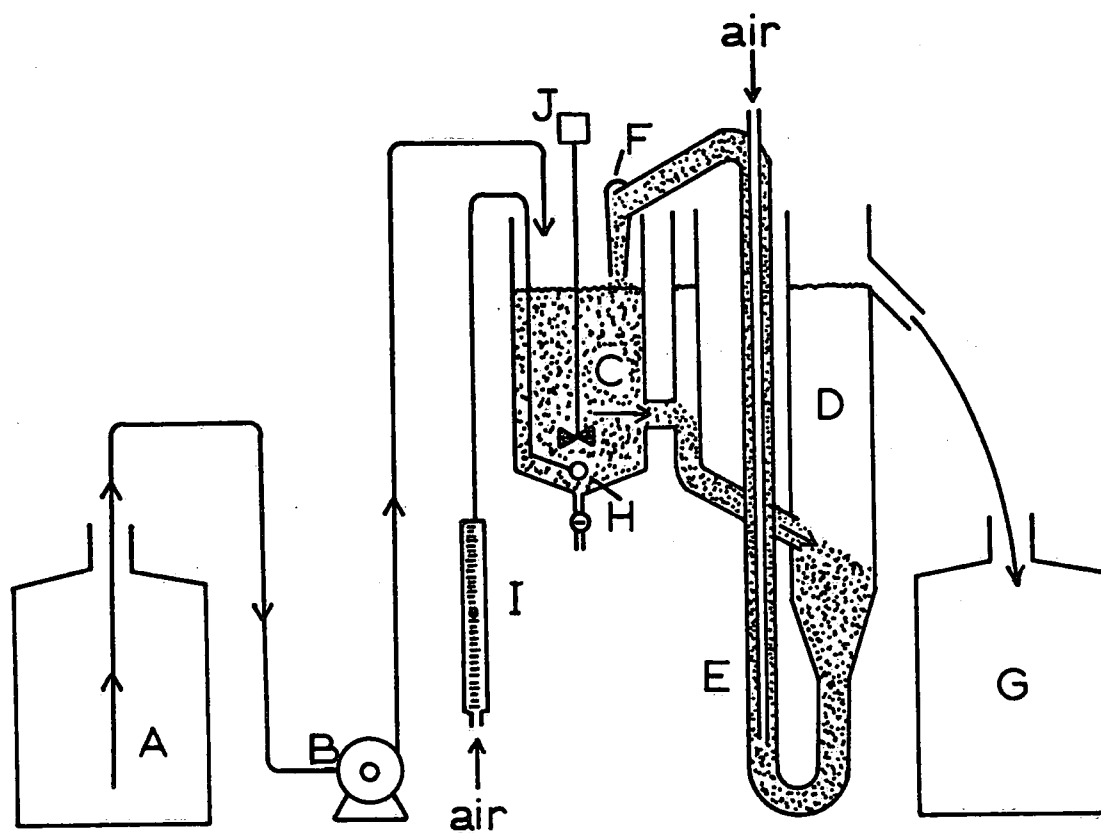


Fig. 1. Bench-scale activated sludge plant.

- | | |
|---------------------|-----------------------|
| A. Storage vessel | F. Downspout |
| B. Dosing pump | G. Collection vessel |
| C. Aeration chamber | H. Aerator |
| D. Settling chamber | I. Air flow meter |
| E. Air lift pump | J. Stirring apparatus |

