

Influence of Natural Reductants on the Abiotic Reduction of Pentachloronitrobenzene in Prairie
Pothole Lakes

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By

Joseph S. Voyles

The Ohio State University

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Approved by



Yu-Ping Chin, Advisor
School of Earth Sciences

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ABSTRACT

Nitroaromatic compounds such as pentachloronitrobenzene ($C_6Cl_5NO_2$) were once widely used as fungicides and pesticides in the United States and some (trifluralin and pendamethilin) are still in circulation. These compounds can contaminate natural systems including surface waters and sediments through overland runoff. In this study, the abiotic reduction of PCNB was examined in controlled laboratory systems containing natural wetland pore waters collected from the Prairie Pothole Lakes (PPL) in North Dakota. The PPL region is dominated by agricultural land use, which can impact the water quality of the lakes due to overland runoff containing organic contaminants. High levels of dissolved organic matter (DOM) and possible reduced sulfur species in pore waters could make the PPL reactive towards organic contaminants entering the system.

Reduction of pentachloronitrobenzene to pentachloroaniline was observed by reaction with natural reductants in PPL pore waters. Iron analysis of the natural pore waters revealed a Fe(II) concentration of $14 \mu M$, which makes it a relatively unimportant reductant. Even when compared to high concentration Fe(II)-only controls, natural pore waters degraded pentachloronitrobenzene at a faster rate. This indicates that the degradation in natural pore waters possibly occurred by reaction with other electron donors such as bisulfide or polysulfides in the presence of dissolved organic matter. Future work is necessary to elucidate the nature of the reductant and the precise role of DOM in the reduction of pentachloronitrobenzene.

CHAPTER 1

INFLUENCE OF NATURAL REDUCTANTS ON THE ABIOTIC REDUCTION OF PENTACHLORONITOBENZENE

1.1. Introduction

Synthetic organic pollutants in wetland sediments are of significant environmental concern. The aquatic food chain is a likely mechanism for bioaccumulation of organic contaminants (USEPA, 2006). Contaminates and their metabolites can then enter humans through the consumption of potable water and fish. Human health consequences as a result of exposure include thyroid hypertrophy, hepatocellular hypertrophy, and hyperplasia (USEPA, 2006). A study by the U.S. Environmental Protection Agency identified 43% of waterways included in the National Sediment Inventory as having probable adverse effects on human health and aquatic life due to contaminants (USEPA, 2004). Nitroaromatic pesticides used to control weed and fungus growth, may be transported to wetland sediments by the deposition of suspended solids to which they are attached (Reigart and Roberts, 1999). Due to their slow

biodegradation and persistence of nitroaromatic compounds, it is probable that surface water may become contaminated (USEPA, 2006), however their fate in sediments are less well understood. Once deposited, the compound then re-equilibrates with surrounding pore water and can undergo abiotic and possibly biotic transformations. Reduction reactions of synthetic organic pollutants can lead to transformation products that are similar to or of greater concern than the parent compounds (Dunnivant et al., 1992).

In controlled studies, nitroaromatic and other organic contaminants have been shown to readily undergo abiotic reductive transformation in the presence of the right reductants. Nitroaromatic compounds were reduced by electron donors such as bisulfide, polysulfides, and Fe(II) in the presence of specific DOM analogs such as hydroquinone and mercaptohydroquinone. These DOM species reduce nitroaromatic compounds at a faster rate because of their role as electron transfer mediators (Dunnivant et al., 1992; Perlinger et al., 1996). An electron transfer mediator such as DOM can be reduced, oxidized, and re-reduced continuously by a bulk reductant e.g., bisulfide essentially supplying a steady-state concentration of reduced DOM.

In sedimentary environments the accumulation of DOM and Fe(II) is significant in the overall fate of organic compounds. In porefluids from different lacustrine environments, levels of Fe(II) and DOM can exceed 1 mM and 3 mM (as dissolved organic carbon, DOC) (Chin et al., 1998; O'Loughlin and Chin, 2004) respectively. At these levels it is likely that the reduction of nitroaromatic compounds by Fe(II) and DOM can occur. In sulfate reducing conditions, natural organic matter and sulfidic compounds (HS^- , S^{2-} , polysulfides) have been shown to transform halogenated organic compounds at environmentally relevant concentrations (Perlinger et al., 1996) and iron is mostly removed as iron sulfides. In hypoxic conditions with reduced sulfur

species (RSS), chloroacetanilide herbicides (Loch et al., 2002) and chloroazine agrochemicals (Lippa et al., 2002) were degraded.

