

A Pollutant Transformation Laboratory Exercise for Environmental Chemistry: The Reduction of Nitrobenzenes by Anaerobic Solutions of Humic Acid

Frank M. Dunnivant* and Mark-Cody Reynolds

Department of Chemistry, Whitman College, Walla Walla, WA 99362; *dunnivfm@whitman.edu

A review of the *JCE* literature from 1999 to 2005 yields over 200 articles pertaining to environmental chemistry. Most of these have appeared after the insightful articles on environmental chemistry education by Tran et al. (1) and Cooper et al. (2). These exercises are encouraging for professors teaching environmental chemistry. Laboratory exercises presented in these articles range from the relatively straightforward environmental monitoring of water (3) to atomic absorption spectrometry (4–5) techniques and analyses using capillary electrophoresis (6–7), ion chromatography (8), and inductively coupled plasma spectrometry (9). Environmental class projects spanning a series of laboratory periods are also described (10–12). Analytical schemes that can be used to teach principles of hazardous waste site remediation are also available (13–20). Two articles provide the instructor with exercises to determine distribution coefficients, an important parameter in understanding the fate and transport pollutants in aquatic environments (11, 21). Two laboratory exercises are relevant to the subject of this article and illustrate chemical transformation of environmental pollutants. These include the hydrolysis of carboxylic esters (an abiotic process) (22) and the microbial degradation of *p*-nitrophenol (23). It is also encouraging that there are currently two commercially available laboratory manuals dedicated to environmental chemistry (24, 25).

Biotic and abiotic pollutant transformation reactions have long been recognized as important in determining the life-cycle toxicity of a compound. This is especially needed today with the intentional design of pesticides to rapidly de-

grade and not accumulate in the environment as pesticides such as DDT did in the past. Both anaerobic and aerobic transformations can occur. Aerobic transformations include the partial degradation of an organic pollutant to byproducts as well as the complete mineralization to carbon dioxide. Anaerobic transformations include dehalogenations, nitro reductions, dealkylations, azo-linkage reductions, and sulfoxide and sulfone reductions. Two excellent reviews of these environmental reactions can be used as preparatory material for students and instructors incorporating this laboratory into their syllabus (25, 26).

The basic abiotic reactions central to anaerobic degradation are similar to the reactions where pollutants are transformed by microorganisms in the environment (refer to Figure 1) (27, 28). However, in the abiotic reactions, natural organic matter (NOM, and in our experiments humic acid) takes the place of microbes to shuttle the electrons from the bulk electron donor (in our case, hydrogen sulfide) to the pollutant (in our case, a nitro group on substituted benzenes). In this reaction, the direct reaction rate, in the absence of NOM, is relatively slow or nonexistent between the pollutant and the bulk electron donor (sulfide). However, the bulk electron donor is oxidized to elemental sulfur or sulfate and rapidly reduces functional groups in the NOM, which in turn rapidly reduces the organic pollutant. The sequence of reductions for nitrobenzene and substituted nitrobenzenes is shown in Scheme I (29). A basic introduction to NOM for the students can be found in Arnold (30) and in most environmental chemistry textbooks. Studies by Dunnivant et al. (31) suggest that the functional groups in NOM (and humic acid) responsible for the transformation reactions are hydroquinone structures.

The laboratory experiment presented here acts as a capstone, integrated lecture–laboratory exercise involving solution preparation, pH buffers, E_H (reduction potential) buffers,¹ organic reaction mechanisms, reaction kinetics, and instrumental analysis. As introductory material to the topic of environmental transformation reactions and before the laboratory exercise, students read and discuss (in lecture) the articles of Macalady et al. (26) and Schwarzenbach et al. (27) since few environmental chemistry textbooks adequately cover

