JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Deodorization of Swine Manure Using Minced Horseradish Roots and Peroxides

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Public concerns about offensive odors from livestock manures are on the rise and so is the pressure to develop practical ways to reduce the odors. The use of minced horseradish (*Armoracia rusticana* L) roots (1:10 w/v plant tissue to swine slurry ratio), with calcium peroxide (CaO_2 at 26 or 34 mM) or hydrogen peroxide (H_2O_2 at 34, 52, or 68 mM) for the deodorization of swine manure, was evaluated through a series of laboratory experiments. The principle underlying this deodorization method is the oxidation of odorants by the concerted action of horseradish peroxidase (present in the plant tissue) and peroxide that serves as an electron acceptor, followed by polymerization of phenolic odorants with a possible copolymerization or adsorption of other odorant compounds. The deodorization effect was assessed by a human panel and gas chromatography (GC). In the case of the GC method, 12 compounds commonly associated with malodor (7 volatile fatty acids or VFAs, 3 phenolic compounds, and 2 indolic compounds) were used as odor indicators. Malodor assessment of the treated slurry by a human panel indicated a 50% reduction in odor intensity. GC results showed 100% removal of all phenolic odorants without reoccurrence for at least 72 h. In view of these data, using plant materials as enzyme carriers and peroxides as electron acceptors emerges as an effective approach to phenolic odor control in animal manure.

KEYWORDS: Volatile fatty acids; phenolic odorants; odor indicators; odor control; swine slurry; horseradish; peroxidase; peroxide

INTRODUCTION

Livestock manures are a nuisance for several reasons, one of which is offensive odors that they emit during storage, transportation, and farmland application, causing societal conflicts. Odor nuisance is generally defined by four factors: intensity, unpleasantness (offensiveness), frequency, and duration. In the past, farm animals were housed in spacious barns where straw bedding absorbed manure and/or they were kept outside, leaving their manure to decay in a pasture. The development of so-called confined animal feeding operations (CAFOs) necessitated complex management systems for the collection, storage, transport, and disposal of animal manure. Farm malodors are among the major problems associated with manure management from CAFOs. Recent evaluation of air emissions from animal feeding operations (AFOs) by the U.S. Environmental Protection Agency (EPA) and the U.S. Department of Agriculture (USDA) (*1*) indicated that odor is primarily of concern in terms of human life quality and of the major importance at local scales (e.g., property line or nearest dwelling). The public, legislators, and environmental regulators have become increasingly concerned with odor because it creates a major threat to the viability and growth of animal industries. As a consequence, reducing the impact of odors on the surrounding community is becoming an essential part of managing livestock enterprises.

Odor sources of livestock production systems include buildings, manure storage, and land application of manure. Almost 50% of all odor complaints are traced back to farmland application of manure and about 45% to animal facilities and manure storage units (*2*). A variety of techniques were proposed

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10.1021/jf0404290 CCC: $30.25
Published on Web 05/19/2005

to control livestock manure odors, ranging from aeration to diet modifications and to the application of manure additives (*3*), but none of these techniques proved to be entirely satisfactory.

Our previous studies (*4*−7) demonstrated that minced horseradish (*Armoracia rusticana* L) roots, potato (*Solanum tuberosum* L) tubers, and white radish (*Raphanus sativus* L) roots combined with small amounts of hydrogen peroxide (H_2O_2) or calcium peroxide (CaO_2) can remove toxic phenols from water and soil due to the activity of peroxidase present in the plant tissue. Horseradish, which contains large quantities of this enzyme, was found to be the most efficient plant material when used for the treatment of an industrial wastewater (*4*). In the study of Roper et al. (*5*), horseradish showed high decontamination potential toward a variety of phenolic contaminants, including pentachlorophenol, phenol, and *p*-cresol.

The underlying phenomenon of horseradish treatment involved enzymatic oxidation of phenolic compounds, leading to the formation of reactive phenoxyl radicals; the subsequent coupling of the oxidation products was completed without further involvement of peroxidase. Through this so-called oxidative coupling, the contaminants were transformed to less toxic polymers or underwent binding to soil organic matter, both of which were expected to reduce the toxicity and mobility of the parent compounds.

