Ozone and Hydrogen Peroxyacetic Acid Treatment To Reduce or Remove EBDCs and ETU Residues in a Solution

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Laboratory studies were conducted in a model system to determine the effects of ozone (1 and 3 ppm) and hydrogen peroxyacetic acid (HPA) (5 and 50 ppm) at pH 4.6, 7.0, and 10.7 and at 10 and 21 °C on the degradation of mancozeb in solution over a 30 min period. All samples were analyzed for residues by GLC and HPLC. Ozonation and HPA treatment were effective in degrading mancozeb in solution. Rate of mancozeb degradation was dependent on pH, with the fastest rate at pH 7.0. Ethylenethiourea (ETU) residue concentrations in the mancozeb solutions were monitored over 60 min. Under controlled conditions, the ETU residue concentrations increased during the 15 min reaction time and then decreased for all three pH values. At 3 ppm of ozone treatment, no ETU residues were detected at all three pH ranges after 15 min of reaction time. Degradation of ETU by HPA was greatest at pH 4.6, and no ETU residues remained after 5 min at either 5 or 50 ppm. The results showed that ozone and HPA gave excellent degradation of pesticide residues depending on pH and temperature. These experiments indicated the potential for the removal of pesticide residues on fruit and in processed products.

Keywords: *EBDC fungicides; mancozeb; ethylenethiourea; ozone; hydrogen peroxyacetic acid*

INTRODUCTION

Ethylene bis(dithiocarbamate)s (EBDCs) are a group of broad-spectrum agricultural chemicals used widely to control ~400 fungal and bacterial pathogens (*1*). After >60 years of use, EBDCs have an impressive safety record when used as directed. The EBDCs registered for food crop uses in the United States are mancozeb, maneb, metiram, nabam, and zineb. Mancozeb is one of the most widely used EBDC fungicides to protect many fruits, vegetables, nuts, and field crops against a wide spectrum of diseases, including scab on apples, potato blight, and leaf spot (*2*).

A major toxicological concern surrounding the EBDCs comes from ethylenethiourea (ETU; Figure 1), an industrial contaminant and a breakdown product of EBDCs. ETU has been classified as a probable human carcinogen by the EPA (*3*).

Ozone (O_3) has been shown to be a more powerful disinfectant than the most commonly used chlorine for deactivation of a very large number of microorganisms and pesticide residues (*4*). It has been used safely and effectively in water treatment and applied in the food industry in Europe for decades, in some cases almost a century (*5*). Ozonation is approved in the United States as generally recognized as safe (GRAS) for treatment of bottled drinking water (*6*). Ozone has certain characteristics that make it attractive as a sanitizer in food processing, and it is safer than many other oxidizing compounds. Applications in the food industry include the use of gaseous ozone for increasing the storage life of foods and dissolved ozone in water for sanitizing the surfaces of fruits, vegetables, and other agricultural products. Ozone does not remain in water or on the surface matrix very long; thus, its use is considered a process rather than a food additive, with no safety concerns about consumption of residual ozone in food products (*5*).

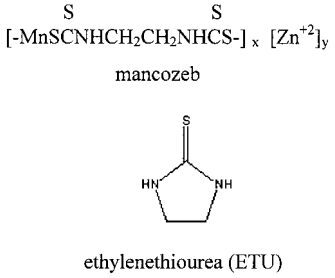

Figure 1. Structures of mancozeb and ethylenethiourea.

A mixture of acetic acid, hydrogen peroxide, and peroxyacetic acid has been shown to have antimicrobial properties. Hydrogen peroxide (H_2O_2) is classified as GRAS for use in food products as a bleaching, oxidizing, reducing, and antimicrobial agent (*7*). Three antimicrobial hydrogen peroxide applications are approved by the U.S. Food and Drug Administration: treatment of milk for use in cheese, preparation of modified whey, and preparation of thermophile-free starch (*7*). Various experimental antimicrobial applications of hydrogen peroxide for foods have been described, including preservation of fresh vegetables and fruits (*8*), control of

