Biodegradation **6**: 329–337, 1995.

Biodegradation of the nitroaromatic herbicide dinoseb (2-*sec*-butyl-4,6-dinitrophenol) under reducing conditions *

Russell H. Kaake,[1,2] Don L. Crawford[1]* & Ronald L. Crawford[1]

[1] *Department of Microbiology, Molecular Biology, and Biochemistry, and The Center for Hazardous Waste Remediation Research, University of Idaho, Moscow, Idaho 83844, USA; ([2] Present address: Research & Development, J.R. Simplot Company, Pocatello, Idaho 83204, USA) (* Corresponding author)*

Accepted 20 February 1995

Key words: nitroaromatics, dinoseb, anaerobic degradation, biodegradation, herbicide, quinones

Abstract

The degradation pathway for dinoseb (2-*sec*-butyl-4,6-dinitrophenol) under reducing conditions was investigated. Cultures were inoculated with a dinoseb-degrading anaerobic enrichment culture used in field studies. Biotransformation intermediates were extracted with ethyl acetate and analyzed by high pressure liquid chromatography, gas chromatography, and mass spectrometry. Dinoseb degradation involves reduction of the nitro groups to amino groups followed by replacement with hydroxyl groups. Depending on the pH and redox potential in the culture, these intermediates may exist as quinones or hydroquinones.

Introduction

Bioremediation of chemical wastes in the environment has received favorable attention as a cost-effective alternative to other remediation strategies in recent years. However, one reason for slow acceptance of some treatment processes has been due to the inability of some processes to demonstrate degradation of the target compound to non-toxic products in the field. For example, degradation of some compounds (e.g. PCE, TCE) result in the production or more toxic products (DiStefano et al. 1991). Others have been unable to detect or identify degradation intermediates with lower toxicity, calling into question the efficacy of the treatment process (Williams et al. 1992). In some cases, apparent degradation may actually be a simple abiotic transformation, polymerization, or immobilization process in which the toxic chemical is bound to some sort of insoluble support (e.g. humus or other soil components). In order for bioremediation processes to become more accepted, it must be proven that each process results in conversion of the target compound to innocuous, readily metabolizable products in the laboratory as well as under field conditions. Here we report a partial degradative pathway for dinoseb (2-*sec*-butyl-4,6-dinitrophenol, DNBP, CAS Registry # 88-85-7) under reducing conditions.

Dinoseb is a herbicide that we used on a wide variety of crops throughout the United States until its recall by the US Environmental Protection Agency (USEPA) in 1986 (Federal Register 1986a, b). Its potent toxicity to animals and fish and teratogenicity in several animal models was the reason its use was banned. No accumulation of dinoseb has been documented at sites where it was applied at recommended application rates. However, due to improper disposal and handling practices, dinoseb is often found as a soil and/or groundwater contaminant at manufacturing facilities and rural airstrips where crop-dusting activities took place. At airstrips, excess dinoseb concentrate that was not applied to crops was often discharged directly to the soil. Due to its resistance to microbial attack, high concentrations of dinoseb can persist in the environment for decades (Kaake et al. 1992).

Several groups in Europe have reported on the degradation of nitrophenols by various bacterial genera. The results from these studies can be summarized into three different pathways. In the path-

* Publication No. 94506 of the Idaho Agricultural Experiment Station

way described by Gunderson and Jensen (1956) and Jensen and Lautrup-Larsen (1967), the nitro substituents are cleaved from some of the nitrophenols that were studied (p-nitrophenol, 2,4-dinitrophenol, 2,4,6-trinitrophenol, and 2-methyl-4,6-dinitrophenol) to form nitrite. The metabolites resulting from these transformations were not followed or identified. However, none of these organisms transformed dinoseb.

A brief communication (Tewfik & Evans 1966) described an aerobic, reductive pathway for the herbicide 2-methyl-4,6-dinotrophenol (DNOC; 3,5-dinitro-*ortho*- cresol) in which the nitro groups were reduced to amino groups and then replaced with hydroxyl groups to form 2,3,5-trihydroxytoluene. However, these data have apparently not been published. A later paper (Hamdi & Tewfik 1970) identified the first intermediate from DNOC as 6-amino-2-methyl-4-nitrophenol. In a separate report (Wallnofer et al. 1978), an *Azotobacter* species was found to produce the same intermediate from DNOC, as well as its analog from dinoseb. In addition the 6-acetamido-derivatives of both DNOC and dinoseb were identified. A partial reductive pathway under anaerobic conditions was reported by Froslie and Karlog (Froslie & Karlog 1970) for DNOC and dinoseb using rumen fluid as inoculum. They identified both the amino and diamino derivatives of both compounds.

More recently, a novel reductive pathway has been proposed for 2,4-dinitrophenol (2,4-DNP) (Lenke et al. 1992) and 2,4,6-trinitrophenol (picric acid) (Lenke & Knackmuss 1992) in which the aromatic nucleus is saturated and then cleaved. This pathway was found to occur as an alternative to the oxidative pathway described above in which nitrite is released into the medium. The resulting products from 2,4-DNP and picric acid were identified as 4,6-dinitrohexanoate and 2,4,6-trinitrohexanone.

