BIODEGRADATION OF NEWS INKS WITH "MODIFIED STURM TEST"

S. Z. Erhan, M. O. Bagby, and T. C. Nelsen

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Abstract: For our previous studies using mixed cultures of soil microorganisms, the biodegradation of ink vehicles and formulated four colored inks was determined gravimetrically. Now, we report results from biodegradation of the four-colored news inks by using the "Modified Sturm Test" (Organization for Economic Cooperation and Development). In this method, test organisms are obtained from activated sludge, and the extent of degradation is determined by measuring carbon dioxide evolution. Biodegradation data for commercial news inks consisting of vehicles containing petroleum resins, and either soybean or mineral oil solvents, and USDA's vehicle containing 100% soy oil, are reported and discussed.

Introduction

The development and use of soybean oil-based ink contributes less to environmental pollution than petroleum-based ink. The enhanced biodegradability of soybean oil as compared with mineral oil (Ticer, 1988; Ellis, 1991) is one of the more significant environmental features associated with the soybean oil-based ink

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Oil Chemical Research, NCAUR, USDA, ARS, 1815 N. University St., Peoria, IL. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

formulations. Soybean oil may be readily broken down or biodegraded by a wide variety of microorganisms and bacteria (Koritala et al., 1987). Previously biodegradation of news ink vehicles (Erhan and Bagby, 1993) and effect of four colored pigments on news ink vehicle biodegradation (Erhan and Bagby, 1994) were reported. In those studies, mixed cultures of soil-borned microorganisms were used, and biodegradation was measured gravimetrically. In this study using "Modified Sturm Test" (OECD, 1981), four colored (black, blue, yellow and red) news inks formulated with three major types of vehicles; (a) petroleum based (Flick, 1985), (b) Newspaper Association of America's (NAA, formerly the American Newspaper Publishers Association, ANPA) hybrid, partial soy oil-based (ANPA, 1988), (c) United States Department of Agriculture's (USDA) 100% soybean oil-based (Erhan and Bagby, 1991) and also, soybean oil-pigment mixed were evaluated for biodegradation. The test organisms were obtained from activated sludge, and the extent of degradation was determined by measuring carbon dioxide evolution.

Experimental

Test organisms as activated sludge were obtained from the Greater Peoria Sanitary District Treatment Plant in Peoria, Illinois. Upon arrival at the laboratory, the activated sludge was aerated for 4 hours and then 750 ml of the liquor was homogenized at medium speed in an Osterizer Blender for 2 min. After settling for at least 30 min, the supernatant was decanted. This procedure was repeated several times to provide sufficient volume of inoculum for 80 reaction bottles. Using dip slides containing TTC (Triphenyl Tetrazolium Chloride) medium, inoculum was tested for microbial numbers, and results showed 10^7 colony forming units per ml. Minimum of 10^6 to 20 X 10^6 colony forming units per ml were needed. The medium in each 1-gallon plastic coated amber glass reaction bottle, consisted of 1 ml each of magnesium sulfate, calcium chloride and ammonium sulfate solution, 2 ml of phosphate buffer solution and 4 ml of ferric chloride solution. The above solutions were prepared as described in the "Modified Sturm Test" 301B method (OECD, 1981). Calcium chloride, dipotassium hydrogen phosphate, disodium hydrogen phosphate and ferric chloride were obtained from Aldrich Chemical Company, Milwaukee, WI. Magnesium sulfate was obtained from EM Industries, Inc., Gibbstown, NJ. Ammonium sulfate and ammonium chloride were obtained from Fisher Scientific Company, St. Louis, MO.

Added to each reaction bottle were 3 ml each of ammonium sulfate, magnesium sulfate and calcium chloride stock solutions, 6 ml of phosphate buffer stock solution, 12 ml of ferric chloride solution, 2943 ml of deionized water and 30 ml of activated sludge inoculum. To purge the system of carbon dioxide, this mixture was aerated with carbon dioxide-free air for 24 hours. Reaction bottles were connected to a carbon dioxide scrubbing apparatus (OECD, 1981) through a series of glass manifolds, glass tubes and tygon tubing. Air pressure was controlled using Matheson tank regulators, Model 3122-590 (Curtin Matheson Scientific, Inc., Houston, TX).

Inks were formulated by mixing each (a) USDA soy news ink vehicle (Gardner Holdt Viscosity of W-X) (Erhan and Bagby, 1991), (b) NAA hybrid soy oil news ink vehicle (ANPA, 1988) (prepared at NCAUR) and (c) petroleum news ink vehicle (Flick, 1985) (prepared at NCAUR) with 18%, 25%, 27% and 9% by weight of black, yellow, red and blue pigment, respectively. Carbon black (Elftex 8) was obtained from Cabot Co. (Boston, MA). Sunbrite Yellow AAA (Sun 273-3556), Lithol Red (Sun 210-4200), Lithol Rubine (Sun 219-0688) and Blue 15 (Sun 249-2083) were purchased from Sun Chemical Co., Cincinnati, OH. In the formulation of red ink, 14.2% Lithol Red and 12.8% Lithol Rubine combination was used. Pigment and vehicle were mixed with a Shar High Speed Dispenser, Model D-10P, at 3000 rpm for 2 hours. Also, above pigments were mixed with alkali refined soybean oil (obtained from Archer Daniels Midland, Decatur, IL) in a beaker for 1 hour by using a magnetic stirrer. Amount of carbon for each sample was determined by elemental carbon-hydrogen analysis. These samples were used for comparison with the ink samples. Three bottles, each containing 100 ml of 0.025N barium hydroxide solution (OECD, 1981), were attached in series to each amber, reaction bottle. Barium hydroxide was obtained from Aldrich Chemical Company, Milwaukee, WI. The test was started by adding test sample (either 10 mg/L or 20 mg/L) and then bubbling CO_2-free air through the solution at a rate of approximately 1-2 bubbles per second. The CO_2 produced by biodegradation of vehicles or oils, in each sample bottle, reacted with the barium hydroxide and precipitated as barium carbonate. The amount of CO_2 produced was determined by titrating the unreacted barium hydroxide with 0.05N standardized hydrochloric acid by using phenolphthalein as an indicator. Periodically (before any barium carbonate precipitate was evident in the second trap), the CO_2 absorber nearest the reaction bottle was removed for titration. The remaining two absorbers were each moved one place closer to the reaction bottle, and a new absorber filled with 100 ml of fresh 0.025N barium hydroxide was placed at the far end of the series. The test was conducted at room temperature in duplicate.