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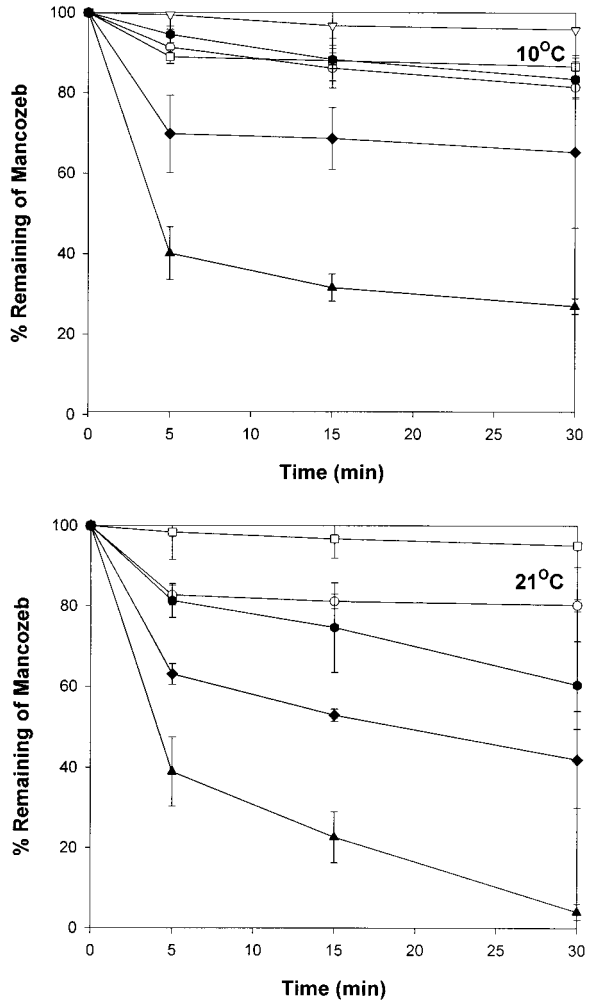

Figure 2. Effect of 1 ppm of O_3 on the degradation of 2 ppm of mancozeb at 10 and 21 °C: (○) control, pH 4.6; (▽) control, pH 7.0; (□) control , pH 10.7; (◆) 1 ppm of O_3, pH 4.6; (▲) 1 ppm of O_3, pH 7.0; (●) 1 ppm of O_3, pH 10.7.

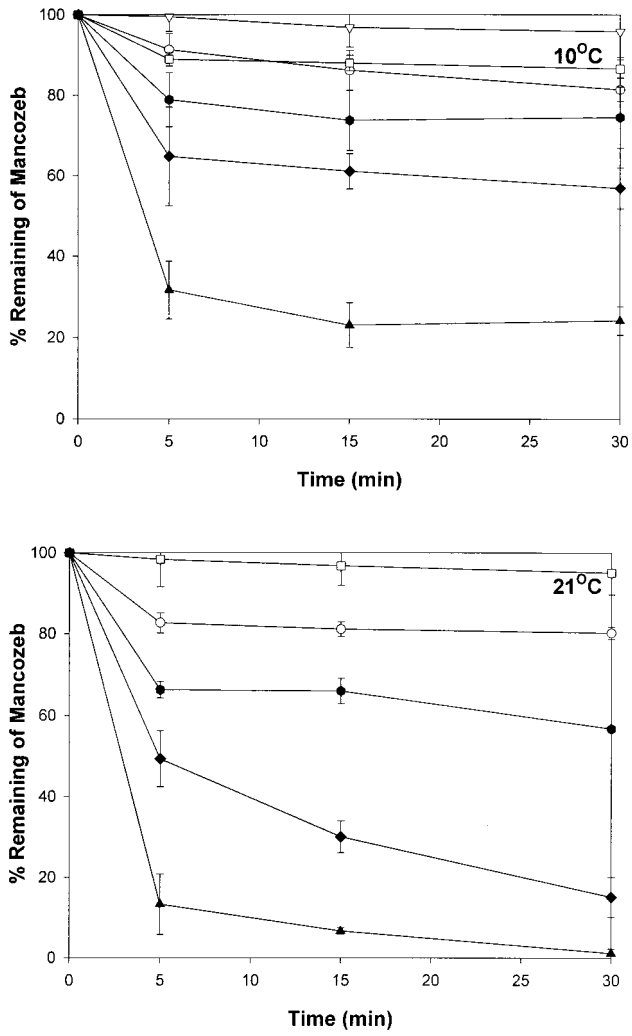

Figure 3. Effect of 3 ppm of O_3 on the degradation of 2 ppm of mancozeb at 10 and 21 °C: (○) control, pH 4.6; (▽) control, pH 7.0; (□) control , pH 10.7; (◆) 3 ppm of O_3, pH 4.6; (▲) 3 ppm of O_3, pH 7.0; (●) 3 ppm of O_3, pH 10.7.

postharvest decay in table grapes (*9*), washing of fresh mushrooms, and preservation of salad vegetables, berries, and fresh-cut melons (*10*). Hydrogen peroxide is unstable in solution but combined with acetic acid, it forms peroxyacetic acid or hydrogen peroxyacetic acid (HPA), which is a fairly stable compound.

