Statistical Evaluation of Biodegradation of News Ink Vehicles and Ink Formulations

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ABSTRACT: Soybean oil, commercial news ink vehicles—consisting of either soy or mineral oil and petroleum resins—and United States Department of Agriculture's (USDA) 100% modified soy oil-based vehicles were subjected to biodegradation. Soybean oil and each vehicle were inoculated with monocultures and a mixed culture of *Aspergillus fumigatus, Penicillium citrinum,* and *Mucor racemosus.* Fermentations were allowed to proceed for 5, 12, and 25 d. Results show that, in 25 d, soy oil was degraded the most, followed by the USDA's ink vehicles (USDA I-III), Newspaper Association of America's (NAA) hybrid soy oil-based and commercial mineral oil-based vehicles. Some differences were found in the abilities of the cultures to degrade the different inks. Color did not appear to affect the degradation rate in soy oil, the USDA inks, or the NAA ink but was a factor in the commercial ink.

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KEY WORDS: Biodegradation, ink vehicle, news ink, statistical, vehicle soybean oil.

Biodegradation plays an important role in the transformation of many organic compounds in the environment. Although several articles speculate that soy oil should biodegrade more readily than mineral oil (1,2), there is a dearth of published experimental data. Cavagnaro and Kaszubowski (3) reviewed the biodegradation of food oils and greases. Knowledge of biodegradation, in terms of rates of degradation, the influence of the resin on soy oil degradation, and resulting products from ink vehicles, is needed. In this study, the following major types of news ink vehicles were evaluated for biodegradation: (i) commercial petroleum-based (4); (ii) Newspaper Association of America (NAA), hybrid soy oil-based (5); (iii) United States Department of Agriculture (USDA), 100% soy oil-based (6); and four colored inks (black, cyan, magenta, yellow), formulated by adding appropriate pigments to the above vehicles (7). Both petroleum-based and hybrid soy oil-based inks are used commercially. Soybean oil with pigments added was used as a control. We selected microorganisms that are commonly found in soil [*Aspergillus fumigatus* (NRRL 163), *Penicillium citrinum* (NRRL 1843), and *Mucor racemosus* (NRRL 5281)]. News ink vehicles and formulations enter the environment mainly during deinking and recycling processes. Their biodegradation by soil microorganisms is important in terms of their being accepted to landfills as waste ink (mixed with fountain solution used to dampen the plate and to keep nonprinting areas from accepting ink) and/or in the form of waste newspaper. Currently, 550 million pounds of news ink annually is used domestically, and we assume that half of that will end up as waste. We already reported preliminary results from this degradation study (8,9). Here we report the final data and the overall evaluation of these results.

EXPERIMENTAL PROCEDURES

Aspergillus fumigatus (NRRL 163), *P. citrinum* (NRRL 1843), and *M. racemosus* (NRRL 5281) were obtained from the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research (NCAUR, Peoria, IL). The synthetic liquid medium used (10) for growing microorganisms contained 2.0 g asparagine, 1.0 g dipotassium hydrogen phosphate, 0.5 g magnesium sulfate, 2.0 g dextrose, 5.0 mg thiamine hydrochloride, 1.45 mg iron (II) sulfate heptahydrate, 0.88 mg zinc sulfate heptahydrate, and 0.23 mg manganese (II) sulfate monohydrate in 1000 mL of distilled water. This medium was adjusted to pH 5.7 with a solution of 1:3 phosphoric acid/water. Thiamine hydrochloride, zinc sulfate heptahydrate, magnesium sulfate, phosphoric acid, and dipotassium hydrogen phosphate were obtained from Aldrich Chemical Company (Milwaukee, WI). Manganese (II) sulfate monohydrate, asparagine, dextrose, and iron (II) sulfate heptahydrate were obtained from Fisher Scientific Company (St. Louis, MO). The culture medium (1000 mL) was sterilized in a 2800-mL flask by autoclaving at 120°C for 30 min. Then, samples (4 g) of substrates were added. Substrates included alkali-refined soybean oil (obtained from Archer Daniels Midland, Decatur, IL), two USDA soy news ink vehicles (Gardner-Holdt viscosity of M-N and W-X) (6), USDA soy news ink vehicle that contained gel (Gardner-Holdt viscosity of W-X) (6), NAA hybrid soy oil news ink vehicle (5) (prepared at NCAUR), or commercial petroleum news ink vehicle (4) (prepared at NCAUR).