Results and Discussion

The amount of CO_2 produced was measured and expressed as percent of the theoretical CO_2, possible from complete oxidation of all available carbon. Percent theoretical CO_2 (TCO_2) was calculated from the carbon content of the test compound. Biodegradation is reported as percent of TCO_2. In this method (OECD, 1981), samples yielding ≥60% of TCO_2 in 28 days are regarded as readily biodegradable. If biodegradation started before day 28 but had not plateaued at day 28, the fermentation should be continued until a plateau is reached. In this study, the experiment was continued for 73 days. Using Eq. 1, percent of CO_2 was calculated for 3, 6, 10, 14, 28, 35, 42, 53, 63 and 73 days.

Eq. 1

$$\% \text{ of } TCO_2 = \frac{\text{mg } CO_2 \text{ produced}}{\text{mg test material added in test} \times \text{mg } TCO_2/\text{mg test material}} \times 100$$

Plots 1 to 4 show the cumulative percent of TCO_2 versus time of 10 mg/L sample of black, blue, yellow and red inks, respectively. Plots 5 to 8 show similar data for 20 mg/L sample of inks.

On these plots, the equation $Y = A\ (1-e^{-bx})$ (Eq. 2) was fit to each curve where:

- Y = % of TCO_2
- X = number of days
- A = the asymptote (highest value for % of TCO_2 where the curve levels out)
- b = a "rate constant" (measures how fast the curve approaches A)

If we assume that this curve describes the relationship between days and % of TCO_2, then we can solve for:

% of TCO_2 at day 28 = $A\ [1 - e^{-b(28)}]$ (Eq. 3) and
Number of day at 60% of TCO_2 = $(1/-b)\ \ln\ (1-60/A)$ (Eq. 4)

Table 1 tabulates the calculated number of days required to reach 60% of TCO_2 and % of TCO_2 at 28 days. By examining Table 1 and the figures, extent of degradation at infinite time can be estimated. For example, from Table 1 and Figure 1 we can select the following data for black ink (10 mg/L sample). Soy oil-pigment reached an asymptote by 83% of TCO_2; USDA ink by 77% of TCO_2; NAA ink by 34% of TCO_2; petroleum ink by 18% of TCO_2.

Similar conclusions can be made for the other samples. Figure 9 shows the number of days required to reach 60% of TCO_2 for all samples at both 10 mg/L and 20 mg/L concentrations. In all cases, it took longer to reach 60% of TCO_2 for the larger samples. This may be related to the low solubility or dispersability of the hydrophobic samples. Differences were compared by t-test of least squares means at $p<0.01$ and show that it was not statistically significant in the case of soy oil-pigment mix and USDA inks, but it was statistically significant for NAA and petroleum inks. Data in Table 1 and Figure 9 show that both NAA and petroleum inks degrade very slowly. Soy oil-pigment mix and USDA inks took an average of 36 and 38 days to reach 60% of TCO_2. NAA and petroleum inks did not yield 60% of TCO_2 even by 73 days, and calculations showed that it would take 240 days for NAA and 420 days for petroleum inks.

High biodegradation results from this test provides evidence that the test compounds are highly biodegradable in aerobic systems. On the contrary, low biodegradation results may reflect numerous causes besides poor biodegradability. Toxic inhibition effects of the inoculum by test compounds is often a cause for low biodegradation. When the toxicity effects occur, reproducibility is poor. In such cases further work is needed to assess the biodegradability of the test compound in systems and at concentrations where inhibition effects are overcome.