The following study focuses on the abiotic reduction of pentachloronitrobenzene (PCNB) by natural reductants in natural sedimentary porefluids in 0.3- μm -filtered solutions. I hypothesize that *PCNB will react with reductants present in natural waters, such as Fe(II) and bisulfide (HS^-) in the presence of dissolved organic matter (DOM).*

1.2. Field Site Description and Sampling

The prairie pothole lakes (PPLs) region of North Dakota is characterized by a high density area of shallow lakes and wetlands. The region is characterized as a glaciated prairie containing an abundance of depressional wetlands in relatively flat outwash plains and hummocky moraines (Winter, 1989). These shallow lakes have a unique water chemistry, containing high concentrations of dissolved organic matter (DOM), and varying concentrations of Fe(II) and RSS, all of which can act as chemical reductants. The PPLs can store surface water, recharge groundwater, supply water to the atmosphere, and be a habitat for wetland animals (LaBaugh et al., 1998). The PPL region can store spring snowmelt varying from days to years (Stewart and Kantrud, 1972). The regional climate and groundwater hydrology cause wet and dry periods, which facilitate varying amounts of reductants in closely-spaced prairie pothole lakes (Kantrud et al., 1989). These chemical reductants promote the transformation of environmental organic contaminants such as pesticides and herbicides.

In 2010, samples were taken from the USGS Cottonwood Lakes Study Area. Samples of sediment pore water and surface water were collected at Lakes P1 and P8 and surface water from P11. The samples from these lakes were used in my research.

1.3. Experimental Details

1.3.1. Chemicals

Recrystallized PCNB (Sigma-Aldrich), pentachloroaniline (PCA; Alfa Aesar), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Sigma-Aldrich), ammonium acetate (Sigma-Aldrich), 1,10 phenanthroline monohydrate (certified ACS, Fisher Scientific), glacial acetic acid (certified ACS Plus, Fisher), $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ (certified ACS, Fisher Scientific), MOPS (3-[N-morpholino] propanesulfonic acid); Sigma-Aldrich, 99%). Acid solutions from HCL (12N, Certified ACS Plus, Fisher Scientific), base solutions NaOH (Fisher Scientific). Solvents used were Milli-Q water (Milli-Q UV Plus, Millipore) and methanol (HPLC Grade, Fisher Scientific).

1.3.2. Reaction Studies- Fe(II)

Reaction samples were prepared in a glovebox (PLAS Labs, Inc.) equipped with an oxygen scrubber. A gas mixture of 95% / 5% nitrogen/hydrogen was used to maintain anoxic conditions for the reaction experiments. An Orion Thermo Aqua Pro pH probe was used to measure pH values for each sample. Reaction media of Milli-Q water, 0.014 M MOPS buffer, 500 μM Fe(II) solution, and NaOH were prepared in glass bottles outside of the glovebox. All reaction media was purged with argon gas for 1 min per each mL of solution. Fe(II) stock solution was acidified (pH~3) prior to purging the solution, once solution was in the glovebox the solution pH was raised to desired level with purged NaOH. The solutions were filtered with a Milli-Q rinsed 0.2 μm filter (Pall Life Sciences IC Acrodisc 25mm syringe filter with Supor (PES) membrane) prior to use. The reaction medium then was transferred into a glass syringe (Popper & Sons, Inc.), and spiked with 20 μL of 1200 μM PCNB stock solution for a $[\text{PCNB}]_0$ of 1 μM . Reactions were performed inside sealed glass syringes with magnetic stir bars and no headspace.

Reaction aliquots were separated at designated time points into 1-ml borosilicate glass autosampler vials (Kimball) preloaded with 30 μL of 2 N HCL to quench the reaction at pH of 2.0 to 3.0. The vials were sealed with plastic caps and loaded into the autosampler for HPLC analysis using a Waters 1525 binary HPLC pump, 2487 dual λ absorbance detector with a 717 Plus autosampler. All compounds were detected at a wavelength of 225 nm. Samples were injected in volumes of 100 μL and compounds were separated through a reverse-phase C_{18} column (Restek Allure C_{18} , 5 μm , 150x3.2 mm) using a 90/10 methanol/water mobile phase. A calibration curve was generated using PCA and PCNB standard solutions ranging in concentration from 0.05 to 1.25 μM and was used to determine the concentration of the two species in the degradation experiments. (Figure 1.2)