A patented biodegradation process has been developed for the remediation of dinoseb (Kaake et al. 1992; Roberts et al. 1993a, b) and other nitroaromatic compounds in soil (Funk et al. 1993) under reduced conditions. The process has been applied at bench, pilot, and full-scale for dinoseb biodegradation. In these studies, unidentified biotransformation products were detected and monitored by HPLC analysis. Degradation was considered complete when no aromatic products could be detected above the detection limit of 1 mg/liter (Kaake et al. 1992). This study provides information on the possible identities of some of these intermediates and proposes a pathway for the degradation of dinoseb under reduced conditions.

Methods and materials

Enrichment culture

An anaerobic enrichment culture isolated from soil which had undergone the bioremediation process previously described was used as inoculum (Kaake et al. 1992). Successive transfers of the culture were made into fresh reduced liquid medium such that there was no soil in the cultures. Cultures were incubated at 30° C.

Growth medium

The culture was maintained on a mineral medium amended with 0.2 to 0.5 g/liter yeast extract, 10 g/liter glucose, and 100 mg/liter dinoseb. The other medium components on a per liter distilled water basis are as follows: 5 g NaCl, 5 g NH_4Cl, 1 g $CaCl_2 \cdot 2H_2O$, 1 g $MgCl_2 \cdot 6H_2O$, 50 mg Na_2SO_4, 50 mg $MgSO_4 \cdot 7H_2O$, 5 mg $FeSO_4 \cdot 7H_2O$, 0.5 mg $MnCl_2 \cdot 4H_2O$, 0.05 mg H_3BO_3, 0.05 mg ZnCl, 0.03 mg $CuCl_2$, 0.01 mg $Na_2MoO_4 \cdot FW2H_2O$, 0.5 mg $CoCl_2 \cdot FW6H_2O$, 0.05 mg $NiCl_2 \cdot 6H_2O$, 0.05 mg Na_2SeO_3, 0.02 mg Biotin, 0.02 mg folic acid, 0.05 mg riboflavin, 0.05 mg thiamine, 0.05 mg nicotinic acid, 0.05 mg pantothenic acid, 0.05 mg cyanocobalamine, 0.05 mg *p*-aminobenzoic acid, 0.06 mg lipoic acid, 5.7 g $NaHCO_3$, 6.48 g K_2HPO_4, 1.96 g KH_2PO_4, and 0.1 g $Na_2S_2O_4$. The medium was prepared anoxically by boiling and cooling under an 80% N_2/20% CO_2 oxygen-free atmosphere. The final pH was 7.0 to 7.2. The medium was reduced with sodium dithionite and dispensed into serum bottles (Sun Brokers, Wilmington, North Carolina) using anaerobic technique.

Metabolite extraction

Cultures were extracted twice with a volume of ethyl acetate equal to half the volume of the culture fluid to be extracted. The pH of the culture fluids ranged from 7 to 7.3. Neither acidic nor basic extractions resulted in greater extraction efficiency. Extractions were performed aerobically in a separatory funnel or anoxically using a liquid-liquid extraction apparatus that was continuously flushed with oxygen-free nitrogen gas. Extracts were dried under aerobic or anoxic conditions with anhydrous sodium sulfate and concentrated by evaporation for HPLC and/or GC/MS analysis.

Analytical procedures

Dinoseb and its biotransformation intermediates were analyzed by high performance liquid chromatography (HPLC) using a 250 × 2 mm Phenomonex (Torrance, Calif.) Spherex 5 μm C_{18} reverse phase column. A Hewlett-Packard model 1090A series II instrument (Palo Alto, Calif.), equipped with a diode-array detector and a computerized data system, was used for the analyses. The column was run with 10% acetonitrile and 90% 0.25 mM phosphate buffer (pH 4.0) for 2 min, then the acetonitrile was increased to 100% over the next 17 min and sustained for four min. The acetonitrile concentration was then decreased back to 10% over 2 min and maintained for 2 more min. The solvent flow rate was 0.2 ml/min, and the column temperature was 40° C. Detection of dinoseb and transformation products was accomplished using the diode-array UV/VIS detector, recording absorption at 210 and 254 nm with continuous scanning of the absorption spectrum of each peak from 190 to 598 nm.

Some aerobic extracts were further purified by preparative thin layer chromatography (TLC). Silica gel plates (0.5 mm thickness) with fluorescent indicator were spotted and developed twice in a 2:1:1 ratio of ethyl acetate:toluene:benzene, respectively. Bands were visualized with a 254 nm UV lamp, scraped, eluted with HPLC grade acetonitrile, and filtered prior to analysis.

