BIODEGRADATION OF NEWS INK VEHICLES

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Abstract: Commercial news ink vehicles consisting of soy or mineral oil and petroleum resins and USDA's 100% modified soy oil based vehicles were subjected to biodegradation. Rate and extent of degradation were compared by inoculating each vehicle with monocultures and mixed cultures of *Aspergillus fumigatus, Penicillium citrinum* and *Mucor racemosus*. Fermentations were allowed to proceed for 5, 12 and 25 days. Results show that USDA's ink vehicles degrade faster and more completely than either commercial mineral oil based or soy-mineral oil based vehicles. In 25 days soybean oil degraded nearly completely, USDA's 100% soy oil-based vehicle degraded 82-92%, and commercial partial soy oil based and petroleum based vehicles degraded 58-68% and 17-27%, respectively.

Introduction

Biodegradation plays an important role in the transformation of many organic compounds in the environment. To our knowledge there is no data published on biodegradation of news ink vehicles. Although several articles speculate that soy oil should biodegrade more readily than mineral oil (Ticer, 1988; Ellis, 1991), there is a dearth of published experimental data. Cavagnaro and Kaszubowski (1988) have reviewed the biodegradation of food oils and greases. Knowledge of biodegradation, rates of degradation and resulting products from ink vehicles are needed, as is the influence of the resin on degradation. In this study, three major types of news ink vehicles; (a) commercial petroleum-based (Flick, 1985), (b) American Newspaper Publishers Association's (ANPA) partial soy oil-based (ANPA, 1988), (c) United States Department of Agriculture's

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(USDA) 100% soy oil-based (Erhan and Bagby, 1991), were evaluated for biodegradation. We selected microorganisms that are commonly found in soil [*Aspergillus fumigatus* (NRRL 163), *Penicillium citrinum* (NRRL 1843) and *Mucor racemosus* (NRRL 5281)]. Here, we report results from that preliminary degradation study.

Experimental

Aspergillus fumigatus (NRRL 163), *Penicillium citrinum* (NRRL 1843) and *Mucor racemosus* (NRRL 5281), used in this study, were obtained from the ARS Culture Collection, National Center for Agricultural Utilization Research (NCAUR). The synthetic liquid medium used (Koritala *et al.*, 1987) 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 using 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. Culture medium (1000 ml) was sterilized in a 2800-ml flask by autoclaving at 120°C. To each were added samples (4 g) of 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) (Erhan and Bagby, 1991), USDA soy news ink vehicle containing gel (Gardner-Holdt viscosity of W-X) (Erhan and Bagby 1991), ANPA soy oil news ink vehicle (ANPA, 1988) (prepared at NCAUR) or commercial petroleum news ink vehicle (Flick, 1985) (prepared at NCAUR). Two loopsful of microorganisms grown on yeast-malt agar slants were transferred to the sterile medium, and then the flask and its contents were shaken (200 rpm) on a rotary shaker at 25°C for 5, 12 and 25 days. Duplicate or triplicate samples were inoculated with either mono- or mixed cultures. The fermentations were terminated by refrigeration at 34°F.

To determine residual lipid material, the fermentation broth was extracted 4 times with diethyl ether (200 ml). Validity of extraction protocol efficiency was established in the absence of microorganisms. 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 (obtained from Whatman Lab Sales, Hillsboro, OR) into tared round-bottomed flasks. To assure quantitative transfer, the sodium sulfate was washed four times with ether. Then the ether was removed with a rotary evaporator, and the flasks and their contents were placed in a vacuum desiccator overnight. Recoveries were determined gravimetrically.

