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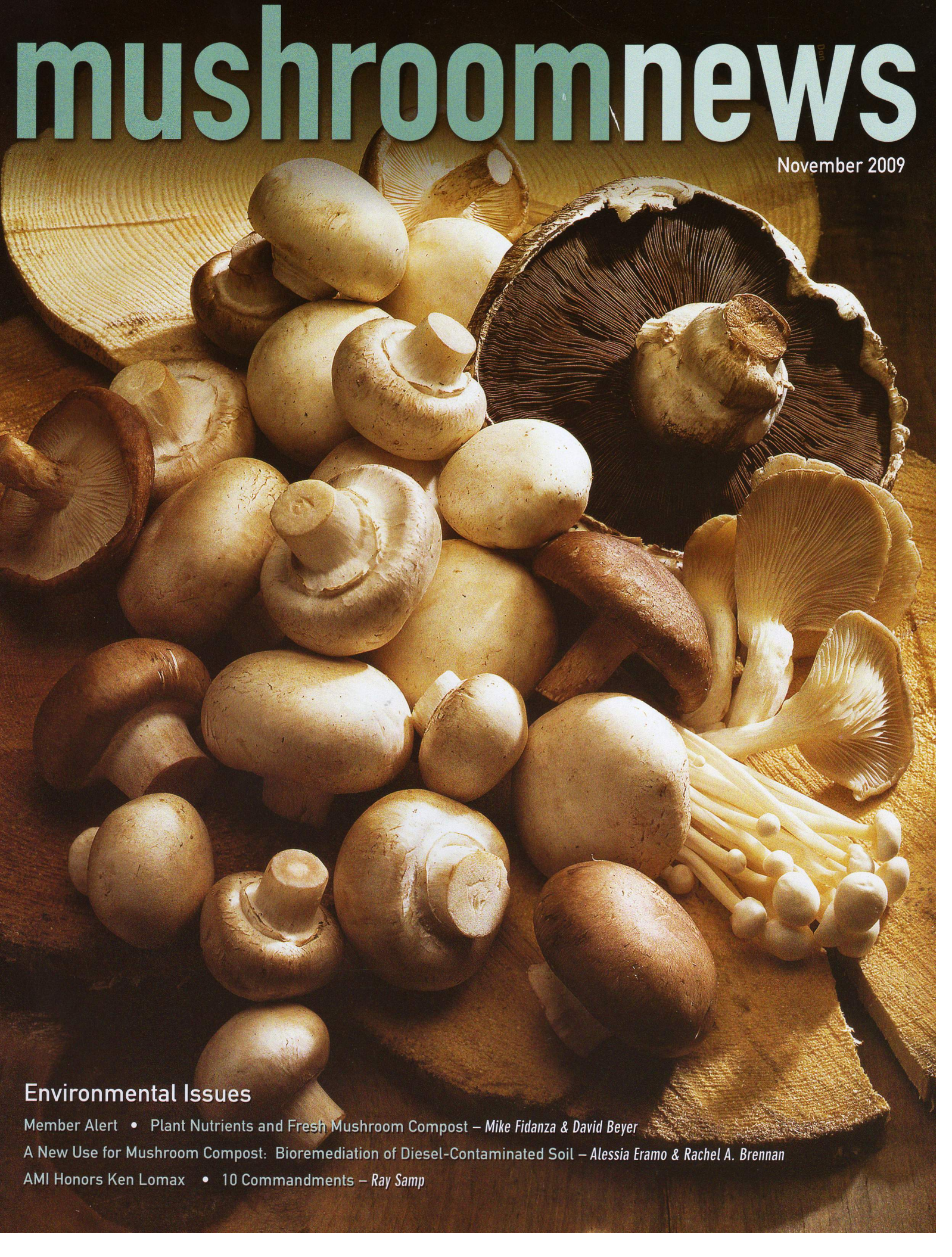

Environmental Issues

A New Use for Mushroom Compost: Bioremediation of Diesel-Contaminated Soil

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Abstract: Composting is a widely recognized remediation strategy for treating soils contaminated with petroleum hydrocarbons; however, the use of mushroom compost (MC), formerly referred to as spent mushroom substrate or SMS, has not been previously reported in the literature as an initial course of remedial action. In this work, a laboratory study was conducted to evaluate the effects of substrate and nutrient addition as well as elevated temperatures on the rate of total petroleum hydrocarbon (TPH) removal from weathered, diesel-contaminated soil over time. High levels of TPH removal were achieved under all MC-amended conditions, and were not significantly enhanced at elevated temperatures or with the addition of nutrients. The greatest decrease in soil TPH was achieved after 160 days of treatment at 30°C, from approximately 1600 to 180 ppm (89 percent removal) at an average rate of 8.9 ppm/day. This treatment level successfully meets the environmental regulations of many regions through the United States, and may be enhanced with further optimization of the conditions tested in this study. These results indicate that MC can be effectively utilized as an inexpensive substrate for the treatment of diesel-contaminated soils, while simultaneously providing a sustainable solution to the ever-present problem of MC disposal.