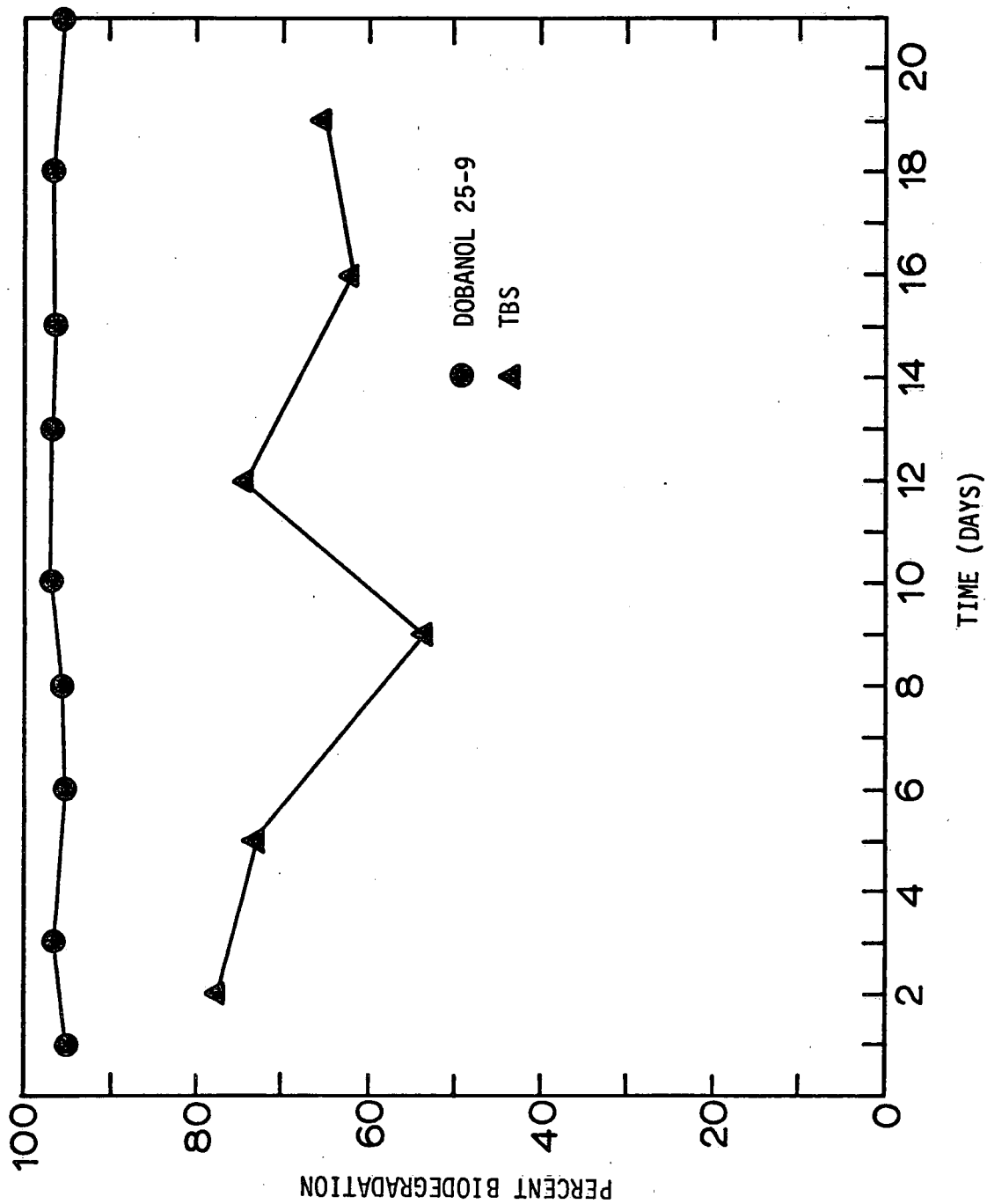
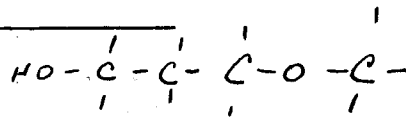
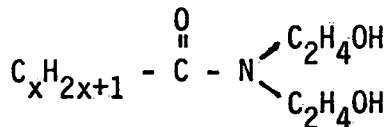
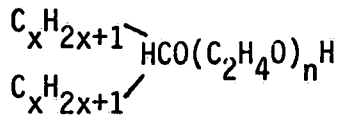
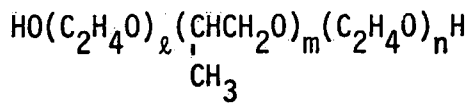


Fig. 2. Confirmatory test for biodegradability of DOBANOL 25-9. Data from Tables 2 and 3 are used.

Appendix "A". Nonionic surfactants in common household use in Canada. A survey* of manufacturers, distributors and blenders of household detergents (laundry, dishwashing and general purpose) in Canada was performed. Results of this survey indicated that there are about four major types of nonionic surfactants which are listed in order of estimated use.

Order	Type	General Structure
1	Linear primary alcohol polyethoxylate	$C_xH_{2x+1}O(C_2H_4O)_n$ 
2	Fatty acid diethanolamides	$C_xH_{2x+1}-C(=O)-N(C_2H_4OH)_2$ 
3	Secondary alcohol polyethoxylates	$C_xH_{2x+1}HCO(C_2H_4O)_nH$ 
4	Polyethoxylate-polypropoxylate block copolymers	$HO(C_2H_4O)_x(CH(CH_3)CH_2O)_m(C_2H_4O)_nH$ 

* Companies participating in the survey:

Admiral Sanitation Ltd, Scarborough
 Amway of Canada Ltd, London
 Bestline Products of Canada Ltd, Toronto
 Canada Packers Ltd, Toronto
 Chempec Ltd, Toronto
 Colgate-Palmolive, Toronto
 Copeland Laboratories Ltd, Toronto
 Diversy Corp. of Canada Ltd, Mississauga
 G.H. Wood and Company Ltd, Toronto
 Lever Detergents, Toronto
 London Soap Company, London
 McKague Chemicals, Scarborough
 Pennwalt of Canada Ltd, Oakville
 Prentice Chemical Company, Hamilton
 Simpsons-Sears, Toronto
 S.F. Lawrason and Company Ltd, London

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