

ANALYSIS OF BIODEGRADATION DATA

Data collected in the biodegradation test program prior to November 16, 1970 were submitted for statistical analysis. The numbers used in the analysis represented the amount of test compound degraded in a 21 hour period. Six materials representing varying degrees of molecular complexity were tested. The conditions for biodegradation were similar for all, except that smaller quantities of the less degradable materials were used.

Student's t Test was used to check for significant evidence of biodegradation. Control charts were plotted on the amount of the test compound present at the beginning and the end of the 21 hour period.

CONCLUSIONS

1. Significant evidence of biodegradability was found for ~~MIPB, HB-40~~ and MCS 1043. Significance level used - 95%.
2. MCS 1016 and MCS 1242 showed some evidence of biodegradation, but neither was significant at 95%.
3. Aroclor 1254 showed no evidence of biodegradation.
4. The compounds studied ranked from the most to the least degradable were: ~~MIPB, HB-40~~, MCS 1043, MCS 1242, MCS 1016, Aroclor 1254. (See Table I)
5. In order to detect biodegradability at the 95% level of significance in 20 observations, approximately one-half of the amount of test material added must be degraded. For MCS 1016 this would require about one week; for Aroclor 1242, about 2 days.
6. The day to day variation in amount of test compound found both before and after the 21 hour degradation period is shown in Figures 1 thru 6.

DISCUSSION

This was a preliminary analysis of existing data. This will be followed by a designed experiment to investigate several variations in experimental technique. The designed experiment is intended to lead to experimental conditions which will provide more consistent results.

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TABLE I
COMPARISON OF TEST MATERIALS
DIFFERENCE BETWEEN FEED SAMPLE + 21 HOUR SAMPLE

<u>Material</u>	<u>Ave. Diff.(mg)</u>	<u>Std. Dev. of Diff.</u>	<u>S/D</u>	<u>Significance</u>
MIPB	-8.7	1.95	0.22	***
HB-40	-7.0	8.80	1.26	*
MCS 1016	0.11	ca 1.0	10	NS
Aroclor 1242	0.30	1.07	3.56	NS
Aroclor 1254	-0.04	ca 1.0	25.0	NS
MCS 1043	0.55	0.95	1.73	*

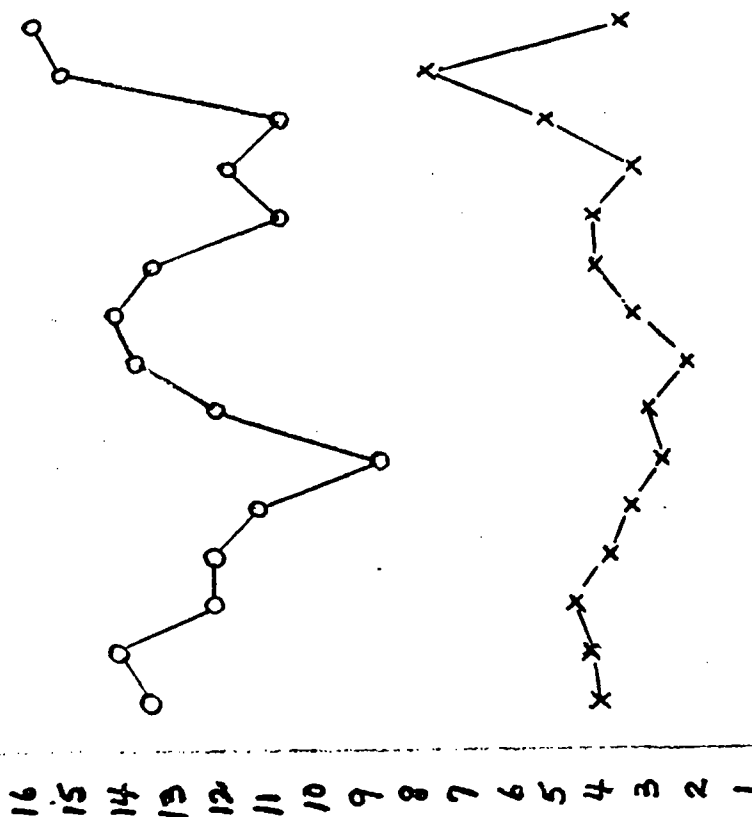
* = 95% Significance

~~***~~ = ~~99.9% Significance~~

NOTE: 10 mg of MIPB and HB-40 were added every 24 hours.
For the other compounds, the addition rate was
1 mg every 24 hours.