High performance liquid chromatography/mass spectrometry (HPLC/MS) was performed using a Hewlett-Packard 1050 HPLC equipped with a UV/VIS detector operating at 210 nm to deliver samples to a particle-beam vacuum desolvation interface. This was connected to a Hewlett-Packard 5989A mass spectrometer with a quadrupole detector. The following adjustments were made to the electron impact sample ionization mode: repeller 7 V, emission 300 V, and electron energy 70 eV. The source temperature was 200–250° C. The MS was calibrated using perfluorotributylamine. A PhaseSep microbore Spherisorb S5 ODS2 (25 × 0.2 mm) C_{18} column (PhaseSep, Clwyd, UK) was used for separation. The solvent flow rate was 0.3 ml/min and consisted of 5% acetonitrile and 95% water which was maintained for 5 min and increased to 100% acetonitrile over 25 min. The rate of increase and length of the analysis were varied in order to maximize separation and resolution of the intermediates present in particular extracts.

Gas chromatography/mass spectrometry (GC/MS) was performed on an HP Series II 5890 gas chromatograph equipped with a capillary fused silica DB-5MS column (25 m × 0.21 mm × 0.33 mm; J & W Scientific, Folsom, Calif.) and MS interface. The interface temperature was set at 280° C. The MS detector was set as it was for the HPLC/MS analyses, with the exception of the source temperature, which was 175 to 200° C. The oven temperature was increased from 100 to 300° C at 10° C/min.

Mass spectra of biotransformation intermediates were compared with standard mass spectra stored in a software library (Wiley & Sons, New York) after background was subtracted. Background was defined as the mass spectrum of an area of the HPLC chromatogram immediately adjacent to the peak to be analyzed. The relative abundances recorded in the adjacent area were then subtracted from those of the peak of interest to obtain the corrected spectrum shown in Fig. 2.

Results

Table 1 lists the compounds which were identified from the enrichment culture supernatants and their mass spectra, with the exception of diacetyl and triacetyl glycerol (molecular weights 176 and 218 amu, respectively). Their fragmentation patterns (mass/charge) and relative intensities (in parentheses) are as follows. Diacetyl glycerol 145(8), 116(4), 103(22), 74(4), 43(100) and triacetyl glycerol 219(1.8×10^{-2}), 145(33), 116(16), 103(41), 43(100). Figure 1 illustrates the proposed degradation pathway for dinoseb under reducing conditions. The presence of phenol (XI) and 6-amino-2-*sec*-butyl-4-nitrophenol (II) was confirmed by matching spectra with standard mass spectra contained in the Wiley software library. Spectra of the remaining biotransformation intermediates were not in the library, nor were certified standards commercially available. Therefore, identities of these intermediates were hypothesized by their molecular weight and fragmentation patterns.

Due to the instability of the diamino compound (III) (Froslie & Karlog 1970), it could not be isolated from aerobic extracts. Verification of (III) required immediate extraction under anoxic conditions and injection to the mass spectrometer.

Although (III), (IV), (V), and (VI) have very similar molecular weights (178–180 amu), the compounds can be distinguished by their mass spectral fragmentation patterns. Compound (III) is the only compound of the four to have a benzene nucleus. Since this structure imparts stability when in the electron beam of the

