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CHEMICAL AND MICROBIOLOGICAL ASSESSMENT OF PENDIMETHALIN-CONTAMINATED SOIL AFTER TREATMENT WITH FENTON'S REAGENT

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Abstract—This study assessed chemical effects and microbial response after Fenton's treatment of pendimethalin contaminated soils. The efficiency of the rapid chemical transformation of pendimethalin varied from 25% to greater than 90%. The highest efficiency was associated with a soil having comparatively low organic matter and low acid neutralizing capacity. This is consistent with the role of organic matter as a free radical scavenger and the optimum formation of free radicals at low pH. Potential heterotrophic activity, as measured by glucose mineralization, decreased with increasing pendimethalin concentration, but this inhibitory effect was removed after Fenton's treatment. Treatment also released BOD, COD, TOC, and nitrate into solution. The organic matter released into solution was biodegradable and served as a substrate for subsequent microbial growth. Analysis of the microbial population growing in the Fenton's treated soil leachates showed an overall decrease in (culturable) heterotrophic diversity, but an increase in the concentration of *Pseudomonas* species. These results suggest that Fenton's treatment of pendimethalin contaminated soil created favorable conditions for microorganisms desirable for bioremediation. Copyright © 1996 Elsevier Science Ltd

Key words—bioremediation, Fenton's reagent, oxidation, pendimethalin, *Pseudomonas*

INTRODUCTION

Soil contamination by toxic pesticides and herbicides is a widespread occurrence (Dasappa and Loehr, 1991). The Iowa Fertilizer and Chemical Association estimates that approximately 90% of agrochemical dealer sites in Iowa have contaminated soil and that 40–50% will require remediation (Freiberg, 1991). Contamination has been found at several sites at levels believed toxic to indigenous microorganisms, potentially reducing the importance of bioremediation as a cleanup alternative (Kelley *et al.*, 1990). There is also concern that groundwater supplies are at risk from these sites. The Kansas Department of Health and Environment estimates that 70–80% of all pesticide contamination in public wells are due to mixing-loading sites, and pesticide detection in Wisconsin wells have been linked to pesticide dealer sites (Habecker, 1989). The need exists for a remediation strategy that is easy, cost-effective, and addresses both chemical and microbiological advantages and constraints.

The mixture of hydrogen peroxide (H_2O_2) and ferrous iron, commonly referred to as Fenton's reagent, produces hydroxyl radical (·OH) a powerful and nonspecific oxidizing agent capable of reacting with many organic compounds (Walling and Johnson, 1975). Recently, several researchers have used Fenton's reagent and chelated iron with hydrogen peroxide to treat pesticide rinsates and pesticide contaminated soils (Kelley *et al.*, 1993; Pignatello, 1992; Yeh and Novak, 1995; Watts *et al.*, 1990). Soil organic carbon had a significant impact on contaminant removal because it can act as a hydroxyl radical sink. Soil organic concentrations greater than 1% significantly decreased the efficiency of Fenton's treatment to degrade pentachlorophenol and hexadecane in soils (Tyre *et al.*, 1991). The effect of pH has also been investigated. Fenton's reagent treatment of pentachlorophenol contaminated soil was most effective at a pH between 2 and 4, followed by steadily decreasing removals from pH 4 to 8 due to decreasing availability of soluble iron (Watts *et al.*, 1990)

Chemical oxidation may not only destroy target compounds, but also reduce toxicity associated with formulation ingredients and active agents (Somich *et al.*, 1990). It can also be an effective pre-treatment step for enhanced bioremediation by (1) transforming

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Table 1. Soil properties*

Parameter	Soil A	Soil B	Soil C
pH	6.7	6.9	7.9
Organic matter (%)	3.4	1.0	0.9
% Sand, silt, and clay	12.5, 50.0, 37.5	12.5, 62.5, 25.0	14.0, 60.5, 25.5
Total Fe (mg/kg)	19.0	23.4	12.2
Total Mn (mg/kg)	5.6	10.7	2.8

*Analysis performed by Minnesota Valley Testing Laboratories (Nevada, Iowa).

constituents to by-products that are more readily biodegradable, and (2) reducing overall toxicity to indigenous microorganisms, allowing them to participate in the remediation process (Kearney *et al.*, 1988; Kelley *et al.*, 1993; Somich *et al.*, 1988, 1990).