Inks were formulated by mixing each of (i) USDA soy news ink vehicle (Gardner-Holdt viscosity of W-X) (6),

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(ii) NAA hybrid soy oil news ink vehicle (5) and commercial petroleum news ink vehicle (4) 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, a combination of 14.2% Lithol Red and 12.8% Lithol Rubine was used. Pigment and vehicle were mixed with a Shar High Speed Disperser (Fort Wayne, IN), Model D-10P, at 3000 rpm for 2 h. Also, the pigments above were mixed with alkali-refined soybean oil (obtained from Archer Daniels Midland with a magnetic stirrer in a beaker for 1 h. These samples were used along with the ink samples for comparison.

Two loops of microorganisms, grown on yeast–malt agar slants, were transferred to the sterile medium. The flasks were shaken (200 rpm) on a rotary shaker at 25°C for 5, 12, and 25 d. Duplicate or triplicate samples were inoculated with either mono- or mixed cultures. The fermentations were terminated by refrigeration at 1.1°C.

To determine residual lipid material, the fermentation broth at termination was extracted four times with diethyl ether (200 mL). The combined ether extract was washed with water (800 mL) and then dried over anhydrous sodium sulfate. The ether solutions were filtered through silicone-coated filter paper (Whatman Lab Sales, Hillsboro, OR) into tared round-bottom flasks. To ensure quantitative transfer, the sodium sulfate was washed four times with ether. The ether was then removed with a rotary evaporator, and the flasks and their contents were placed in a vacuum desiccator overnight. Recoveries were determined gravimetrically. Validity of extraction protocol efficiency was established in the absence of microorganisms.

Percentage degradation was examined by analysis of variance (ANOVA) and analysis of covariance (ANCOVA) models (11); all mean comparisons were done by *t*-test comparison of least-squares means.

RESULTS AND DISCUSSION

Biodegradation of ink vehicles. The extent of biodegradation of the soybean oil and the ink vehicles was determined by differences between the amount added to the fermentation and the amount recovered at termination (8). The results from monocultures are tabulated in Table 1. Percentage degradation of all tested vehicles and soybean oil in mixed cultures of NRRL 163 and 1843, NRRL 163 and 5281, and NRRL 1843 and 5281 organisms is shown in Table 2. Table 2 also tabulates the percentage degradation when all three microorganisms—NRRL 163, 1843, and 5281—were present. Each oil within each group of the microorganism data sets was analyzed in an ANOVA model which tested time as the main effect. Comparisons between days were made by protected least significant difference (LSD at $P < 0.05$).

Tables 3–5 tabulate the percentage degradation and significant differences for all vehicles and soybean oil with mono- and mixed cultures after 5, 12, and 25 d of fermentation, respectively. Percentage biodegradation was examined by an ANOVA model that included fungal treatment (NRRL 163, NRRL 1843, NRRL 5281, NRRL 163 and 1843, NRRL 163 and 5281, NRRL 1843 and 5281, or NRRL 163 and 1843 and

TABLE 1
Percentage Degradation for Different Ink Vehicles and Soybean Oil After 5, 12, and 25 Days of Fermentation with Monocultures[a]

