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Project Report

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Project #: 1951

Biodegradation Testing of Five Printing Products

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Project Summary

Six samples of printing products were tested for biodegradability under aerobic conditions according to standard method OECD 310. Most of the products contained a significant proportion of inorganic material and water. This portion could be considered to be in mineral form to begin with, and cannot be further biodegraded. The environmental fate of this material was not investigated. The scope of this project was to determine the biodegradability of the organic fraction of the five products. All products were mineralized to some extent, as indicated by conversion of their organic components to CO_2. Extrapolation of these results, assuming that all organic contents degrade uniformly, suggests that the environmental persistence of the products ranges from approximately 1.4 to 4.6 years.

We conclude that none of the materials meet the OECD definition of "readily biodegradable." Samples A, B, and C appear to meet the definition of "inherently biodegradable." Samples D, E, and F appear to be "partially biodegradable."

Project Description

Six samples of printing products, submitted by The Artcraft Company, were subjected to biodegradation testing by the standard OECD method 310 closed-bottle aerobic biodegradation assay. This protocol simulates the environment of an aerobic sewage treatment process and is considered indicative of biodegradability in all aerobic environments.

Sample Description

Six samples were received in the laboratory on 14 July, 2008. They consisted of printing inks and related products, as described below.

Figure 1. Appearance of Unexposed Sample

Table 1. Descriptions of Samples

Sample	Description	Label Information
1951A	Red Viscous Paste	PMS 485
1951B	Red Viscous Paste	PMS 485 soy
1951C	White Powder	#118 Gloss thermo powder
1951D	Blue Viscous Liquid	Ropes Blue 2800 W Buzz
1951 E	Black Viscous Liquid	A 4440 Black Faust
1951 F	Plastic Foil one side silver, one side mustard	ITW Foils 06-105-385

Because the samples were largely insoluble in water, insoluble reference materials were selected. Reference samples consisted of granular cellulose, (Sigma-Aldrich C6413) as a reference or positive control and polyethylene as a negative control.

Sample Preparation

Samples were analyzed for total C, H, and N content, using a Leco CHN analyzer. This was necessary to determine how gas production in

experiments relates to percent biodegradation of the product, and for standardization of the experiments.

Results were as follows:

Table 2. Elemental Composition of Samples and Controls

	Percent by weight: Carbon	Hydrogen	Nitrogen	Ash
Sample 1951A	75.48	9.91	2.25	2.6
Sample 1951B	75.6	9.92	2.04	2.7
Sample 1951C	77.11	11.20	6.25	0
Sample 1951D	15.66	3.10	2.29	37.1
Sample 1951 E	18.66	4.78	0.09	0
Sample 1951 F	61.1	4.59	0.63	0
Cellulose	44.4	6.2	0.0	0
PET	62.5	4.2	0.0	0

Samples D and E were miscible in water, but the rest of the samples were not. Because it was necessary to dilute the material and dispense it into microcosm bottles, other means of handling the materials had to be devised.

Samples A and B, the red inks, were immiscible in water or acetone, but were miscible in methylene chloride. Aliquots of these samples were dissolved in methylene chloride (1.65 g sample per 5 ml solvent) and portion containing 50 mg of carbon were pipetted onto a series of glass-fiber filters. The solvent was allowed to evaporate, and the filters were inserted into microcosm bottles. (Figure 2.)