While several studies have focused on specific aspects of Fenton's reagent use in soil remediation, studies involving a comprehensive chemical and microbiological evaluation of these systems after Fenton's reagent application are needed. This paper addresses both chemical changes and microbial response after Fenton's reagent treatment of pendimethalin contaminated soil. Both treatment potential and ancillary benefits associated with Fenton's reagent use are addressed. Pendimethalin was chosen as a model contaminant because of its relatively long persistence in soil (Zimdahl *et al.*, 1984) and because it has been detected in soil at several pesticide dealerships (Gannon, 1992).

MATERIALS AND METHODS

Three uncontaminated soils with varying properties (Table 1) were obtained from pesticide dealership sites (soil horizon A) and stored at 4°C. Pendimethalin, the active ingredient (a.i.) in Prowl™, was purchased from Chem Service, Inc. Its chemical structure and properties are shown in Fig. 1 and Table 2 respectively. Prowl™ commercial formulation (4 lb a.i/gal) was obtained from a local pesticide dealership. Soils were contaminated to desired levels by dissolving herbicide in ethyl acetate, dosing the soil, and then allowing the ethyl acetate to evaporate.

Soil treatment experiments with Fenton's reagent were conducted at 20°C in triplicate 120-mL serum bottles, shielded from light with aluminum foil. Each batch reactor consisted of 10 g of soil and 50 mL of solution (1:5 w/v) with varying amounts of hydrogen peroxide (150–360 g/kg soil) and a fixed ferrous iron (made using ferrous sulfate) concentration of 2 g (Fe(II)/kg soil. Ferrous iron, supplied using a solution of ferrous sulfate, was an important component because treatment with hydrogen peroxide alone (150–360 g/kg soil) did not remove pendimethalin. The pH of the treatment solution was between 2 and 3 before addition to soil. To mimic field applications, no attempt was made to control pH after reagent addition. The remaining pendimethalin was extracted with ethyl acetate and measured 48 h after addition of the appropriate reagents, during which greater than 95% of the hydrogen peroxide decomposed. At this time, the soil slurry pH was measured and the slurry was allowed to settle. The water phase (leachate) was withdrawn and filtered (0.45 μm), and the remaining soil was air-dried, prior to further testing. Larger scale treatment experiments at the same soil to water ratio as above (150 g soil and 750 mL solution, 1:5 w/v) were also conducted to obtain greater volumes of leachate for further analysis. These experiments were allowed to progress for two weeks before leachate separation. This was done to insure that hydrogen peroxide decomposition was essentially complete and would not interfere with leachate analysis.

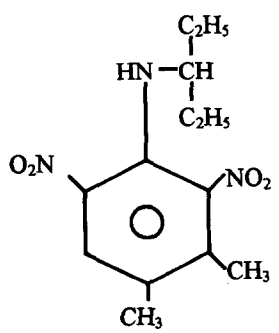

Fig. 1. Chemical structure of pendimethalin.

Microbial degradation of [U-^{14}C] glucose to $^{14}CO_2$ was measured in soil microcosms to assess the effect of herbicide concentration and Fenton's reagent treatment on heterotrophic activity. The effect of pendimethalin concentration was examined by mixing 5 g of uncontaminated soil (which served as a microbial seed) and 5 g of soil that had been sterilized with hydrogen peroxide and contaminated with pendimethalin and Prowl™ to a desired level (0–100 mg a.i./kg soil). For the Fenton's reagent experiments, an additional external microbial seed was required because microorganisms were unable to survive the harsh conditions created during Fenton's reagent treatment. That is, no colony growth was observed on tryptic soy agar (TSA). The microcosms were prepared by mixing 5 g of uncontaminated soil (microbial seed) and 5 g of Fenton's reagent-treated, air-dried soil. [U-^{14}C] glucose (2 mL at 1.45 mg/L) was added after 2 days, and $^{14}CO_2$ production was monitored over time. Test tubes containing 2 mL of 1 M NaOH were used to trap $^{14}CO_2$. Three microcosms were analyzed at each time interval. Glucose degradation or sorption was not observed on hydrogen peroxide sterilized controls.