Days	Soybean oil[b]	USDA I[c]	USDA II[d]	USDA III[e]	NAA[f]	Commercial[g]
			Aspergillus fumigatus (NRRL 163)			
5	90.6[b]	58.8[b]	50.3[c]	52.7[c]	30.8[c]	11.7[b]
12	92.1[b]	83.9[a]	69.6[b]	73.2[b]	54.6[b]	13.2[b]
25	97.8[a]	87.3[a]	86.5[a]	86.5[a]	58.1[a]	19.2[a]
			Penicillium citrinum (NRRL 1843)			
5	90.1[c]	69.3[c]	68.8[c]	69.5[c]	29.5[c]	13.0[b]
12	95.5[b]	79.7[b]	77.1[b]	80.8[b]	52.4[b]	19.7[a]
25	98.4[a]	91.9[a]	85.5[a]	86.6[a]	65.5[a]	22.8[a]
			Mucor racemosus (NRRL 5281)			
5	90.1[c]	71.6[c]	61.5[c]	63.5[c]	35.4[c]	14.8[a]
12	94.7[b]	79.6[b]	74.4[b]	74.9[b]	50.6[b]	23.4[a]
25	97.5[a]	88.5[a]	82.0[a]	83.0[a]	61.9[a]	27.3[a]

[a]Means within a column and within each group of data set with a different superscript are different at $P < 0.05$ by least significant difference (LSD) tests.
[b]Alkali-refined soybean oil.
[c]USDA I: 100% soy oil-based vehicle (Type I); Gardner-Holdt Viscosity M-N.
[d]USDA II: 100% soy oil-based vehicle (Type I); Gardner-Holdt Viscosity W-X.
[e]USDA III: 100% soy oil-based vehicle (Type II); Gardner-Holdt Viscosity W-X.
[f]NAA: hybrid soy oil-based vehicle.
[g]Commercial petroleum oil-based vehicle.

TABLE 2
Percentage Degradation for Different Vehicles and Soybean Oil After 5, 12, and 25 Days of Fermentation with Mixed Cultures[a]

Days	Soybean oil[b]	USDA I[c]	USDA II[d]	USDA III[e]	NAA[f]	Commercial[g]
			Aspergillus fumigatus (NRRL 163) and *Penicillium citrinum* (NRRL 1843)			
5	89.4^{c}	66.2^{c}	69.4^{c}	68.2^{c}	32.9^{c}	11.0^{b}
12	93.4^{b}	79.4^{b}	75.9^{b}	72.5^{b}	59.1^{b}	14.1^{ab}
25	97.8^{a}	89.2^{a}	85.9^{a}	81.6^{a}	68.2^{a}	22.0^{a}
			A. fumigatus (NRRL 163) and *Mucor racemosus* (NRRL 5281)			
5	90.1^{b}	73.1^{b}	68.6^{a}	62.5^{b}	46.2^{a}	6.2^{b}
12	92.1^{b}	83.1^{a}	73.3^{a}	82.5^{a}	55.9^{a}	13.8^{a}
25	97.1^{a}	89.6^{a}	86.8^{a}	83.7^{a}	57.6^{a}	16.6^{a}
			P. citrinum (NRRL 1843) and *M. racemosus* (NRRL 5281)			
5	93.7^{b}	72.6^{c}	65.8^{b}	69.2^{b}	50.6^{a}	13.8^{b}
12	97.7^{a}	81.7^{b}	78.2^{a}	76.1^{ab}	54.6^{a}	17.7^{ab}
25	98.8^{a}	89.0^{a}	84.9^{a}	82.8^{a}	63.6^{a}	18.1^{a}
			A. fumigatus (NRRL 163), *P. citrinum* (NRRL 1843), and *M. racemosus* (NRRL 5281)			
5	92.7^{c}	72.9^{b}	65.3^{c}	65.2^{c}	30.5^{c}	12.2^{c}
12	96.3^{b}	77.4^{b}	70.1^{b}	73.1^{b}	54.9^{b}	18.0^{b}
25	97.2^{a}	89.3^{a}	83.3^{a}	85.2^{a}	65.2^{a}	22.7^{a}

[a]Means within a column and within each group of data set with a different superscript are different at $P < 0.05$ by LSD tests. For footnotes *b–g,* see Table 1.