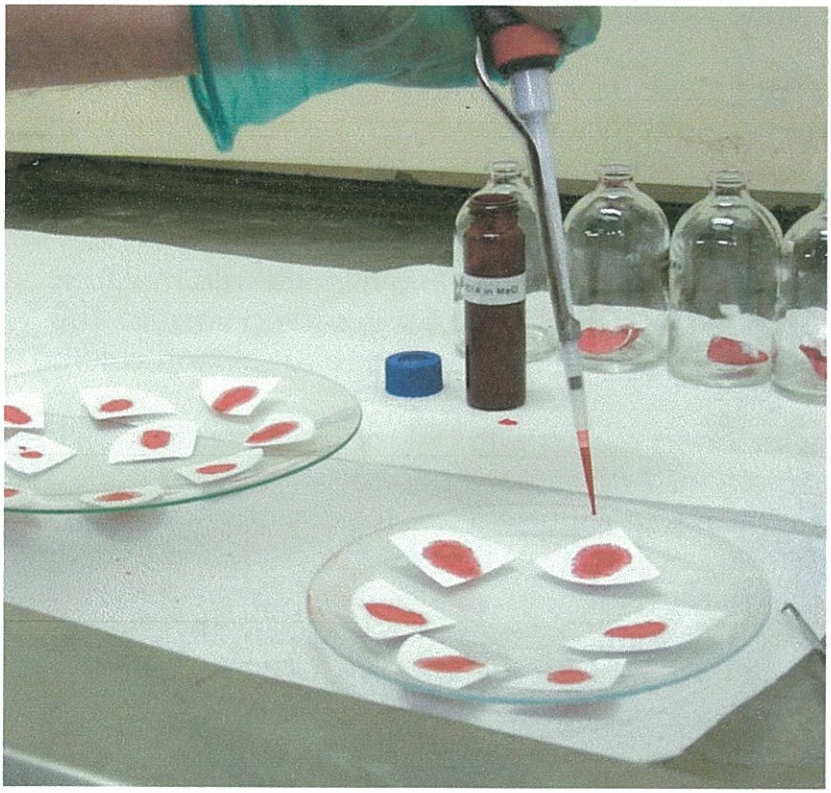

Figure 2. Sample preparation for 1951A and 1951B

Sample C, the white powder, was weighed out and added directly to microcosm bottles in 0.06 g aliquots.

Samples D and E, the blue and black inks, were diluted in water, with constant stirring, and aliquots containing 50 mg of carbon were pipetted directly into microcosm bottles.

Sample F, the plastic foil, was run through a clean office paper shredder, and 0.08 g aliquots were weighed out and added directly to microcosm bottles.

Task 1. Aerobic Biodegradation by Sewage Microorganisms OECD 310.

This experiment measured the mineralization of the test sample to CO_2 in aerobic microcosms that simulated an aerobic waste water treatment environment. This is considered representative of most aerobic environments that are likely to receive waste materials.

Samples were incubated at 35°C in a mineral salts medium containing mature activated sewage solids in sealed vessels. The headspace was air and the ratio of headspace to solution volume was 3:2.

Treatments included media with test material, media with reference material, or media alone. Background controls contained non-degradable polyethylene tetraphtalate. A toxicity control treatment was used as a test of potential toxicity of the test material, and included both test material and reference material. Sterile controls, were included to test for abiotic decomposition. Additional controls contained only the glass fiber filters used as carriers for samples A and B. Individual microcosms were periodically sacrificed and analyzed for inorganic carbon. Data were used to calculate the rate and cumulative amount of CO_2 production. Assuming that the CO_2 production in control vessels represented the background rate, these data were used to calculate percent biodegradation of the test materials.

Experimental Protocol

An inoculum of activated "mixed liquor" sewage effluent was obtained from a municipal sewage treatment plant in The Dalles, Oregon. This material was screened to remove large particles and conditioned in the laboratory for 24 hours by bubbling CO_2-free air through it. This process removed inorganic carbon and minimized background carbon. The inoculum was diluted 1:10 in a mineral salts solution containing, per liter:

KH_2PO_4	0.0850g
K_2HPO_4	0.2175g
$Na_2HPO_4{\cdot}2H_2O$	0.3340g
NH_4Cl	0.0050g
$CaCl_2{\cdot}7H_2O$	0.0364g
$MgSO_4{\cdot}7H_2O$	0.0225g
$FeCl_3{\cdot}6H_2O$	0.00025g

Each sample was added to microcosms in amounts that yielded approximately 50 mg of carbon.