The objective of this study was to determine the effectiveness of ozone and hydrogen peroxyacetic acid treatments on the degradation of mancozeb and ETU in aqueous solution.

MATERIALS AND METHODS

Materials. Mancozeb standard was obtained from Rohm & Haas (Philadelphia, PA). ETU standard was obtained from Aldrich Co. (Milwaukee, WI). The stock solutions of mancozeb and ETU were prepared in distilled water at a concentration of 100 μg/100 mL. The standards were protected from light and stored in the refrigerator at 4 °C. Sodium thiosulfate, sodium sulfate, potassium iodide, and potassium indigo trisulfonate were all of reagent grade. All organic solvents used for preparation of stock solution and HPLC were of distilled-in-glass grade.

Methods. Solution studies were conducted in a model system to determine the effect of (1) ozone at two concentrations (1 and 3 ppm) and HPA at two concentrations (5 and 50 ppm); (2) three pH values of 4.6, 7.0, and 10.7; and (3) two temperatures of 10 °C and ambient (21 °C).

Aqueous solutions buffered at pH 4.6 (0.2 M sodium acetate), pH 7.0 (0.2 M sodium phosphate), and pH 10.7 (0.2 M carbonate−bicarbonate) were prepared. Degradation of mancozeb was studied over a 30 min period because the typical water contact time for apples in a commercial plant is ∼10−15 min and under normal conditions would rarely exceed 30 min. There were three replications per treatment.

A. Ozone and HPA Solution Preparation. For the ozonation study, ozone was bubbled through a glass sparger (i.e., bubbles of ∼10 mm i.d.) into 990 mL of distilled water at the appropriate temperature adjusted by a circulating water bath and the pH adjusted by the addition of standard buffer solutions under 25 psi at 15 standard cubic feet per hour (SCFH) of oxygen until the desired ozone concentration (1 or 3 ppm) was attained. One hundred milliliters of ozonated water was spiked with mancozeb to give a final concentration of 2 ppm. Ozone detection and monitoring were performed using the indigo colorimetric method as described in *Standard Methods for the Examination of Water and Wastewater* (*11*). All reagents were prepared just prior to use. The ozone concentrations were monitored before and after each sampling run. The ozonated water was collected into a 100 mL volumetric flask containing 10 mL of the indigo reagent to minimize loss of ozone. A separate volumetric flask was filled with distilled water containing 10 mL of indigo reagent to serve as a blank. The solutions were mixed thoroughly, and the absorbance of each solution was immediately measured at 600 nm in a 1 cm cell. The concentration of ozone, in