TABLE 3
Percentage Degradation for Different Vehicles and Soybean Oil After 5 Days of Fermentation with Mono- and Mixed Cultures[a]

Microorganism	Soybean oil[b]	USDA I[c]	USDA II[d]	USDA III[e]	NAA[f]	Commercial[g]
NRRL 163	90.6^{b}	58.8^{c}	50.3^{b}	52.7^{d}	30.8^{c}	11.7^{ab}
NRRL 1843	90.1^{b}	69.3^{ab}	68.8^{a}	69.5^{a}	29.5^{c}	13.0^{ab}
NRRL 5281	90.1^{b}	71.6^{a}	61.5^{a}	63.5^{c}	35.5^{bc}	14.8^{a}
NRRL (163 + 1843)	89.4^{b}	66.2^{b}	69.4^{a}	68.2^{ab}	32.9^{c}	11.0^{ab}
NRRL (163 + 5281)	90.1^{b}	73.1^{a}	68.6^{a}	62.5^{c}	46.2^{a}	6.2^{b}
NRRL (1843 + 5281)	93.7^{a}	73.2^{a}	65.8^{a}	67.5^{ab}	41.3^{ab}	13.8^{a}
NRRL (163 + 1843 + 5281)	92.7^{a}	72.9^{a}	65.3^{a}	64.7^{bc}	30.5^{c}	12.2^{ab}

[a]Means within a column with different superscripts are different at $P < 0.05$ by *t*-test comparisons of least square means. For footnotes *b–g,* see Table 1.

TABLE 4
Percentage Degradation for Different Vehicles and Soybean Oil After 12 Days of Fermentation with Mono- and Mixed Cultures[a]

Microorganism	Soybean oil[b]	USDA I[c]	USDA II[d]	USDA III[e]	NAA[f]	Commercial[g]
NRRL 163	92.1^{d}	83.9^{a}	69.6^{b}	73.2^{bc}	54.6^{b}	13.2^{c}
NRRL 1843	95.5^{b}	79.7^{bc}	77.1^{a}	80.8^{a}	52.4^{cd}	19.7^{b}
NRRL 5281	94.7^{bc}	79.6^{bc}	74.4^{ab}	74.9^{bc}	50.6^{d}	23.4^{a}
NRRL (163 + 1843)	93.4^{cd}	79.4^{bc}	75.9^{a}	72.5^{c}	59.1^{a}	14.1^{c}
NRRL (163 + 5281)	92.1^{d}	83.1^{ab}	73.3^{ab}	82.5^{a}	55.9^{ab}	13.9^{c}
NRRL (1843 + 5281)	97.7^{a}	81.7^{a}	78.2^{a}	76.1^{b}	54.6^{b}	17.7^{b}
NRRL (163 + 1843 + 5281)	96.3^{ab}	77.4^{c}	68.8^{b}	73.1^{bc}	54.9^{bc}	18.0^{b}

[a]Means within a column with different superscripts are different at $P < 0.05$ by *t*-test comparisons of least square means. For footnotes *b–g,* see Table 1.

TABLE 5
Percentage Degradation for Different Vehicles and Soybean Oil After 25 Days of Fermentation with Mono- and Mixed Cultures[a]

Microorganism	Soybean oil[b]	USDA I[c]	USDA II[d]	USDA III[e]	NAA[f]	Commercial[g]
NRRL 163	97.8^{bc}	87.3^{b}	86.5^{a}	86.5^{a}	58.1^{cd}	19.2^{bc}
NRRL 1843	98.4^{ab}	91.9^{a}	85.5^{a}	86.6^{a}	65.5^{ab}	22.8^{ab}
NRRL 5281	97.5^{b}	88.5^{ab}	82.0^{c}	83.0^{bc}	61.9^{bc}	27.3^{a}
NRRL (163 + 1843)	97.8^{b}	89.2^{ab}	85.9^{ab}	81.6^{c}	68.2^{a}	22.0^{b}
NRRL (163 + 5281)	97.1^{c}	89.6^{ab}	86.8^{a}	83.7^{bc}	57.6^{d}	16.6^{c}
NRRL (1843 + 5281)	98.8^{a}	89.0^{ab}	85.0^{ac}	82.8^{bc}	63.3^{b}	18.1^{bc}
NRRL (163 + 1843 + 5281)	97.2^{c}	89.3^{ab}	83.3^{bc}	85.2^{ab}	65.2^{ab}	22.7^{a}

[a]Means within a column with different superscripts are different at $P < 0.05$ by *t*-test comparisons of least square means. For footnotes *b–g,* see Table 1.