Table 3. Design of Aerobic Biodegradation Experiment

treatment	amendment	amount	mg C	number
T_A test	1951A	.06 g	50	30
T_B test	1951B	.06 g	50	30
T_C test	1951C	.06 g	45.5	30
T_D test	1951D	.16 g	44.25*	30
T_E test	1951E	.134g	44*	30
T_F test	1951F	.08 g	50	30
R reference	Cellulose	.11 g	49	30
C control	none	-	0	30
IA inhibition	1951A + Cellulose	.06 + .11	100	6
IB inhibition	1951B + Cellulose	.06 + .11	100	6
IC inhibition	1951C + Cellulose	.06 + .11	95.5	6
ID inhibition	1951D + Cellulose	.16 + .11	95.25*	6
IE inhibition	1951E + Cellulose	.134 + .11	94*	6
IF inhibition	1951F + Cellulose	.08 + .11	99	6
S_A sterile	1951A	.06 g	50	6
S_B sterile	1951B	.06 g	50	6
S_C sterile	1951C	.06 g	45.5	6
S_D sterile	1951D	.16 g	44.25*	6
S_E sterile	1951E	.134g	44*	6
S_F sterile	1951F	.08 g	50	6
GF filter	glass fiber filters	0	0	6

* corrected for inorganic carbon content

Figure 3. Closed-Bottle Microcosms

Each bottle was then inoculated with 50 ml of diluted effluent solution. Test vessels were sealed with butyl rubber stoppers and aluminum seals. Test vessels were incubated in the dark at 35°C with daily agitation.

At each measurement time, three bottles of each treatment were removed from the incubator and acidified to ph <2 by the injection of 0.5 ml 10% H_2SO_4 with a hypodermic needle and syringe. After agitation and equilibration for one hour, headspace samples were removed with a gas-tight syringe and hypodermic needle, and analyzed by gas chromatography. The instrument used was a Hewlett-Packard 5880A equipped with dual packed columns (carboseive II, Supelco, Inc.) and a two-channel thermal conductivity detector, or a Carle GC 8700 equipped with a packed column (carboseive II, Supelco, Inc.) and a single thermal conductivity detector. Carrier and reference gases were helium. Instruments were calibrated using mixed-gas standards. The amount of

CO2 produced in each microcosm was used to calculate the percentage of the test substrate that was mineralized by microorganisms.

$$\%D = \frac{(TIC_t - TIC_b)}{TOC} \times 100$$

Where TICt = mg inorganic carbon in test bottle at time t
TICb = mg inorganic carbon in blank bottles at time t
TOC = mg organic carbon added initially to the test vessel

Results

All of the test samples produced CO_2 at rates greater than the controls, indicating that they were all at least partially mineralized. The following discussion assumes that each sample was a pure substance, but see the "discussion of results" section for caveats. CO_2 production data are shown in Table 4.

Table 4. CO_2 production in project 1951 microcosms

	Percent of Carbon							
Days	C	R	1951A	1951B	1951C	1951D	1951E	1951F
0	0.0000	0.0000	0.0000	0.0000	0.0000	11.4921	11.9750	0.0000
4	0.2708	1.2125	1.6777	1.5377	0.8197	13.7715	13.1662	0.6652
9	0.3175	1.7051	3.4822	3.2826	1.6294	14.8790	13.8970	0.9405
15	0.2563	5.2142	2.8447	2.3828	0.6877	13.9067	12.6592	1.8194
23	0.2016	5.2352	3.6689	3.6931	1.4733	13.4254	14.1884	1.8113
30	0.2338	7.1717	3.8057	4.3402	1.4041	12.5513	15.7321	1.9369
41	0.3545	13.9920	6.8996	7.9636	2.9510	15.9639	15.9784	2.4455