Results and Discussion

The extent of biodegradation of the soybean oil and the ink vehicles was determined by diffrences between that added to the fermentation and that recovered at termination. The relative rates of degradation is ascertained from the extent of degradation at the 5, 12 and 25 day intervals. The results from using mono cultures are tabulated in Tables 1, 2 and 3. Table 1 shows the percent biodegradation with *Aspergillus fumigatus* (NRRL 163). Average percent degradations of the three USDA vehicles were 24, 21 and 29% more than that for the ANPA vehicle and 42, 62 and 68% more than that for the commercial petroleum based vehicle at 5, 12 and 25 days, respectively. The biodegradation results with *Penicillium citrinum* (NRRL 1843) are tabulated in Table 2. USDA vehicles' average degradation was 40, 27 and 21% greater than that for ANPA vehicle and 56, 60 and 63% more than that for commercial petroleum based vehicle at 5, 12 and 25 days, respectively. Table 3 shows the percent degradation with *Mucor racemosus* (NRRL 5281). The average degradation of USDA vehicles was 30, 26 and 23% greater than that for ANPA vehicle and 51, 53 and 57% greater than that for petroleum based vehicle at 5, 12 and 25 days, respectively.

As expected, the soybean oil was nearly completely degraded in 25 days by all organisms. *Aspergillus fumigatus* was somewhat less effective in degrading the vehicles than *Penicillium citrinum* and

TABLE 1
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Aspergillus fumigatus* (NRRL 163)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	90.3	58.8	50.3	52.7	29.9	11.7
12	92.1	83.9	69.6	73.2	54.6	13.2
25	97.8	87.4	86.5	86.5	58.1	19.2

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soy oil based vehicle.

[f]Commercial petroleum oil based vehicle.

TABLE 2
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Penicillium citrinum* (NRRL 1843)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	90.1	69.3	68.8	69.5	29.5	12.9
12	95.5	79.7	77.1	80.8	52.4	19.7
25	98.4	91.9	85.5	86.6	65.5	22.8

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soy oil based vehicle.

[f]Commercial petroleum based vehicle.

TABLE 3
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Mucor racemosus* (NRRL 5281)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	90.1	71.6	61.5	63.5	35.5	14.8
12	94.7	79.6	74.4	74.9	50.6	23.4
25	97.5	88.5	82.1	83.0	61.9	27.3

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soy oil based vehicle.

[f]Commercial petroleum based vehicle.

Mucor racemosus, after 5 days, but the results were somewhat closer at 12 and 25 days.

As expected, time did not greatly increase the degradation of petroleum based vehicle. Even at 25 days only 19-27% degradation took place. ANPA vehicle degraded only 30-36% at 5 days but after 25 days 58-66% degradation was seen. This result was expected because 30-33% of the vehicle is petroleum based resin, and the remainder is soybean oil.

Percent degradation of all tested vehicles and soybean oil by using mixed cultures of NRRL 163 and 1843, NRRL 163 and 5281 and NRRL 1843 and 5281 organisms are shown in Tables 4, 5 and 6, respectively. Table 7 tabulates the percent degradations when all three microorganisms NRRL 163, 1843 and 5281 were present. Percent degradations obtained with mixed cultures were very similar to those with monocultures.

Figure 1 plots the composite percent degradation data of all tested vehicles and soybean oil with mono and mixed cultures. This figure allows for an efficient overview of the results. It is clearly seen that the microorganisms consume the various substrates at markedly different rates and with differing levels of completeness. After 25 days, soybean oil is nearly completely degraded; while, USDA's 100% soy-oil based vehicle degraded 82 to 92%, commercial partial soy oil based and petroleum based vehicles degraded 58 to 68%, and 17 to 27%, respectively. Thus, the USDA vehicles offer environmental advantage as well as, the other superior qualities previously described, i.e., no petroleum, less ruboff and lower cost than any commercial ink including the ANPA partial soybean oil ink.

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TABLE 4
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Aspergillus fumigatus* (NRRL 163) and *Penicillium citrinum* (NRRL 1843)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	89.2	66.2	69.4	68.3	32.9	10.9
12	92.8	78.0	75.9	72.5	56.6	14.1
25	97.8	89.2	85.9	81.6	68.2	22.0

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soyoil based vehicle.

[f]Commercial petroleum based vehicle.

TABLE 5
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Aspergillus fumigatus* (NRRL 163) and *Mucor racemosus* (NRRL 5281)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	90.1	73.1	68.6	62.5	46.2	6.2
12	92.1	83.8	73.3	82.5	55.9	13.8
25	97.1	89.6	86.8	83.7	57.6	16.6

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soy oil based vehicle.

[f]Commercial petroleum based vehicle.

