

BIODEGRADATION (OECD 301E)

TEST SUBSTANCE

Identity: A mixture containing perfluorooctanesulfonate, which may also be referred to as PFOS, FC-95, or as a component of FC-203. (1-Octanesulfonic acid) (CAS # 2795-39-3).

Remarks: The 3M production lot numbers were Antwerp, Belgium 9/82 and US 3072. The test sample is FC-203 produced in Antwerp, Belgium. Current information indicates FC-203 is a mixture of 1.34% PFOS, 35% diethylene glycol butyl ether, 37.85% water, 20% ethylene glycol, 2.66 % Sultone foamer, 3% sodium octyl sulfate, 0.1% sodium lauryl sulfate, and 0.05% tolyltriazole.

The following summary applies to a mixture with incompletely characterized concentrations of impurities. Data may not accurately reflect degradation potential of the fluorochemical component of the test sample.

METHOD

Method: Modified OECD Screening Test, OECD 301E, with DOC Analysis

Test type: Ready Biodegradability

GLP: No

Year Completed: 1983

Analytical monitoring: Dissolved organic carbon (DOC)

Statistical methods: Results were determined by calculation of the % DOC removal and graphic interpretation.

Test organism source: A 50:50 mix of soil extract and secondary effluent. Fresh sandy loam agricultural soils from Ramsey and Washington Counties, MN. Secondary effluent, supernatant of activated sludge mixed liquor aeration basin, obtained from Metrow Wastewater Treatment Plant, St. Paul, MN.

Test condition:

Dilution water: Deionized water

Mineral Nutrient Medium: OECD medium using yeast extract, OECD medium using synthetic vitamin solution and OECD medium with reduced nitrogen content but using yeast extract.

Reference and test solution preparation: Test and reference solutions were prepared for each of the three mineral nutrient mediums, except US 3072 only in OECD with yeast extract. Solutions were split into aliquots and put into Erlenmeyer flasks. Test materials prepared to give final test concentration of 40 mg DOC/L, reference standards at 20 mg DOC/L and 15 mg DOC/L for sodium benzoate and LAS respectively.

Test vessels: One L Erlenmeyer flasks containing 500 mL test solution and capped with foam plugs.

Incubation conditions: Continuous dark conditions

Temperature: 23.5 – 24°C

Agitation: Continuous

Number of concentrations: One plus references and blank, all in duplicate for each nutrient medium type.

Inoculum condition on test initiation: Not taken.

Element Basis: Decrease in dissolved organic carbon compared to blanks.

Test substance flasks conditions: Not recorded.

RESULTS

Nominal concentrations: Test materials at ~40 mg DOC/L, sodium benzoate at ~20 mg DOC/L, LAS at ~15 mg DOC/L and Blank controls.

Element values:

FC-203 Antwerp 9/82

28-Day Degradation = 94% mean DOC removal (yeast)

28-Day Degradation = 89% mean DOC removal (vitamin)

28-Day Degradation = 35% mean DOC removal (low nitrogen)

FC-203 US 3072

28-Day Degradation = 97% mean DOC removal (yeast)

Remarks: Testing was conducted on the mixture as described in the Test Substance Remarks field. The values reported apply to that mixture and not the fluorochemical proportion alone.

CONCLUSIONS

FC-203 Antwerp 9/82, mean degradation based on DOC removal ranged from 35% for the low nitrogen series to 94% for the yeast series, with 89% mean DOC removal for the vitamin series after 28 days.

FC-203 US 3072, degradation based on DOC removal was 97% after 28 days.

Submitter: 3M Company, Environmental Laboratory, P.O. Box 33331, St. Paul, Minnesota, 55133

DATA QUALITY

Reliability: Klimisch ranking = 2. This study meets the criteria for quality testing. However, the sample purities were not properly characterized and the study lacks analytical confirmation of the amount of fluorochemical proportion in the solution.

REFERENCES

The studies were conducted by the 3M Company, Environmental Laboratory,
3M Lab Request number 9611, 1983.

OTHER

Last changed: 6/28/00

TECHNICAL REPORT SUMMARY

TO: TECHNICAL COMMUNICATIONS CENTER - 201-2C-12

(Important – If report is printed on both sides of paper, send two copies to TCC.) Guidelines on reverse side.

Division		Dept. Number
Environmental Laboratory (EE & PC) St. Paul, Minnesota		0535
Project		Project Number
International		9970010100
Report Title		Report Number
Modified OECD Screening Test on EC-203, EC-203A, and EC-600		#003
To		Period Covered or Date
D. Reese, Toxicology, 3M Germany		7/9/84
Author(s)		Employee Number(s)
E. A. Reiner - Environmental Laboratory - 21-2W-05		47816
Notebook Reference		No. of Pages Including Coversheet
None - See Environmental Lab Request #9611		12

[illegible]

cc: R.L.Bohon-21-2W-05
D.R.Ricker-236-1B-10
D.W.Kent-236-2A-04

Information Liaison
Initials: *PL*

3M CONFIDENTIAL

000481

MODIFIED OECD SCREENING TEST ON FC-203, FC-203A, AND FC-600

INTRODUCTION

This study was undertaken to demonstrate the biodegradability of two Light Water (L/W) products, FC-600 and FC-203, using the internationally recognized Modified OECD Screening Test⁽¹⁾. This testing was requested by 3M Germany because of inconsistency in the results of past biodegradation tests done on these products.