Since phenols that were target pollutants in the above-discussed decontamination investigations are also known as major odorants in swine slurry, the objective of this study was to evaluate the application of horseradish treatment to odor removal. Specifically, the study was aimed at evaluating the immediate (after 2 h) and post-treatment (after 24 and 72 h) effectiveness of minced horseradish roots in reducing the concentrations of three phenolic compounds (phenol, *p*-cresol, and *p*-ethylphenol), seven volatile fatty acids or VFAs (*n*-butyric acid, *n*-caproic acid, isobutyric acid, isocaproic acid, isovaleric acid, propionic acid, *n*-valeric acid), and two indolic compounds (indole and skatole). These target chemicals were chosen because they were found to be positively correlated with malodors from animal manure (*8*).

MATERIALS AND METHODS

Swine Manure Treatment with Horseradish and Peroxides. Horseradish roots (HR) were bought at a local vegetable market and stored at 4 °C until used. Immediately before the experiments, they were washed with water and cut into pieces using a laboratory blender. CaO_2 (powder, 75% w/w) and H_2O_2 (35% solution) were purchased from Sigma-Aldrich (St. Louis, MO).

Swine manure slurry samples were collected from a concrete swine slurry storage pit (capacity: 150 000 L) at the Swine Center operated by the Department of Dairy and Animal Science at The Pennsylvania State University. The samples were collected after at least half an hour of mixing (i.e., homogenization) of slurry in the storage pit.

Samples of swine slurry (30 mL) were distributed in 125 mL Erlenmeyer flasks and amended with 3 g of minced horseradish roots (HR) (10% w/v or 1:10 HR to swine slurry ratio), and the reaction was initiated with the addition of a specified amount of peroxides (P) in the form of either H_2O_2 (HP) or CaO_2 (CP). Six combinations of the reaction components were used as treatments and controls: HR&CP [30 mL of swine slurry + 3 g of HR + 34 or 26 mM CP], HR&HP [30 mL of swine slurry + 3 g of HR + 34, 52, or 68 mM HP], CP [30 mL of swine slurry + 26 or 34 mM CP], HP [30 mL of swine slurry + 34, 52, or 68 mM HP], HR [30 mL of swine slurry + 3 g of HR], and NoHR&P [30 mL of swine slurry with no HR and peroxide].

If not specified otherwise, the treatment time was 2 h. The samples were incubated statically with only occasional hand swirling at 25 °C in the dark. In an initial experiment involving 10% horseradish and 34 mM H_2O_2, the samples were analyzed by a panel of trained odor evaluators (sniffing). During three subsequent experiments, gas chromatography (GC) measurements were used to evaluate changes in the concentration of odor indicators. The first two experiments were run under the same conditions except for the concentration of peroxides (34 mM H_2O_2 or CaO_2; and 68 mM H_2O_2 or 34 mM CaO_2, respectively). They were carried out under a completely random design in which treatments and controls were randomly assigned in triplicate to 30 mL swine slurry samples in 125 mL Erlenmeyer flasks. In the third experiment that assessed the effect of post-treatment on odor concentration, the samples were incubated for 2, 24, and 72 h. In this experiment, a 6 × 3 factorial design with six deodorization treatments (HR&CP, HR&HP, HR, HP, CP, NoHR&P) and three time periods (2, 24, and 72 h) was run to assess the post-treatment effect. **Table 1** presents a list of all the experiments carried out in this study.