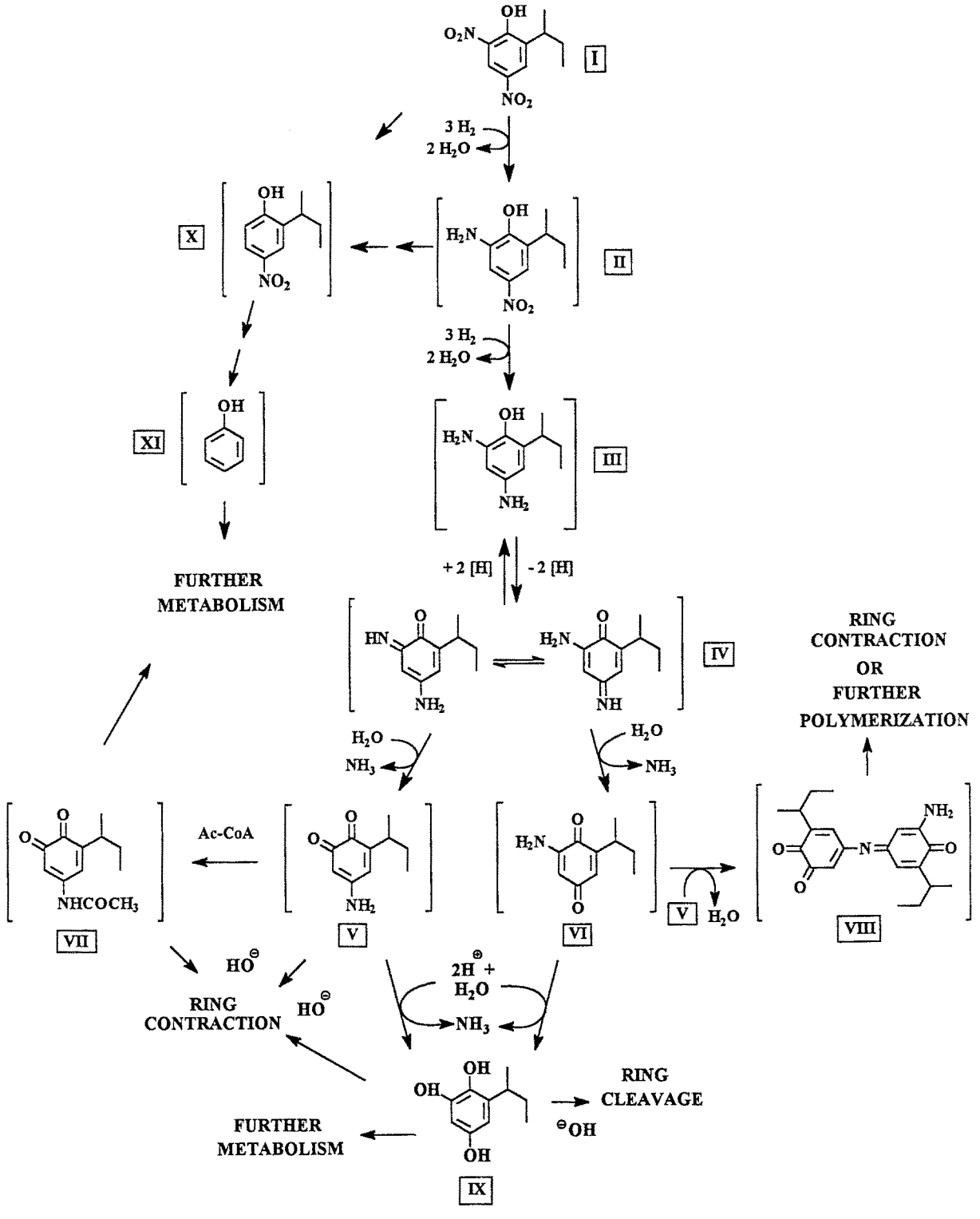

Fig. 1. Proposed biodegradation pathway for dinoseb under reducing conditions. Brackets indicate compounds that were tentatively identified by mass spectrometry.