Modified OECD Screening Tests conducted in 1980 by the 3M Environmental Laboratory showed that three L/W products were highly biodegradable⁽²⁾. The products tested were FC-203A (previously identified as FC-203 NFP), FC-206, and FC-3017 (FC-206 freeze protected). Greater than 90% of the dissolved organic carbon (DOC) of these 3 products was removed during the 1980 testing. See Table 1 for a summary of these results.

Table 1

Results of 1980 Testing on Light Water Products

Product	% Degradation at Day:				
	<u>7</u>	<u>14</u>	<u>21</u>	<u>27</u>	<u>28</u>
FC-203 NFP ¹	13	85	93	91	93
FC-206	23	90	94	93	92
FC-3017 ²	37	94	96	96	95
Hydroquinone ³	89	92	97	94	93

Footnotes:

¹ FC-203 NFP is nonfreeze protected FC-203 and is equivalent to FC-203A.

² FC-3017 is freeze protected FC-206.

³ Hydroquinone was the reference product.

These 1980 results contradict those of H. Hellmann and D. Muller⁽³⁾ who used a similar test method, the TOC Degradation Test⁽⁴⁾. Hellmann's TOC (total organic carbon) test results, however, are an exception from normally obtained results. His finding of 8% TOC removal for FC-3017 and 6% TOC removal for FC-206 in the 21-day long TOC Degradation Test contradict his own BOD₅/COD data. His 5-day biochemical oxygen demand (BOD) results were 50% and 40% of the chemical oxygen demand (COD) of FC-3017 and FC-206, respectively. This is a significant level of degradation for a 5-day BOD test. These opposite results within his own paper clearly are an inexplicable discrepancy.

Hellmann's TOC Degradation Test results are also inconsistent with BOD₂₀/COD measurements made by the 3M Environmental Laboratory and by other independent laboratories on 3M AFFF products. Such results typically show BOD₂₀ values to range between 60% and 90% of the COD. For example, a collection of such data on 3 U.S. military specification AFFF products manufactured by 3M is shown in Table 2.

Table 2

BOD, Carbonaceous BOD, and COD Data on Mil-Spec AFFF Product

Lab	BOD ₂₀ (mg/kg)	C-BOD ₂₀ (mg/kg)	COD (mg/kg)	BOD ₂₀ COD	C-BOD ₂₀ COD
<u>FC-203 C Lot 501</u>					
3M	580,000	600,000	730,000 740,000	.79	.81
Pace	800,000*	730,000*	810,000 730,000		
Serco	440,000	440,000	737,000	.60	.60
Capsule	570,000	620,000	950,000	.6	.65
Galbraith inoculum #1	616,000	671,000	776,450	.79	.86
Galbraith inoculum #2	670,000	641,000	776,450	.86	.82
Average	575,000	594,000	782,000	.73	.75
S. D.	85,000	90,000	80,000	.12	.12

* Calculated BOD's from different dilutions were very inconsistent.

<u>FC-206C Lot 502</u>					
3M	290,000	290,000	380,000 360,000	0.78	0.78
Pace	350,000	370,000	450,000	0.78	0.82
Serco	240,000	260,000	370,000	0.65	0.70
Capsule	260,000	320,000	410,000	0.63	0.78
Galbraith inoculum #1	416,500	411,000	407,250	1.02	1.01
Galbraith inoculum #2	395,500	374,000	407,250	.97	.92
Average	325,000	338,000	396,000	.81	.83
S. D.	73,000	57,000	33,000	.16	.11

<u>FC-780B Lot 501</u>					
3M	240,000	230,000	320,000 330,000	.74	.71
Pace	250,000	250,000	350,000	.71	.71
Serco	210,000	220,000	287,000	.73	.77
Capsule	240,000	230,000	360,000	.67	.64
Average	235,000	232,000	329,000	.71	.71
S. D.	17,000	13,000	28,000	.03	.05

METHODS AND MATERIALS

1. Procedure

Testing was performed in accordance with the OECD Guidelines for testing of chemicals adopted May 12, 1981.⁽¹⁾

2. Chemicals

The reference materials used were laboratory grade sodium benzoate, ($C_7H_5NaO_2$) F.W. = 144.11 and linear alkylbenzene sulfonate (LAS). The LAS solution used was a 5.68% active solution supplied by the U.S. Environmental Protection Agency as a reference material for biodegradation testing. The Light Water (L/W) test materials used were U.S. FC-203 Lot 3072 provided in 1983 by the Commercial Chemicals Division, FC-203 and FC-600 samples provided by E.T.S.L. Antwerp in 9/82 and FC-203 NFP provided by E.T.S.L. in 1980. Antwerp provided no lot numbers with their samples. FC-203 NFP (nonfreeze protected) is equivalent to FC-203A.

Prior to testing, the L/W and reference materials were dissolved in the OECD nutrient solution and brought to the following initial DOC concentrations: sodium benzoate 20 mg/l, LAS approximately 15 mg/l, and L/W products approximately 40 mg/l. The L/W TOC was higher than that used in previous testing (20 mg/l) because our experience has shown that L/W at 40 mg/l is not toxic. Also higher concentrations improve the accuracy of the TOC analysis.

For this study, nutrient solutions were prepared using both vitamin sources allowed by the OECD Screening Test, yeast extract and synthetic vitamin solution. This was done to determine if the vitamin source affected the extent of L/W degradation.

INOCULUM

The inoculum was a 50/50 mixture of soil extract and secondary effluent prepared in accordance with OECD procedures. The soil, which was extracted, was a mixture of fresh, sandy loam garden soils obtained from Ramsey and Washington Counties in Minnesota. The secondary effluent sample was the supernatant of an activated sludge mixed liquor sample taken from an aeration basin at the St. Paul Metro Wastewater Treatment Plant. The combined inoculum was passed through Whatman 54 filter paper prior to usage.