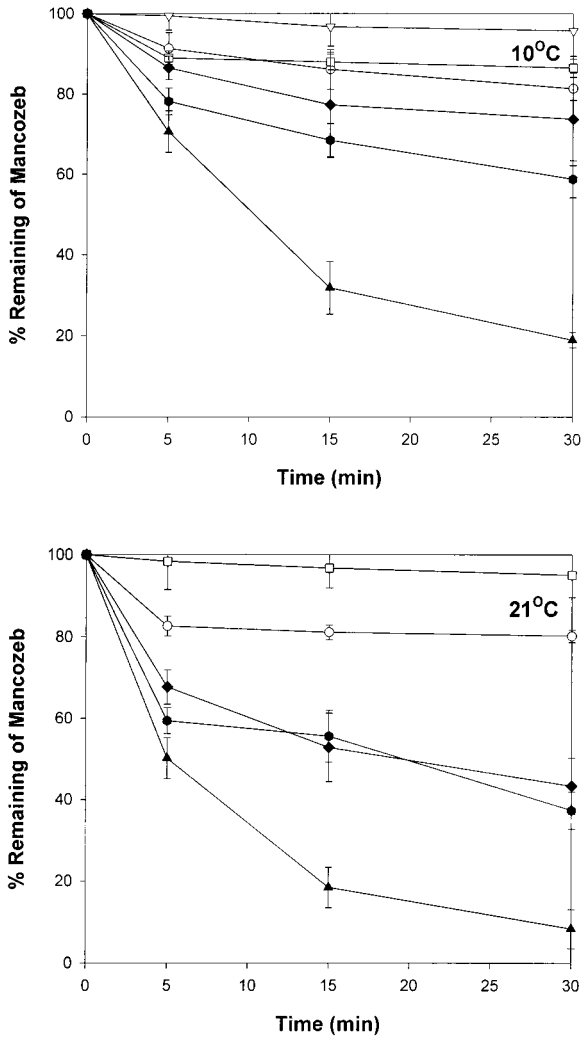

Figure 4. Effect of 5 ppm of HPA on the degradation of 2 ppm of mancozeb at 10 and 21 °C: (○) control, pH 4.6; (▽) control, pH 7.0; (□) control , pH 10.7; (◆) 5 ppm of HPA, pH 4.6; (▲) 5 ppm of HPA, pH 7.0; (●) 5 ppm of HPA, pH 10.7.

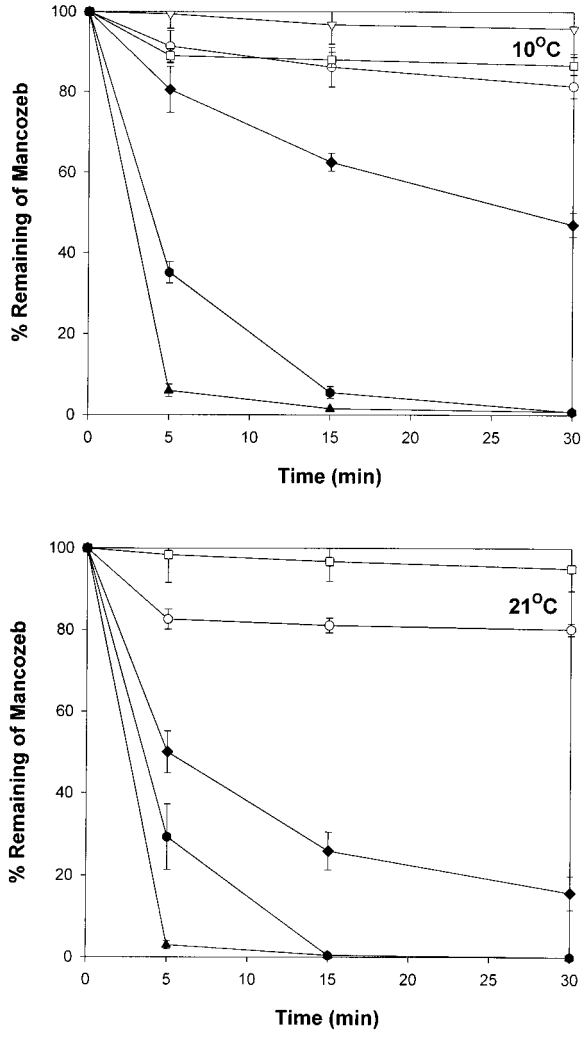

Figure 5. Effect of 50 ppm of HPA on the degradation of 2 ppm of mancozeb at 10 and 21 °C: (○) control, pH 4.6; (▽) control, pH 7.0; (□) control , pH 10.7; (◆) 50 ppm of HPA, pH 4.6; (▲) 50 ppm of HPA, pH 7.0; (●) 50 ppm of HPA, pH 10.7.

milligrams per liter, was calculated using the formula

$$\text{mg of } O_3/\text{L} = (1000A)/(fbV)$$

where A was the difference in absorbance between sample and blank solution, b was the path length (1 cm), V was the volume of the sample (90 mL), and f was a constant with a value of 0.42.

For HPA treatment, an appropriate amount of HPA stock solution was added to each pH solution to bring the final concentration to 5 or 50 ppm. Each pH solution was spiked with mancozeb stock solution to give a final concentration of 2 ppm. Total residual HPA was measured using a test kit (Ecolab Inc.).

B. Mancozeb and ETU Residue Analyses. Mancozeb residues were analyzed as carbon disulfide (CS_2) by gas−liquid chromatographic headspace analysis (*12*). Twenty milliliters of sample was transferred at 0, 5, 15, and 30 min intervals into sample bottles. A 0.1 M sodium thiosulfate solution was added to the samples at the appropriate time to quench the reaction. Forty milliliters of 1.5% stannous chloride in 5 M HCl was added and immediately sealed with a crimped septum. Fifty microliters of a 1 mg/mL thiophene solution was injected as an internal standard into each bottle and incubated at 70−80 °C in a water bath for 15 min. Bottles were removed and agitated for 2 min by hand. Bottles were replaced in the water bath with repeated shaking for 1 h. A 100 μL sample was removed with a gastight syringe from the bottle headspace and injected into the GC.