FIG. 1. Comparison of degradation by *Aspergillus fumigatus* (NRRL 163), *Penicillium citrinum* (NRRL 1843), and *Mucor racemosus* (NRRL 5281) microorganisms. (Percentage degradation is an average of all inks tested.)

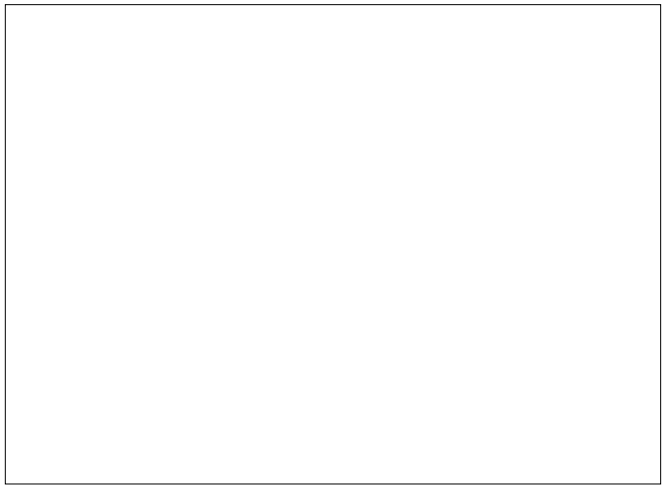

FIG. 2. Effect of monocultured and mixed cultured microorganisms on biodegradation. (Percentage degradation is an average of all inks tested.) NRRL 163: —·-·—; NRRL 163 + NRRL 5281 + NRRL 1843: ------; NRRL 1843 + NRRL 5281: — — — —; NRRL 5281: ————; NRRL 1843: —·-·—·-; NRRL 163 + NRRL 1843: —— ——; NRRL 163 + NRRL 5281: — — — —.

5281), time (5, 12, or 25 d) and vehicle type (soy oil, 100% soy USDA I-III, hybrid soy NAA, commercial petroleum) as main effect sources of variation as well as all possible two-way interactions. Figure 1 shows that when *A. fumigatus* (NRRL 163) was used, degradation averaged across all inks was less ($P < 0.05$) than when *P. citrinum* (NRRL 1843) or *M. racemosus* (NRRL 5281) was used. Average percentage degradations were 62.1, 66.5, and 65.3, respectively. When *A. fumigatus* was combined with *P. citrinum, M. racemosus,* or both the percentage degradation was higher ($P < 0.05$) than with *A. fumigatus* alone but was not higher ($P > 0.10$) than either *P. citrinum* or *M. racemosus* alone. Percentage degradation rose ($P < 0.01$) from day 5 (56.0%) to day 12 (66.2%) and again ($P < 0.01$) to day 25 (73.4%) (Fig. 2).

Overall, on all three days, with any combination of tested microorganisms the soy oil degraded the most (94.6%), followed by USDA's 100% soy-based vehicles (USDA I = 79.8%, USDA II = 74.4%, and USDA = 74.8%), NAA's hybrid soy vehicle (50.9%), and the commercial petroleum-based vehicle (16.7%) (Fig. 3). All differences were signifi-

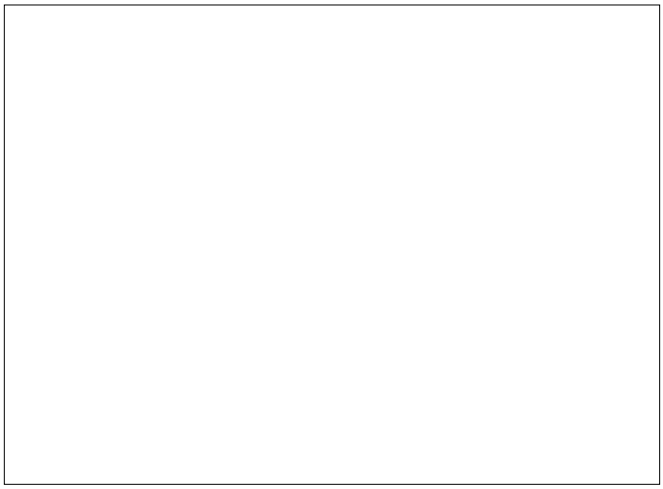

FIG. 3. Biodegradation of soybean oil and ink vehicles. Abbreviations: USDA: United States Department of Agriculture; NAA: Newspaper Association of America.