INSTRUMENT

The organic carbon analyzer used was a Dohman DC-52A. Its sensitivity limit is approximately 2 mg of carbon per liter.

SAMPLE HANDLING AND STORAGE

The sample preservation method used was that prescribed by the manufacturer of the organic carbon analyzer.⁽⁵⁾ The method involves adding 1 drop of concentrated HCl per 10 ml of filtered sample. This brings the samples to less than pH 2. The samples were stored under refrigeration in vials with aluminum foil lined caps. Our experience is that this storage method can preserve L/W containing samples for several months without affecting the DOC. All DOC analyses on samples from this study were done within one week of its completion.

RESULTS AND DISCUSSION

Table 3 summarizes the results of this study. This table shows that sodium benzoate, the reference compound, was nearly completely degraded (95%) within 7 days. Every replicate of all of the L/W products showed greater than 80% TOC removal within the 28-day test period. Seven of the ten test flasks containing the various L/W products showed greater than 90% TOC removal. The average DOC removal for all four L/W product was greater than 90%.

The results of this testing are shown graphically in Figures 1 and 2. These results are averaged, and because of this, do not show the actual pattern of degradation in the individual cultures. Figure 3 is an example graph of 2 cultures degrading the same product, FC-203 NFP. It shows that once degradation of this product began that it was very rapid, but that the lag prior to the onset of degradation was variable. The reason for this variability between the duplicate cultures was not known nor was it expected. It may have been due to the fact that the cultures received different levels of viable microorganisms capable of degrading the major components of the Light Water products. Thus, the population of bacteria with these capabilities had to build up in the cultures prior to the onset of rapid (exponential) degradation.

Review of the data in Table 3 indicates that results of replicate testing of the other L/W products also varied considerably at days 7, 14, and 21. Again, this variation appears to be due primarily to the length of the lag phase prior to the onset of biodegradation. However, due to the small number of samples taken, one frequently could not tell how long biodegradation of individual L/W containing cultures took following the initial lag.

Table 3

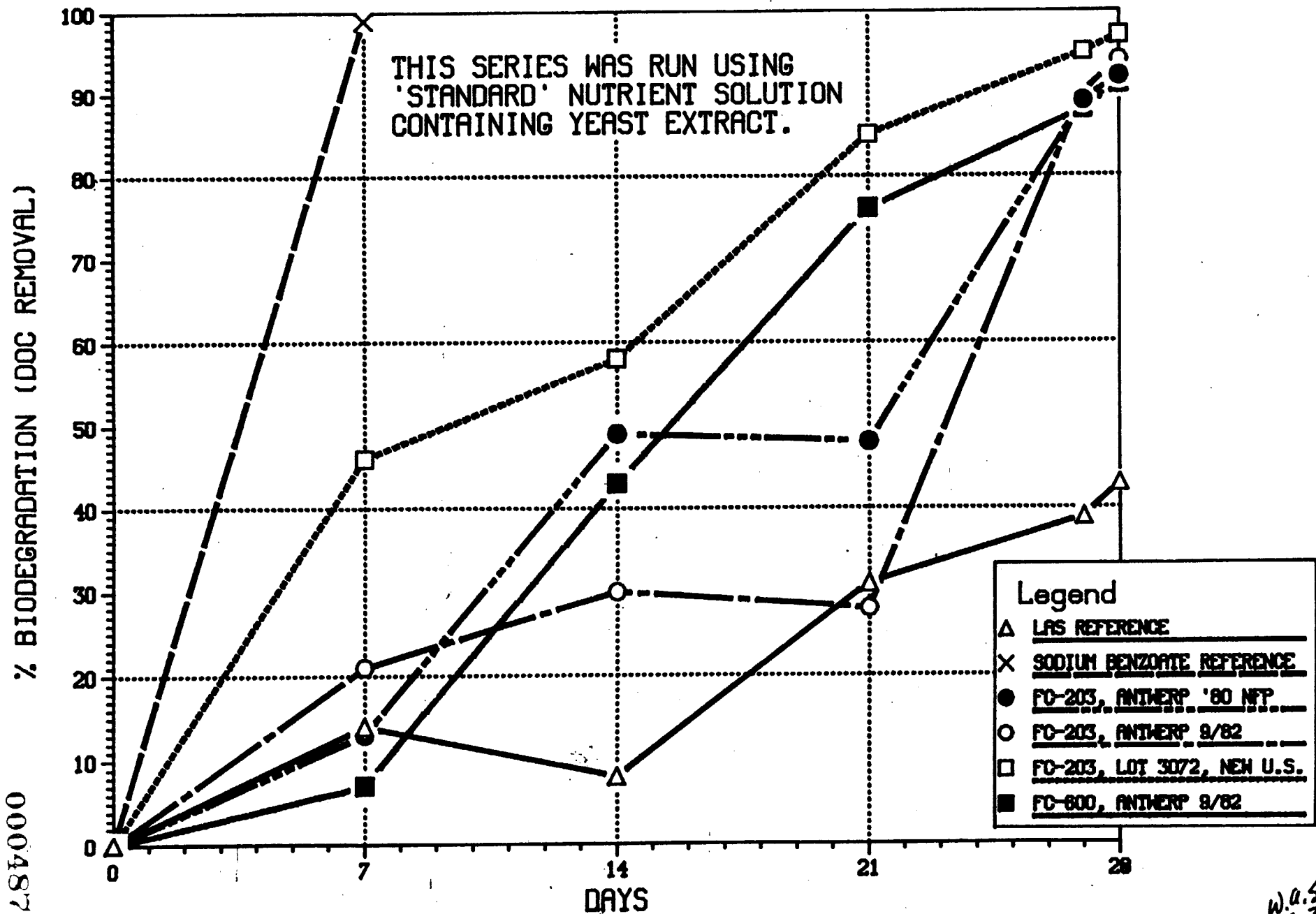
Biodegradation of LIGHT WATER Products and Reference Materials