INTRODUCTION

Environmental contamination by petroleum hydrocarbons is caused by leakage from underground storage tanks and pipelines, accidental spills, improper waste disposal practices, and leaching landfills (Yadav and Reddy, 1993). Leaking underground storage tanks, one of the largest sources of soil pollution, can also negatively affect adjacent groundwater supplies. As of September 2006, the Environmental Protection Agency (EPA) had confirmed over 460,000 UST releases, and it is estimated that there are still 113,000 that remain to be cleaned up (EPA Office of Solid Waste and Emergency Response). Due to the sheer number of sites contaminated by petroleum hydrocarbons, and their potential for causing a myriad of medical problems in humans, they are among the most frequently treated contaminants at U.S. Environmental Protection Agency (USEPA) Superfund sites (Zytner *et al.*, 2001). On-site biological treatment is becoming an increasingly used alternative for the remediation of petroleum hydrocarbons due to its low cost and capacity for complete destruction of contaminants.

Composting has emerged as a favorable technology for the bioremediation of hydrocarbon-contaminated soils because it has relatively low capital and operating costs, simple operation and design, and relatively high treatment efficiencies (Namkoong *et al.*, 2002). Typical organic amendments include manure, sewage sludge, bark chips, yard waste, and food processing wastes (Singh *et al.*, 2005). Another abundant, but often overlooked, organic soil amendment is mushroom compost (MC). MC is produced in copious amounts by the mushroom industry, with 5 kg of MC generated for every 1 kg of mushrooms produced (Lau *et al.*, 2003). The uses of MC have generally been limited to soil conditioning and fertilizing, with the majority of the product being disposed of in landfills, often with significant transportation and disposal fees (Chiu *et al.*, 1998; Chiu *et al.*, 2000). However, MC contains high levels of residual nutrients and enzymes, which may be beneficial for stimulating microbial degradation of organopollutants like hydrocarbons (Chiu *et al.*, 1998; Khammuang and Sarnthima, 2007; Lau *et al.*, 2003). Despite this, only one report was found in the literature in which MC was used to stimulate the degradation of fuel-contaminated sediment, and that was only after a two-year pretreatment with land farming (Harmsen *et al.*, 1999).

To our knowledge, MC has never been reported in the literature as an initial course of

remedial action for treating petroleum-contaminated soil. The over-abundance of MC makes the development of sustainable management practices and new uses for MC of prime importance to the mushroom industry (Ntougias *et al.*, 2004). The purpose of this preliminary investigation was to evaluate the ability of mushroom compost to serve as an alternative substrate for the remediation of diesel-contaminated soil.

MATERIALS & METHODS

Soil & Substrates

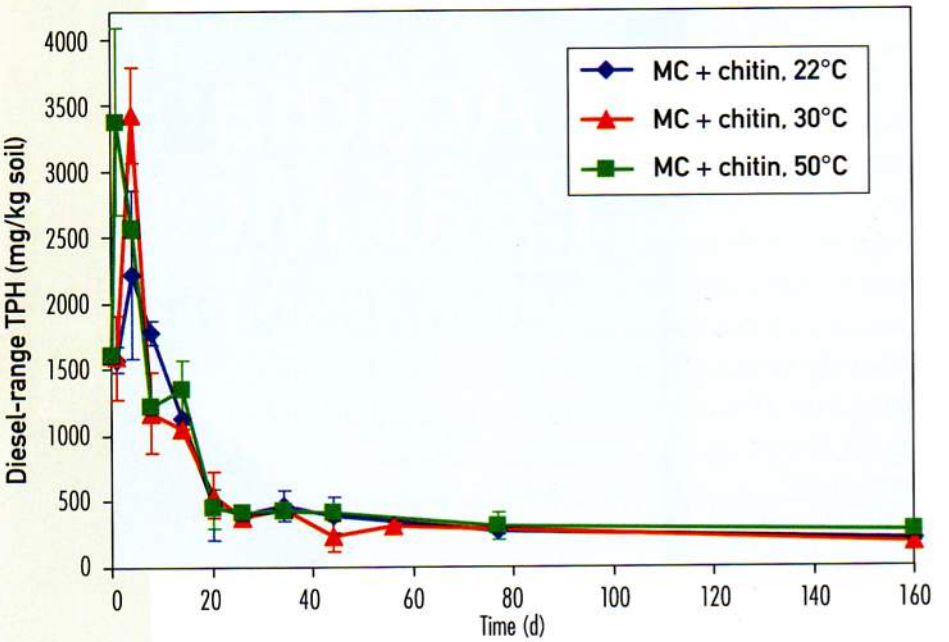
Mushroom compost, uncontaminated soil, and a core of hydrocarbon-contaminated soil were shipped from The California Mushroom Farm (Ventura, CA) to The Pennsylvania State University on ice and then stored at 4°C in the dark until microcosm establishment. The MC was ground and sieved to eliminate or break down fractions > 4.75 mm, and the soil was sieved to eliminate rocks and pebbles > 2 mm.