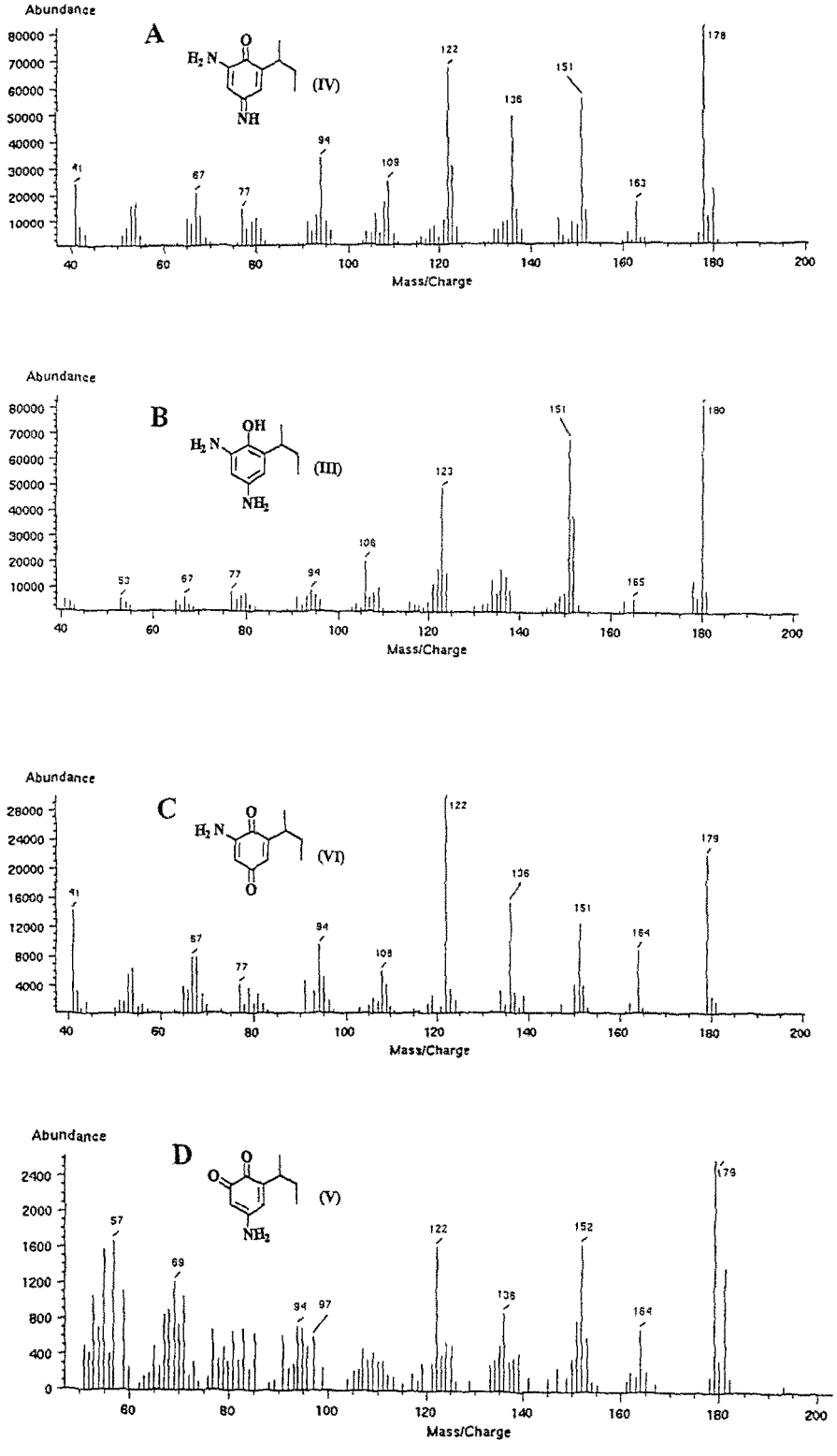

Fig. 2. Mass spectra of selected compounds isolated from culture supernatants.

Table 1. Physical data for dinoseb and compounds isolated from culture supernatants.

Compound	Molecular weight (amu)	Mass spectrum (mass/charge) (relative intensity)	U.V. maxima* (nm)
Dinoseb (I)	240	240(19), 211(100), 163(48), 147(31), 117(22), 89(10), 77(4)	213, 267, 363(sh)**, 410(sh)
6-amino-2-sec-butyl-4-nitrophenol (II)	210	210(37), 181(100), 194(9), 151(83), 135(43)	226, 256, 323
2,4-diamino-6-sec-butylphenol (III)	180	182(0.7), 180(100), 151(83), 123(60), 106(25)	na
4-amino-6-sec-butyl-1,2-quinonimine or 2-amino-6-sec-butyl-1,4-quinonimine (IV)***	178	180(26), 178(100), 151(66), 122(87), 94(29), 41(46)	na
4-amino-6-sec-butyl-1,2-dibenzoquinone (V)	179	181(6), 179(73), 164(27), 152(63), 136(34), 122(63), 69(47), 57(64)	280, 472
2-amino-6-sec-butyl-1,4-benzoquinone (VI)	179	181(6), 179(73), 164(30), 151(43), 136(50), 122(100), 94(32), 41(48)	280, 472
4-acetamido-6-sec-butyl-1,2-benzoquinone (VII)	221	223(17), 221(100), 192(34), 181(59), 179(33), 152(31), 151(28), 122(28), 68(18)	218, 277, 378(sh)
(VIII)	340	340(46), 284(19), 177(100), 164(53), 161(59), 149(42), 121(18), 57(8)	na
2-sec-butyl-4-nitrophenol (X)	195	195(21), 166(15), 139(45), 138(100), 110(17), 69(20), 41(16)	na
phenol (XI)	94	94(100), 66(52), 65(40)	270

* UV spectral data from diode array detector. Solvent was acetonitrile:water mixture. Solvent ratio dependent upon retention time. ** (sh) – shoulder. *** HPLC and GC/MS retention times were indistinguishable. na – data not available.

mass spectrometer, the fragments from (III) consist of the intact aromatic ring with whole or portions of functional groups removed (Fig. 2b).

However, quinones are not aromatic (Berger & Rieker 1974) and their ring structures are less stable. Consequently, the quinone ring is subject to fragmentation in addition to the functional groups. Quinones typically show loss of carbon monoxide (mass/charge 28). This results in an increase in the number of major fragmentation peaks (mass/charge 136 and 164) and a change in the relative intensities of the peaks (compare Fig. 2b and d).

Differentiation of the aminoquinones (V and VI) can be accomplished by closely examining the mass spectra (Fig. 2c and d). Quinones and their corresponding hydroquinones are in an equilibrium which is dependent upon pH and redox potential. This tautomerization can also occur within the inlet port or solvation chamber of the MS if trace amounts of water are present or if the residence time is increased (Zeller 1974). Upon reduction of the quinone in the mass spectrometer, a peak 2 amu higher than the molecular ion (an [M + 2] peak) is observed. For *o*-quinones, the [M + 2] peak is much more pronounced than in *p*-quinones due to their higher redox potential (Zeller 1974). The intensity of the [M + 2] peak has been used as a characteristic in differentiating 1,2- and 1,4-isomers of several types of quinonoid compounds (Zeller 1974).

Figure 2c and d demonstrate this change in fragmentation patterns. Both (V) and (VI) have a molecular weight of 179 amu. However, by looking at the relative intensity of the [M + 2] peak at 181 amu (Table 1), the two isomers can be differentiated. The presence of the two isomers in culture supernatants is supported by the fact that they could be resolved on the HPLC and GC, but had identical UV/VIS spectra.

Although the two isomers of (IV) could not be resolved by chromatography, it is surmised that each form can easily tautomerize to the other. Upon analyzing the mass spectrum of the peak corresponding to (IV), the [M + 2] peak was found to vary in intensity throughout the HPLC peak. This suggests that both isomers of (IV) coeluted from the column.