ETU residues were determined using a modification of the HPLC method published by Ahmad et al. (*12*). Twenty milliliters of sample was weighed into an Erlenmeyer flask, and then 8 g of potassium fluoride and 0.6 g of ammonium chloride were added. This mixture was extracted with 50 mL of dichloromethane two times. The dichloromethane layer was passed through a bed of 25 g of anhydrous sodium sulfate, collected in a round-bottom flask, and evaporated to dryness on an automated Zymark Turbovap evaporator at 40 °C. The residue was dissolved in 3 mL of distilled water, and 50 μL was injected into an HPLC column.

C. Chromatographic Analyses. Mancozeb residues as CS_2 were detected and quantified using a Hewlett-Packard series II 5890 gas chromatograph (GC) equipped with a flame photometric detector (FPD) in the sulfur mode. The GC was equipped with a Supel-Q-Plot fused silica capillary column (30 m long × 0.53 mm i.d.) with a film thickness of 0.25 μm (Supelco Inc., Bellefonte, PA). The oven temperature was 80 °C, and the injector and detector temperatures were 230 and 300 °C, respectively. Helium and nitrogen were used as the GC carrier gas and makeup gas, respectively. Carrier gas flow through the column was 20 mL/min. Integration was carried out with HP Chemstation software interfaced to the GC.

ETU residues were detected and quantified using a liquid chromatograph with a Hypersil BDS C_{18} column (250 mm × 4.6 mm, 5 μm particles), a Hypersil BDS C_{18} guard column

(10 mm × 4.6 mm, 5 μm particles), and a UV detector set at 240 nm. The mobile phase was 0.72% butylamine in distilled water at pH 3.0−3.2. An M-45 Waters HPLC pump (Waters Associates, Inc., Milford, MA) was used for solvent delivery at a flow rate of 0.5 mL/min. After the system was stabilized, 50 μL samples were injected.

The method of detection limits (MDL) for mancozeb and ETU were determined to be 0.01 and 0.005 μg/mL, respectively.

D. Statistical Analysis. All determinations were replicated three times. Means, standard deviations (SD), mean square errors (SE), two-factor ANOVA, correlation, and interaction of main effects were calculated using Sigma Stat computer software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using the Student−Newman−Keuls method for multiple comparisons. A $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Degradation of Mancozeb by Ozone. In the GC analysis, carbon disulfide appeared as a single sharp peak with a retention time of 5.1 min. Mancozeb was stable at pH 7.0 at both 10 and 21 °C with very little degradation due to hydrolysis; between 95 and 99% (at 10 °C) and between 95 and 97% (at 21 °C) residual mancozeb remained after 30 min. Mancozeb was relatively less stable at pH 4.6 and 10.7, with about 78 and 80% remaining, respectively, after 30 min at ambient temperature (Figure 2). This indicates mancozeb is less stable under basic and acidic conditions than neutral condition.

Degradation of mancozeb by ozone was greatest at pH 7.0 and decreased with increasing pH. The ozone treatment at pH 10.7 was the least effective at both 10 and 21 °C. Its degradation was only about 10 and 18% after 5 and 30 min, respectively, at 21 °C (Figures 2 and 3). In 1 ppm of ozone treatment, almost 96% of the initial amount of mancozeb was degraded after 30 min at pH 7.0 and ambient temperature (Figure 2). Ozonation at 3 ppm significantly ($p < 0.05$) increased the rate of degradation of mancozeb in pH 4.6 and 7.0 treatments at ambient temperature. Only ∼1% of mancozeb remained at pH 7.0 after 30 min at 21 °C. At pH 7.0, almost 65% of the initial amount of mancozeb was degraded after only 5 min in a 3 ppm of ozone concentration (Figure 3). Ozone degraded the mancozeb residues within the first 5 min. This has important implications for practical situations because the control time required to lower the concentration of any pesticide will affect cost. The most effective treatment was ozonation at 3 ppm in the pH 7.0 solution, whereas pH 10.7 was the least effective treatment.

Many factors govern the solubility of ozone in water, one being temperature. Ozone is partially soluble in water and, like most gases, increases in solubility as the water temperature decreases. The solubility of ozone in water is 0.003 g/L (3 ppm) at 20 °C (*13*). Dissolved ozone also decreases with increasing temperature, due to thermal decomposition (*14*), which could adversely affect the overall degradation process. In this study, two temperatures, 10 and 21 °C, were used.