Crab-shells, a sustainable byproduct of the shellfish industry, were used in the microcosms as an additional source of nutrients to help counteract the nutrient deficiency that often limits degrading microbial communities in heavily contaminated environments (Tiquia *et al.*, 2002; Waybrant *et al.*, 2002). The crab-shell used in these experiments was derived from Dungeness crab (ChitoRem® SC-20, JRW Bioremediation, LLC, Lenexa, KS) with a typical composition (according to the provider) of: 20 – 25 percent chitin, 35 – 50 percent protein, 25 – 35 percent mineral matter ($CaCO_3$), and <10 percent moisture. Particle sizes between 850 µm and 2 mm were utilized in this study.

Experimental Setup & Sampling

The removal of TPH using MC as a substrate was monitored under three temperature conditions typically encountered during composting: 22°C; 30°C; and 50°C. Twenty sacrificial microcosms were established for each of the temperature conditions in 160 ml glass serum bottles with Teflon-lined stoppers and aluminum crimp caps. Each active microcosm contained laboratory air, 4.44 g soil, 2.22 g MC, and 0.16 g crab shells (wet weights, 5 g total dry weight) to yield a 1:0.5 mass:mass ratio (1:1 volume:volume ratio) of MC:contaminated soil. This soil:substrate wet mass ratio of 1:0.5 was intentionally designed to correspond with ratios recommended in the literature (Namkoong *et al.* 2002). Active microcosms were

Figure 1: Treatment of diesel-contaminated soil in microcosm tests with MC + crab-shell chitin under different temperature conditions. Data points are duplicate averages; error bars represent one standard deviation.



adjusted to a final moisture content of 50 percent by adding 3.34 mL distilled deionized (DDI) water. Controls with 4.44 g of soil only (no substrate) were also established. The moisture content of controls was not adjusted so that hydrocarbon degradation under natural soil conditions could be reported.

Microcosms were periodically sacrificed in duplicate over 160 days based on the observed rate of remediation. Throughout the experiment, oxygen (O_2) and carbon dioxide (CO_2) concentrations in the headspace were closely monitored to ensure that the molar ratio of O_2:CO_2 did not fall below 1.5, which is the stoichiometric minimum requirement for aerobic degradation of diesel range alkanes (Baker *et al.*, 2000; Van de Steene and Verplancke, 2007). If this was detected for any of the conditions at any time, all remaining bottles were flushed with lab air for 15-20 minutes to fully purge the microcosm headspace and replenish oxygen levels.

ANALYSIS

Headspace Gas Quantification

CO_2 concentrations were quantified using a SRI 310 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD), a Porapak Q column (Agilent J&W), and helium as a carrier gas. O_2 concentrations were quantified using a SRI 8610 B gas chromatograph (GC) equipped with a TCD, a Molesieve 5A molecular sieve column (Alltech), and argon as a carrier gas.

Hydrocarbon Extraction & Quantification

Solid samples (1 g for controls or 1.5 g for actives) from each microcosm were directly extracted with acetone in 2 mL centrifuge tubes with 0.4 g sodium sulfate ($NaSO_4$) to absorb moisture. The tubes were vortexed for 1 minute to mix, sonicated for 5 minutes, and centrifuged for 10 minutes at 10,000 rpm. The TPH concentration of the resulting extracts was determined by injecting 2 uL onto an Agilent model 6890 N gas chromatograph (GC) with a flame ionization detector (FID), an HP-5 capillary column (J&W Scientific, 25 m x 0.32 mm x 0.52 µm), and helium as a carrier gas.

Statistics

Analysis of variance (ANOVA) was used to determine if the relationships between treatment conditions were statistically significant ($p > 0.05$) at various time points during the experiments. Tukey 99.0 percent (_ = .01) simultaneous confidence intervals were used for this analysis and results were generated using the Minitab Statistical Software ® Program (Minitab Inc., State College, PA).