Biochemical oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), nitrate, and Microtox™ measurements were used to characterize untreated and Fenton's treated leachates from pendimethalin and Prowl™ contaminated soil. The ability of microorganisms to grow on soluble products of pendimethalin and Prowl™ Fenton's treatment was determined in nephlo flasks containing 99 mL of filtered leachate (adjusted to pH 7 by addition of NaOH) and 1 mL of microbial seed solution, incubated at 24 ± 1°C with constant rotary shaking at 100 rpm. The seed solution was prepared by diluting 10 g soil in 100 mL sterile mineral

Table 2. Pendimethalin chemical properties*

Parameter	Value
Molecular weight	281
Molecular surface area	0.81 nm^2
Water solubility (at 20°C)	0.5 mg/L
Vapor pressure	30×10^{-6} mm Hg
Soil log K_{OC}	4.39

*Weber J. B. (1990).

media and shaking vigorously for 15 min. Microbial growth was monitored by periodic measurement of optical density at 600 nm (OD_{600}) on a Bausch and Lomb Spectronic 20. The microbial diversity resulting after growth in the seeded leachates was evaluated by counting the number of colony types (NCT) grown on TSA and Bacto® *Pseudomonas* Isolation Agar (PIA). Microbial colony types were determined by differences in color, configuration, margins, elevation, and texture. Leachate pH neutralization was performed in the lab to enable further studies of microbial response. Leachate neutralization is a likely phenomenon in the field as well, where the buffering capacity of soils can raise the pH of treatment leachates (Aiken and Costaris, 1995).

Leachates from soils not exposed to Fenton's reagent (deionized water only) were collected to establish baseline BOD, COD, TOC, nitrate, and Microtox™ values. Leachate collected from soil that had been spiked with ethyl acetate alone was used as a control to examine the effect of the soil contamination procedure on leachate makeup and its potential contribution to microbial growth.

Analysis of pendimethalin was by gas chromatography (GC) with an electron capture detector. Pendimethalin was extracted from the soil by mixing the soil slurry with 50 mL of ethyl acetate and using a wrist-action shaker for 4 h. Ethyl acetate was separated from the soil slurry by centrifugation and filtering (0.22 μm) prior to analysis. The overall extraction efficiency of pendimethalin from the soil was 91.8 $\pm$ 5.2% (n = 12). The pendimethalin concentration in water was determined using solid phase extraction and then analysis by GC. Hydrogen peroxide was measured by iodometric titration (Kieber and Helz, 1986). NaOH trapped $^{14}CO_2$ was measured using liquid scintillation counting with a Beckman LS6000IC. BOD, COD, and TOC were analyzed using standard methods (American Public Health Association, 1992). Ultimate BOD (BOD_L) was determined using the nonlinear curve fit function provided by Sigma Plot™. Microtox™ assay was used to measure the relative toxicity of soil leachates. Nitrate was measured by ion chromatography with a Dionex Model 2000. Isolated microorganisms were identified using a Biolog™ bacterial identification system.

RESULTS AND DISCUSSION

Pendimethalin removal by Fenton's reagent

The removal of pendimethalin increased with increasing hydrogen peroxide dose, with removals up to 99% efficiency on both soils A and B at the highest dose of 360 g/kg soil (Fig. 2). Fenton's treatment was most effective on soil B, with greater than 90% removal achieved at the lowest hydrogen peroxide dose of 150 g/kg soil. Pendimethalin removal from soil A was significantly lower than from soil B at the lowest hydrogen peroxide dose (Fig. 1). This is likely attributable to the lower organic content of soil B (1% vs 3.4% for soil A) and a lower final soil slurry pH of 3–4, both factors which have been observed to lead to greater contaminant removal efficiencies with Fenton's reagent (Tyre *et al.*, 1991; Watts *et al.*, 1990). This is consistent with both the role of organic matter as a free radical scavenger, which would therefore compete with pendimethalin, and the mechanism of hydroxyl radical formation which is favored at lower pH values.