Ozone has the property of autodecomposition, producing numerous free radical species, the most prominent in reaction with water being the hydroxyl radical ($OH^{\bullet}$). As the pH of solutions containing dissolved ozone increases, the rate of decomposition of molecular ozone to produce hydroxyl free radicals also increases, so that at a pH of ∼10, ozone decomposes rapidly (*5*). Kearney et al. (*15*) found that ozonation at high pH was fairly ineffective, due to its instability. This is due to the catalytic effect of hydroxyl ions on the ozone decomposition process. At pH 10, the half-life for ozone in pure water is ∼30 s (*16*). Therefore, an increase in pH reduced the effect of ozone on the degradation of mancozeb, whereas the effect of hydrolysis increased slightly.

Table 1. ETU Conversion from Samples Fortified with Mancozeb (after 15 min of Reaction Time)

treatment	pH	EBDC added (ppm)	ETU found[a] (ppb)	% conversion[b]
control	4.6	2.0	14.77 ± 0.91	0.74
	7.0	2.0	21.93 ± 1.11	1.10
	10.7	2.0	19.07 ± 0.75	0.95
1 ppm of O_3	4.6	2.0	ND[c]	
	7.0	2.0	7.60 ± 0.89	0.38
	10.7	2.0	9.53 ± 0.67	0.48
3 ppm of O_3	4.6	2.0	ND	
	7.0	2.0	ND	
	10.7	2.0	5.20 ± 0.44	0.26
5 ppm of HPA	4.6	2.0	ND	
	7.0	2.0	ND	
	10.7	2.0	8.10 ± 0.72	0.41
50 ppm of HPA	4.6	2.0	ND	
	7.0	2.0	ND	
	10.7	2.0	5.80 ± 0.56	0.29

[a] Means with the same superscript are not significantly different ($p > 0.05$); means ± standard deviations; $n = 3$ for all treatment. [b] Percent ETU conversion was calculated as (g of ETU/g of EBDC) × 100. [c] ND, none detected. This represents a value <5 ng/g, which is the detection limit for the method employed for ETU in solutions.

Degradation of Mancozeb by HPA. Maximum degradation of mancozeb by HPA was observed at pH 7.0 (Figures 4 and 5). In the 5 ppm of HPA treatment, between 50 and 70% of mancozeb remained after 5 min at both 10 and 21 °C at pH 7.0. Treatments at pH 4.6 and 10.7 were less effective than that at pH 7.0. Degradation of mancozeb at pH 7.0 at both 10 and 21 °C was significantly ($p < 0.05$) different from that at pH 4.6 (Figure 4). The HPA treatment at pH 4.6 was the least effective at both 10 and 21 °C with 45−75% degradation after 30 min. HPA treatment at 50 ppm for the degradation of mancozeb was much more effective than 5 ppm of HPA in all three pH treatments and at both temperatures. Also, increased temperature (i.e. 21 °C) caused complete degradation of mancozeb after 15 min in 50 ppm of HPA (Figure 5). HPA treatment at neutral pH was more effective than alkaline or acidic conditions. This related to the stability of HPA at various pH ranges.

Conversion of Mancozeb into ETU. Conversion of mancozeb into ETU was studied at 21 °C. In the HPLC analysis, ETU appeared as a peak with a retention time of 10.4 min. The degradation of mancozeb to ETU in solution due to hydrolysis, ozonation, and HPA treatment is shown in Table 1 and Figures 6 and 7. It was found that the rate of decomposition of mancozeb to ETU was influenced by pH. The total yield of ETU was decreased when the pH was lowered from 7.0 or 10.7 to 4.6. At pH 7.0, the initial ETU concentration was 17.3 ppb, which increased to 21.9 ppb after 15 min and then decreased to 12.3 ppb after 60 min. At pH 10.7, the initial ETU concentration was 15.0 ppb, which increased to 19.1 ppb after 15 min and then decreased to 12.0 ppb after 60 min. At pH 4.6, the initial ETU concentration