RESULTS & DISCUSSION

Hydrocarbon Removal under Various Temperature Conditions

All active microcosms displayed an apparent rapid increase in TPH concentration during the first 1 – 4 days of sampling, the timing and concentration of which was inversely related to temperature (Figure 1). This occurrence has been observed by others, and may have been caused by GC detection of some intermediate or metabolite (Zytner *et al.*, 2006). Following this initial peak event, TPH concentrations decreased by 1121 ± 54 mg/kg relative to starting values during the first 20 days in all active microcosms. The average first order TPH degradation rate constant (k_{TPH}) during this initial period was -0.0944 ± 0.001 d^{-1}, which is within the range of values reported by others (Namkoong *et al.*, 2002; Nocentini *et al.*, 2000; Van Gestel *et al.*, 2003; Zytner *et al.*, 2006). After the first 20 days of rapid degradation, the rate of TPH removal slowed considerably as the lower weight and more bioavailable compounds were removed (Figure 2).

Despite frequent aeration and monitoring over the next 140 days, the TPH levels seemed to stabilize on a residual concentration, which is known to be dependant on the soil characteristics and age of contamination (Nocentini *et al.*, 2000).

Overall removal was greatest in 30°C active microcosms (89 percent) and lowest in 30°C control microcosms (37 percent) (Table 1). The decrease in TPH concentrations observed in the controls may be due to loss through evaporation as the headspace of the bottles was being flushed with air. Although active microcosms showed different TPH removal trends at early times depending on temperature, final TPH concentrations in microcosms at the various temperatures were not significantly different ($p = 0.175$ at the 95 percent confidence interval, Table 1). These results compare well to studies where a range of temperatures, such as 35 and 50°C (Hogan *et al.*, 1989) resulted in similar removals of aliphatic and polycyclic aromatic hydrocarbons.

Table 1: Initial TPH, final TPH, and percent TPH removal in diesel microcosms after 160 days of treatment.

Condition	Initial TPH (mg TPH/kg soil)	Final TPH on day 160 (mg TPH/kg soil)	Overall Removal* (%)
Active 22°C	1610	210	87
Active 30°C	1610	181	89
Active 50°C	1610	270	83
Control 22°C	1530	530	65
Control 30°C	1530	960	37
Control 50°C	1530	390	74

*(Initial – Final)/Initial

Respiration Rates

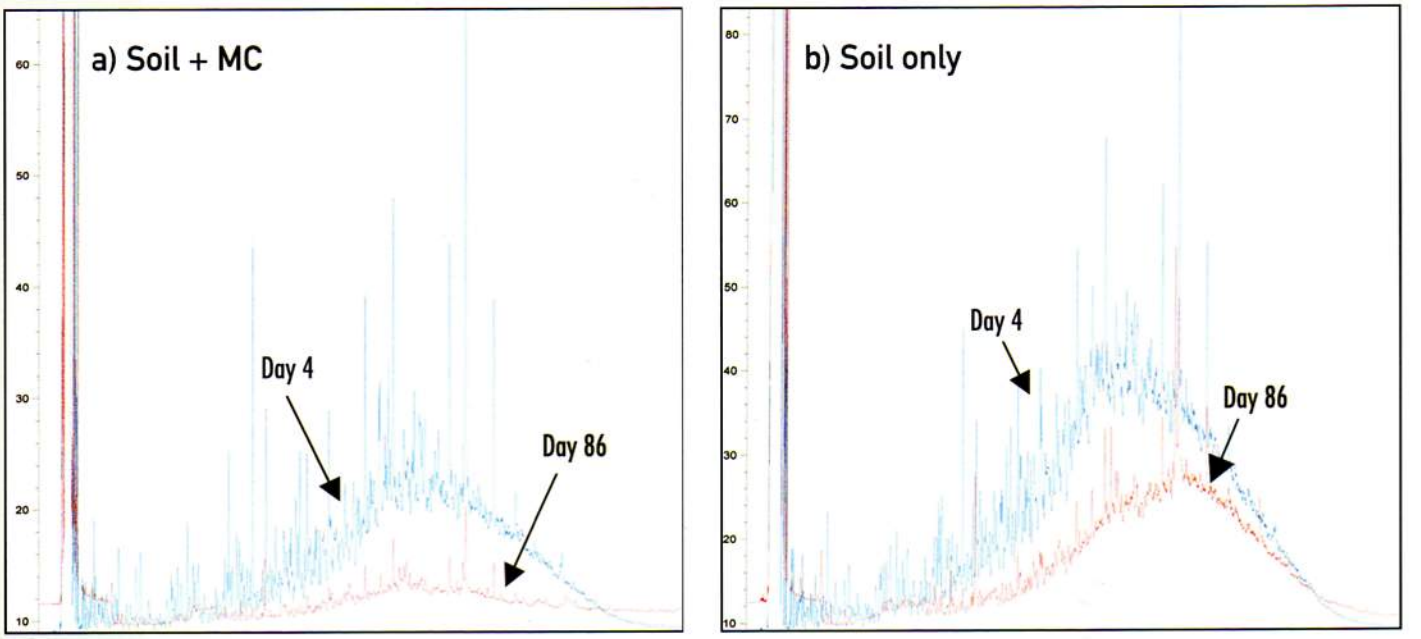
The trends observed with TPH removal (Figure 1) resembled those of CO_2 production (data not shown), in that both began high and tapered towards the end of the experiment. The molar rate of CO_2 produced per day was highest during the first days of experimentation, dropped dramatically, and decreased unsteadily until it plateaued later in the experiment. Carbon dioxide production was highest in the 50°C microcosms at first (164 umol/d) but quickly dropped and was surpassed by the 30°C microcosms before day 26; however, for most of the experiment, CO_2 production remained close in the 30 and 50°C microcosms. Rates were lowest in the 22°C microcosms for the majority of the experiment (18 – 63 umol/d). In the controls, CO_2 production was minor, as would be expected in the absence of substrate.