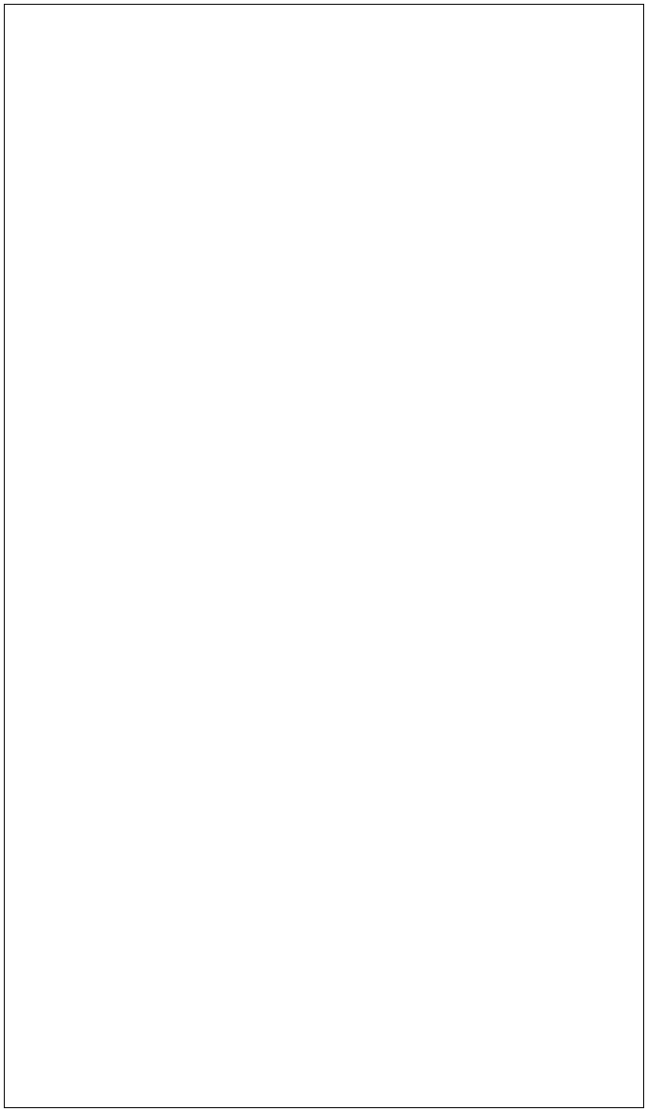

FIG. 4. Comparison of degradation of four colored inks from different sources at 5, 12, and 25 d of fermentation. Cross-hatched: NAA; open, (National Association of Printing Ink Manufacturers); solid: Soy oil; horizontally lined: USDA. For abbreviations, see Figure 3.