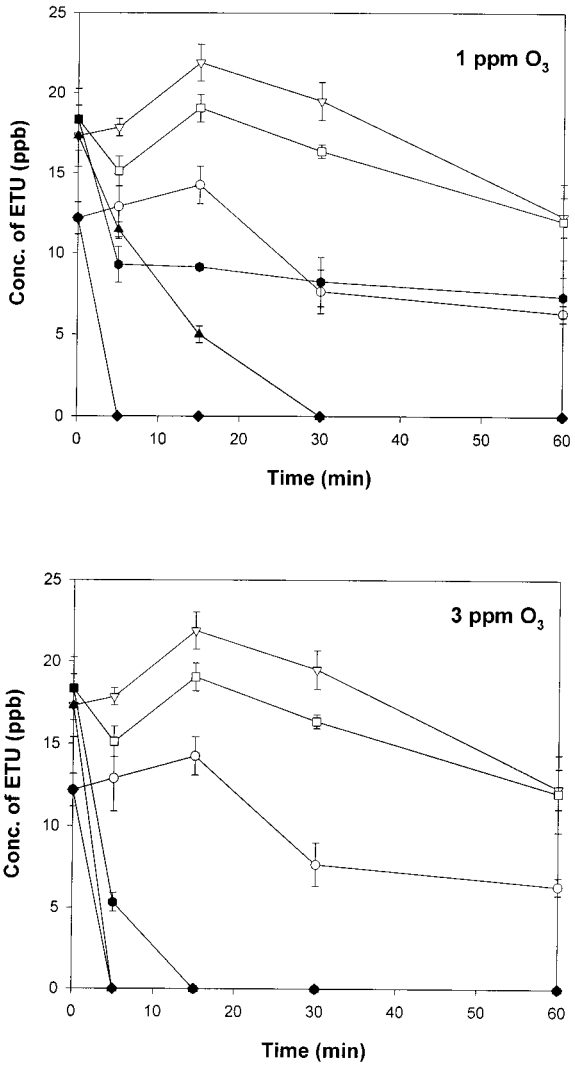

Figure 6. Effect of O_3 on the concentration of ETU with time at 21 °C: (○) control, pH 4.6; (▽) control, pH 7.0; (□) control , pH 10.7; (◆) O_3, pH 4.6; (▲) O_3, pH 7.0; (●) O_3, pH 10.7.

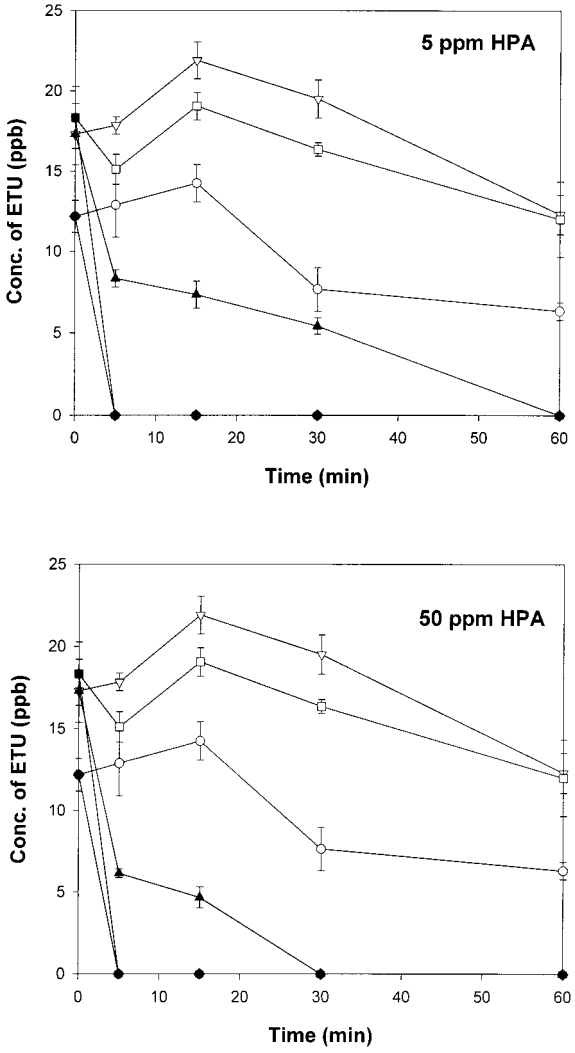

Figure 7. Effect of HPA on the concentration of ETU with time at 21 °C: (○) control, pH 4.6; (▽) control, pH 7.0; (□) control , pH 10.7; (◆) HPA, pH 4.6; (▲) HPA, pH 7.0; (●) HPA, pH 10.7.

was 11.9 ppb, which increased to 14.3 ppb after 15 min and then decreased to 5.3 ppb after 60 min. This indicates that an acidic pH of 4.6 was much more effective in reducing the conversion rate of mancozeb into ETU compared with neutral or alkaline pH. In the processing of fruits or vegetables, acidic treatment can be a preventative method in ETU production. Engst and Schnaak (*17*) reported that ethylenebisdithiocarbamic acid readily forms ETU under highly alkaline conditions (pH 10.5). As shown in Figures 6 and 7, conversion of mancozeb to ETU reached a maximum at 15 min of reaction time and then decreased for all three pH ranges. Ozone and HPA treatments were effective in reducing ETU residue levels. With 3 ppm of ozone at pH 4.6 and 7.0, ETU residues were below the detection limit. Degradation of ETU by HPA was greatest at pH 4.6, and no ETU residues remained after 5 min at both 5 and 50 ppm.