<u>Product/Vitamin Source</u>	<u>% DOC Removal After X Days</u>					
	<u>7</u>	<u>14</u>	<u>17</u>	<u>21</u>	<u>27</u>	<u>28</u>
Sodium Benzoate/Yeast-1 ⁽¹⁾	98.8					
Sodium Benzoate/Yeast-2 ⁽²⁾	99.6					
Sodium Benzoate/Vit.-1	94.4					
Sodium Benzoate/Vit.-2	88.6					
Mean	95					
FC-203 ⁽³⁾ /Yeast-1	11.7	30.5	-- ⁽⁴⁾	28.4	89.8	95.3
FC-203/Yeast-2	29.5	29.1	--	27.0	91.0	92.1
FC-203/Vit.-1	29.4	32.6	--	27.7	80.5	83.6
FC-203/Vit.-2	24.8	88.6	--	90.2	95.6	95.2
Mean						92
U.S. FC-203/Yeast-1	26.9	25.1	--	79.2	95.5	96.5
U.S. FC-203/Yeast-2	64.3	90.8	--	90.7	96.0	97.5
Mean						97
FC-203A ⁽⁵⁾ /Yeast-1	12.6	8.5	--	11.0	86.4	87.2
FC-203A/Yeast-2	13.2	89.1	--	85.1	92.5	96.0
Mean						92
FC-600 ³ /Yeast-1	6.6	75.5	--	-- ⁽⁶⁾	89.3	94.2
FC-600/Yeast-2	7.7	10.5	--	76.3	87.0	88.8
Mean						91
LAS/Yeast-1	22.8	7.8	25.1	30.8	49.1	52.1
LAS/Yeast-2	5.9	8.8	20.4	-- ⁽⁶⁾	29.7	33.4
Mean						43

- Footnotes:
- (1) Used yeast extract as vitamin source.
 - (2) Used OECD synthetic vitamin solution as vitamin source.
 - (3) From Antwerp 1982.
 - (4) 17-day samples were only taken for LAS.
 - (5) From Antwerp 1980. Originally labelled FC-203 NFP.
 - (6) Sample contaminated, not used.

Figure 1

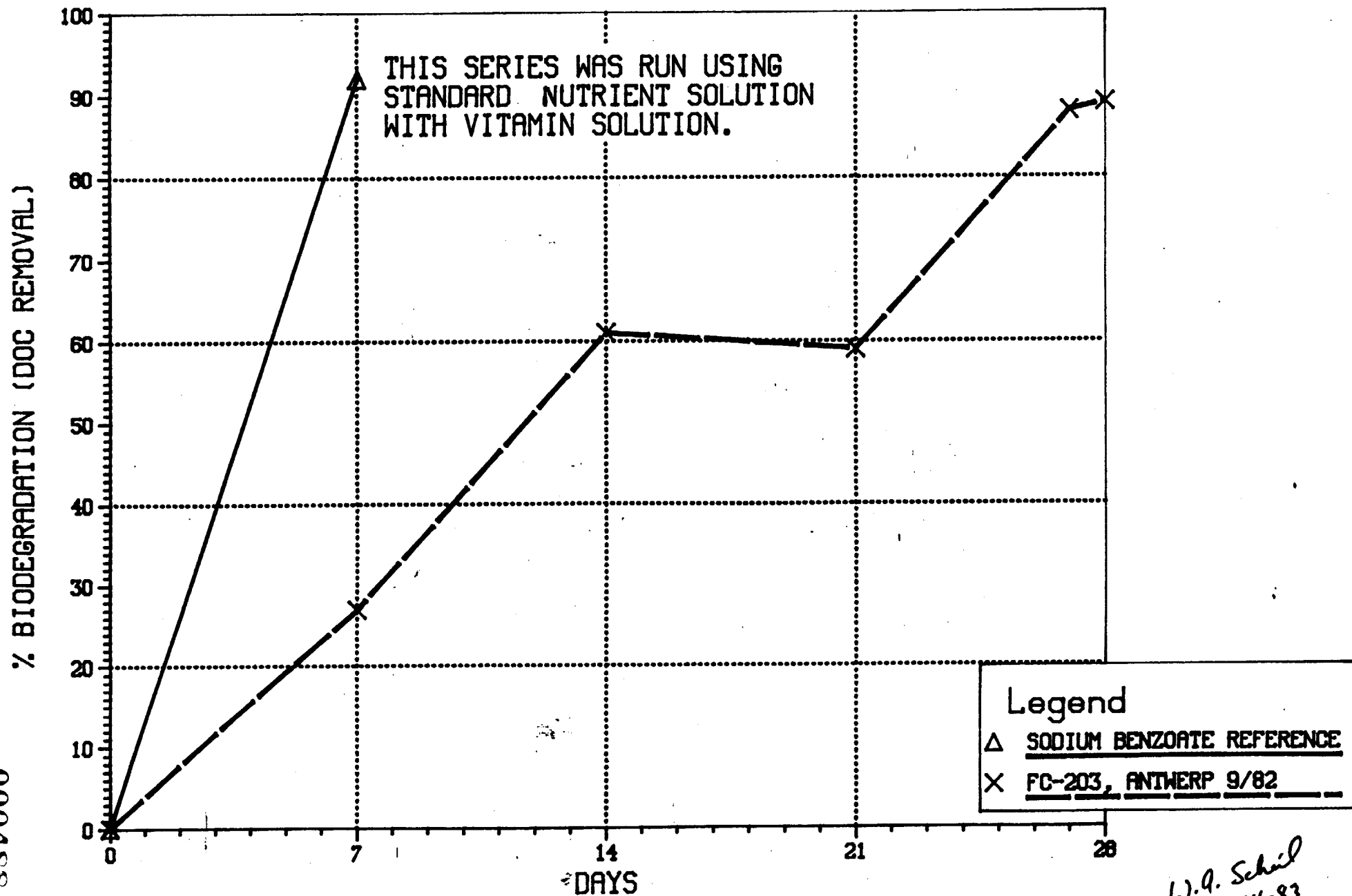
READY BIODEGRADABILITY: Modified Screening Test OECD 301E -- Adopted 12 May 81 Environmental Lab Request No. 9611