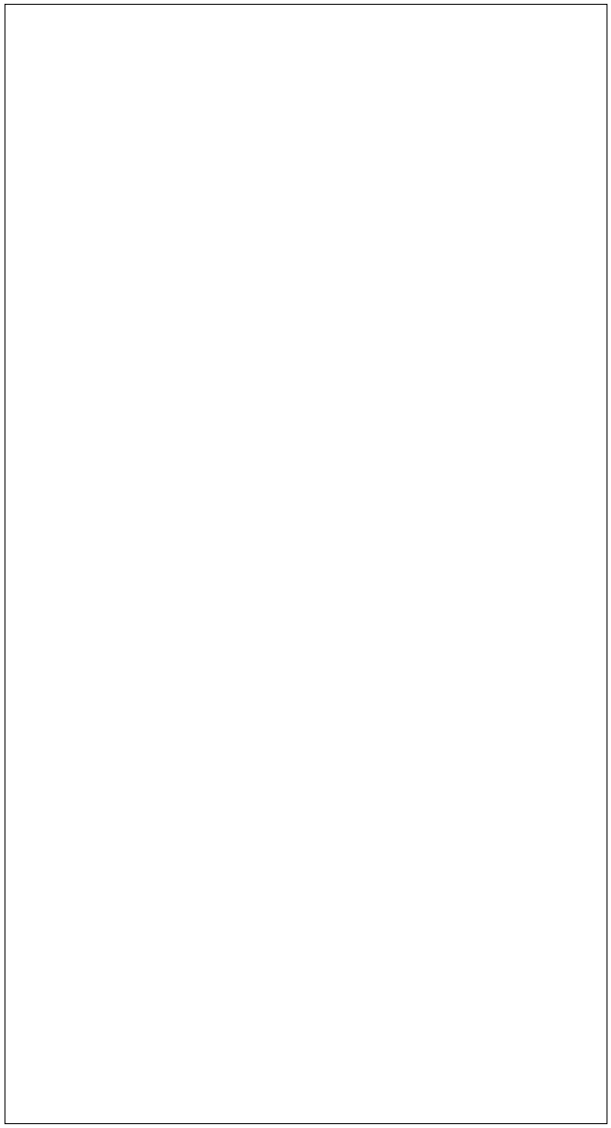

FIG. 5. Comparison of degradation of soy oil–pigment mix, USDA, NAA hybrid soy, and commercial petroleum inks in four colors at 5, 12, and 25 d of fermentation. For abbreviations see Figures 3 and 4. Cross-hatched: black; open: blue; solid: red; horizontally-lined: yellow.

cant ($P < 0.05$), except for the comparison of USDA II and USDA III. Percentage degradation with any combination of tested microorganisms after 25 d of fermentation was 97.8% for soy oil, and 89.3, 85.0, and 84.2% for USDA's 100% soy-based vehicles (USDA I, II, and III), respectively. NAA's hybrid soy vehicle degraded 62.6%, and commercial petroleum-based vehicle degraded 21.1% in 25 d. The data at 25 d show the practical differences between the various vehicles. Thus, it is clearly demonstrated that, in terms of biodegradation, the soy vehicles are far superior to the hybrid soy, which, in turn, is superior to the 100% petroleum vehicle. Examination of significant microorganisms by time, microorganism by vehicle, and time by vehicle interactions suggested that the interactions were caused by changes in degree of difference rather than changes in rank.

Biodegradation of formulated inks. Biodegradation of the soybean oil–pigment mix and the news inks was determined by differences between amounts added to the fermentation and amounts recovered at termination. Calculations were based on vehicle contents in the formulated inks (9).

An initial ANOVA for percentage degradation in all possible two-way interactions and the three-way interaction were run with sources of variation (color, vehicle, time) (Fig. 4). All were significant ($P < 0.01$), so the analysis was broken into subsets to best describe the differences in degradation. The effects of time (d) were considered for each vehicle and for each color within the vehicle by means of ANCOVA (Fig. 5). In the USDA ink, there were no differences among colors, and the degradation rate was 1.01% per day. In the National Association of Printing Ink Manufacturers (NAPIM)

ink, there were no differences among colors, and the degradation rate was 0.44% per day. In the soy oil the overall degradation rate was 0.67% per day, with the blue ink tending to degrade slightly faster than the others. In the American Newspaper Publishers Association (ANPA) ink, the degradation rates were 1.70, 1.57, 0.92, and 0.54% per day for yellow, blue, red, and black, respectively.

At each day, the vehicles were ranked soy oil > USDA > NAA > commercial, and all differences were significant ($P < 0.05$). At day 5, there were no degradation differences among colors. At day 12, vehicles with the blue pigment had degraded more ($P < 0.01$) than with other colors. At day 25, vehicle containing blue and yellow had degraded to a similar degree, which was greater than with red, which in turn was greater than with black ($P < 0.01$).

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