Removal efficiencies from soil C were the lowest, with less than 25% pendimethalin removed for all hydrogen peroxide dosages. Soil C has low organic

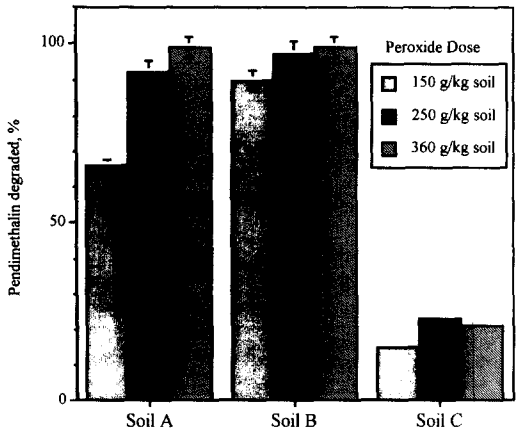

Fig. 2. Fenton's treatment of pendimethalin in various soils at a fixed ferous iron dosage of 2 g Fe(II)/kg soil and variable hydrogen peroxide addition. Batch reactors contained 10 g of soil in 50 mL of solution with an initial pendimethalin concentration of 100 mg/kg. The initial and final pH of the reactors was between 2 and 3, except for soil C which had a final pH of 7. Reported values are the mean $\pm$ one standard deviation (n = 3). Error bars not shown when smaller than bar thickness.

matter (0.9%), similar to soil B, but the final soil slurry pH was 7 (as opposed to 3–4 for soil B slurry). Since soils B and C were similar in organic matter content and received identical treatments, the difference in pendimethalin removal between the two (>90% vs <25%) was likely due to the difference in final soil slurry pH. The higher soil slurry pH (i.e. higher soil buffering capacity), probably reduced iron solubility and hydroxyl radical production, which would result in a reduction in removal efficiency. This notion is supported by the high removal efficiencies in soil A (soil slurry pH 3–4), which were greater than 66% despite having 3.4% soil organic matter.

Glucose mineralization in microcosms prepared with soil B

Approximately 40% of the added ^{14}C-labeled glucose was mineralized to $^{14}CO_2$ in the absence of pendimethalin (Fig. 3). Other studies have shown anywhere from 35 to 60% mineralization (Seto and Alexander, 1985). Glucose mineralization decreased with increasing pendimethalin concentration to a minimum of 13% at 100 mg/kg (Fig. 3). Using glucose mineralization as an indicator of potential heterotrophic microbial activity (Parsons and Smith, 1989), the reduction in activity shows that pendimethalin could be inhibitory to soil microorganisms at concentrations typical of those measured at contaminated sites (Freiberg, 1991).

Following Fenton's treatment of the pendimethalin contaminated soil, slurries were seeded with soil microorganisms to evaluate the effect of treatment on potential heterotrophic activity. The extent of glucose mineralization recovered to the level observed with uncontaminated soil (Fig. 4). Therefore, the inhibitory effect of pendimethalin on heterotrophic activity

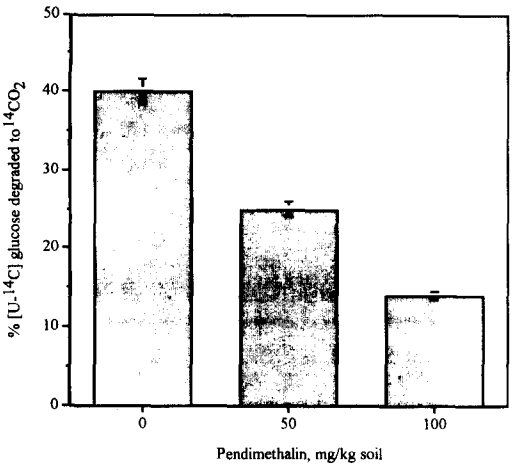

Fig. 3. Glucose degradation to carbon dioxide in soil B exposed to various pendimethalin concentrations (mg/kg). Measurements were taken after 6 days, when greater than 98% of the added glucose was degraded. Reported values are the mean ± one standard deviation ($n = 3$).

was removed by Fenton's treatment. Prowl™ contaminated soil also showed an increase in glucose mineralization after Fenton's treatment, but not to the same level as the pendimethalin contaminated soil. Since both soils (pendimethalin and Prowl™) were treated similarly and had similar pendimethalin removal (95%), the difference is likely associated with the Prowl™ formulation ingredients or treatment byproducts.