FIGURE I MIPB

O = FEED SAMPLE
X = 21 HR SAMPLE



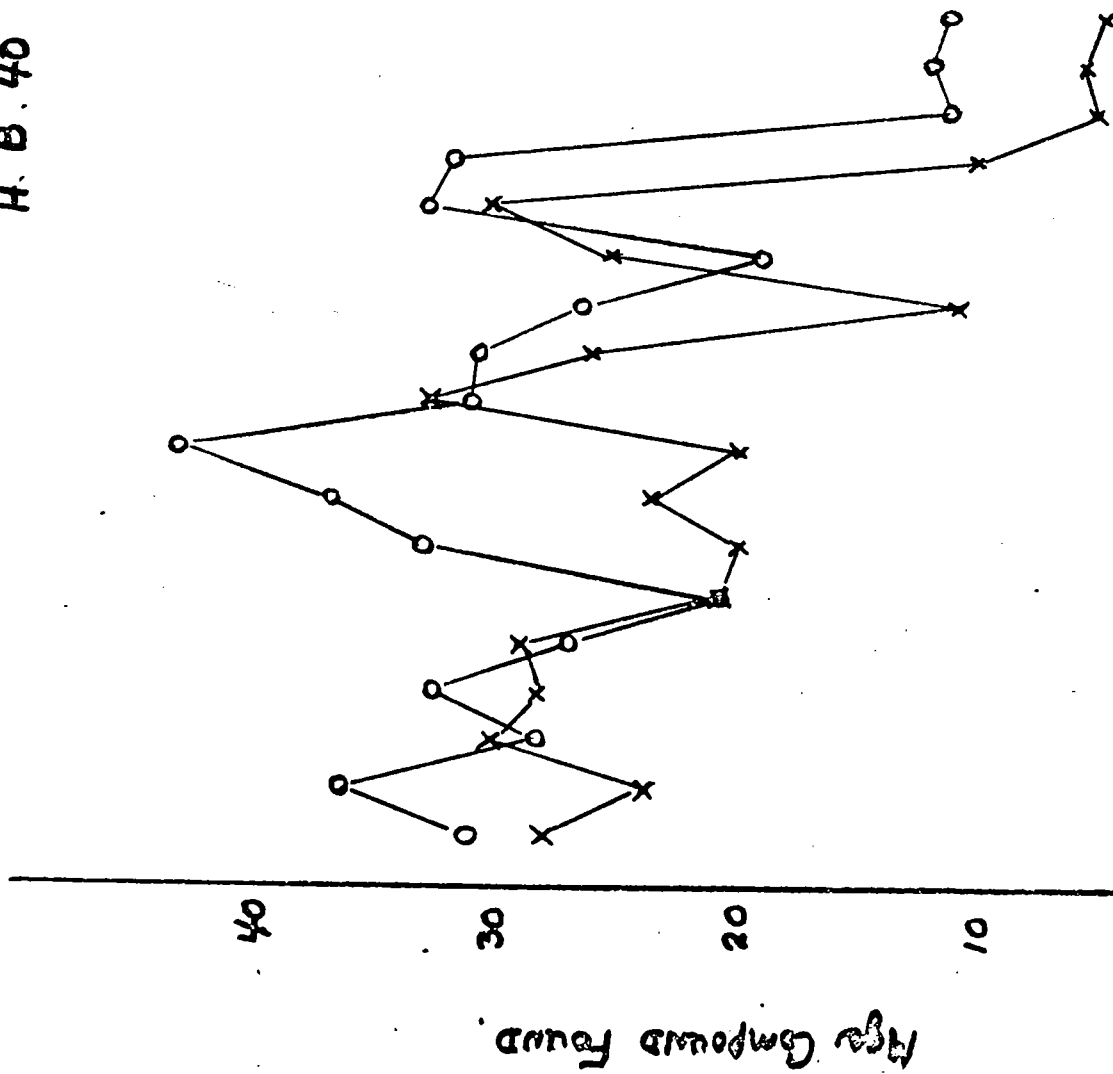
Mg. Compound Found

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FIGURE 2