1.3.3. Reaction Studies- PPL surface and pore water

Reaction samples were prepared in a glovebox (PLAS Labs, Inc.) equipped with an oxygen scrubber. A gas mixture of 95% / 5% nitrogen/hydrogen was used to maintain anoxic conditions for the reaction experiments. PPL surface water (P11), and PPL pore water (P8) were filtered in the glovebox using a 0.3 μm glass fiber syringe-tip filter (Sterlitech) before being transferred to a glass syringe. Each syringe was spiked with 20 μL of 1200 μM PCNB stock solution for an approximate $[\text{PCNB}]_0$ of 1 μM . Reaction aliquots were taken at designated time intervals, injected directly into pre-weighed 1-ml borosilicate glass autosampler containing 30 μL of 2 N HCl to quench the reaction, and subsequently analyzed by HPLC equipped with a UV/Vis detector set to measure absorbance at 225 nm. Samples were injected in volumes of 100 μL and compounds were separated through a reverse-phase C_{18} column (Restek Allure C_{18} , 5 μm , 150x3.2 mm) using a 90/10 methanol/water mobile phase. A calibration curve was generated using PCA and PCNB standard solutions ranging in concentration from 0.05 to 1.25

μM and was used to determine the concentration of the two species in the degradation experiments. (Figure 1.2)

1.3.4. Fe(II) and TOC Measurements

Prior to reaction with PCNB, aliquots for Fe(II) measurements were taken and acidified with 2 N HCL. 1 mL of the aliquot was reacted with 1.0 mL of an aqueous 1,10-phenanthroline solution ($\sim 5.0\text{M}$), 0.5 mL ammonium acetate buffer ($\sim 3.8\text{M}$) and 2.5 mL Milli-Q water. The Fe(II) 1,10 phenanthroline complex absorbance was measured in plastic cuvettes at a wavelength of 508 nm (Varian Cary 13 or 50) zeroed to a Fe(II)-free blank containing the colorimetric reagents. Acidified Fe(II) standard solutions ranging in concentration of 10-500 ppm made from $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ were analyzed in an identical fashion and were used to create a calibration curve. (Figure 1.3)

Diluted pore and surface water samples were measured with a Shimadzu TOC 5000 after filtering the sample with a $0.3\ \mu\text{m}$ borosilicate glass fiber filter. (Figure 1.6)

1.4. Results and Discussion

1.4.1. Reduction of PCNB in Fe(II)-only Reaction Media

In the Fe(II) + MOPS solution control reactions, reduction of PCNB to PCA was observed (Figure 1.4). Approximately 36% of PCNB was degraded to PCA over a period of 2 hours with $406.9\ \mu\text{M}$ Fe(II) present. There was no degradation observed in the PCNB MOPS blank reaction. Hakala et al., (2007) observed pseudo-first-order kinetics and complete degradation of $1\ \mu\text{M}$ PCNB at 50 minutes with $390\ \mu\text{M}$ Fe(II) present. When compared to Klupinski et al. (2004) Fe(II)-only experiments, they observed a slow reduction of PCNB but

explain that the reaction proceeds through surface catalysis of iron oxide due trace O₂ in their reaction system. They explain that their pseudo-zero-order kinetics reaction proceeds through a short-lived intermediate that limits the rate at which PCNB can react with relatively low concentration of iron oxide surface species. Due to difficulties in maintaining anoxic conditions, reduction in my present work may have occurred by a similar mechanism in which the different levels of reactive surface species cause variation of PCNB degradation in Fe(II)-only reactions. However, there was insufficient reactive Fe(II) present to rapidly degrade PCNB to the extent observed by others (Hakala et al., 2007)

1.4.2. Reduction of PCNB in PPL pore and surface water

Rapid reduction of PCNB to PCA was observed in reaction media containing PPL pore water only. The PCA byproduct was observed to be fluctuating at a low concentrations (<.05µM), indicating a subsequent covalent binding of the aromatic amine to organic matter species, confirming observations by Weber et al. (1996). As observed by Weber et al.,(1996) humic acid is more reactive towards aniline than fulvic acid. Due to the low concentrations of PCA there may be more humic acid in solution that is able to react with PCA and removing it from solution. The mass balance equation is given by:

$$[\text{PCNB}]_0 = [\text{PCNB}] + [\text{PCA}] + [\text{PCA-DOM}].$$

The initial concentration of PCNB is equal to the product of the concentration of unreacted PCNB, PCA, and covalently bonded PCA-DOM. In the PCNB P8 pore water solution, 100% of PCNB was degraded over a period of 9 hours with 14 µM Fe(II) present (Figure 1.5). Because of the low levels of Fe(II), other reductants like bisulfide are likely responsible. The pore water samples exhibited a sulfur odor. In the PCNB P11 surface water there was very little to no

degradation of PCNB with 2.35 μM Fe(II) present. The [DOC] of 108.3 mg·C/L in the P8 pore water (Figure 1.6) represents the amount of DOM that can be reduced and re-reduced continuously by a bulk reductant (Dunnivant et al., 1992; Perlinger et al., 1996). The reduced DOM then acts as an electron mediator, reducing PCNB to PCA. The bulk reductant in the PPL pore water is suspected to be reduced sulfur species.