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Table 1. Calculated Values % of TCO_2 at 28 days and Number of Days at 60% of TCO_2

Source and color of ink	Concentration of inks (mg/L)	A[a]	% of TCO_2 at 28 days	Days to reach 60% of TCO_2
Soy oil-Black	10	83	52	36
USDA-Black	10	77	50	40
NAA-Black	10	34	14	--[b]
Petroleum-Black	10	18	3	--
Soyoil-Black	20	75	50	40
USDA-Black	20	81	49	40
NAA-Black	20	218	7	275
Petroleum-Black	20	70	5	690
Soyoil-Blue	10	114	58	29
USDA-Blue	10	81	54	35
NAA-Blue	10	430	15	114
Petroleum-Blue	10	32	11	--
Soyoil-Blue	20	90	53	35
USDA-Blue	20	79	51	39
NAA-Blue	20	172	7	309
Petroleum-Blue	20	27	7	--

Table 1. Continued

Source and color of ink	Concentration of inks (mg/L)	A[a]	% of TCO_2 at 28 days	Days to reach 60% of TCO_2
Soyoil-Red	10	105	53	34
USDA-Red	10	86	53	34
NAA-Red	10	799	13	136
Petroleum-Red	10	160	6	337
Soy oil-Red	20	80	50	39
USDA-Red	20	77	51	39
NAA-Red	20	194	7	281
Petroleum-Red	20	175	6	338
Soy oil-Yellow	10	95	55	33
USDA-Yellow	10	73	54	36
NAA-Yellow	10	245	16	115
Petroleum-Yellow	10	47	15	--
Soyoil-Yellow	20	81	50	39
USDA-Yellow	20	80	50	40
NAA-Yellow	20	282	9	201
Petroleum-Yellow	20	136	7	314

Table 1. Footnotes

[a]The asymphote--the highest value % of TCO_2 where the curve levels off (Eq. 2).

[b] -- will never reach 60% of TCO_2.

Figure Legends

Figures 1-8.

1. Cumulative CO_2 evolution of black news inks (10 mg/L)

2. Cumulative of CO_2 evolution of blue news inks (10 mg/L)

3. Cumulative of CO_2 evolution of yellow news inks (10 mg/L)

4. Cumulative of CO_2 evolution of red news inks (10 mg/L)

5. Cumulative CO_2 evolution of black news inks (20 mg/L)

6. Cumulative of CO_2 evolution of blue news inks (20 mg/L)

7. Cumulative of CO_2 evolution of yellow news inks (20 mg/L)

8. Cumulative of CO_2 evolution of red news inks (20 mg/L)

○ Alkali refined soybean oil--pigment mixture (Erhan and Bagby, 1991)

Δ USDA's ink-formulated with 100% soybean oil-based vehicle (Type 1) (Erhan and Bagby, 1991)

◇ NAA's (ANPA) ink-formulated with partial soybean oil-based vehicle (ANPA, 1988)

□ Petroleum ink-formulated with petroleum oil-based vehicle (Flick, 1985)

Figure 9. Average number of days required to reach 60% of theoretical CO_2.

☐ 10 mg/L

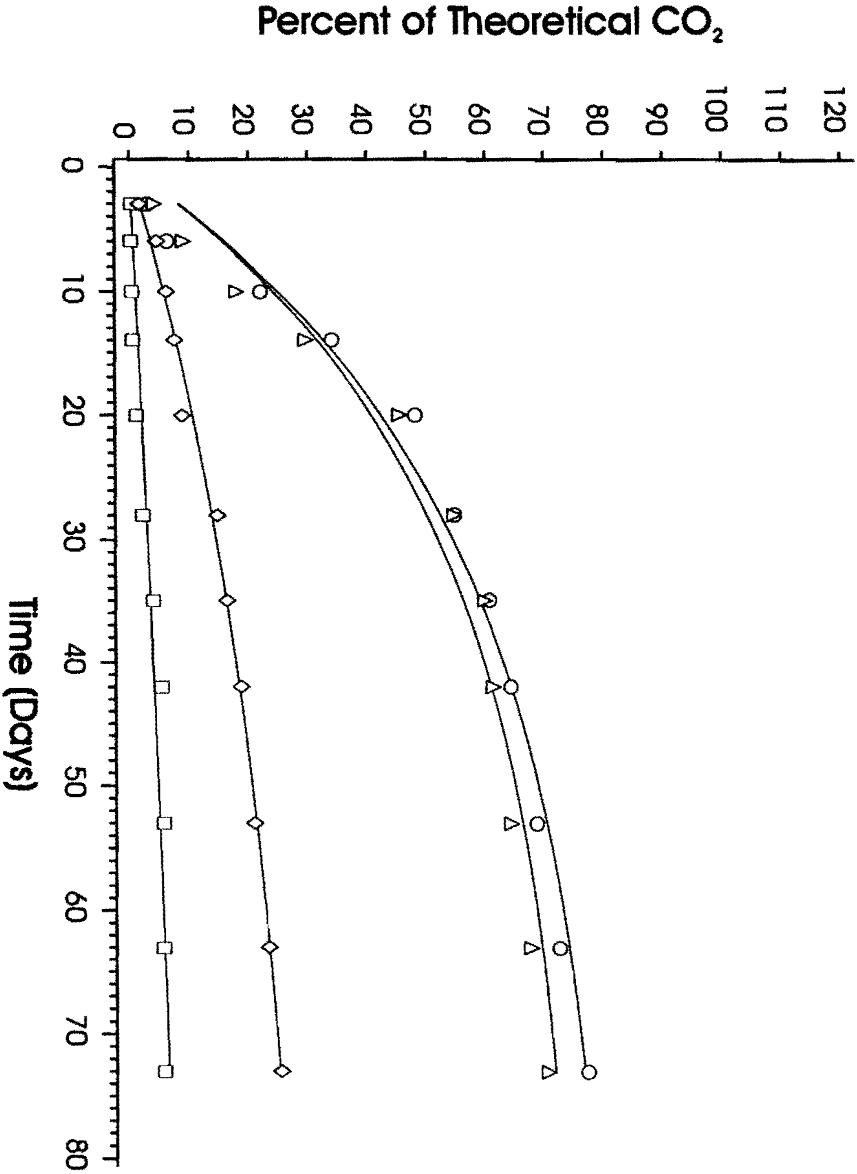
■ 20 mg/L

Soy--alkali refined oil-pigment mixture (Erhan and Bagby, 1991).

