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Potential of agricultural by-products in the bioremediation of fuel spills

AL Pometto III[1], CS Oulman[2], AA DiSpirito[3], KE Johnson[1] and S Baranow[3]

[1]*Department of Food Science and Human Nutrition;* [2]*Department of Civil and Construction Engineering;* [3]*Department of Microbiology, Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011, USA*

Respirometer studies were used to determine the benefits of mixing soybean hulls with gravel contaminated with petroleum products. The aerobic conditions prevailing in the respirometer maintained significant microbiological activity. A reduction in petrochemical concentrations was detected, whether the contaminated gravel was treated with agricultural by-products or not, but at a slightly faster rate with the treatment.

Keywords: biodegradation; soybean hulls; petrochemical oxidation

Introduction

The heavy use of petroleum products as fuels has been attended by a corresponding release of crude oil and other petroleum products in the terrestrial environment. Under suitable conditions, bioremediation has been demonstrated to be a potential technique for the removal of degradable hydrocarbons from contaminated soils. Indeed, there are many contaminated sites currently undergoing bioremediation [1]. Several recent comprehensive reviews on bioremediation have been compiled [2,8,9].

Various techniques that have been employed to enhance biodegradation of hydrocarbons during *ex situ* bioremediation include fertilization, aeration and dilution. Song *et al* [7] demonstrated that biodegradation of diesel fuel, heating oil, and jet fuel in soil was enhanced by using tillage, fertilization and liming. Troy *et al* [9] remediated soil contaminated with diesel fuel by land farming. Field studies of the *Exxon Valdez* crude oil spill in Alaska [6] demonstrated that bioremediation of the surface of the shore line materials was accelerated by treatment with fertilizer but that degradation below the surface was variable.

Two of us (ALP and CSO) have participated in a variety of currently unpublished bench scale and field tests designed to determine the efficacy of admixing various agricultural by-products with soil contaminated with petroleum products to stimulate microbiological activity. The working hypothesis in these tests was that the natural products would provide a delivery medium for nutrients, moisture, physical support for increased aeration, and microorganisms needed in degrading the petroleum products. The results indicated that bioremediation was stimulated in both the control samples and in the treated samples. Under the conditions of the tests reported in this paper, bioremediation was accelerated by providing an aerobic environment where microorganisms introduced with the petroleum products could thrive and consume the carbon source.

Correspondence: AL Pometto III, Department of Food Science and Human Nutrition, Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011, USA
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Materials and methods

Preparation of treatments

Contaminated gravel used in the study was prepared by mixing petroleum products with pea gravel to produce a level of contamination of about 2000 ppm by weight. The pea gravel had a mean particle size of about 8 mm and was prepared by sieving to remove fines (bulk density of 1612 g L^{-1}). The soybean hulls were obtained from a soybean processing plant (Cargill Soy Processing Plant, Iowa Falls, IA, USA) and had a bulk density of 130 g L^{-1} and a Kjeldahl nitrogen of 9.8%. The soybean hulls were blended with gravel to produce a mixture containing 60% gravel by volume. Moisture content was adjusted to 22% (w/w) prior to use.

Four petroleum products were initially included in the study: crude oil, No. 2 diesel fuel, unleaded gasoline, and motor oil. The crude oil was a sample of Alaska Valdez North Slope crude oil supplied by EXXON. The diesel fuel and gasoline were obtained from a local fuel service station. The motor oil was a 10/40 grade of used motor oil.

Absorption capacity for soybean hulls was determined for each petroleum product and water. Soybean hulls were oven dried at 70°C, equilibrated to room temperature and humidity, then a 3-g sample was added to a 4.5-cm Buchner porcelain funnel with a clamped silicone tubing on the outlet. The soybean hulls were completely covered with petroleum product (~50-ml) and allowed to stand at room temperature for 15 min. Then excess unabsorbed petrochemical was removed by aspiration for 10 min followed by centrifugation. The retained material to be tested was transferred quantitatively into two preweighed modified glass funnels, placed in a 55-ml stainless steel centrifuge tube, and centrifuged at $480 \times g$ for 10 min at 4°C. The filter funnels were 15-ml 20-C Kimax glass funnels with fritted discs that were modified by cutting off a portion of the bottom stem and top. A slit was cut into the remaining length of the bottom stem to enhance petrochemical displacement. After centri-

fugation, any petroleum product present in the stem was absorbed with a paper towel and each funnel was then reweighed. The percentage absorption of soybean hulls for each petrochemical and water was determined on the basis of three replicates.