Evaluation of Odor Changes by a Human Panel. The effect of horseradish treatment on odor perception was estimated according to the procedure developed and validated by Green and Flammer (*9*) and Green et al. (*10*) and utilized by Heinemann et al. (*11*), Wood and Wheeler (*12*), and Wheeler et al. (*13*). Briefly, the panel consisted of six trained evaluators from whom the identities of the samples were withdrawn and who independently recorded their estimates of odor intensity and pleasantness using qualitative scales on a set of computer displays (*9*−*11*). The scale for odor intensity ranged from strongest odor imaginable to very strong, strong, moderate, weak, and no odor, and that for odor pleasantness ranged from extremely unpleasant to neutral and extremely pleasant. Having been recorded, the estimates were electronically assigned with numerical values, ranging from 100 (strongest odor imaginable) to 0 (no odor) and from −11 (most unpleasant) to +11 (most pleasant) (*10*). The samples were presented to panelists in a random order (sniffing order) 2 h after horseradish treatment (30 mL of swine slurry + 3 g of HR + 34 mM H_2O_2). All panelists evaluated each sample three times (sniffing replication by panelist) during individual sessions.

Odorant Extraction and Quantification. By use of the modified procedure of Ohta and Ikeda (*14*), 10 mL aliquots of the slurry sample were withdrawn from the incubation flask and acidified with 2.0 mL of 1 M HCl. The odorants were extracted (for 4 h at 4 °C) into a 2.5 mL layer of diethyl ether placed on the top of the acidified slurry and quantified by gas chromatography using a Hewlett-Packard 5890 chromatograph with a flame ionization detector (FID) and an HP G1030A ChemStation controller. The injection volume was 1 μL. The separation of the compounds was achieved using a RESTEK capillary column: Rtx-1 (30 m, 0.32 mm i.d., 4.0 μm df). The GC-FID conditions are presented in **Table 2**.

The extracted odorants were tentatively identified on the basis of the identity of their retention times with the retention times of 12 chemicals that served as malodor indicators. The standards of these chemicals (propionic acid, isobutyric acid, *n*-butyric acid, isovaleric acid, *n*-valeric acid, isocaproic acid, *n*-caproic acid, phenol, *p*-cresol, *p*-ethylphenol, indole, and skatole) were purchased from Sigma-Aldrich (St. Louis, MO). External standard calibration procedure was used. Briefly, primary stock standard solutions for each odorant were prepared in methanol using pure reagents. A composite stock standard solution was then prepared by mixing individual primary stock standard solutions and diluting them with diethyl ether. By use of the composite stock standard solution, triplicate calibration standards were prepared at five different concentrations.

Calibration curves for each malodor indicator were obtained by a linear regression of the detector response (i.e., peak area versus the concentration of the calibration standard). Where no signal was detectable in the ether extract from swine slurry samples, the absence of the compound was assumed. The retention times (min), R^2 values of calibration curves, percent odorant recoveries, and precision of odorant measurements are given in **Table 3**.

Statistical Analysis. One-way analysis of variance (one-way ANOVA) was used to test the equality of means of the deodorization treatments using the SAS statistical package (*15*). After the *F* test in the ANOVA was found to be significant, Fisher's (protected) least significant difference (LSD) test (*16*) at 5% significance level was applied to determine which treatment mean values were significantly different. The main effects and first order (two-factor) interactions

Table 1. List of All Experiments Involving Swine Slurry, Horseradish, and Peroxides (P)