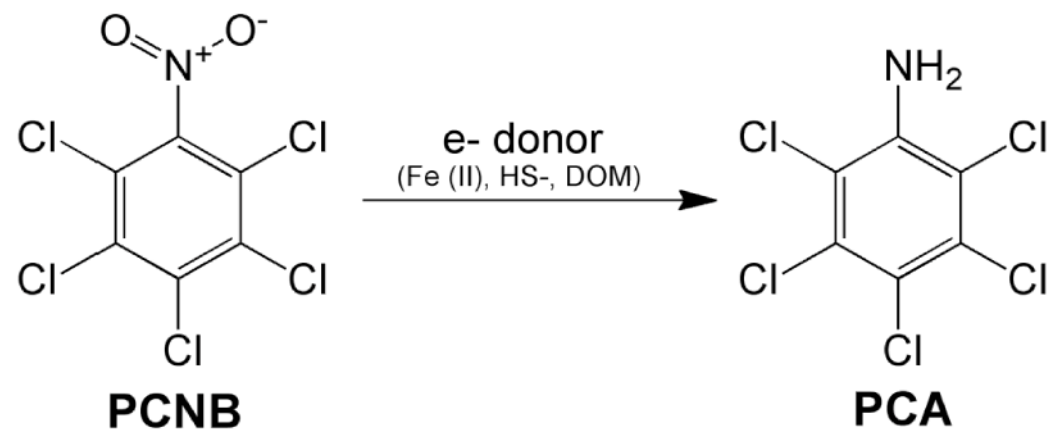
1.5. Conclusions

Pentachloronitrobenzene (PCNB) was degraded in controlled systems containing Fe(II) and natural wetland pore waters. Reduction in controlled systems containing high [Fe(II)] was most likely through surface catalysis of iron oxide due trace O_2 in their reaction system. In the natural pore waters with very low [Fe(II)], PCNB was degraded over a period of 9 hours. The degradation product, PCA, covalently bonded to DOM. By the observance of a sulfur odor, it is likely that bisulfide or polysulfides are responsible for the degradation of PCNB. The DOM can be reduced continuously by the bulk reductant (bisulfide or polysulfides) which then reduces PCNB to PCA. In the surface water, there was very little to no degradation of PCNB with 2.35 μM Fe(II) present. Future work is necessary to elucidate the reductant and the precise nature of DOM's role in the reduction of pentachloronitrobenzene.

1.6. Future Work

The actual reductant responsible for the degradation of pentachloronitrobenzene remains unclear. Determination of a reduced sulfur species, bisulfide, by spectrophotometric methylene blue method would narrow the possible electron donors in the natural pore waters. Other reduced sulfur compounds include polysulfides and organic sulfur. However, these species may be extremely difficult due to the handling of the oxygen-sensitive chemicals involved. Once the

reductant is determined, further studies with varying pH of reaction media, concentration of reductant and DOM can be used to probe the reductive capacity of the naturally occurring reductant. Investigating other natural pore waters in nearby vicinity to sampling area can provide a more detailed picture of the reductant distribution in the PPL region.



Pentachloronitrobenzene		Pentachloroaniline	
Molecular Weight:	295.34 g/mol	Molecular Weight:	265.35 g/mol
Aqueous Solubility:	0.44 mg/L	Aqueous Solubility:	0.0298 mg/L

Figure 1.1: Structures and properties of PCNB and PCA.