USDA--formulated with 100% soybean oil-based vehicle (Type 1) (Erhan and Bagby, 1991).

NAA (ANPA)--formulated with partial soybean oil-based vehicle (ANPA, 1988).

Petroleum--formulated with petroleum oil-based vehicle (Flick, 1985).



Percent of Theoretical CO_2
0
10
20
30
40
50
60
70
80
90
100
110
120
Time (Days)
0
10
20
30
40
50
60
70
80

Figure 1

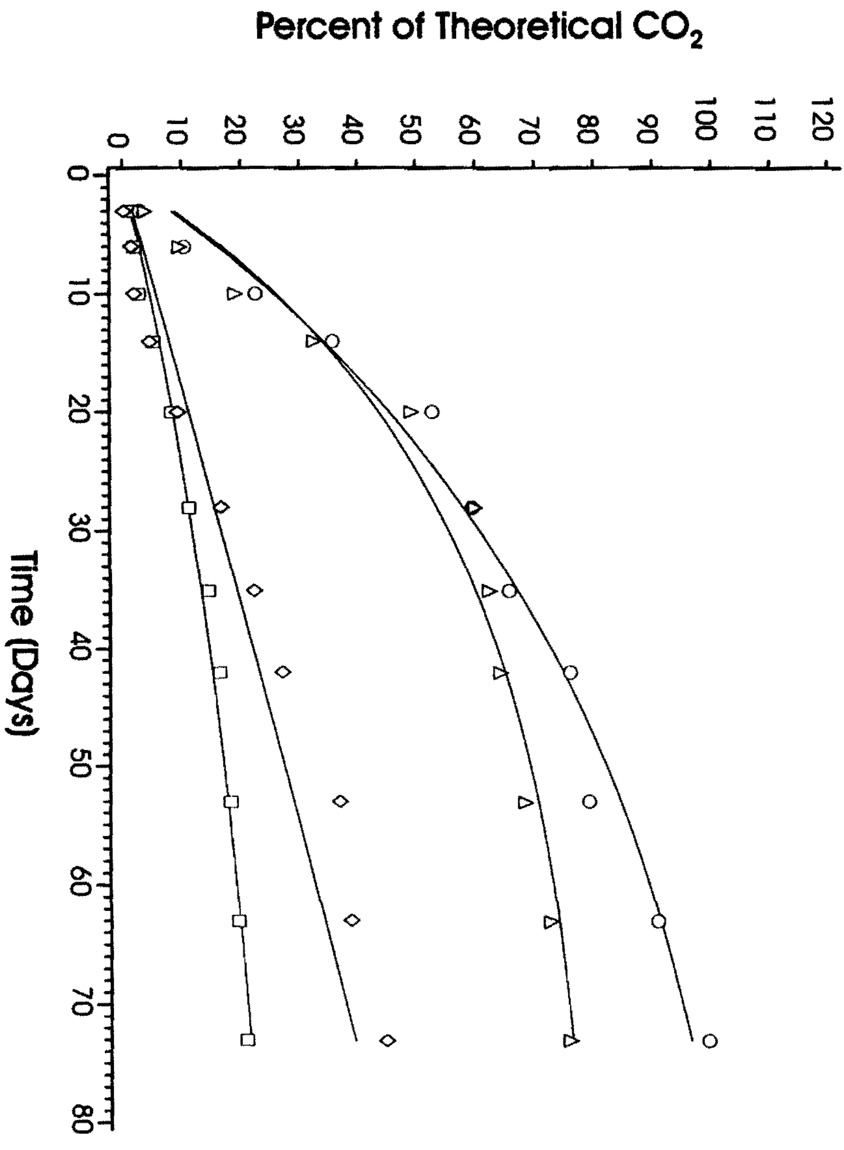

Percent of Theoretical CO_2