Characterization of leachates from soil B

The leachates from untreated and Fenton's treated soil B were analyzed (BOD, COD, TOC, and nitrate) to determine to what extent pendimethalin, formulation ingredients from Prowl™, and treatment

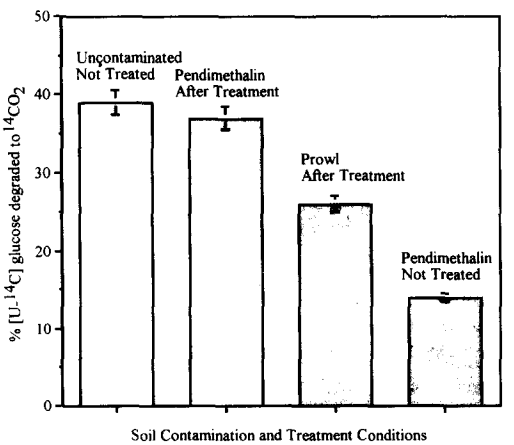

Fig. 4. Effect of Fenton's treatment on glucose degradation to carbon dioxide in soil B. The soil was treated with 2 g Fe(II)/kg and 180 g peroxide/kg. Contaminated soils had an initial concentration of 100 mg a.i/kg. This concentration was reduced from 100 to 5 mg/kg due to Fenton's treatment. Measurements were taken after 6 days, when greater than 98% of the added glucose was degraded. Reported values are the mean ± one standard deviation ($n = 3$).

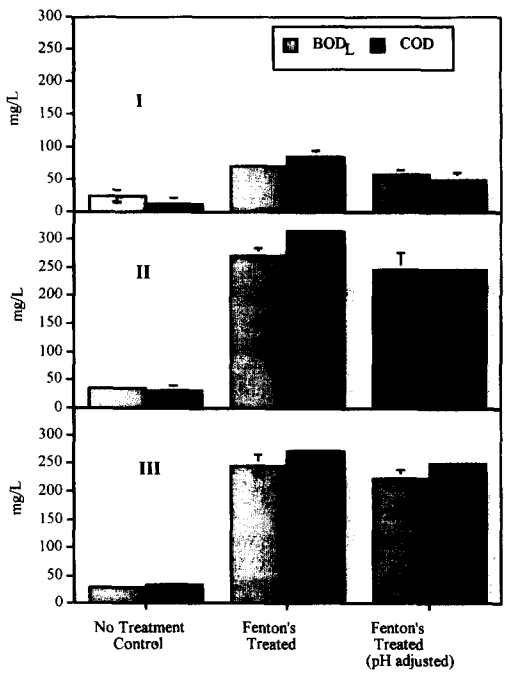

Fig. 5. Leachate characterization before and after Fenton's treatment (hydrogen peroxide 180 g/kg ferrous iron 2 g/kg) of soil B that was (I) uncontaminated, (II) contaminated with pendimethalin (100 mg/kg), or (III) contaminated with Prowl™ (100 mg a.i/kg). Reported values are the mean ± one standard deviation ($n = 3$). Error bars not shown when smaller than bar thickness.

by-products are released into solution. Soil B was selected for this evaluation because significant removal of pendimethalin occurred after Fenton's reagent addition (>90% was observed at all hydrogen peroxide doses), which should potentially produce the greatest amount of treatment by-products. For this evaluation, Fenton's treatment (hydrogen peroxide of 180 g/kg and ferrous iron of 2 g/kg) of soil B contaminated with pendimethalin (100 mg/kg) and Prowl™ (100 mg a.i./kg) resulted in greater than 95% removal of pendimethalin from the soil. Less than 0.1 mg/pendimethalin/L was measured in the soil leachates after treatment.

Treatment with Fenton's reagent greatly increased the BOD_L, COD, and TOC of soil leachates from both contaminated and uncontaminated soils. The increase was greater for the pendimethalin and Prowl™ contaminated soils (Fig. 5). Adjustment of pH and removal of precipitate decreased the BOD_L, COD, and TOC of the leachates. This was expected, because many inorganic and organic compounds become less soluble and precipitate at higher pH values (Bradley and Chapelle, 1995), and sorb to precipitates.