Two other quinone by-products were isolated (Fig. 1 VII and VIII). Acetylation is a common mechanism cells use to detoxify or inactivate some substrates (Cundliffe 1989) including aromatic amines (McCormick et al. 1981). Compound (VII) may be

formed in an alternate pathway which may serve to decrease the toxicity of compound (V). This compound was found to accumulate in reduced liquid cultures. However, in open soil cultures in the laboratory and field, (VII) only transiently appeared and was removed.

Compound (VIII) was isolated from in an aerobic extract of the enrichment culture. Linkages of this type are common among *p*-quinones and anilines (Berger & Rieker 1974). Implications of this condensation reaction will be addressed in the discussion.

Isolation of trace amounts of (X) and (XI) from some culture extracts suggest an alternate pathway. Formation of (X) suggests the presence of an oxidative pathway similar to that proposed by Gunderson and Jensen (1956) and Jensen and Lautrup-Larsen (1967). However, both (X) and (XI) (phenol) were isolated sporadically in only trace amounts on the GC/MS. Neither compound was identified upon direct HPLC analysis of aqueous samples nor was the production of nitrite measured.

Quantitative analysis of the compounds was not possible due to their instability and unavailability from commercial sources. However, in a typical culture supernatant midway through degradation, compounds (V), (VI), and (VII) each made up 33, 21, and 25% of the total non-dinoseb aromatic products as determined by peak area at 210 nm, respectively.

Discussion

Because the degradation of dinoseb and its metabolites takes place at such low redox potentials (about - 200 mV), the difference in redox potential during degradation compared to that at the time the metabolites were extracted and analyzed must be considered. During the extraction process, strict attention was paid to exclude oxygen. However, even if anoxic conditions were maintained, the pH and redox potential would be affected by the loss of carbon dioxide and hydrogen sulfide during the isolation and purification steps. Although Fig. 1 does not explicitly illustrate all the possible tautomeric forms of each compound (i.e. IV through IX), it is reasonable to assume that an equilibrium between the quinone and hydroquinone is reached. This equilibrium is likely to be dependent upon the redox potential. Typically, hydroquinones are colorless while their quinone derivatives are colored (Berger & Rieker 1974). In these experiments, the culture supernatants, which were colorless when under anaerobic conditions, turned color upon exposure to air, suggesting the conversion of hydroquinones to their corresponding quinones. The high reactivity of these compounds made their isolation difficult. Froslie and Karlog (1970) had similar difficulties in isolating (III) and had to chemically oxidize (III) to the more stable (IV) for analysis. The quinone metabolites could exist in their hydroquinone forms under the conditions in which the culture is grown and spontaneously oxidize upon exposure to oxygen or during extraction.

The primary reason for investigating anaerobic bioremediation processes for nitroaromatics was to determine if polymerization of the amino intermediates that form during both oxic (Gundersen & Jensen 1956; Tewfik & Evans 1977; Hamdi & Tewfik 1970; Won et al. 1974; Wallnofer et al. 1978; Tan et al. 1992; U.S. Army Environmental Center 1993) and anoxic (Froslie & Karlog 1970; Tratnyek & Macalady 1989; Boopathy et al. 1993; Gorontzy et al. 1993) incubations could be prevented. Under oxic conditions, these aminoaromatic compounds can react readily with soil organic matter and other aminoaromatics to form insoluble polymeric materials (Erdtman & Granath 1954; Brown 1967; Musso 1967; U.S. Army Environmental Center 1993). The observation of these polymerization reactions is supported by the isolation of (VIII). While polymerization of aminoaromatics may result in reduced toxicity (Tan et al. 1992; U.S. Army Environmental Center 1993), their environmental fate is unclear. Observations in our laboratory suggest that aminoaromatic polymers can be resolubilized and degraded under reduced conditions. However, it is desirable to prevent polymerization reactions from occurring by maintaining reduced conditions and allowing degradation to continue.

While not isolated, compound (IX) (6-*sec*-butylhydroxyhydroquinone) is a logical continuation of the proposed pathway. Failure to isolate this compound may be due to its high susceptibility to chemical decomposition and microbial degradation. A review by Hodge (1974), stated that hydroxyhydroquinones spontaneously undergo complex hydrolytic reactions under even mild conditions. These reactions involve ring cleavage (most often with 1,4-benzoquinones) and ring contraction reactions (common for 1,2-benzoquinones). These ring contractions are a variation of the well-known reaction named the benzilic acid rearrangement discovered in 1838 (Selman & Eastham 1960). The reaction involves the introduction of a hydroxyl group into a diketone followed by a molecular rearrangement to form a 5 membered cyclic

alpha-hydroxy acid which subsequently decarboxylates to form a 1,3-cyclopentadione. Musso and Bormann (1965) conducted studies of these reactions on compounds closely related to (IX). While substituents on the ring affect the types of ring contractions that occur, it is possible that compounds (VII) and (VIII) could also undergo chemical decompositions similar to (IX).