Sterile Controls

	Percent of Carbon							
Days	C	R	1951A	1951B	1951C	1951D	1951E	1951F
0	0.0000	0.0000	0.0000	0.0000	0.0000	11.4900	11.9700	0.0000
4	-0.0978	-0.0978	-0.0978	-0.0978	-0.0978	11.4921	11.9750	-0.0978
30	0.0905	0.1453	0.2467	0.0277	0.1533	11.4100	11.8076	0.6362

Inhibition Tests and other Controls

	Percent of Carbon						
Days	1951A	1951B	1951C	1951D	1951E	1951F	filters
0	0.0000	0.0000	0.0000	11.4900	11.9700	0.0000	0.0000
4	1.6777	1.5377	0.8197	13.7715	13.1662	0.6652	0.3250
30	8.6349	7.1717	7.6256	23.4040	21.3114	6.2928	0.4790

C = Control
R = Reference
Filters = glass fiber filters used for A and B

Overall biodegradation results are shown in Figure 4. No significant CO_2 production occurred in unamended control microcosms, and the reference material was strongly mineralized, indicating that a valid assay

was conducted. Note that a slowly degrading reference material (cellulose) was selected to match the expected rates of the test material, rather than a "readily biodegradable" reference.

For most of the test substances, the biodegradation rate was faster in the first ten to fourteen days, and thereafter a lower rate was observed. This may be a consequence of multiple ingredients that degrade at different rates. Samples A,B,D, and E were partially miscible in the test medium, causing a light tint of their respective colors in the liquid. However most of the material, and all of samples C and F, remained in a separate phase at the bottom of the test vessel. This generally leads to relatively low rates of biodegradation.