W.A. Schind
 9-30-83

Figure 2

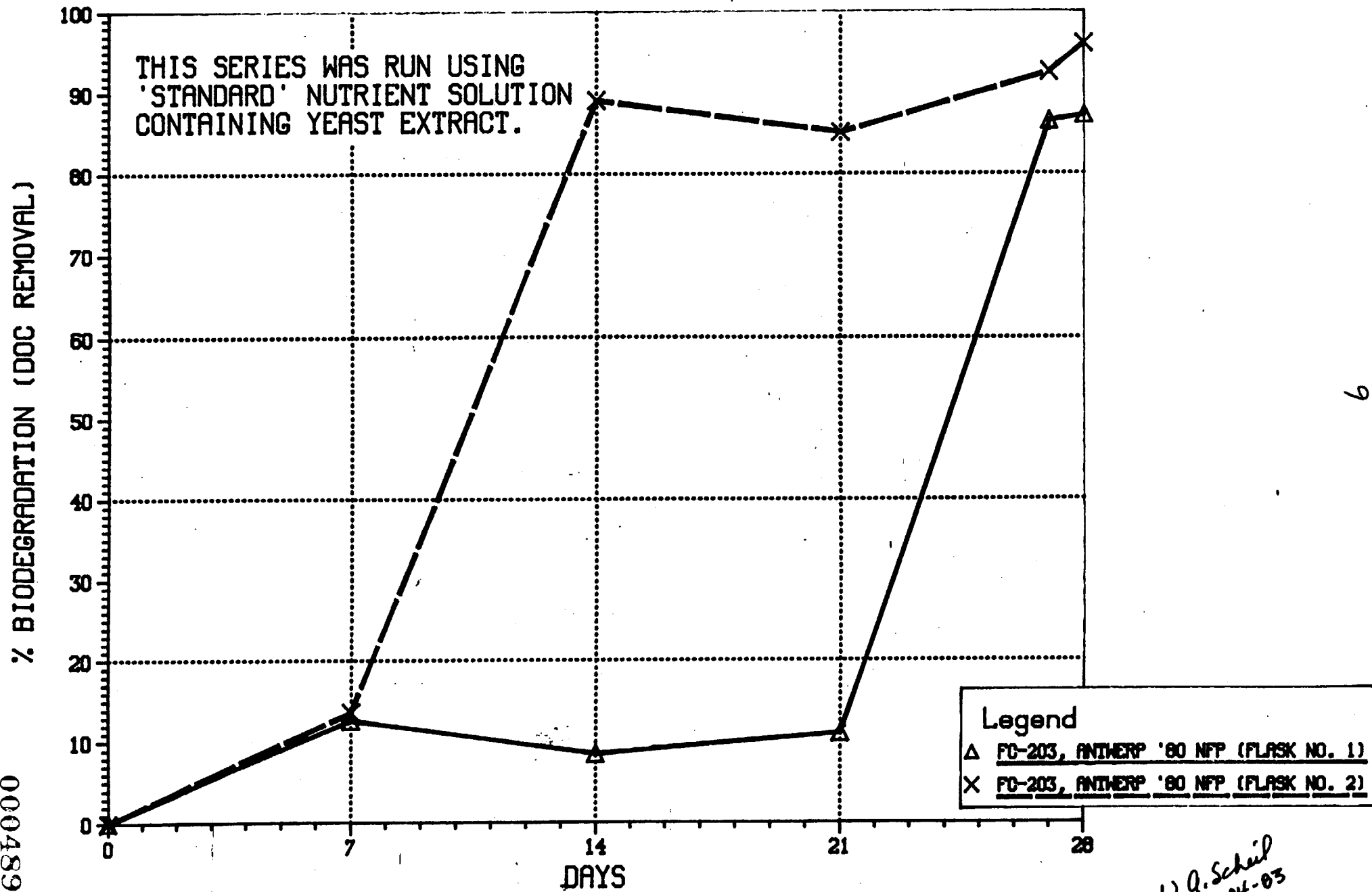
READY BIODEGRADABILITY: Modified Screening Test OECD 301E -- Adopted 12 May 81 Environmental Lab Request No. 9611



W. Q. Schind
 10-04-83

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Figure 3
READY BIODEGRADABILITY: Modified Screening Test
OECD 301E -- Adopted 12 May 81
Environmental Lab Request No. 9611