Strong evidence also exists for the microbial degradation of (IX) under reduced conditions. Several studies have documented complete degradation of several di- and tri-hydroxy substituted benzenes under reducing conditions by several bacterial genera (Balba & Evans 1980; Patel et al. 1981; Szewzyk et al. 1985; Krumholz et al. 1987; Schnell et al. 1989, 1991; Brune & Schink 1992; Brackmann & Fuchs 1993). Schink and co-workers (Schnell et al. 1991; Brune et al. 1992) identified a *Pelobacter* sp. that degraded hydroxyhydroquinone to acetate via phloroglucinol (1,3,5-trihydroxybenzene). Stevens (1989) identified ^{14}C-acetate in an anaerobic dinoseb-degrading culture fed [U-^{14}C]-dinoseb. A similar pathway may occur in this enrichment culture. This would necessitate the formation of (IX), which then is likely metabolized to acetate and/or TCA cycle intermediates. Diacetyl and triacetyl glycerol may also be intermediates in this pathway.

The chemical instability and commercial unavailability of these proposed intermediates poses a difficult problem in elucidating the biodegradation pathway of dinoseb under reducing conditions. However, these data suggest that degradation of dinoseb under reducing conditions continues beyond the formation of aminoaromatic intermediates and prevents their polymerization. The replacement of amino groups with hydroxyl groups may play an important role in the degradation of dinoseb to non-toxic products.

Acknowledgements

This work was supported by the J.R. Simplot Company and the U.S. Environmental Protection Agency under the Emerging Technologies Program (USEPA Award # CR816818010). The authors would like to thank Dr. Andrzej Paszczynski for his help with the mass spectrometer and interpretation of mass spectra.

References

Balba MT & Evans WC (1980) The methanogenic biodegradation of catechol by a microbial consortium: evidence for the production of phenol through *cis*-benzenediol. Biochem. Soc. Trans. 8: 452–453

Berger St & Rieker A (1974) Identification and determination of quinones. In: Saul Patai (Ed) The Chemistry of the Quinonoid Compounds. John Wiley & Sons, London

Boopathy R, Kulpa CF & Wilson M (1993) Metabolism of 2,4,6-trinitrotoluene (TNT) by *Desulfovibrio* sp. (B strain). Appl. Microbiol. Biotechnol. 39: 270–275

Brackmann R & Fuchs G (1993) Enzymes of anaerobic metabolism of phenolic compounds 4-hydroxybenzoyl-CoA reductase (dehydroxylating) from a denitrifying *Pseudomonas* species. Eur. J. Biochem. 213: 563–571

Brown BR (1967) Biochemical aspects of oxidative coupling of phenols. In: Taylor WI & Battersby AR (Eds) Oxidative Coupling of Phenols, vol. 1 (pp. 167–202). Marcel Dekker, Inc., New York

Brune A & Schink B (1992) Phloroglucinol pathway in the strictly anaerobic *Pelobacter acidigallici*: fermentation of trihydroxybenzenes to acetate via triacetic acid. Arch. Microbiol. 157: 417–424

Brune A, Schnell S & Schink B (1992) Sequential transhydroxylations converting hydroxyhydroquinone to phloroglucinol in the strictly anaerobic, fermentative bacterium *Pelobacter massiliensis*. Appl. Environ. Microbiol. 58: 1861–1868

Cundliffe E (1989) How antibiotic-producing organisms avoid suicide. Annu. Rev. Microbiol. 43: 207–233

DiStefano TD, Gossett JM & Zinder SH (1991) Reductive dechlorination of high concentrations of tetrachlorethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. Appl. Environ. Microbiol. 57: 2287–2292

Erdtman H & Granath M (1954) Studies on humic acids V. The reaction of *p*-benzoquinone with alkali. Acta Chem. Scand. 8: 811–816

Environmental Protection Agency. Federal Register vol. 51, no. 198, pp. 36634–36650

— Federal Register vol. 51, no. 198, pp. 36650–36661

Froslie A & Karlog O (1970) Ruminal metabolism of DNOC and DNBP. Acta Veterinaria Scandinavica 11: 114–132

Funk SB, Roberts DJ, Crawford DL & Crawford RL (1993) Initial-phase optimization for bioremediation of munition compound-contaminated soils. Appl. Environ. Microbiol. 59: 2171–2177

Gorontzy T, Kuver J & Blotevogel K (1993) Microbial transformation of nitroaromatic compounds under anaerobic conditions. J. Gen. Microbiol. 139: 1331–1336

Gundersen K & Jensen HL (1956) A soil bacterium decomposing organic nitro-compounds. Acta Agric. Scand. 6: 100–114

Hamdi YA & Tewfik MS (1970) Degradation of 3,5-dinitro-*o*-cresol by *Rhizobium* and *Azotobacter* spp. Soil Biol. Biochem. 2: 163–166

Hodge P (1974) Fragmentation reactions of quinones. pp. 579–616. Wiley & Sons, London

Jensen HL & Lautrup-Larsen (1967) Microorganisms that decompose nitro-aromatic compounds with special reference to dinitro-*ortho*-cresol. Acta Agric. Scand. 17: 115–126