Time (Days)
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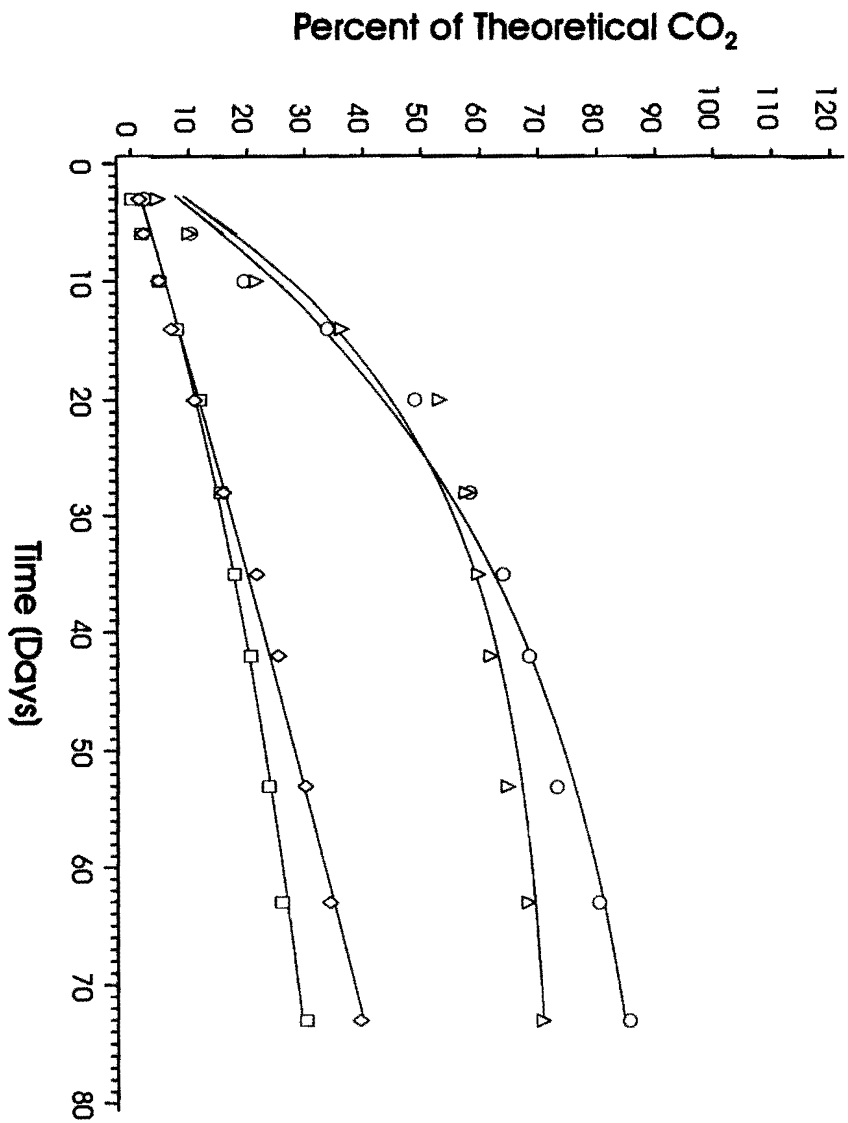
Figure 2



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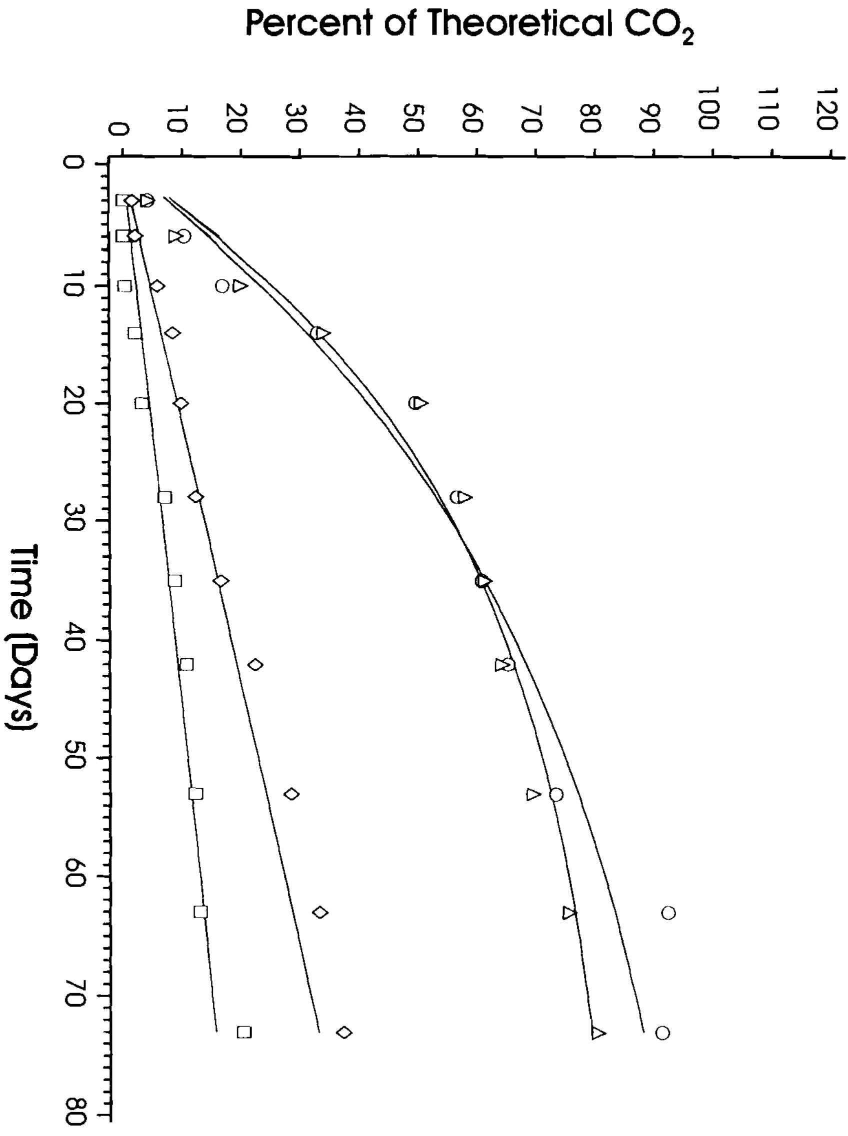
Percent of Theoretical CO_2
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110
120
Time (Days)
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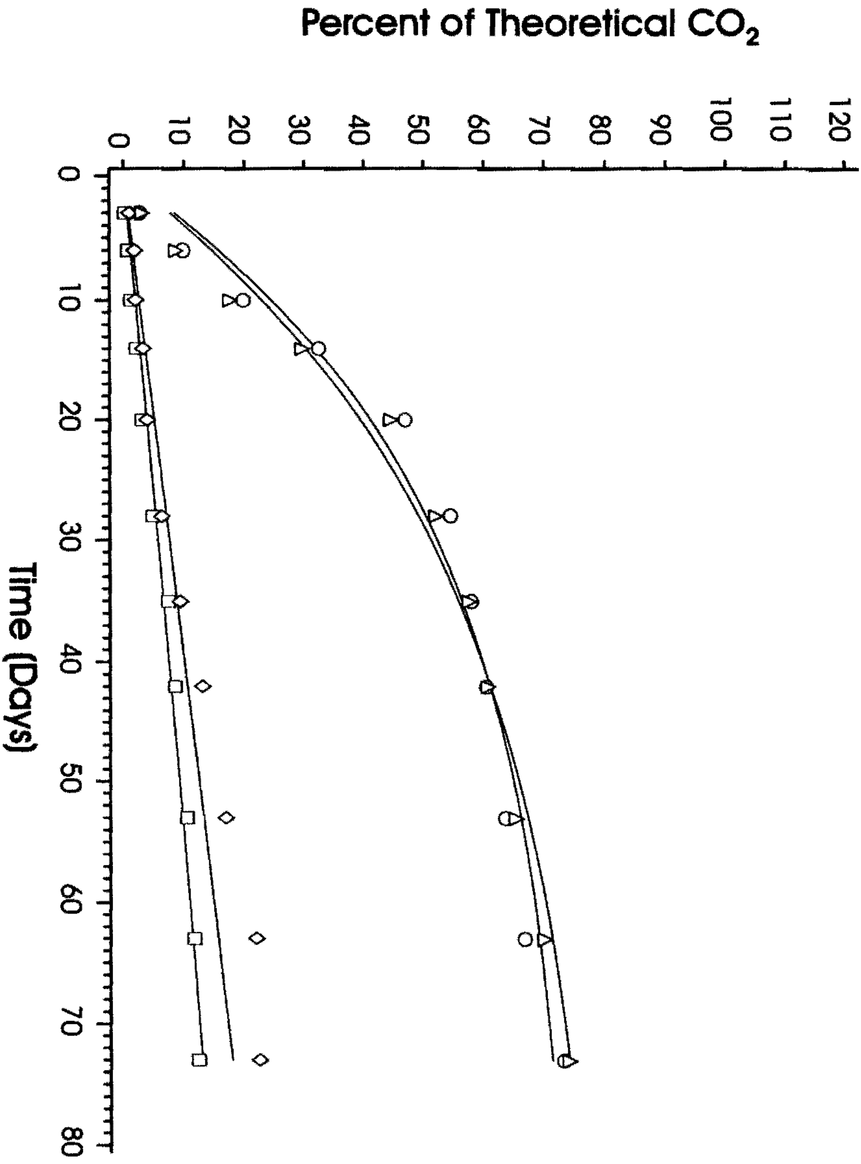
80