H. B. 40

OZ FEED SAMPLE
X = 21 HR SAMPLE

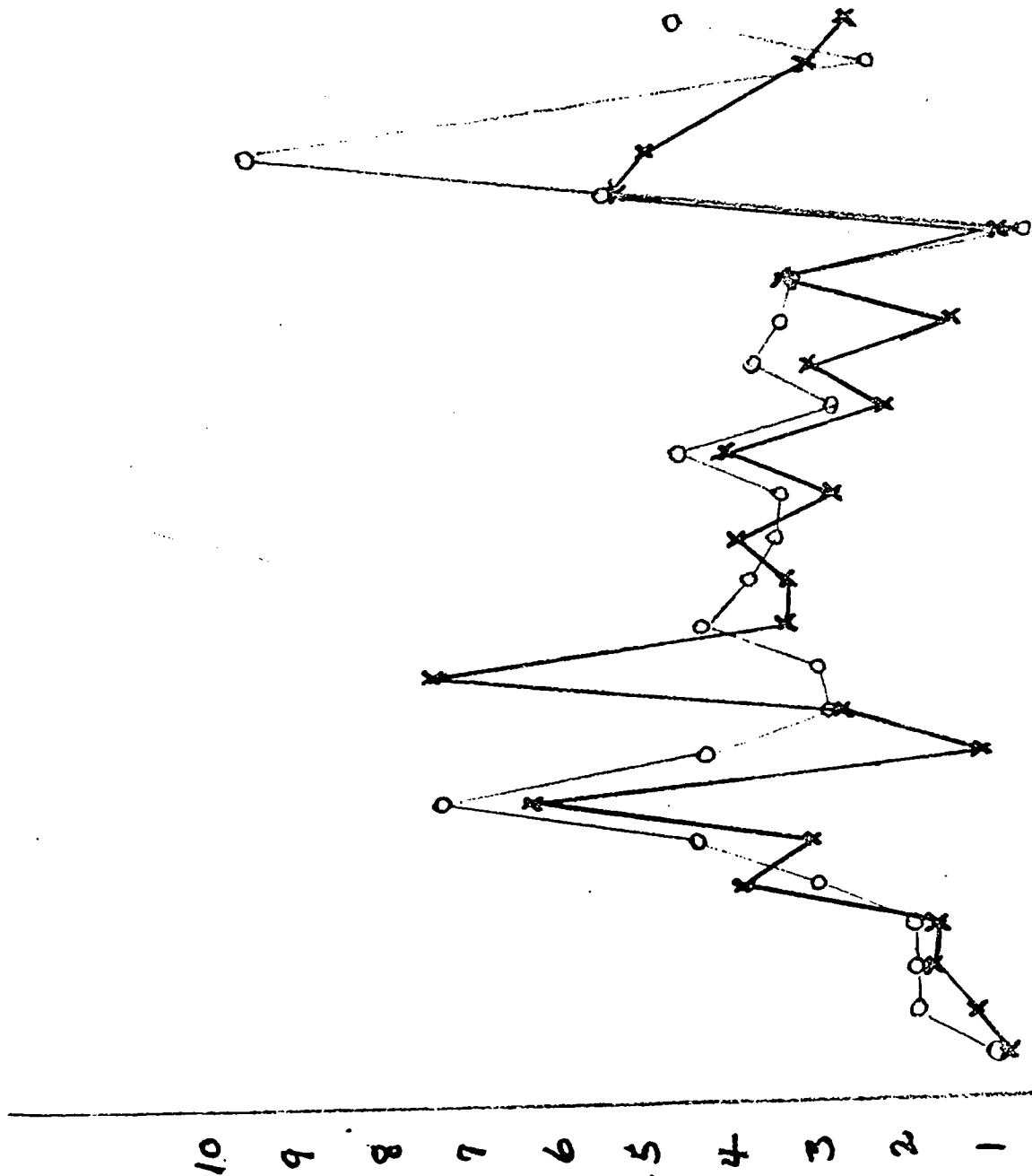
DELETE



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FIGURE 3