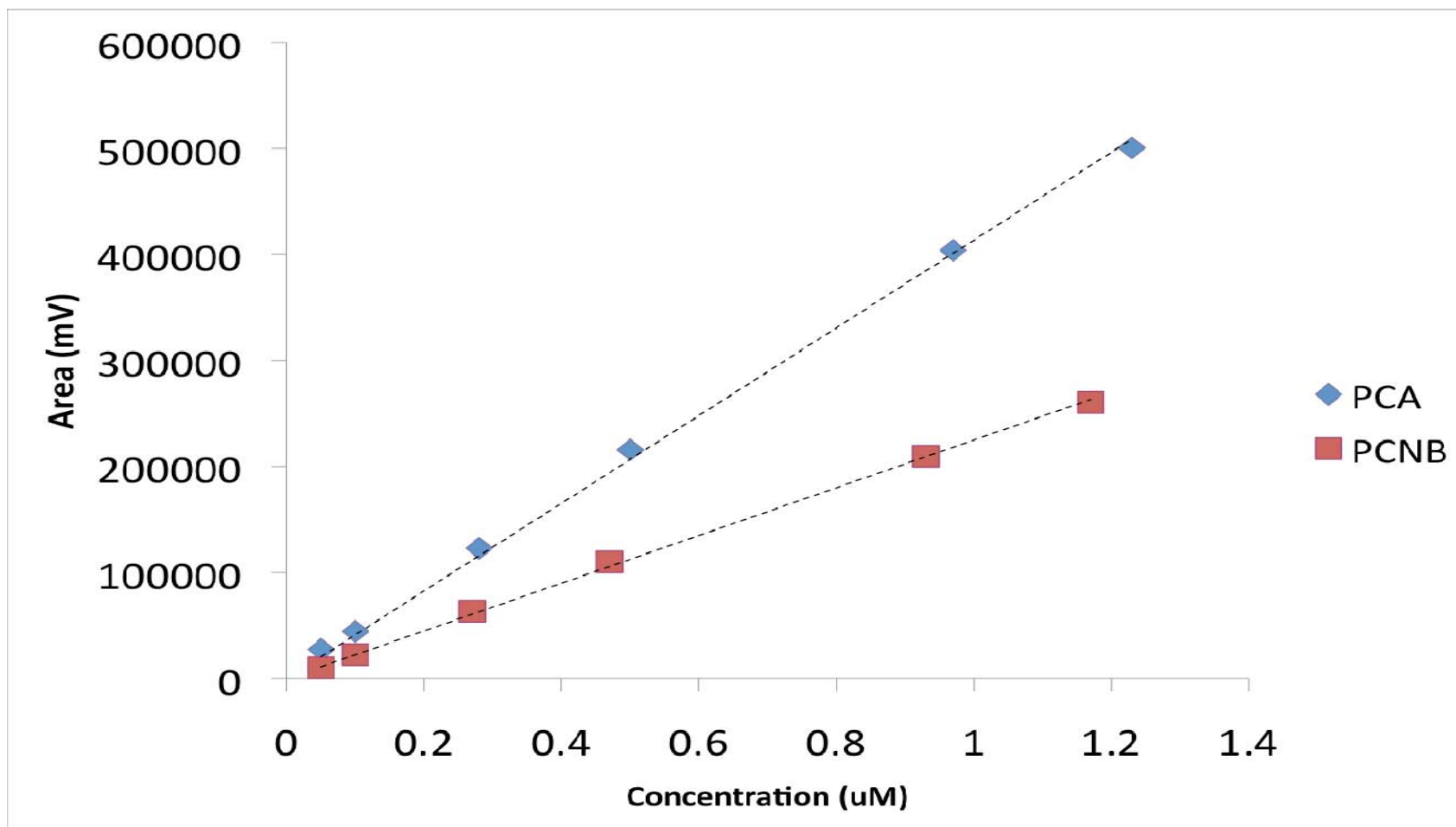


Figure 1.2: A calibration curve was generated using PCA and PCNB standard solutions ranging in concentration from 0.05 to 1.25 μM and was used to determine the concentration of the two species in the degradation experiments.