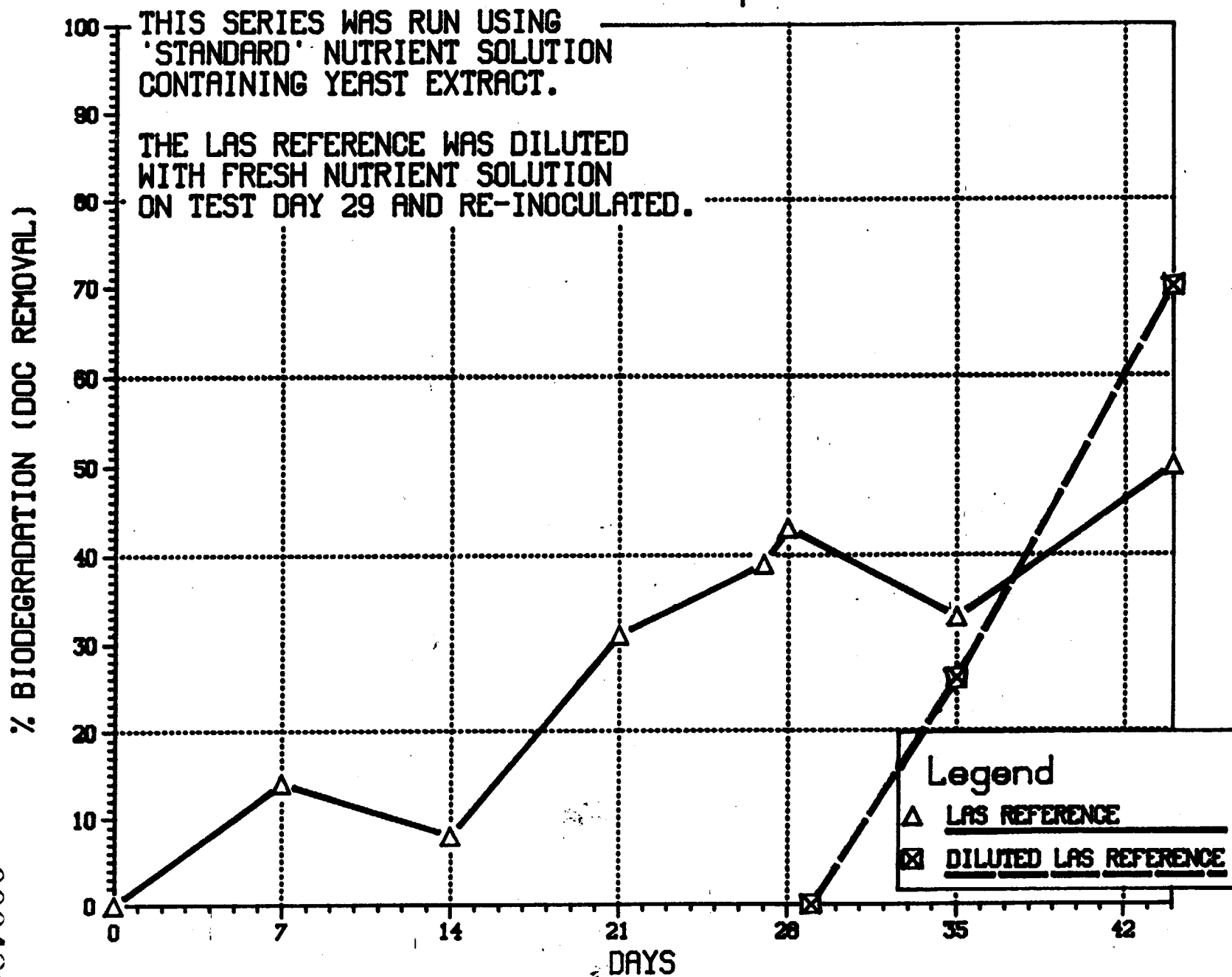


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W.A. Scheil
10-04-83

Figure 4

READY BIODEGRADABILITY: Modified Screening Test
OECD 301E -- Adopted 12 May 81
Environmental Lab Request No. 9611



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The modified OECD Screening Test is intended for use on single chemical substances. L/W products, on the other hand, are aqueous solutions containing a mixture of chemicals. Pure chemicals, which have greater than 70% loss of DOC within 28 days, are regarded by the OECD guidelines as being readily biodegradable. Also, this result has to be reached within 10 days of the DOC removal first exceeding 10%. Only one of the replicates of each of the four L/W products tested met this second OECD requirement of 70% degradation within 10 days of first reaching 10% degradation. This requirement, however, is not realistically applicable to L/W or other formulated products (mixtures). The chemicals within mixtures will frequently have differing susceptibilities to biodegradation. Readily degradable chemical components of such products may start to degrade immediately, while the onset of degradation of other chemicals in these products may be delayed. Thus, disregarding this second criteria, which is not reasonable for mixtures of chemicals, the results show that the mixture of chemical components in each of these products is readily biodegradable.

Cultures tested with the synthetic vitamin solution showed, on average, a slightly lower level of DOC removed than cultures using yeast extract as the vitamin source. Future OECD screening tests should thus use yeast extract since the synthetic vitamin solution is more difficult to prepare and does not give improved degradation.