The observed increases in BOD_L, COD, and TOC following Fenton's reagent treatment of pendimethalin contaminated soil are greater than expected and are difficult to explain. A portion of the BOD_L, COD, and TOC increase can be attributed to the release of natural soil organics. Solubilization of

organic matter commonly occurs after treatment with hydrogen peroxide (Griffith and Schnitzer, 1977). Based upon results of uncontaminated controls, increases of 60 mg/L BOD_L, 80 mg/L COD, and 35 mg/L TOC would be expected from the release of soil organics without considering the effect of the added herbicide. The observed increases, however, were much larger (270 mg/L BOD_L, 310 mg/L COD, and 132 mg/L TOC).

The additional increases cannot be solely attributed to the organic content of added pendimethalin, Prowl™, or ethyl acetate. The theoretical COD of the pendimethalin added was only 25 mg/L (11 mg/L as TOC), and ethyl acetate was shown to completely evaporate during soil preparation. Although the BOD_L, COD, and TOC of the formulation ingredients in Prowl™ are not known, based on untreated controls, they did not contribute greatly in excess of pendimethalin. Therefore, even if pendimethalin or its by-products were completely solubilized, they would only account for about 10% of the BOD_L, COD, and TOC increases.

Galil and Novak (1995) reported TOC release following PCP addition to soil and attributed this to competitive sorption between pentachlorophenol and soil organic matter resulting in displacement of soil organic matter to solution. They also observed TOC increases much greater than the amount of PCP that was added (on an equivalent carbon basis). Thus, competitive sorption may partly explain the relatively large increase in BOD_L, COD, and TOC in the soils with added pendimethalin and Prowl™. Specifically, sorption of pendimethalin or its treatment by-products may have displaced soil organic matter into solution, increasing the BOD_L, COD, and TOC of the leachate.

Nitrate in the soil leachates was also measured. The untreated soil leachates had less than 2.0 mg/L nitrate in solution. Fenton's treatment of uncontaminated soil released approximately 7 mg/L of additional nitrate (Table 3), while treatment of pendimethalin- and Prowl™ contaminated soil further increased nitrate levels by 14 and 12 mg/L, respectively. If the 7 mg/L nitrate release by uncontaminated soil is considered to be the background contribution of the soil, then the increases attributable to treatment of pendimethalin and Prowl™ are 7 and 5 mg/L, respectively. Considering that the added pendimethalin was 95% degraded, and assuming all of its nitro groups were converted to nitrate, the theoretical increase in nitrate concentration would be 13 mg/L. Although the observed nitrate concentration increases attributable to pendimethalin and Prowl™ are lower than this value, they indicate a substantial release of nitrate from pendimethalin. Regardless of its origin (soil or pendimethalin transformation), nitrate can be used as a nitrogen source for microbial growth or as an electron acceptor by facultative denitrifiers and could promote increased microbial activity in soil leachates and support further degradation of treatment by-products by microorganisms (Alvarez and Vogel, 1995).

Microtox™ assay and comparison of leachate BOD_L to COD ratios were used as indicators of the relative ability of Fenton's reagent to reduce toxicity and increase biodegradability. No significant difference in the relative toxicity (95% confidence) of the untreated and Fenton's treated leachates was measured using Microtox™ assay (all leachates had <10 toxicity units). The BOD_L to COD ratios were near unity in all cases (Fig. 5), which indicates that the by-products of treatment are biodegradable and could potentially be used for microbial growth. These results indicate that treatment does not release highly toxic compounds in solution and that the by-products are potential substrates for microorganisms.