Two of the petroleum products were ultimately eliminated from the study. The gasoline was eliminated because of its high volatility. Most of the gasoline was lost to evaporation during the respirometer tests. The motor oil was eliminated because it is apparent that the samples prepared with this petroleum product were not adequately mixed. The amount of motor oil recovered from gravel samples before treatment was only 10–30% of the amount that was added. The results included in this paper are for gravel contaminated with crude oil, with diesel fuel, and uncontaminated gravel.

In one part of the study, the gravel and soybean hulls were heat sterilized at 121°C and mixed aseptically with the two petroleum products. The gravel was sterilized for 1 h and the soybean hulls were sterilized for 15 min. About five parts by volume of sterile water were added to 100 parts of the soybean hulls after sterilization to make up for water lost during the heat treatment. Samples prepared using the sterilized materials were expected to discriminate between the effects caused by microorganisms in the agricultural by-product and gravel from those caused by microorganisms in the petroleum products.

Analytical procedures

Two analytical procedures were used to measure the extent of bioremediation in the samples of contaminated gravel. Respirometry was used as a measure of biological activity and gas chromatography performed on solvent extracts recovered from the gravel was used in determining the removal of hydrocarbons.

The respirometers used in the study were sterile 473-ml glass wide-mouth square-bottles sealed with stoppers containing an inlet and outlet tube for continuous aeration. The sample sizes used in the respirometer were about 150 ml of gravel or 250 ml of gravel and soybean hull mixture. The respirometers were weighed before and after filling to determine the initial weight of sample being tested.

Filtered, CO_2 free air was supplied to the respirometers and the outlet air was passed through a trap containing granular activated carbon to collect volatile petrochemicals and 10 ml of 4 N NaOH to trap CO_2. Silicone stoppers and tubing were used in the apparatus to minimize absorption of hydrocarbons which can occur with rubber or plastic materials.

Carbon dioxide traps were replaced periodically with fresh traps. The entire 10 ml of NaOH in each trap was titrated with 0.1 N HCl to determine the amount of CO_2 that had been collected. The capacity of each CO_2 trap was 880 mg of carbon dioxide.

Intially, five respirometers from each treatment were randomly selected and the contents were extracted with methylene chloride to determine the starting concentration of petroleum product. On days 7, 14, 28, and 56 three additional bottles from each treatment were randomly selected and the contents were extracted with methylene chloride to determine the remaining concentration of petrochemicals. The corresponding activated carbon traps for each respirometer were also solvent-extracted at the same time.

A single extraction using methylene chloride was performed on the contents of the respirometer by adding 150 ml of methylene chloride (Fisher Chemical Co, Fairlawn, NJ, USA) into the bottle, stoppering the mouth with an inverted 50-ml Erlenmeyer flask, wrapping the flask and neck of the bottle with Parafilm to seal the system, and agitating the assembly on a shaker at 125 rpm for 16 h at room temperature to extract residual petrochemicals. The methylene chloride level was marked on the side of the bottle before shaking to indicate whether solvent losses had occurred. Test runs on control bottles prepared and handled the same but containing methylene chloride only indicated that the glassware and Parafilm did not introduce interfering substances into the extracts.

A 4–5 ml sample from each extract was filtered (0.45 μm PTFE Arcodisc CR filters, Fisher Scientific) and was sealed in a 5-ml Type-1 glass serum vial with some anhydrous sodium sulfate to absorb any residual water. Each vial was sealed with a silicone/Teflon septum (Teflon side toward the methylene chloride extract) with a metal capping unit. The vials containing extract were stored prior to analysis at −18°C.

The amount of residual hydrocarbons in the extracts was measured with a gas chromatograph (Carlo Erba, Peabody, MA, or Varian model 3400, Walnut Creek, CA) equipped with a flame ionization detector and a 30-m DB-5 narrowbore (0.25 mm ID) capillary column (J & W Scientific, Folsom, CA, USA). The carrier gas was hydrogen at a column flow rate of 2 ml per min and a split injection ratio of 30 : 1. The injector and detector temperatures were 300°C. The initial column oven temperature was held at 35°C for 2 min and was increased at 12°C per min to a final temperature of 260°C that was maintained for 5 min. Concentrations were determined by comparing the area under the chromatogram for each sample with the area under the standard curve obtained for the corresponding petroleum product.