MCS 1043

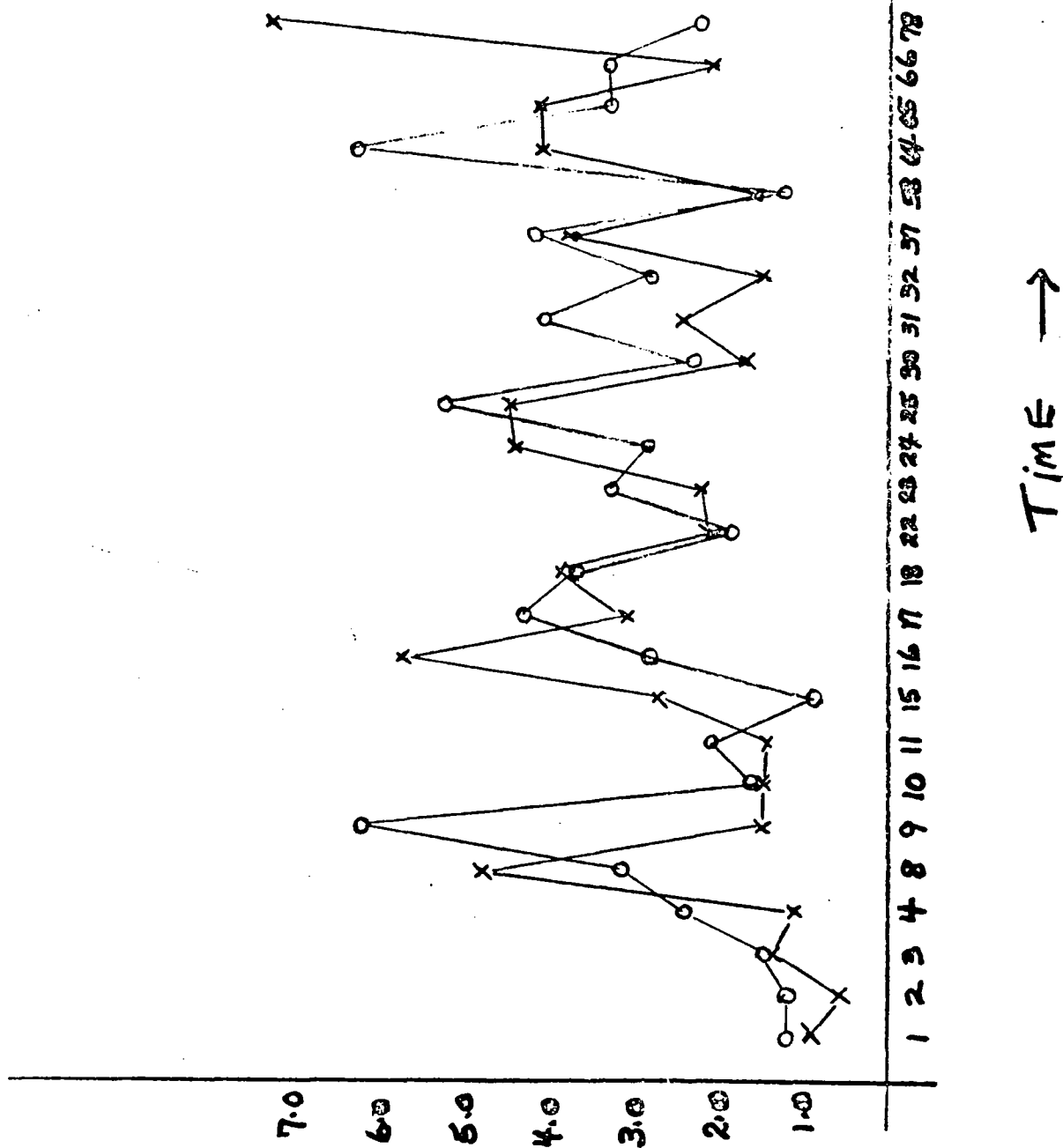


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FIGURE 4
 Reactor 1242
 Amount Found