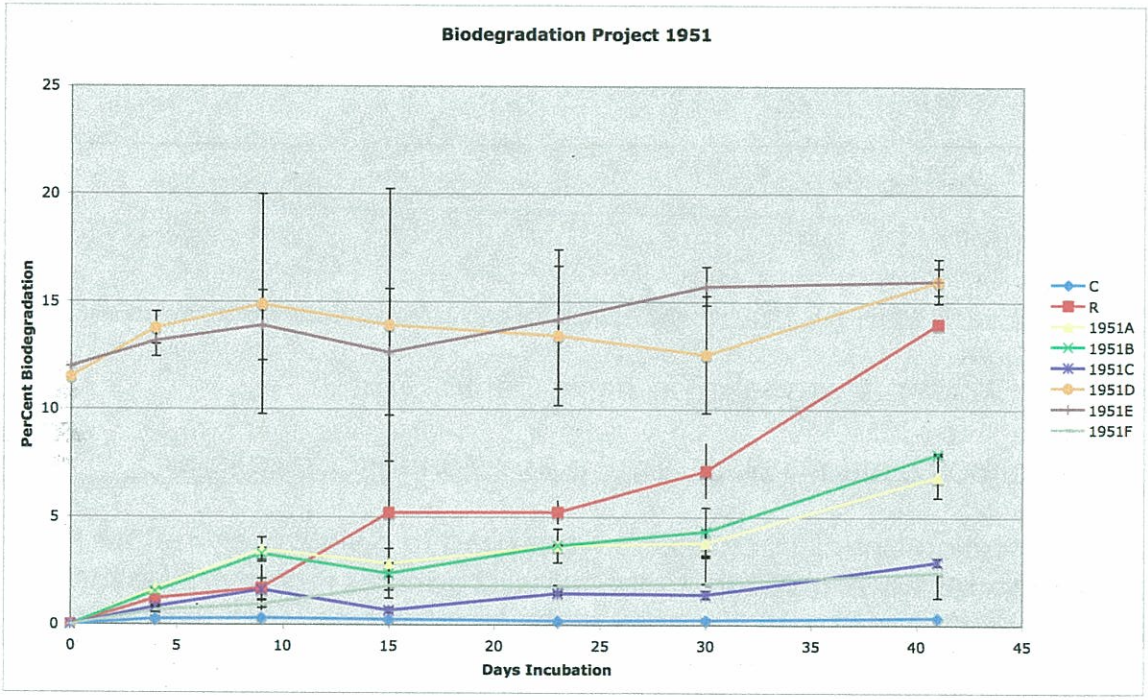

Figure 4. Biodegradation Results Summary, Project 1951

Symbols = mean values Error bars = standard deviation

Sterile microcosms were used to determine whether abiotic degradation occurred. Inorganic carbon did not significantly increase over time, indicating that abiotic reactions did not occur. Samples D and E had a high initial DIC content, as shown by both sterile and test microcosm results. This inorganic fraction appears to represent about 11.5 to 12 percent of the carbon in these samples. For rate calculations, this

amount was subtracted from the total carbon added. The high background of inorganic carbon in these two samples evidently caused some interference with CO_2 measurements, resulting in relatively large standard error at most intermediate measurements.

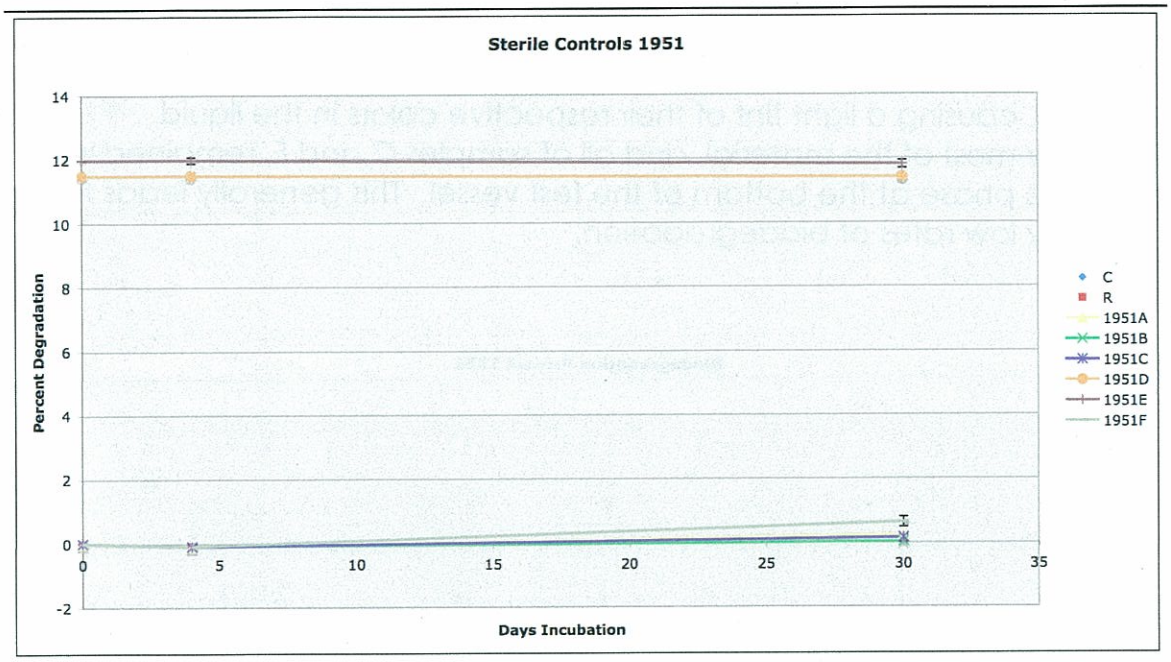

Figure 5. Abiotic Degradation in Sterile Control Vessels

Symbols = mean values Error bars = standard deviations

Inhibition-check microcosms were used to determine whether the test substrates were inhibitory to the microorganisms that catalyze the biodegradation process. Because of the high carbon loading in these microcosms, and the long incubation period, they ran out of oxygen at about day 25. However until that time, CO_2 production rates were similar to those in reference microcosms, so no significant toxicity of the test substances to microorganisms was observed.