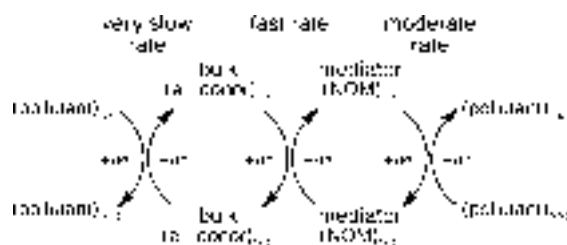
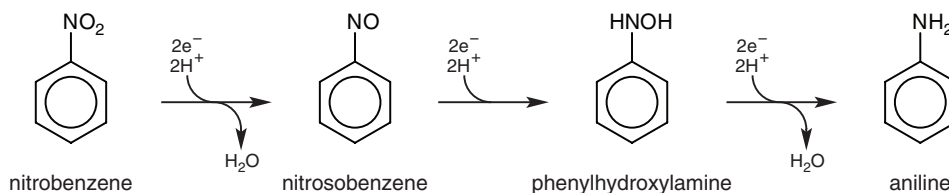


Figure 1. Conceptual representation of the electron shuttle system modified from Glass (28).



Scheme I. Reaction mechanism for the reduction nitrobenzene to aniline (modified from ref 29).

these reactions and processes. In the laboratory experiment following the lecture, students perform one or more of the chemical reduction exercises presented in the Supplemental Material^W and below.

Materials and Methods

There are a variety of experiments that can be conducted with this experiment setup, all of these use kinetic interpretations to study the collected data. We suggest using the most basic experiment, the reduction of a substituted nitrobenzene, that uses sample solutions made in 50-mL serum vials, containing 5.0 mM H_2S , 0.050 M HEPES pH 7.2 buffer, a 100 μM substituted nitrobenzene (recommended compound: 3-chloronitrobenzene), sufficient HCl to neutralize the OH^- generated by the hydrolysis of S^{2-} , deionized H_2O , and 15 mg/L Fluka humic acid. Final sample volumes are 4.0×10^1 mL. Experiments are run in duplicate, at a minimum, so two vials containing all of the chemicals are required. If an autosampler is available, we suggest that each pair of students prepare a vial containing all chemicals and follow it through the experiment. Samples are then taken at predetermined intervals (suggested sampling times are given in the online instructions) to monitor the concentration of substituted nitrobenzene analyte through time. Blanks, solutions containing everything but the NOM, are run to show the lack of a reaction without NOM being present.

- For GC analysis: Ethyl acetate (0.50 mL per sample) is used for extraction of samples and spiked to a level of $1.00 \times 10^2 \mu\text{M}$ unsubstituted nitrobenzene (an internal standard) as described in the Supplemental Material.^W
- For HPLC analysis: Ethyl acetate (0.50 mL per sample; no internal standard) is used for extraction of samples.

A summary of the GC and HPLC procedures, conditions, and analyte retention times are given in the Supplemental Material.^W

If the students work in groups and prepare all solutions, this laboratory exercise will take approximately three, three-hour lab periods and can be used as a class project exercise. If stock solutions are prepared in advance (recommended), the time requirement is reduced to two lab periods. Preparation of sample solutions in serum vials and sampling during

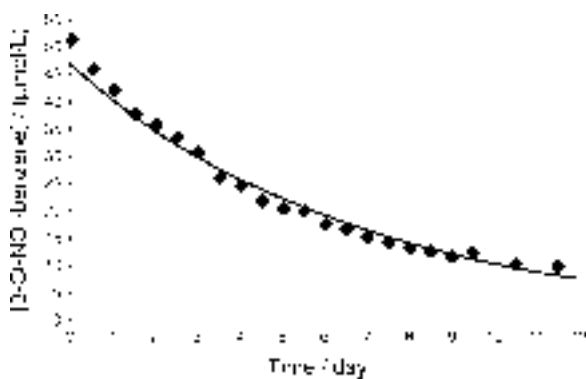


Figure 2. Illustration of the first-order degradation of 3-chloronitrobenzene in 5 mM S^{2-} , at a pH value of 7.2, and in 15 mg/L Fluka natural organic matter (student results).

the following week account for the first laboratory period. HPLC analysis will take an additional lab period (approximately 10 minutes per run). GC analysis will take approximately 30 minutes per sample and standard, so an autosampler is suggested.