The activated carbon traps were extracted with continuous exchanges of 10 ml of methylene chloride for a total of 50 ml of solvent added, which was confirmed to be sufficient to extract all the petroleum product trapped. Tests run on these extracts, however, indicated that the activated carbon traps were ineffective at the concentrations of petroleum product released.

Results and discussion

Soybean hull absorption capacities

The absorption capacity of soybean hulls illustrated different preferences for the petroleum product and water (Table 1). Soybean hulls, however, absorbed significantly more water than petrochemicals. Absorption of petroleum products might have a significant effect on biodegradation by moving the contaminating compound from the inert soil to an organic material that potentially can stimulate biodegradation, whereas absorption of water will stimulate microbial activity. This benefit was illustrated by Ho *et al* [3,4] with plastic composite-supports used for lactic acid biofilm bioreactors in which the incorporation of 35–45%

Table 1 Percent absorption of different petroleum products and water by oven-dried soybean hulls[a]

Product	Soybean hulls (%)
Crude oil	16
Diesel	9
Used motor oil	28
Water	191

[a]Each value represents an average of three replicates. Percent absorption was calculated by dividing the weight of each soybean hull after petrochemical treatment by the starting material weight × 100%.

(w/w) ground soybean hulls into these materials significantly improved bacterial attachment to the support, slow release of nutrients and increased support porosity. These benefits and others significantly improved lactic acid production in this novel bioreactor and soybean hulls were an essential element for this improvement [5].

Biological activity

Figure 1 is a plot of cumulative CO_2 evolution for the uncontaminated gravel and for the gravel contaminated with crude oil and diesel fuel in the samples that were not treated with soybean hulls. The CO_2 evolution was practically the same in all samples. Therefore, gravel alone did not stimulate biodegradation of the petrochemical, and any reduction in the crude oil or diesel fuel was attributed to nonbiological mechanism(s) (ie, air scrubbing, volatilization, etc).

Figure 2 is a plot of cumulative CO_2 evolution for crude oil- and diesel-contaminated gravel treated with soybean hulls. The cumulative CO_2 evolution was much higher than the gravel alone and was obviously related to biodegradation of the soybean hulls. A significant reduction in biological activity was observed when petroleum product was added, which suggests some toxic effect(s) on microbial activity. The source of microorganisms responsible for the CO_2 evolution was associated with fuel, gravel, and/or the soybean hulls. When the gravel and soybean hulls were sterilized, the cumulative CO_2 evolution for crude oil- and diesel-contaminated gravel treated with soybean hulls was initially lower than it was with the non-sterilized materials. It is apparent that the microorganisms introduced with the crude oil or diesel fuel were able to utilize the soybean hulls as a carbon and nitrogen source. As expected, soybean hulls provided some readily degradable carbon compounds, which was illustrated by the cumulative CO_2 evolution curve for the soybean hulls alone, which was rapid and steady for 56 days.

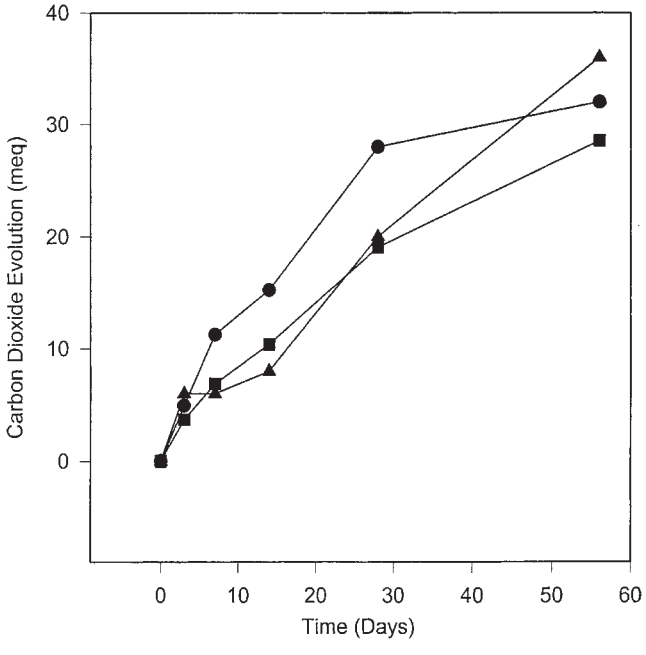

Figure 1 Cumulative CO_2 evolution from gravel alone (—●—) and from gravel contaminated with crude oil (—■—) or diesel fuel (—▲—). Standard deviation was 0.65–2.1 meq of CO_2.