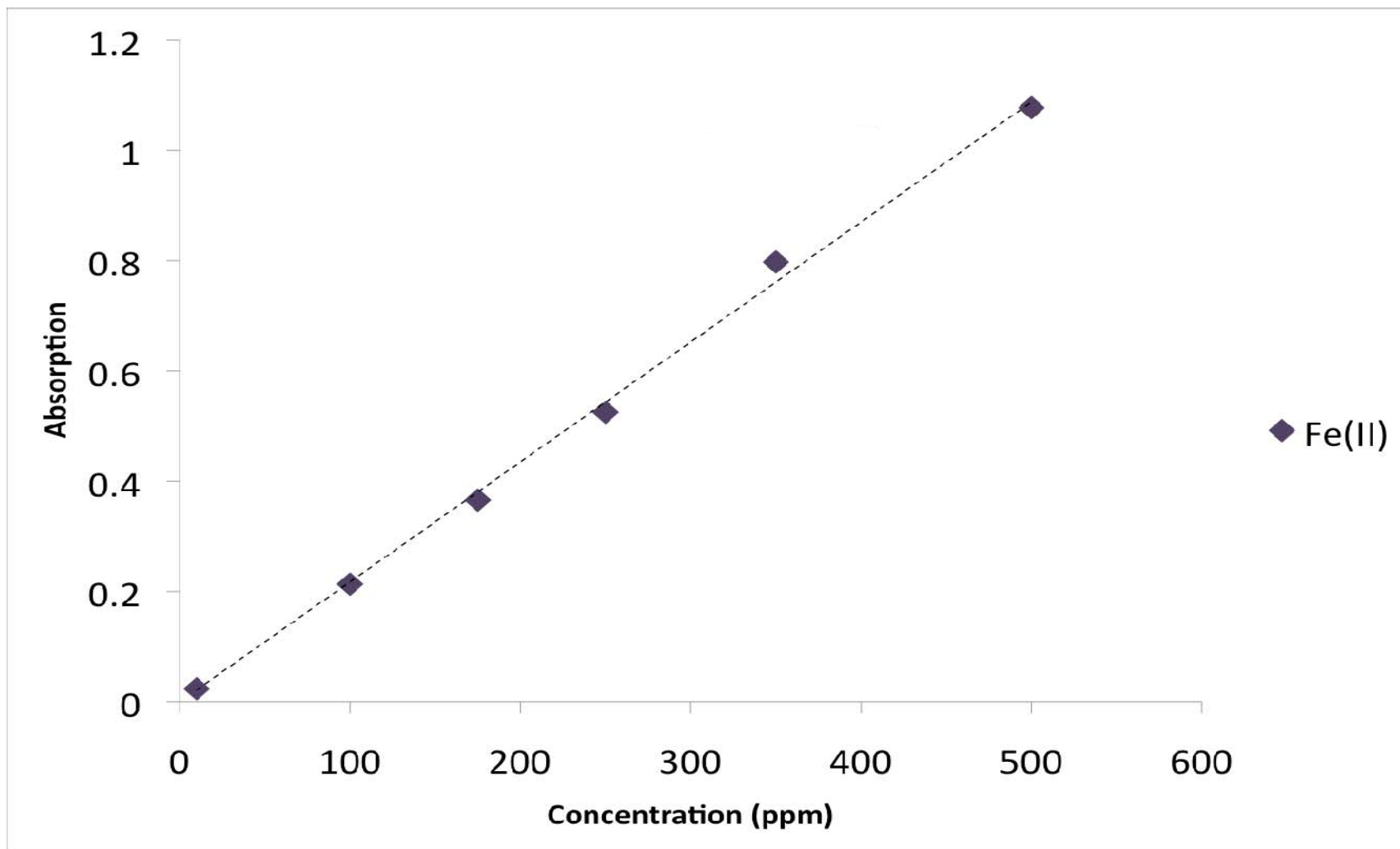


Figure 1.3: Acidified Fe(II) standard solutions ranging in concentration of 10-500 ppm made from $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ were analyzed in an identical fashion and were used to create a calibration curve.