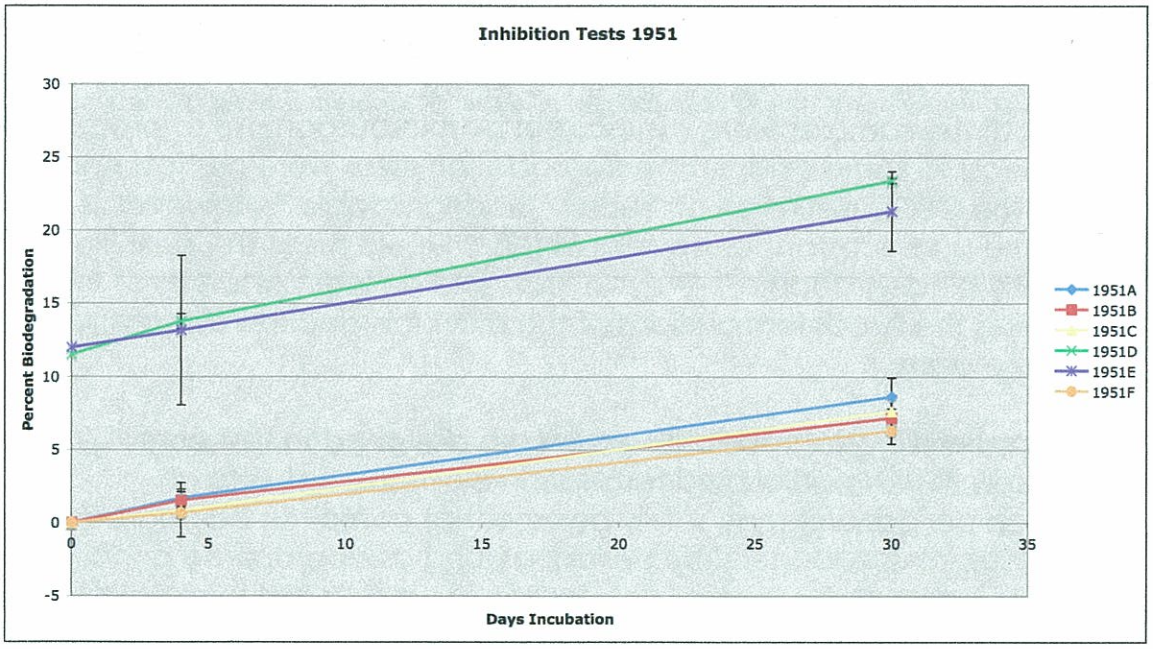

Figure 6. Inhibition Test Results for Project 1951

The controls containing glass fiber filter paper alone were not significantly different from controls with no addition. This indicated that the carriers used for samples A and B did not affect the results.

Discussion of Results

All of the samples were mineralized by microorganisms to produce CO_2, at least to some extent. The degradation rates were not fast enough to meet the OECD definition of "readily biodegradable." However, if these were pure substances, several of them would meet the definition of "inherently biodegradable." The results should be interpreted by considering the exact formulations of the products and the assumptions behind the assay.

Standard biodegradability assays are designed to test pure substances. When partial biodegradation is observed during an incubation period, one may safely assume that complete biodegradation would occur over a longer time period, as long as appropriate conditions persist. However, these assays are commonly used to test formulated products that contain multiple components. In this case, the results must be interpreted based on the product formulation. For example, if a product contains 50% component A and 50% component B, and 55% biodegradation is observed, one can conclude that both components are inherently biodegradable. However, if only 45% biodegradation is observed, one cannot conclude that the entire product is inherently biodegradable, without further analysis. For mixtures of chemically similar compounds, OECD test results are usually accepted as if they were pure substances.