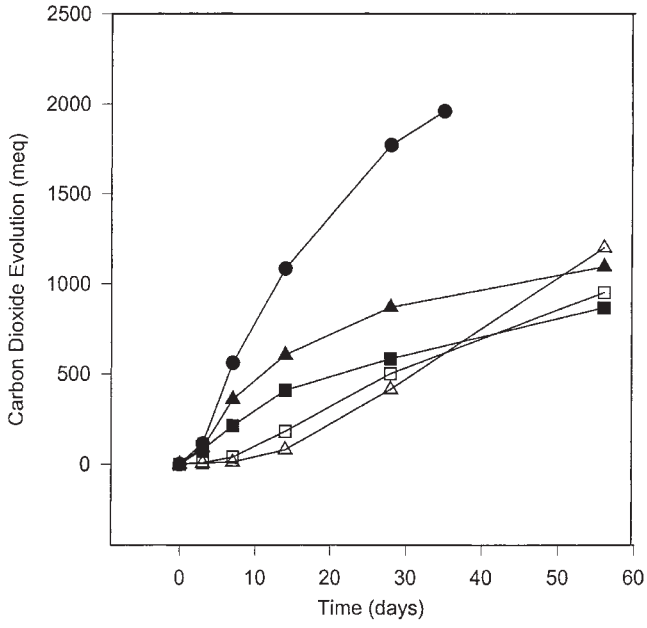

Figure 2 Cumulative CO_2 evolution for gravel containing soybean hulls (soyhull,—●—), crude oil with soybean hulls (crude, —■—), diesel fuel with soybean hulls (diesel,—▲—), crude oil with heat-treated soybean hull (HT-soyhull-crude,—□—) and diesel fuel with heat-treated soybean hull (HT-soyhull-diesel,—△—). Standard deviation was 3.4–11.6 meq of CO_2.

Hydrocarbon removal

Based on the data shown in Table 2, the crude oil and diesel fuel recovered from the gravel immediately after contamination was less than 2000 ppm, the desired amount that was added in all of the samples, except for the diesel fuel samples treated with soybean hulls (2290 ppm). Part of the petroleum product ended up on the walls of the mixing equipment or on other apparatus used in preparing the samples. There was visibly less petroleum product residue on the mixing chamber walls when soybean hulls were added to petroleum product-contaminated gravel because of the absorptive capacity of the soybean hulls for these hydrocarbons. This observation was supported by the 30% higher zero-time concentrations for the soybean hull-treated gravel than the gravel alone.

Significant reductions in the hydrocarbon residual were observed in all of the samples tested by the end of the 8-

Table 2 Summary concentration of residual crude oil or diesel fuel (ppm) recovered from contaminated gravel with and without treatment with soybean hulls with standard errors (se) and percentage loss[a]

Treatment	Zero time	7 days	14 days	28 days	56 days
			Crude oil		
Gravel alone	1141 se 310	363 se 57 (68%)	398 se 6 (65%)	813 se 340 (30%)	324 se 108 (72%)
Soybean hulls	1479 se 125	651 se 66 (56%)	428 se 33 (71%)	306 se 62 (79%)	87 se 35 (94%)
			Diesel		
Gravel alone	1277 se 128	673 se 143 (47%)	686 se 170 (46%)	1530 se 115 (0%)	551 se 23 (57%)
Soybean hulls	2287 se 423	1197 se 261 (48%)	704 se 129 (69%)	802 se 213 (65%)	ND[b]

[a]All values are a mean of three replicates. Target value for initial crude oil or diesel fuel concentration was 2000 ppm. The percentage loss is in parentheses and is calculated based on the zero time value for each treatment and fuel. Methylene chloride extraction of soybean hull alone demonstrated an average background of 91 ppm ± 26.8 solvent extractables.
[b]Not determined because sample was lost.

week study period whether the soybean hulls were added or not (Table 2). Treated samples, however, demonstrated >94% reductions in petroleum product by week 8. The aerobic environment provided in the respirometer appears to have been a major factor in promoting remediation whether biologically or nonbiologically. This tends to support the results of our field studies on *ex situ* bioremediation in which removal of petrochemicals occurred in the controls as well as in the treated samples. Therefore, the overall outcome of the treated and nontreated contaminated gravel was the same, but the rate of bioremediation was accelerated by the addition of soybean hulls. These results suggest a need to evaluate other similar agriculture by-products (ie, rice hulls, oat hulls, corn fiber, etc) for their bioremediation potential and the need to evaluate soybean hulls at even higher concentrations of petroleum product-contaminated systems.

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