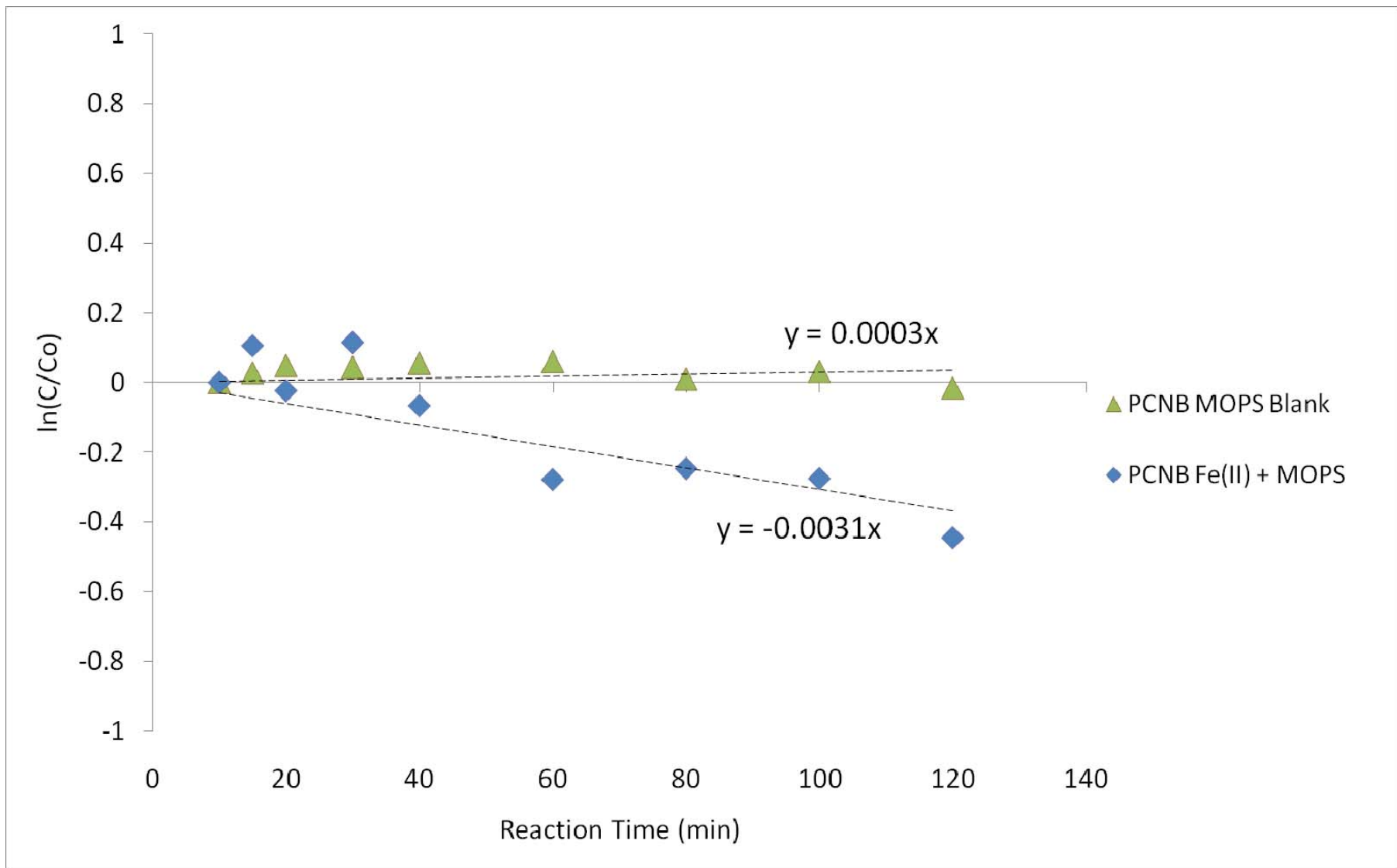


Figure 1.4: In the PCNB Fe(II) + MOPS solution, approximately 36% of PCNB was degraded over a period of 2 hours. There was no degradation observed in the PCNB MOPS Blank reaction. The rate constant for PCNB degradation is $k = 0.0031 \text{ min}^{-1}$