Figure 4


Percent of Theoretical CO_2
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Time (Days)
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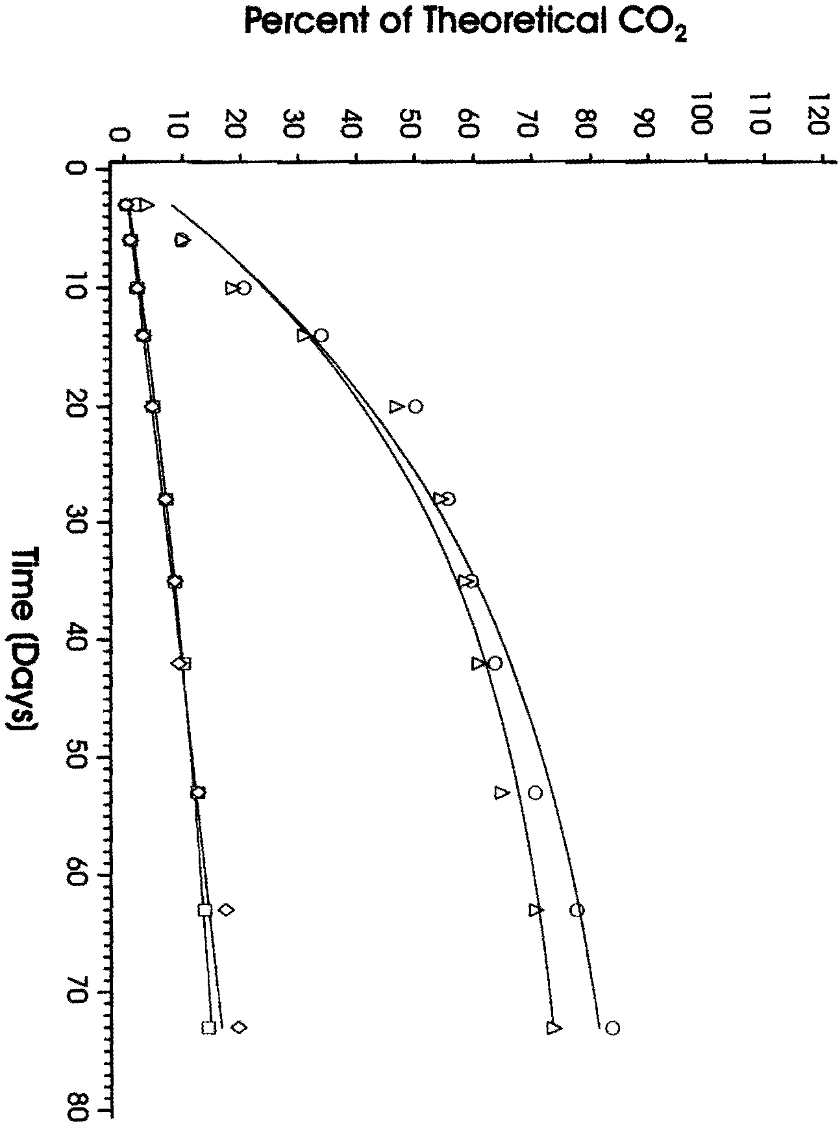
Figure 5