LAS, which is a readily biodegradable and commonly used surfactant in detergents, did not degrade fully under these test conditions. This occurred in spite of the fact that its concentration was only 15 mg/l of DOC compared to the 40 mg/l of DOC used for the Light Water products. The reason for the slow rate of LAS biodegradation appears to be that LAS inhibited microbial degradation at the test concentration⁽⁶⁾. To check this hypothesis, at the end of the 28-day test period, the remaining LAS containing test cultures were split and half were diluted to twice their volume with fresh inoculated nutrient media. At this point, the rate of degradation in the diluted cultures increased substantially. Seventy percent of the DOC remaining at the time of dilution was removed within 15 days. Results of this work on LAS are shown graphically in Figure 4. Another researcher, has similarly reported a low level of biodegradation of LAS in the modified OECD screening test. D. Liu found that Marlon A (dodecylbenzene sulfonate) had a half life of 39 days.⁽⁷⁾

CONCLUSIONS

The present studies repeated the findings of previous OECD Screening Tests on Light Water products done by the 3M Environmental Laboratory. Both sets of tests showed that L/W products are "readily biodegradable." In all cases, the average DOC removal for L/W products was greater than 90% after 28 days. L/W products were much more degradable than the very commonly used and "readily biodegradable" surfactant, LAS.

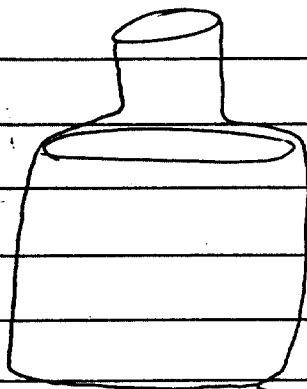
REFERENCES

- (1) Organization for Economic Cooperation and Development, Guidelines For Testing of Chemicals, Section 301 E, Ready Biodegradability: Modified OECD Screening Test, May 12, 1981.
- (2) E. A. Reiner, Biodegradation of "LIGHT WATER" Products in OECD Test 6/80, 3M Technical Report No. 40, Dept. 0535, Project 9970012600, 6/30/80.
- (3) Dr. H. Hellmann and D. Muller, "Environmental Impact of Fire Extinguishing Foam Compounds (Umweltgefahr durch Feuerlosch-Schaummittel). The study was carried out by the West German Institute of Hydrology (Bundesanstalt für Gewässerkunde), April 18, 1979.
- (4) D. Muller and T. Tittizer, "The TOC Degradation Test - A Process to Test Organic Materials for Complete Biodegradation (Der TOC - Abbaustest - ein Verfahren zur Prüfung von Organischen Substanzen auf Vollständigen Biologischen Abbau). Developed at the Bundesanstalt für Gewässerkunde, Koblenz, Z. F. Wasser-und Abwasser-Forschung (1979).
- (5) Dohrman DC-52A Operating Manual, 4th Ed., 1978.
- (6) R. D. Swisher, Surfactant Biodegradation, pages 16-17 and 147, Marcel Dekker, Inc., 1970.
- (7) D. Liu, Study on Biodegradation of Aniline and Marlon A, Biodegradation 5, Edited by T. A. Oxley and S. Barry, 1983.

Lab Request No. 9611

Environmental Lab - Work Sheet

Sample Description

Date: 7/26/83Analyst: W.A. SchielPreparation of Nutrient SolutionsFill carboy ~ 10 L with DI H₂O19 ml MgSO₄ Solution19 ml CaCl₂ Solution19 ml FeCl₃ Solution

19 ml Trace Element Solution

Dilute to 19 L with deionized water

Standard Nitrogen
with YeastLow Nitrogen
with YeastStandard Nitrogen
with Vitamin Solution

8.0 ml Standard K-buffer

8.0 ml yeast extract soln.

Dilute to 8.0 L with above
solution.

6.0 ml Low-N K-buffer

6.0 ml yeast extract soln.

Dilute to 6.0 L with above
soln.

4.0 ml Vitamin Sol

Dilute to 4.0

with above
solution

Lab Request No. 9611

Environmental Lab — Work Sheet

Sample Description

Date: 7-26-83Analyst: W.A. SchipPreparation of Test Solutions

Dilute (X) grams of test and reference material with 1.0 L of nutrient solution.

<u>(X) grams</u>	<u>Material</u>
0.144	FC-203 Lot 3072
0.154	FC-203 (ANTWERP 9/82)
0.261	FC-203 (ANTWERP 1980 NFD)
0.0343	Sodium benzoate
0.515	LAS (5.68% Active)
0.486	FC-600 (ANTWERP 9/82)

Solutions were split into 500 ml aliquots and put into 1.0 L Erlenmeyer flasks. Each flask was then spiked in WAS7/20

Inoculum:

A 50:50 mix of soil extract and secondary effluent was prepared using wastewater from the Mpls. / St. Paul Metro Wastewater Plant (Pigeye) and soil a 50:50 mixture of soil from EAR's garden and sandy-loam soil from Washington County, Minnesota.

Environmental Lab - Work Sheet

Sample Description

Date: 8-24-83Analyst: W. A. SchindDilution of LAS Test Solutions on Test Day 29Scope:

Two LAS reference compound test solutions and two parallel seeded blanks from the "STANDARD" series were diluted with equal portions of fresh nutrient solution and re-inoculated.

Procedure:

Nutrient solution was prepared using twice the yeast extract concentration. 100-ml of S-BK-1, S-BK-2, S-LAS-1 and S-LAS-2 were transferred to separate flasks. An additional 100ml of the freshly prepared nutrient solution was added. Each of the new solutions were re-inoculated with 0.5ml inoculum per liter of solution (0.1 ml/200ml). Note: The yeast extract concentration was doubled to bring the final concentration of the diluted solutions back to the original starting concentration, assuming degradation of the original yeast extract.

Seed source: 50:50 mix of secondary effluent and soil extract. The effluent was obtained from Elk River Treatment Plant (rotating biological disk) and the soil was a 50:50 mixture of EAE's garden soil and sandy loam soil from Washington County, MD.