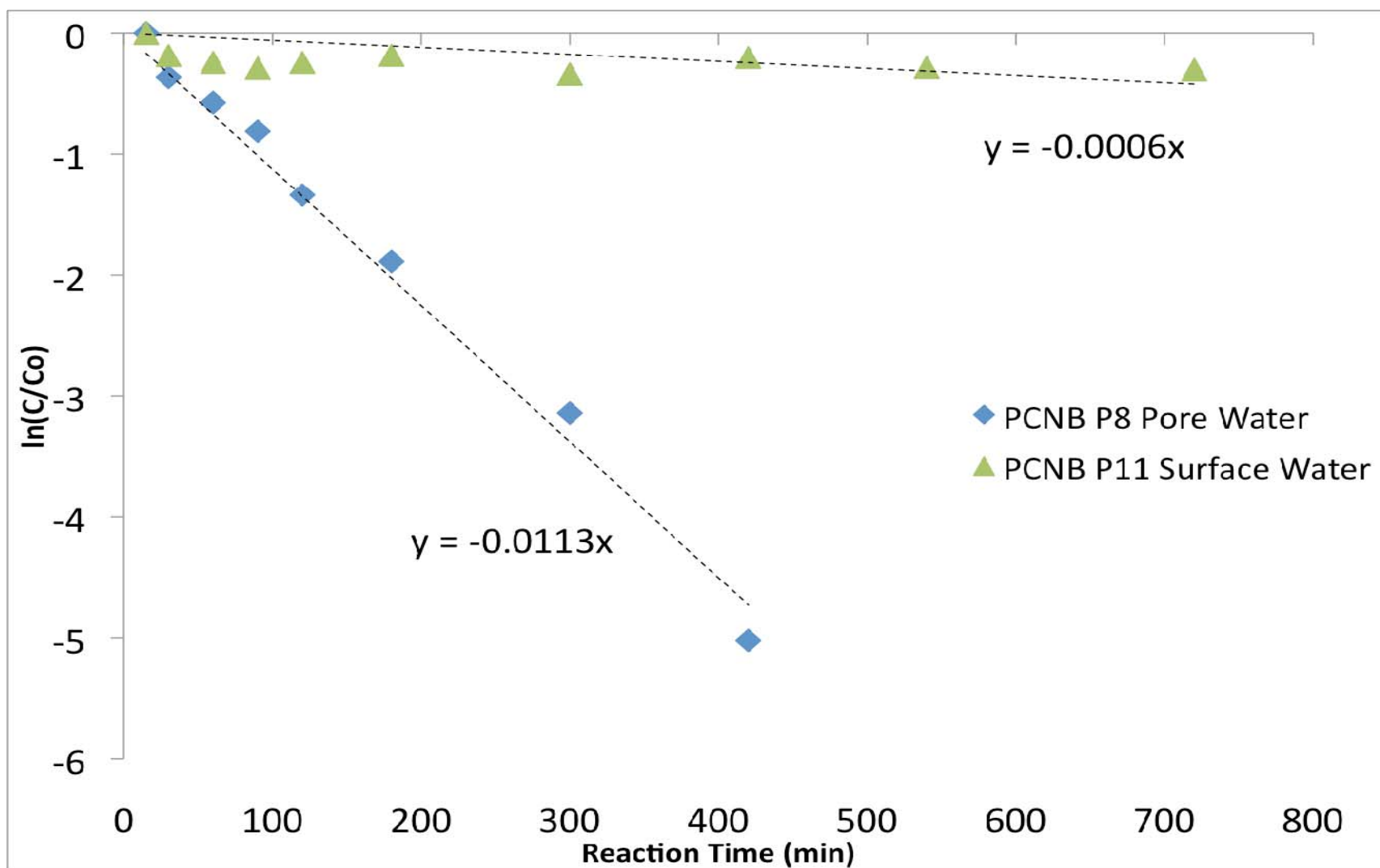


Figure 1.5: In the PCNB P8 pore water solution, 100% of PCNB was degraded over a period of 9 hours. There is not a linear increase in [PCA], this is because of subsequent reaction to organic matter species (Weber et al., 1996). The rate constant for PCNB degradation is $k=0.0113 \text{ min}^{-1}$

Table 1.6: TOC analysis and UV/VIS Scans

Sample	Actual [C] mg·C/L	Abs 280	Abs 254
P11 surface water	43.4	0.0565	0.0885
P8 Pore water	108.3	0.1523	0.2577

1.7. References

- Dunnivant, F. M.; Schwarzenbach, R.P.; Macalady, D. L. (1992) Reduction of substituted nitrobenzenes in aqueous solutions containing natural organic matter. *Environ Sci. Technol.*, **26**, 2133-2141
- LaBaugh, J. W., T. C. Winter, D. O. Rosenberry. Hydrologic Functions of Prairie Wetlands. Great Plains Research 1998, 8, 17-37.
- Klupinski, T. P.; Chin, Y. P.; Traina, S. J. Abiotic degradation of pentachloronitobenzene by Fe(II): Reactions on goethite and iron oxide nanoparticles. (2004) *Environ. Sci. Technol.*, **38**, 4353-4360.
- Lippa, K.A.; and, Roberts A.L. Nucleophilic Aromatic Substitution Reactions of Chloroazines with Bisulfide (HS-) and Polysulfides (Sn²⁻). *Environmental Sci & Technol.*, 2002 **36** (9), 2008-2018
- Loch, A. R.; Lippa, K. A.; Carlson, D.L.; Chin, Y.P.; Traina S. J.; and Roberts. Nucleophilic Aliphatic Substitution Reactions of Propachlor, Alachlor, and Metolachlor with Bisulfide (HS-) and Polysulfides (Sn²⁻) *Environmental Science & Technology* 2002 **36** (19), 4065-4073
- Perlinger, J. A., W. Angst, and R.P Schwarzenbach, "Kinetics of the reduction of hexachloroethane by juglone in solutions containing hydrogen sulfide", *Environ. Sci. Technol.*, **30**, 3408-3417 (1996)
- Stewart, R. E., Kantrund, H. A. "Vegetation of prairie potholes in North Dakota, in relation to quality of water and other environmental factors." USGS Professional Paper 585-D. Washington, DC: US Government Printing Office: 1972.
- Weber, E.J.; Spidle, D.J.; Thorn, K.A. (1996) Covalent binding of aniline to humic substances. 1. Kinetic studies. *Environ. Sci. Technol.*, **30**, 2755-2763
- Winter, T.C. Hydrologic studies of wetlands in the northern prairie. *Northern Prairie Wetlands*, Ed. A.G.van der Valk. Ames, Iowa: Iowa State University Press: 1989, 16-54.

United States. Environmental Protection Agency. The Incidence and Severity of Sediment Contamination in Surface Waters of the United States, 2nd ed.: National Sediment Quality Survey; EPA 823-R-04-007; U.S. Environmental Protection Agency, Office of Science and Technology: Washington, DC. Electronic document available: <http://www.epa.gov/waterscience/cs/report/2004/index.htm>, 2004.

United States. Environmental Protection Agency. Registration Eligibility Decision for Pentachloronitrobenzene; EPA OPP-2006-XXX; U.S. Environmental Protection Agency, Office of Science and Technology: Washington, DC. Electronic document available: www.epa.gov/oppsrrd1/REDS/pcnb_red.pdf, 2006.