"Biodegradation" generally refers to organic materials – those that are composed of carbon bonded to carbon, hydrogen, and other elements. Microbes can also chemically transform some other kinds of materials, but those processes are outside the scope of standard biodegradability tests. Thus, discussion of biodegradability refers only to the organic fraction of test samples. Other components of a product, such as water or salts, are not considered biodegradable, however water is usually considered benign and not counted as a persistent residue. Other components that are already in stable mineral form might also, arguably be treated in this way.

If we assume that the organic fraction of each of the samples is a pure substance, or at least that it is degraded homogenously, we can calculate biodegradation rates and estimate the environmental persistence of each product. This is shown in Table 5.

Table 5. Biodegradation Rate Summary

Sample	% Carbon	% degraded	Apparent Rate (per day)	Persistance (days)	label
Reference	44	14	0.00341	293	cellulose
1951A	75.5	6.9	0.00168	594	PMS 485
1951B	75.6	8	0.00194	515	PMS 485 Soy
1951C	77.1	3	0.00072	1389	#118 Gloss thermo powder
1951D	13.9*	5.1	0.00113	882	Ropes Blue 2800 W Buzz
1951E	16.5*	4.5	0.00100	998	A 4440 Black Faust
1951F	61.1	2.5	0.00059	1679	ITW Foils 06-105-385

Samples A and B behaved similarly in this assay. Both were composed almost entirely of organic material. Steady rates of biodegradation, with no plateau, were observed throughout the incubation period. Half-life kinetics were not observed. As expected, due to their low solubility in water, rates of biodegradation were low, and 7 to 8 percent of the sample was mineralized to CO_2 during the assay. Control experiments indicate that the glass fiber filters used in sample preparation did not contribute CO_2 or interfere with the assay. If we assume that these products are pure substances, we can extrapolate that 500 to 600 days would be required for their complete biodegradation. They would be considered "inherently biodegradable" but not "readily biodegradable."

Sample C was also composed almost entirely of organic matter, but was an insoluble solid. As might be expected, a very low rate of biodegradation was observed, although CO_2 production was clearly in excess of the negative controls. Three percent of the product was mineralized to CO_2 during the assay. If we assume that this is a pure substance, we can project that nearly 1400 days would be required for its complete biodegradation. If so, it would be considered "inherently biodegradable" but not "readily biodegradable."

Sample D contained only 15.7% carbon. Of this, 11.5% was inorganic carbon, so the actual content of organic carbon was 13.9%. In addition, 37% of this sample was incombustible, or "ash." The composition of this fraction is unknown, but it cannot be considered biodegradable. (If it were an inert mineral substance, one might be able to argue that it is environmentally benign, like water, and subtract it from the analysis. However, that is outside the scope of this study.) Approximately 27% was

water. Of the organic fraction, 5.1% was degraded during the assay period. We can extrapolate this to estimate that nearly 900 days would be required for the complete biodegradation of the organic fraction. The overall product would be considered "partially biodegradable."

Sample E contained only 18.7% carbon. Of this, 12% was inorganic carbon, so the actual content of organic carbon was 16.5%. The water content was approximately 30%. Of the organic fraction, 4.5% was degraded during the assay period. We can extrapolate this to estimate that nearly 1000 days would be required for degradation of the organic content. The mass balance is rather anomalous, because only 50% of the mass is accounted for by organic material and water. Assuming oxidation of about 15%, this leaves around 35% of the mass unaccounted. (This is similar to the ash content of sample D.) With the current information, this product would be considered "partially biodegradable."

Sample F was an insoluble solid film that contained 61% carbon. There was evidently a large percentage of inorganic material – apparently metallic – that would be considered non-biodegradable. A low but significant rate of biodegradation of the organic fraction was observed. If the organic fraction was a pure substance, we can extrapolate that nearly 1700 days would be required for its complete biodegradation. The overall product would be considered "partially biodegradable."