Microbial growth and diversity after exposure to soil B leachates

The potential impact and fate of treatment by-products that might migrate off site in the soil leachates was further investigated. Specifically, the ability of microorganisms to degrade soluble material created by soil treatment and the subsequent population changes in the microbial consortium in the soil leachates was tested. No measurable growth was observed in any of the untreated soil leachates

Table 3. TOC and nitrate measured in soil leachates before and after Fenton's reagent addition to soil B. The soil was treated with 2 g Fe(II)/kg and 180 g peroxide/kg. Fenton's treated leachates were analyzed before and after pH adjustment to pH 7

Soil leachate type	Fenton's treatment	pH adjustment	pH	TOC* (mg/L)	Nitrate* (mg/L-NO_3^-)
Uncontaminated	No	Yes	7.5	4.0 ± 0.0	<2.0
	Yes	No	3.7	40.1 ± 0.3	8.7 ± 1.2
	Yes	Yes	7.5	18.7 ± 0.3	10.2 ± 1.0
Pendimethalin contaminated	No	No	7.5	20.0 ± 0.1	<2.0
	Yes	No	3.7	132.0 ± 0.2	16.3 ± 1.5
	Yes	Yes	7.5	102.1 ± 1.0	13.2 ± 1.6
Prowl™ contaminated	No	No	7.2	24.6 ± 0.1	<2.0
	Yes	No	3.8	120.1 ± 0.4	13.0 ± 0.5
	Yes	Yes	7.5	101.3 ± 0.7	12.8 ± 0.7

*TOC and nitrate values given as mean ± one standard deviation of duplicate measurements.

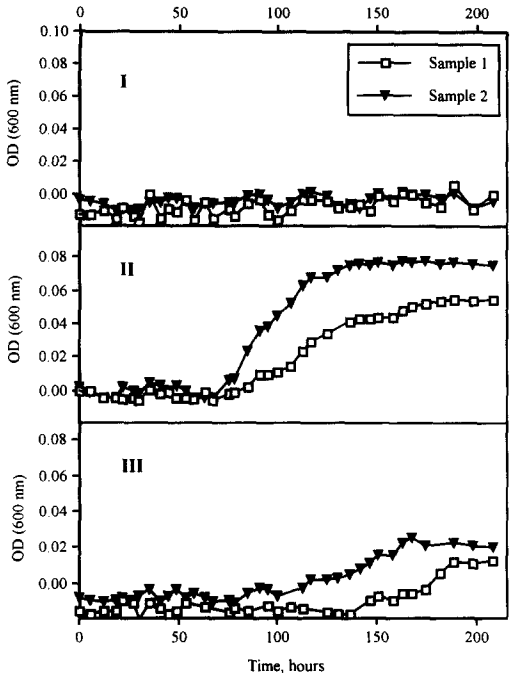

Fig. 6. Growth of bacteria on Fenton's treated leachates (pH 7.5) from soil B that were (I) uncontaminated, (II) pendimethalin contaminated (100 mg/kg), or (III) Prowl™ contaminated (100 mg a.i/kg). Data depicted is from two separate reactors and reported values are the mean ± one standard deviation ($n = 3$).

(contaminated or uncontaminated) or in leachates from uncontaminated soils treated with Fenton's reagent. This was not unexpected for the contaminated leachates given the low measured concentration of pendimethalin in solution (<0.1 mg/L). Following Fenton's reagent treatment, however, growth was observed in both the pendimethalin and Prowl™ contaminated soil leachates (Fig. 6). Growth on the Fenton's treated pendimethalin contaminated soil leachate shows that microorganisms degraded treatment by-products associated with the herbicide. A longer lag period (125 vs 75 h) and lower growth yield (0.03 vs 0.08 max ΔOD_{600}) was observed in the Prowl™ contaminated soil leachate, which reiterates a potential inhibitory effect associated with the formulation ingredients. Microbial growth in all cases increased with increasing substrate availability (Figs 5 and 6). These results are consistent with Fenton reaction studies involving other nitroaromatic compounds which have shown oxidative cleavage (N-dealkylation) of nitro groups and enhanced solubility and biodegradability (Lipczynska-Kochany, 1992; Schwarzenbach *et al.*, 1993).

The increases in OD_{600} were checked for compatibility based on expected cell yields. A laboratory derived correlation between absorbance and total suspended solids (TSS) with Polyseed™ (Polybac Corporation) fed glucose was used for this purpose (i.e. $TSS = -4 + 1453*OD_{600}$; $r^2 = 0.987$). For example, an OD_{600} increase of 0.08 (as observed for Fenton's treated pendimethalin contaminated soil leachate) corresponds to a TSS increase of 112 mg/L. Assuming volatile suspended solids (VSS) = 0.8*TSS (Metcalf & Eddy, Inc., 1991), VSS = 90 mg/L. Using this value and the BOD_5 available for growth (181 mg/L), the cell yield would be approximately 90/181 = 0.5 g-VSS/g-BOD_5. This value is quite reasonable (Metcalf & Eddy, Inc., 1991).