Lab Request No. 9611

Sample Des

Results on diluted
LAS test solutions
will be reported separate.

1983

Date: 8-24-83Analyst: W. A. SchilW. A. Schil
10/4/83on Test Day 29

Form 24698-PWO

Scope:

Two LAS reference compound test solutions and two parallel seeded blanks from the "STANDARD" series were diluted with equal portions of fresh nutrient solution and re-inoculated.

Procedure:

Nutrient solution was prepared using twice the yeast extract concentration. 100-ml of S-BK-1, S-BK-2, S-LAS-1 and S-LAS-2 were transferred to separate flasks. An additional 100ml of the freshly prepared nutrient solution was added. Each of the new solutions were re-inoculated with 0.5 ml inoculum per liter of solution (0.1 ml/200ml). Note: The yeast extract concentration was doubled to bring the final concentration of the diluted solutions back to the original starting concentration, assuming degradation of the original yeast extract.

Seed source: 50:50 mix of secondary effluent and soil extract. The effluent was obtained from Elk River Treatment Plant (rotating biological disk) and the soil was a 50:50 mixture of EAR's garden soil and sandy loam soil from Washington County, MA.

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EAP

Fuji

Wade 8/24/83

Environmental Lab - Work Sheet

Lab Request No. 9611

Sample Description

Date: 8-24-83

Analyst: W. A. Scheid

Dilution of LAS Test Solutions on Test Day 29Scope:

Two LAS reference compound test solutions and two parallel seeded blanks from the "STANDARD" series were diluted with equal portions of fresh nutrient solution and re-inoculated.

Procedure:

Nutrient solution was prepared using twice the yeast extract concentration. 100-ml of S-BK-1, S-BK-2, S-LAS-1 and S-LAS-2 were transferred to separate flasks. An additional 100ml of the freshly prepared nutrient solution was added. Each of the new solutions were re-inoculated with 0.5ml inoculum per liter of solution (0.1 ml/200ml). Note: The yeast extract concentration was doubled to bring the final concentration of the diluted solutions back to the original starting concentration, assuming degradation of the original yeast extract.

Seed source: 50:50 mix of secondary effluent and soil extract. The effluent was obtained from Elk River Treatment Plant (rotating biological disk) and the soil was a 50:50 mixture of EAP's garden soil and sandy loam soil from Washington County, MA.

000497

dilution increase
degradation

Lab Request No. 9611

Environmental Lab - Work Sheet

Sample Description

Date: 8-24-83

Analyst: W. A. Schind

Dilution of LAS Test Solutions on Test Day 29Scope:

Two LAS reference compound test solutions and two parallel seeded blanks from the "STANDARD" series were diluted with equal portions of fresh nutrient solution and re-inoculated.

Procedure:

Nutrient solution was prepared using twice the yeast extract concentration. 100-ml of S-BK-1, S-BK-2, S-LAS-1 and S-LAS-2 were transferred to separate flasks. An additional 100ml of the freshly prepared nutrient solution was added. Each of the new solutions were re-inoculated with 0.5 ml inoculum per liter of solution (0.1 ml/200ml). Note: The yeast extract concentration was doubled to bring the final concentration of the diluted solutions back to the original starting concentration, assuming degradation of the original yeast extract.

Seed source: 50:50 mix of secondary effluent and soil extract. The effluent was obtained from Elk River Treatment Plant (rotating biological disk) and the soil was a 50:50 mixture of EAE's garden soil and sandy loam soil from Washington County, MN.

000498

Environmental Lab - Work Sheet

Sample Description

Date: 10-5-83Analyst: W. A. SchiefNotes

The scope of lab request no. 9611 is to perform biodegradation testing on various FC materials and referenced using OECD method 301E, "Ready Biodegradability: Modified OECD Screening Test." Several different ~~nutrient~~ solution variations are also to be used for comparison of nitrogen requirements and also for comparison of growth factor effects from use of yeast extract versus synthetic vitamin solutions.

Three ~~three~~ series of test solutions were prepared with different nutrient solutions as follows: 1) nutrient solution containing yeast extract ~~as~~ as a substitute for vitamin solution (S); 2) nutrient solution containing synthetic vitamin solution (S), and; 3) ~~nutrient~~ nutrient solution containing yeast extract as a substitute for vitamin solution (S) and a 91.5% lower nitrogen content in the phosphate buffer solution (a) - $1.7g NH_4Cl$ used.

The various ~~nutrient~~ solutions were prepared, using identical components with the exception of the stated variations. See attached sheet titled "Preparation of Nutrient Solutions."

continue - Notes

Environmental Lab - Work Sheet

Sample Description

Date: 10-5-83

Analyst: W. G. Scheif

Notes - continued

It was noted that test results of duplicate test solutions varied considerably during intermediate sampling periods (days 7, 14 and 21). This variation mainly occurred in the length of the lag phase of the biodegradation curve. Once the exponential or log phase is reached, the degradation rate of the duplicate solutions is very similar.

Because of the exponential growth rate of bacteria, during the start of the exponential or log phase of biodegradation just before ~~or just after~~ a sampling day could reflect considerable variation in the amount of degradation measured compared to a duplicate test solution which for example started the exponential phase just after the sampling day.

Because of infrequent sampling periods (every ~7 days), the start of the exponential phase is not clearly defined, leaving room for considerable variation in the amount of degradation of duplicate test solutions even though the start of the exponential phase may only be several days apart. Once the exponential phase is reached, this variation in results is diminished.