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Appendix: Larger Data Tables

Table 6. Raw Data: CO_2 Peak Areas from Gas Chromatograph

day	blank	blank	blank	reference	reference	reference	1951A	1951A	1951A
0	0	0	0	0	0	0	0	0	0
4	0.0073	0.0078	0.0078	0.0286	0.0253	0.0275	0.0358	0.0383	0.0362
9	0.0093	0.0093	0.0072	0.0642	0.0181	0.0297	0.0901	0.0663	0.066
15	0.0051	0.0064	0.0105	0.072	0.1799	0.0781	0.0602	0.0599	0.0627
23	0.004	0.0056	0.009	0.1033	0.1022	0.1258	0.0967	0.0602	0.0771
30	0.0062	0.0071	0.0073	0.1465	0.1241	0.181	0.0686	0.0761	0.0978
41	0.0082	0.0095	0.0104	0.2945	0.2885	0.2923	0.12	0.1629	0.1518

Days	1951B	1951B	1951B	1951C	1951C	1951C	1951D	1951D	1951D
0	0	0	0	0	0	0	0.224	0.2391	0.2451
4	0.036	0.0338	0.0318	0.0183	0.0189	0.0198	0.267	0.2939	0.3007
9	0.0789	0.0647	0.0664	0.0255	0.032	0.0498	0.1938	0.3297	0.4069
15	0.0305	0.0433	0.0803	0.0216	0.0133	0.0139	0.1395	0.3385	0.392
23	0.0812	0.0761	0.0782	0.0362	0.0325	0.0289	0.2003	0.3196	0.3202
30	0.1048	0.0631	0.1078	0.0375	0.0288	0.027	0.2477	0.3262	0.2119
41	0.1664	0.1662	0.1682	0.0692	0.0583	0.0619	0.3433	0.3156	0.3389

Days	1951E	1951E	1951E	1951F	1951F	1951F
0	0.251	0.2481	0.2499	0	0	0
4	0.2551	0.2867	0.2822	0.0205	0.0146	0.0123
9	0.3254	0.2902	0.2538	0.0271	0.0164	0.021
15	0.325	0.2681	0.1994	0.0471	0.0371	0.0349
23	0.3682	0.2314	0.2879	0.0512	0.0303	0.0371
30	0.3177	0.3143	0.3514	0.0446	0.0392	0.0426
41	0.3116	0.3576	0.3295	0.0329	0.0826	0.0425

Table 6. Continued

Sterile Controls

Days	blank	blank	blank	ref	ref	ref	1951A	1951A	1951A
4	0.011	0.0069	0.007	0.0096	0.0086	0.0087	0.0111	0.0081	0.0064
30	0.0041	0.003	0.0046	0.0039	0.0055	0.0057	0.0053	0.0069	0.0092

Days	1951B	1951B	1951B	1951C	1951C	1951C	1951D	1951D	1951D
4	0.0056	0.0056	0.0113	0.008	0.0084	0.0083	0.1343	0.2138	0.2416
30	0.0009	0.0033	0.0036	0.0059	0.0048	0.0049	0.198	0.268	0.2489

Days	1951E	1951E	1951E	1951F	1951F	1951F
4	0.2418	0.2427	0.2545	0.0128	0.0095	0.0097
30	0.242	0.2511	0.2465	0.012	0.0213	0.0123

Inhibition Checks

Days	1951A	1951A	1951A	1951B	1951B	1951B	1951C	1951C	1951C
4	0.0358	0.0383	0.0362	0.036	0.0338	0.0318	0.0183	0.0189	0.0198
30	0.1862	0.1761	0.1802	0.1465	0.1241	0.181	0.16	0.17	0.1498

Days	1951D	1951D	1951D	1951E	1951E	1951E	1951F	1951F	1951F
4	0.267	0.2939	0.3007	0.2551	0.2867	0.2822	0.0205	0.0146	0.0123
30	0.38	0.5	0.58	0.48	0.42	0.43	0.16	0.116	0.121