Conclusions. The objective of this study was to determine the effectiveness of ozone and HPA treatments on the dissipation of mancozeb and ETU in buffered solution. It was found that ozone and HPA treatments were effective in reducing or eliminating ETU residues as well as mancozeb. Mancozeb residues in model system solutions decreased by 40−95% with ozone treatment and by 50−95% with HPA treatment. The rate of degradation of mancozeb increased at neutral pH and high temperature. ETU residue was quickly degraded in acidic conditions in combination with ozone and HPA treatments. The best combination of temperature, pH, and HPA or ozone for the degradation of both mancozeb and ETU was developed.

LITERATURE CITED

(1) EPA. *Preliminary Risk Assessment*; EPA Env. Sci. Div. Reg. 5−85173535598; U.S. GPO: Washington, DC, 1989; pp 8−19.
(2) DuPont. *Effective Basic Disease Control*; DuPont Biochemical Department: Wilmington, DE, 1992.
(3) U.S. EPA. Ethylene bisdithiocarbamates (EBDCs); Notice of intent to cancel and conclusion of Special Review. *Fed. Regist.* **1992**, *57* (41), 7434−7530.
(4) Ong, K. C.; Cash, J. N.; Zabik, M. J.; Siddiq, M.; Jones, A. L. Chlorine and ozone washes for pesticide removal from apples and processed apple sauce. *Food Chem.* **1996**, *55*, 153−160.
(5) Graham, D. M. Use of ozone for food processing. *Food Technol.* **1997**, *51*, 72−75.
(6) FDA. Beverages: Bottled water; Final rule. *Fed. Regist.* **1995**, *60*, 57075−57130.
(7) Saper, G. M.; Simmons, G. F. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. *Food Technol.* **1998**, *52* (2), 48−52.

(8) Honnay, R. Process for improving the preservation of fresh vegetables and fruits. Eur. Patent 0255814, 1988.
(9) Forney, C. F.; Rij, R. E.; Denis-Arrue, R.; Smilanick, J. L. Vapor phase hydrogen peroxide inhibits postharvest decay of table grapes. *HortScience* **1991**, *26*, 1512−1214.
(10) Saper, G. M.; Miller, R. L.; Simmons, G. F. Effects of hydrogen peroxide treatment on fresh-cut fruits and vegetables. Presented at the Annual Meeting of the Institute of Food Technologists, Anaheim, CA, June 3−7, 1995.
(11) *Standard Methods for Examination of Water and Wastewater*, 17th ed.; American Public Health Association: New York, 1987; pp 162−165.
(12) Ahmad, N.; Guo, L.; Mandarakas, P.; Appleby, S. Determination of dithiocarbamate and its breakdown product ethylenethiourea in fruits and vegetables. *J. Assoc. Off. Anal. Chem*. **1995**, *78*, 1238−1243.
(13) *Harkin Atlas Ozone. Material Safety Data Sheet*; Harkin Ozone System: Mountainview, CA, 1999.
(14) Kearney, P. C.; Ruth, J. M.; Zeng, Q.; Mazzocchi, P. UV-Ozonation of paraquat. *J. Agric. Food Chem*. **1985**, *33*, 953−957.
(15) Kearney, P. C.; Muldoon, M. T.; Somich, C. J.; Ruth, J. M.; Voaden, J. Biodegradation of ozonated atrazine as a wastewater disposal system. *J. Agric. Food Chem*. **1988**, *36*, 1301−1306.
(16) Masten, S. J.; Davies, S. H. R. The use of ozonation to degrade organic contaminants in wastewaters. *Environ. Sci. Technol*. **1994**, *28*, 180A−185A.
(17) Engst, R.; Schnaak, W. Residues of dithiocarbamate fungicides and their metabolites on plant foods. *Residue Rev*. **1974**, *52*, 45−67.

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