TABLE 6
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Penicillium citrinum* (NRRL 1843) and *Mucor racemosus* (NRRL 5281)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	93.7	72.6	65.8	66.6	41.3	13.8
12	97.7	81.7	78.2	76.1	54.7	17.7
25	98.8	89.0	84.9	82.8	63.3	18.1

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soyoil based vehicle.

[f]Commercial petroleum based vehicle.

TABLE 7
Percent Degradation for Different Vehicles and Soybean Oil After 5, 12 and 25 days of Fermentation with *Aspergillus fumigatus* (NRRL 163), *Penicillium citrinum* (NRRL 1843) and *Mucor racemosus* (NRRL 5281)

# of Days	Percent degradation					
	Soybean oil[a]	USDA I[b]	USDA II[c]	USDA III[d]	ANPA[e]	Commercial[f]
5	92.7	72.9	65.6	64.7	29.7	12.2
12	96.3	77.5	70.1	73.1	54.9	18.0
25	97.3	89.3	83.3	85.2	65.2	22.7

[a]Alkali refined soybean oil.

[b]USDA I - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity M-N.

[c]USDA II - 100% soy oil-based vehicle (Type I) - Gardner Holdt Viscosity W-X.

[d]USDA III - 100% soy oil-based vehicle (Type II) - Gardner Holdt Viscosity W-X.

[e]ANPA - Partial soy oil based vehicle.

[f]Commercial petroleum oil based vehicle.

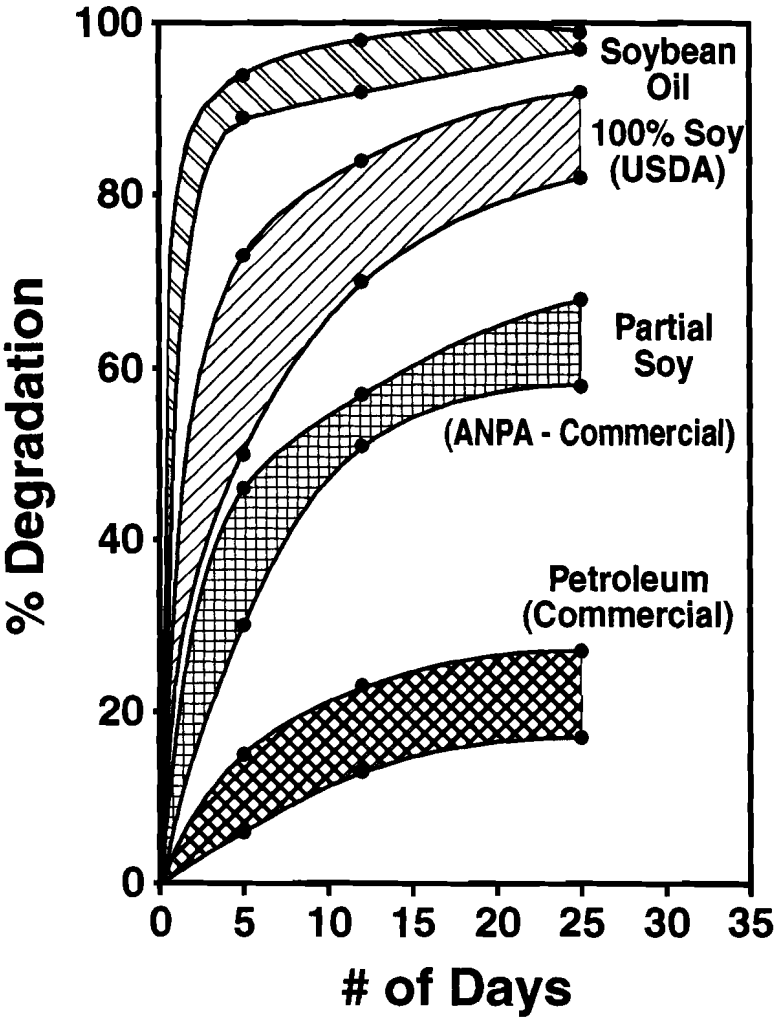

Figure 1. Biodegradation of Soybean Oil and Ink Vehicles (Broad Plots Represents All Data Ranging from Min. to Max.)

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