Percent of Theoretical CO_2
0
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120
Time (Days)
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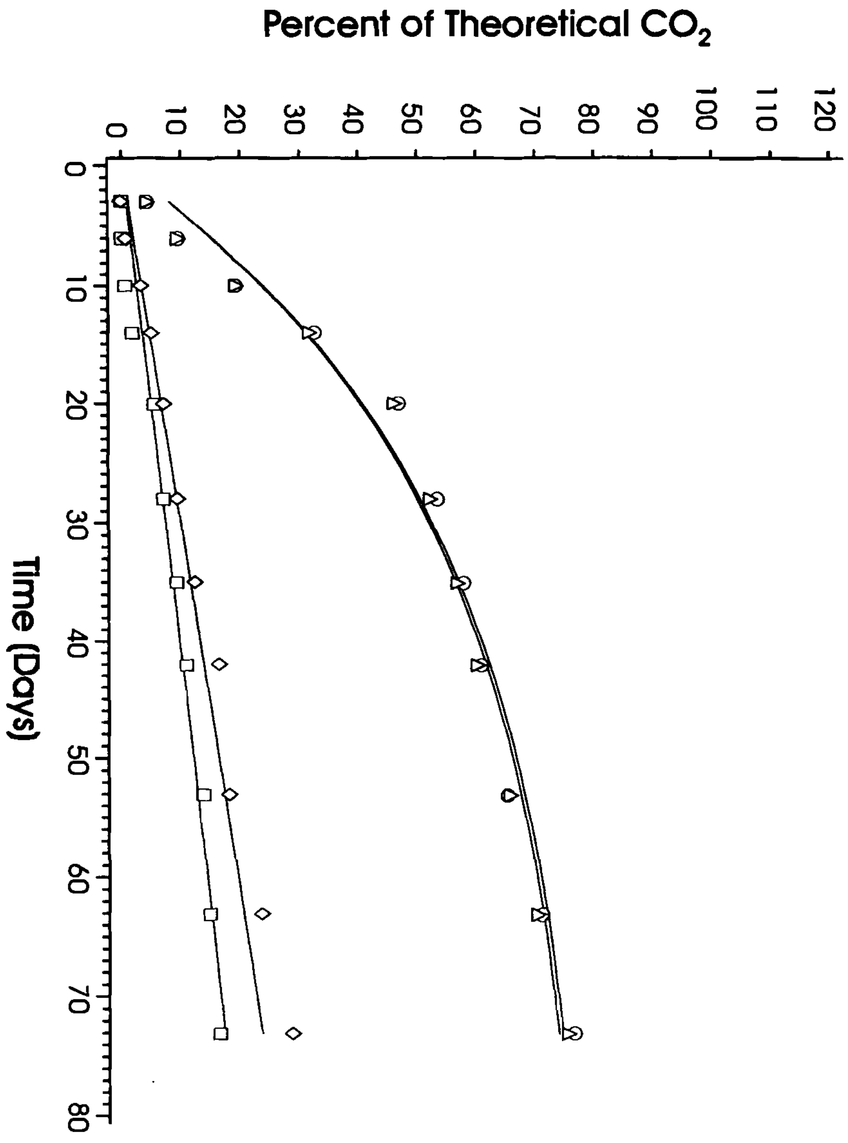
70
80