Further evaluation of the microbial community grown on the leachates was made by characterizing colony forming units (cfu) grown on TSA and PIA. The number of colony types (NCT) growing on TSA decreased with both contamination and treatment. Six different colony types were dominant in the uncontaminated soil leachate, but only three colony types were dominant in the Fenton's reagent treated pendimethalin and Prowl™ contaminated soil leachates. Nevertheless, the concentration of *Pseudomonas* spp. increased following Fenton's reagent treatment (from 10^3 to 10^5 cfu/mL). Only one PIA colony type was dominant in the uncontaminated soil leachate, while two to three colony types were dominant in the Fenton's treated soil leachates. Biolog™ was used to identify selected bacteria isolates after growth on the treated soil leachates (*Pseudomonas putida* strains were the predominant species). These species have wide catabolic capacity, capable of utilizing over 100 organic compounds as carbon sources and as electron donors for energy generation (Brock *et al.*, 1994).

The decrease in colony types isolated from the treated leachates on TSA indicates that treatment may create conditions that decrease the diversity of the succeeding (culturable) heterotrophic consortium. This could be due to release of growth substrates that provide a competitive advantage to fast-growing microbial species (Atlas and Bartha, 1993). Indeed, the one hundred-fold increase in the number of microorganisms growing on PIA (from 10^3 to 10^5 cfu/mL) shows that Fenton's reagent treatment of pendimethalin contaminated soil selected for *Pseudomonas* spp. Bacteria belonging to this genus are desirable for bioremediation because of their wide catabolic capacity. Furthermore, they are both r-strategists and Gram negative (Atlas and Bartha, 1993). As r-strategists, *Pseudomonas* spp. can take-over through rapid growth rate and dominate situations in which resources are abundant, such as leachates from Fenton's treated soil (i.e. increased BOD, COD, TOC, and nitrate levels). As Gram negative species, they may have a physiological advantage for protection against toxic substances in treated soil leachates because their lipopolysaccaride of the outer membrane reduces the concentration of any toxic substances in or near the cytoplasmic membrane (Nickens and Hageman, 1989). Therefore, the proliferation of *Pseudomonas* spp. suggests that Fenton's treatment of pendimethalin contaminated soil may create favorable conditions for the potential

participation of highly competent microorganisms in the cleanup process.

SUMMARY AND CONCLUSIONS

This study assessed the chemical and microbiological effects of Fenton's reagent treatment of pendimethalin contaminated soil in batch soil systems. On the basis of the results of this study, the following conclusions were made:

- Fenton's reagent addition could remove up to 99% of the pendimethalin originally present in soil. Differences in removal efficiencies between soils were consistent with known effects of pH and soil organic matter (i.e. removal efficiencies decreased with increasing organic content and increasing pH).
- Potential microbial heterotrophic activity, assayed by glucose mineralization, showed that pendimethalin has an inhibitory effect that was removed after Fenton's treatment. Treatment of Prowl™ contaminated soil, however, did not increase the extent of glucose mineralization to the level observed with uncontaminated soil. This difference suggests the potential inhibitory role of Prowl™ formulation ingredients.
- Fenton's reagent treatment released BOD, COD, TOC, and nitrate to solution. The compounds released into solution were biodegradable, as evidenced by microbial growth in the presence of the leachates and higher BOD:COD ratios.
- Diversity of the (culturable) heterotrophic microbial consortium decreased after growth in the Fenton's treated soil leachates. Nevertheless, the concentration of *Pseudomonas* species increased (from 10^3 to 10^5 cfu/mL), suggesting selection for microorganisms desirable for bioremediation.

In summary, converging lines of evidence suggest that treatment of pendimethalin contaminated soil with Fenton's reagent can reduce soil levels and enhance the potential for subsequent microbial degradation.

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