Initially, headspace O_2 levels were depleted in as little as two days. Over time, however, headspace flushing of the microcosm bottles became a less frequent requirement, as the molar ratio between O_2 and CO_2 remained high enough to support microbial degradation of organic contaminants. Similarly, others have noticed that O_2 utilization rates are highest at the onset of composting and then taper off quite drastically (Nocentini *et al.*, 2000, Van de Steene and Verplancke, 2007, Van Gestel *et al.*, 2003). Coincident with CO_2 production, O_2 utilization was highest in the 50°C microcosms, followed by the 30°C microcosms and 22°C microcosms, respectively.

The Use of Crab-Shell Chitin as a Fractional Nutrient Addition

In these tests, MC was augmented with crab-shell chitin as nutrient amendment because it was shown to increase TPH removal at the onset of treatment in previous tests in our laboratory. Through further testing, however, it was determined that a fractional amendment of chitin did not significantly enhance the overall remediation of diesel-contaminated soil when compared to microcosms amended with MC only ($p = 0.855$ at the 95 percent confidence interval). The nitrogen in the tested MC may therefore be

Figure 2: Comparison of chromatograms in microcosms containing (a) diesel-contaminated soil + MC and (b) diesel-contaminated soil only (control) over time. The preferential degradation of lower-molecular weight compounds is apparent by the decrease in faster-eluting peaks on the left side of the chromatograms.



sufficient to support microbial activity. The use of chitin in composting systems should not be discredited, however, as it may provide nitrogen in beneficial form to compost systems that are nitrogen deficient.

CONCLUSIONS

The results of this investigation indicate the following conclusions regarding biodegradation of diesel-contaminated soil using mushroom compost:

1) ***Mushroom compost is a viable substrate to enhance diesel degradation in contaminated soils.***
   - The results indicate that the majority of remediation occurs within the first few weeks of treatment and follows first order decay, followed by an extended period in which little degradation occurs. However, active monitoring and maintenance of the composting system and further optimization of the conditions tested in this study may enable more complete remediation of the contaminants.

2) ***Temperature does not markedly affect remediation efficiency.***
   - Nearly equivalent diesel remediation was observed at the three temperatures tested (22°C, 30°C, and 50°C); therefore, unless temperatures within the composting system fall below 22°C during the course of full-scale remediation, it should not be necessary to enhance the temperature of the system. The ability to conduct hydrocarbon composting at ambient temperatures may provide an additional cost benefit for full-scale implementation.

3) ***The inclusion of a fractional amount of crab shells does not enhance the ultimate extent of TPH remediation under aerobic conditions, but may offer other benefits.***
   - Although there was no significant increase in TPH removal in the presence of crab shells, their inclusion may prevent nutrient limitation with extended incubation times.

Thus, composting of diesel contaminated soils is a versatile strategy that has high potential to provide a low cost, low energy-demand solution for a multitude of petroleum contaminated sites. The use of MC in a full-scale composting treatment could create a beneficial use of this largely produced waste product and may help resolve the long-standing environmental problem of MC disposal.

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