O = FEED
 X = 24 HR

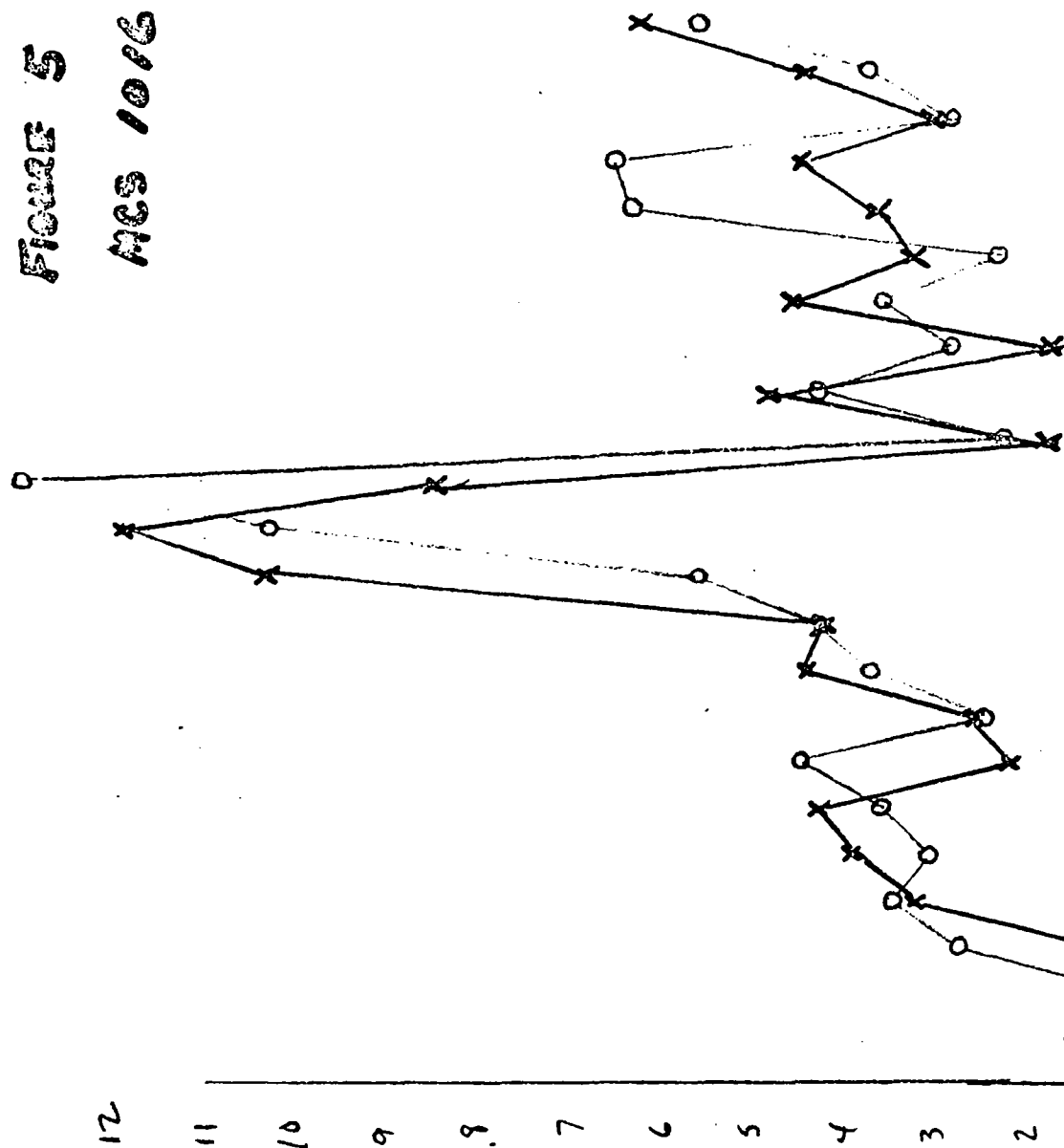


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FIGURE 5

MCS 1016



Time →

O = Pressure

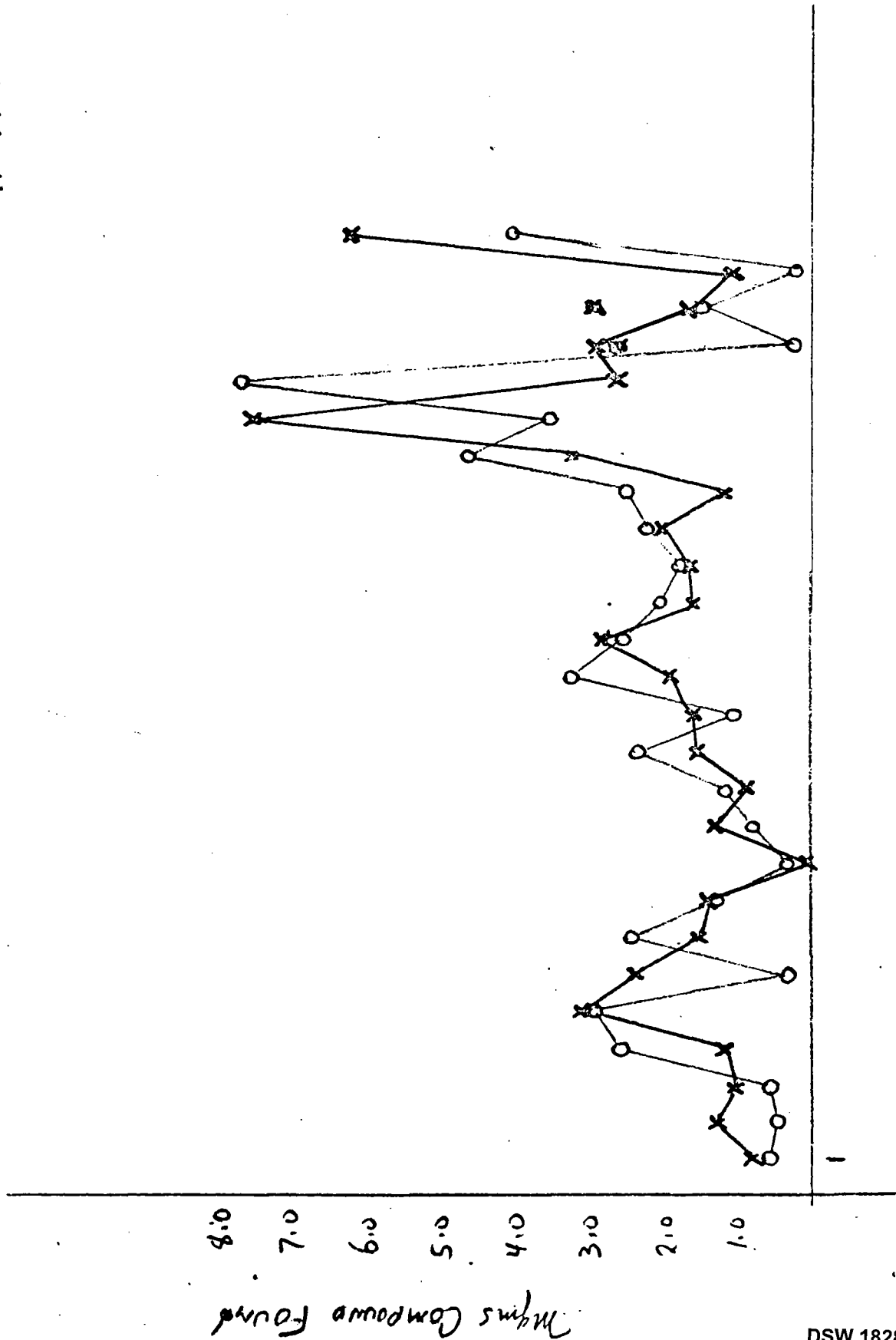
X = 21 HRS.

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Figure 6

Aceton 1254

O = FEED SAMPLE
X = 21 HR SAMPLE



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STUDY OF OPERATING VARIABLES IN THE
ACTIVATED SLUDGE BIODEGRADEABILITY TEST

INTRODUCTION

In order to provide more meaningful information on the biodegradation of Monsanto products, several of the operating variables in the activated sludge test procedure were studied. These variables were time cycle, suspended solids, amount of test material fed, method of feeding, aeration rate, method of sampling and concentration of test material in the solvent. The effects of these variables on the percent disappearance of the test material during the time period involved were sought. Also of interest was the variability of the calculated percent disappearance. Three test materials (biphenyl, 2,4'-polychlorinated biphenyl and 2,2',4,4',6,6'-polychlorinated biphenyl) were used.