Kaake RH, Roberts DJ, Stevens TO, Crawford RL & Crawford DL (1992) Bioremediation of soils contaminated with 2-*sec*-butyl-4,6-dinitrophenol (dinoseb). Appl. Environ. Microbiol. 58: 1683–1689

Krumholz LR, Crawford RL, Hemling ME & Bryant MP (1987) Metabolism of gallate and phloroglucinol in *Eubacterium oxi-*

doreducens via 3-hydroxy-5-oxohexanoate. J. Bacteriol. 169: 1886–1890

Lenke H & Knackmuss H (1992) Initial hydrogenation during catabolism of picric acid by *Rhodococcus erythropolis* HL 24-2. Appl. Environ. Microbiol. 58: 2933–2937

Lenke H, Pieper DH, Bruhn C & Knackmuss H (1992) Degradation of 2,4-dinitrophenol by two *Rhodococcus erythropolis* strains, HL 24-1 and HL 24-2. Appl. Environ. Microbiol. 58: 2928–2932

McCormick NG, Cornell JH & Kaplan AM (1981) Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. Appl. Environ. Microbiol. 42: 817–823

Musso H (1967) Phenol coupling. In: Taylor WI & Battersby AR (Eds) Oxidative Coupling of Phenols, vol. 1 (pp. 1–94). Marcel Dekker, Inc., New York

Musso H & Bormann D (1965) Die Autoxydation des 5-*tert*-Butyl-resorcins. Chem. Ber. 98: 2774–2796

Patel TR, Jure KG & Jones GA (1981) Catabolism of phloroglucinol by the rumen anaerobe *Coprococcus*. Appl. Environ. Microbiol. 42: 1010–1017

Roberts DJ, Kaake RH, Funk SB, Crawford DL & Crawford RL (1993a) Anaerobic remediation of dinoseb from contaminated soil: An on-site demonstration. Appl. Biochem. Biotechnol. 39/40: 781–789

— (1993b) Field scale anaerobic bioremediation of dinoseb-contaminated soils. In: Levin MA & Gealt MA (Eds) Biotreatment of Industrial and Hazardous Waste. McGraw-Hill, New York

Schnell S, Bak F & Pfennig N (1989) Anaerobic degradation of aniline and dihydroxybenzenes by newly isolated sulfate-reducing bacteria and description of *Desulfobacterium anilini*. Arch. Microbiol. 152: 556–563

Schnell S, Brune A & Schink B (1991) Degradation of hydroxyhydroquinone by the strictly anaerobic fermenting bacterium *Pelobacter massiliensis* sp. nov. Arch. Microbiol. 155: 511–516

Selman S & Eastham JF (1960) Benzilic acid and related rearrangements. Q. Rev. Chem. Soc. 14: 221–235

Stevens TO (1989) Ph.D. thesis, Biodegradation of dinoseb (2-sec-butyl-4,6-dinitrophenol) and bioremediation of dinoseb-contaminated soils. University of Idaho

Szewzyk U, Szewzyk R & Schink B (1985) Methanogenic degradation of hydroquinone and catechol via reductive dehydroxylation to phenol. FEMS Microbiol. Ecol. 31: 79–87

Tan EL, Ho CH, Griest WH & Tyndall RL (1992) Mutagenicity of trinitrotoluene and its metabolites formed during composting. J. Tox. Environ. Health 36: 165–175

Tewfik MS & Evans WC (1966) The metabolism of 3,5-dinitro-*o*-cresol (DNOC) by soil micro-organisms. Biochem. J. 99: 31 pp.

Tratnyek PG & Macalady DL (1989) Abiotic reduction of nitro aromatic pesticides in anaerobic laboratory systems. J. Agric. Food Chem. 37: 248–254

U.S. Army Environmental Center. Windrow composting demonstration for explosives-contaminated soils at the Umatilla depot activity Hermiston, Oregon. Report # CETHA-TS-CR-93043. Prepared for USAEC Aberdeen Proving Ground, MD, 21010-5401 by Roy F. Weston, Inc., 1 Weston Way, West Chester, PA 19380-1499

— Windrow Composting Engineering/Economic Evaluation. Report # CETHA-TS-CR-93050. Prepared for USAEC, Aberdeen Proving Ground, MD, 21010-5401 by Roy F. Weston, Inc., 1 Weston Way, West Chester, PA 19380-1499

Wallnofer PR, Ziegler W, Engelhardt G & Rothmeier H (1978) Transformation of dinitrophenol-herbicides by *Azotobacter* sp. Chemosphere 12: 967–972

Williams RT, Ziegenfuss PS & Sisk WE (1992) Composting of explosives and propellant contaminated soils under thermophilic and mesophilic conditions. J. Ind. Microbiol. 9: 137–144

Won WD, Heckly RJ, Glover DJ & Hoffsomer JC (1974) Metabolic disposition of 2,4,6-trinitrotoluene. Appl. Environ. Microbiol. 27: 513–516

Zeller KP (1974) Mass spectra of quinones. In: Saul Patai (Ed) The Chemistry of the Quinonoid Compounds. John Wiley & Sons, London