Figure 6


Percent of Theoretical CO_2
0
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Time (Days)
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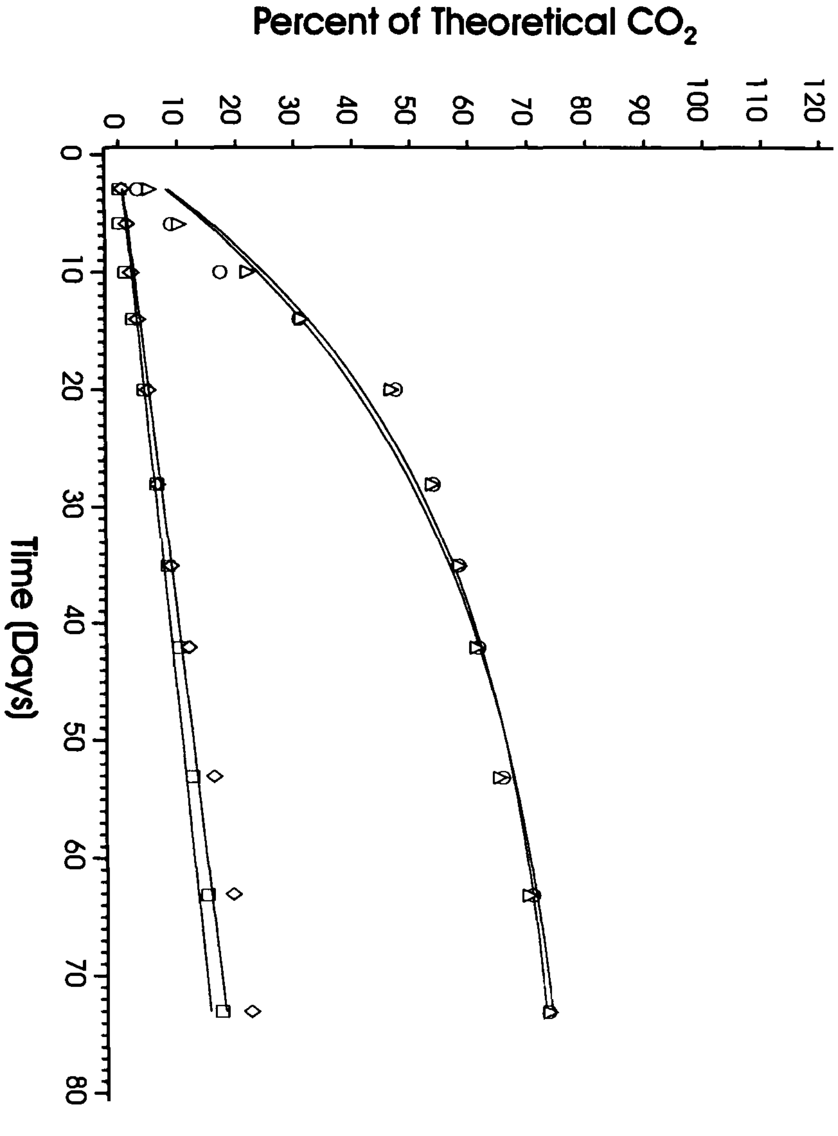
Figure 7



Percent of Theoretical CO_2
0
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80
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110
120
Time (Days)
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10
20
30
40
50
60
70
80

Figure 8

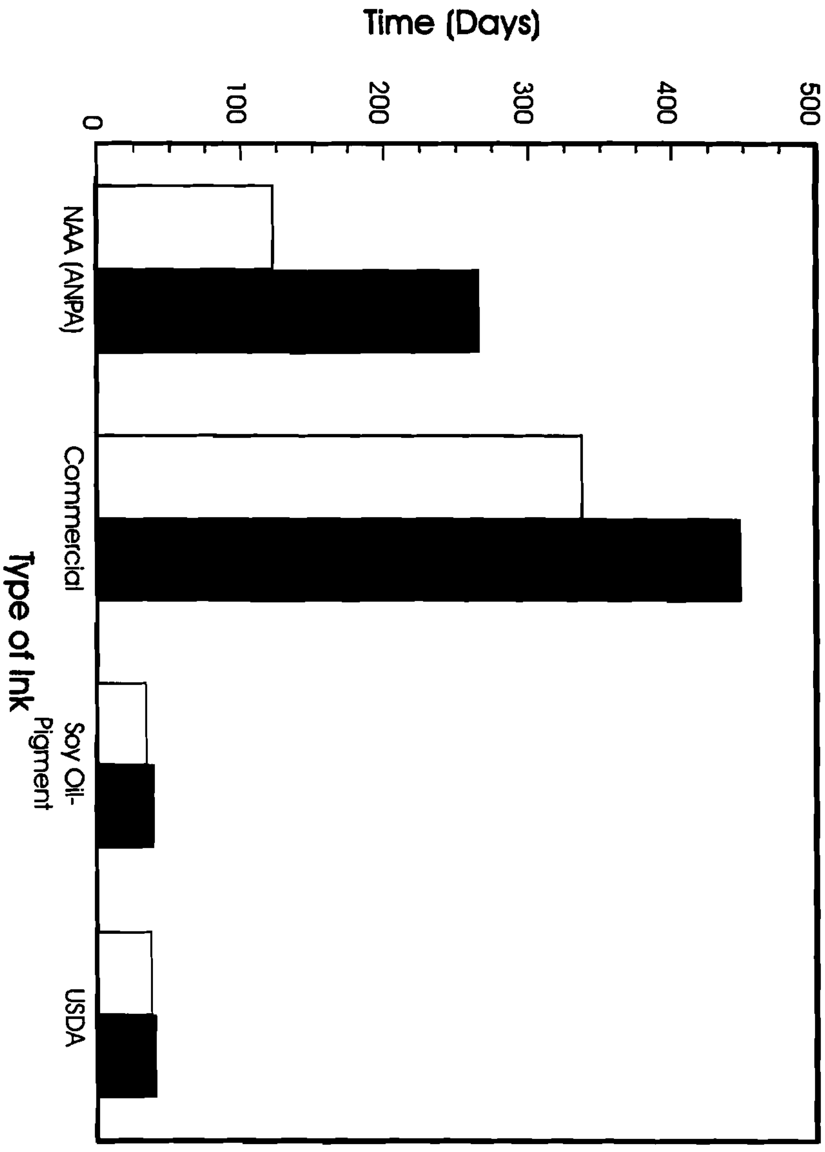
Time (Days)
0
100
200
300
400
500
NAA (ANPA)
Commercial
Soy Oil-Pigment
USDA
Type of Ink

Figure 9