CONCLUSIONS

1. Higher percent disappearance was obtained at longer time cycles, lower suspended solids, higher amount of test material fed, surface feeding and sidearm sampling (biphenyl and 2,4'-PCB).
2. On the hexachlorinated material deep injection feeding and low concentration in solvent gave higher results.
3. At the levels studied, aeration rate has no effect.
4. The longer time cycle and lower suspended solids gave more reproducible result.

DATA AND OBSERVATIONS

The experimental design used was a Plackett and Burman screening design which permits study of seven main effects in eight experimental runs. The factors and levels were as follows:

<u>Factor</u>	<u>Low Level</u>	<u>High Level</u>
Time Cycle	24 hrs.	48 hrs.
Suspended Solids	1250 g	2500 g
Amt. of Material Fed	1 mg	5 mg
Method of Feed	Surface	Deep Injection
Aeration Rate	1/2 cu ft/min	1 cu ft/min
Method of Sampling	Sidearm	Surface
Concentration in solvent	1/2 max.	max.

The experimental design is shown below:

<u>Run No.</u>	<u>Time Cycle</u>	<u>Suspended Solids</u>	<u>Amt. of Material</u>	<u>Method of Feed</u>	<u>Aeration Rate</u>	<u>Method of Sampling</u>	<u>Concentration in Solvent</u>
1	24	1250	1 mg	Surface	1 cu ft/min	Sidearm	1/2 max
2	24	1250	5 mg	Surface	1/2 "	Surface	max
3	24	2500	1 mg	Injection	1 "	Surface	max
4	24	2500	5 mg	Injection	1/2 "	Sidearm	1/2 max
5	48	1250	1 mg	Injection	1/2 "	Sidearm	max
6	48	1250	5 mg	Injection	1 "	Surface	1/2 max
7	48	2500	1 mg	Surface	1/2 "	Surface	1/2 max
8	48	2500	5 mg	Surface	1 "	Sidearm	max

The differences between factor levels were calculated for each test material by subtracting the results of the runs at the low level of each factor from the results at the high level of each factor. The response was % disappearance of the test material during the time period under study. Runs 1 through 4 had four results each and runs 5 through 8 had two results each. The differences given below are based on the average percent disappearance for each run.

Differences Between Factor Levels

	Biphenyl	2,4'-PCB	(¹) 2,2',4,4',6,6'-PCB	Total
Time Cycle	5.6	39.5***	3.3	48.4***
Suspended Solids	-11.4**	-34.5***	-2.3	-43.6***
Amt. Material	10.2*	- 2.2	8.1	16.1
Method of Feed	3.6	33.1***	-12.1**	24.6
Aeration Rate	- 3.5	1.2	-1.7	- 4.0
Method of Sampling	- 8.4*	- 3.4	6.1	- 5.7
Conc. in Solvent	- 1.6	- 3.3	-15.6***	-20.5

(¹) (One wild value in run 8 was replaced by a more reasonable result.)

For each material an analysis of variance was run on the factors giving the three highest differences as shown above. The asterisks show statistical significance at 90% (one asterisk) and 95% (two asterisks).

Reproducibility of the data, expressed as estimated standard deviation, was calculated for each run as shown below.

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<u>Run No.</u>	<u>Biphenyl</u>	<u>2,4'-PCB</u>	<u>2,2',4,4',6,6'-PCB</u>	<u>Ave.</u>
1	4.0	0.8	17.3	9.8
2	0.3	8.7	9.3	6.1
3	5.3	10.7	23.9	13.3
4	6.7	8.1	16.1	10.3
5	0.0	0.1	0.2	0.1
6	0.0	0.0	6.6	2.2
7	10.6	3.3	6.4	6.8
8	1.2	1.2	11.2	4.5

Ave. Std. Deviation

<u>Factor</u>	<u>Low Level</u>	<u>High Level</u>
Time	9.9	3.4
Suspended Solids	4.3	8.7
Sample Size	7.5	5.8

RECOMMENDATIONS

The following conditions were recommended for adoption in routine degradation testing: 48 hr cycle, low suspended solids, surface feed, 5 mg sample, sidearm sampling and low concentration in solvent.

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