Hazards

Nitrobenzene and substituted nitrobenzenes are inhalation hazards that may cause methemoglobinemia when high solutions are used and may cause eye irritation. Students are only given a methanolic 0.1 M solution and subsequently work with 100 μM solutions, which are to be used in the fume hood to avoid exposure hazards. HEPES may cause irritation to sensitive individuals. Sodium sulfide is corrosive. Stockroom personnel should consult the MSDS for this chemical and only use it in a fume hood. Avoid the addition of acid to the solution since it will generate large quantities of hydrogen sulfide (this is especially important in concentrated solutions and during waste storage and disposal). The students will use a 0.5 M sulfide solution, but it should only be used in the fume hood.

Other standard precautions, such as splash goggles, proper gloves, lab coat, and adequate ventilation, should be used in handling of chemicals in this exercise and in disposing of organic wastes. Three disposal containers should be present in a fume hood, including one for sulfide waste, one for inorganic chemicals and HEPES waste, and one for nitrobenzene-containing solutions.

Results and Discussion

Because of the complex nature of this experiment and the variety of experiments that can be performed, here we present typical student results for the basic experiment. The remaining experiments are available in the Supplemental Material.^W

The most basic and suggested approach is to study the rate-order dependence of the nitro reduction. This is shown in Figure 2 for the reduction of 3-chloronitrobenzene in a solution containing 15 mg/L Fluka NOM, a pH value of 7.2, and S^{2-} of 5.0 mM. This is a moderate concentration of NOM, and faster rates can be obtained by using a higher concentration of NOM. We have the students plot the data using zero-, first-, and second-order plots to estimate the rate order. The exact rate order is not always a clear exercise and, thus, students learn the error normally present in experimental data. In order to clarify and determine the rate order, the experiments should be conducted over at least three half-lives. As seen from the data in Figure 2, the reduction of 3-chloronitrobenzene generally follows first-order kinetics, which is not surprising given the relatively high concentration of bulk electron donors and NOM, but these are typical relative concentrations found in the many environmental settings. After the students are familiar with the basic experiment they can proceed with more complicated experiments: the dependence of k on NOM concentrations, pH, and substitute pattern of the nitrobenzene (details in the Supplemental Material^W). If time does not permit then we suggest that data from these experiments, Figures 4–8 in the Supplemental Materials^W, be given to the students and that a class dis-

cussion of each of these experiment parameters follows. This class discussion emphasizes the importance of the diversity of solution properties on reaction rate.

This is a highly involved and advanced laboratory exercise and we have found that students understand the reactions and details of the procedures better if they have first read and discussed the articles of Macalady et al. (26) or Schwarzenbach et al. (27). An additional reference is the textbook of Schwarzenbach et al. (32). These articles present the variety of abiotic reactions that can occur in polluted environmental systems such as hydrolysis, nucleophilic substitutions, dehalogenations, reductions of aromatics, and reduction of azo compounds. Finally, as a "recap exercise" the students are given the research article by Dunnivant et al. (23) as an applied summary of the laboratory exercise.

Summary

This article presents a capstone laboratory exercise for environmental chemistry. Students are introduced to the concept of pollutant transformation in the lecture portion of the class, where they read and discuss the articles of Macalady et al. (26) and Schwarzenbach et al. (27). In lab, the students can prepare all of the solutions necessary for the experiment, plan sampling strategies, sample over the period of one week, analyze the samples on an HPLC or GC, estimate the reaction order, and calculate the degradation rate constant. Students completing the lecture and laboratory exercises gain a thorough understanding of the effect of solution chemistry properties on abiotic transformation reactions in the environment. They see and appreciate master variables, such as pH and E_H , as well as reactant concentrations and substitute patterns.

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Supplemental Material

List of the material and supplies, instructions for solution preparation, instrument conditions, and detailed procedures for the basic and advanced experiments are available in this issue of *JCE Online*.

Note

1. An E_H buffer is used to control the reduction potential, just as a pH buffer is used to control the pH of the solution. The E_H in water under normal environmental conditions can range from +0.80 V (very oxidized) to as low as -0.25 V in very anoxic waters.

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