treatment	description
	Initial Experiment with Samples Analyzed by Panel of Trained Odor Evaluators
NoHR&P	30 mL of swine slurry, 2 h static incubation
HR	30 mL of swine slurry + 3 g of HR, 2 h static incubation
HP	30 mL of swine slurry + 34 mM HP, 2 h static incubation
HR&HP	30 mL of swine slurry + 3 g of HR + 34 mM HP, 2 h static incubation
	First Experiment with Samples Analyzed by Gas Chromatography
NoHR&P	30 mL of swine slurry, 2 h static incubation
HR	30 mL of swine slurry + 3 g of HR, 2 h static incubation
HP	30 mL of swine slurry + 34 mM HP, 2 h static incubation
CP	30 mL of swine slurry + 34 mM CP, 2 h static incubation
HR&HP	30 mL of swine slurry + 3 g of HR + 34 mM HP, 2 h static incubation
HR&CP	30 mL of swine slurry + 3 g of HR + 34 mM CP, 2 h static incubation
	Second Experiment with Samples Analyzed by Gas Chromatography
NoHR&P	30 mL of swine slurry, 2 h static incubation
HR	30 mL of swine slurry + 3 g of HR, 2 h static incubation
HP	30 mL of swine slurry + 68 mM HP, 2 h static incubation
CP	30 mL of swine slurry + 34 mM CP, 2 h static incubation
HR&HP	30 mL of swine slurry + 3 g of HR + 68 mM HP, 2 h static incubation
HR&CP	30 mL of swine slurry + 3 g of HR + 34 mM CP, 2 h static incubation
	Third Experiment with Samples Analyzed by Gas Chromatography
NoHR&P	30 mL of swine slurry, 2, 24, and 72 h of static incubation
HR	30 mL of swine slurry + 3 g of HR, 2, 24, and 72 h of static incubation
HP	30 mL of swine slurry + 52 mM HP, 2, 24, and 72 h of static incubation
CP	30 mL of swine slurry + 26 mM CP, 2, 24, and 72 h of static incubation
HR&HP	30 mL of swine slurry + 3 g of HR + 52 mM HP, 2, 24, and 72 h of static incubation
HR&CP	30 mL of swine slurry + 3 g of HR + 26 mM CP, 2, 24, and 72 h of static incubation

Table 2. GC Conditions Used for Determining the Concentration of the 12 Malodor Indicators (VFAs, Phenols, and Indoles) in Swine Manure Extracts

injector temp	220 °C
column temp parameters	initial temp, 35 °C for 1 min first gradient, 15 °C/min for 7.67 min and held at 150 °C for 2 min second gradient, 25 °C/min for 2.4 min to 210 °C final temp, 210 °C for 5 min total time elapsed – 27 min
detector temp	220 °C
flow rates	He column flow, 6.8 mL/min; split flow, 37.1 mL/min; split ratio, 6.0; H_2, 30 mL/min; air, 400 mL/min

Table 3. Quality Assessment Parameters for the GC Method Used in This Study

test odorants	mean retention times (min) ($n = 4$)	mean R^2 values for calibration curves ($n = 4$)	mean recovery of odorants (%) from spiked swine slurry extracts ($n = 3$)	mean precision (%) for odorant measurements in swine slurry extracts ($n = 3$)
propionic acid	5.649	0.9997	99.5	98.5
isobutyric acid	6.726	0.9997	109.5	97.9
n-butyric acid	7.159	0.9987	107.0	97.9
isovaleric acid	8.043	0.9985	110.5	98.1
n-valeric acid	8.656	0.9981	101.5	99.0
isocaproic acid	9.836	0.9957	102.5	99.0
n-caproic acid	10.284	0.9983	112.5	96.9
phenol	10.656	0.9972	98.5	100.0
p-cresol	12.298	0.9967	95.5	98.0
p-ethylphenol	13.577	0.9981	95.5	98.5
indole	15.626	0.9974	99.0	106.1
skatole	17.506	0.9970	100.5	99.5

between treatments and incubation time were analyzed using the generalized linear model (GLM) procedures of SAS (*15*).

RESULTS

Evaluation of Odor Changes by a Human Panel after Horseradish Treatment. The average odor intensity and pleasantness values from a human panel that evaluated the swine slurry samples treated with 10% (w/v) horseradish and 34 mM H_2O_2 (HR&HP) are presented in **Figure 1**. The odor intensity of untreated sample (NoHR&P) was about 34 units, while that for the HR&HP treatment was about 18 units out of a possible 100 units (the strongest odor imaginable). This indicated a decrease in the intensity of the malodor of about 50% (**Figure 1**). Unpleasantness of the odor was also reduced: from −6 units for the control to −3 units for the HR&HP treatment on a scale from −11 units (most unpleasant) to 11 units (most pleasant). Some reductions in odor intensity (20−30%) and unpleasantness

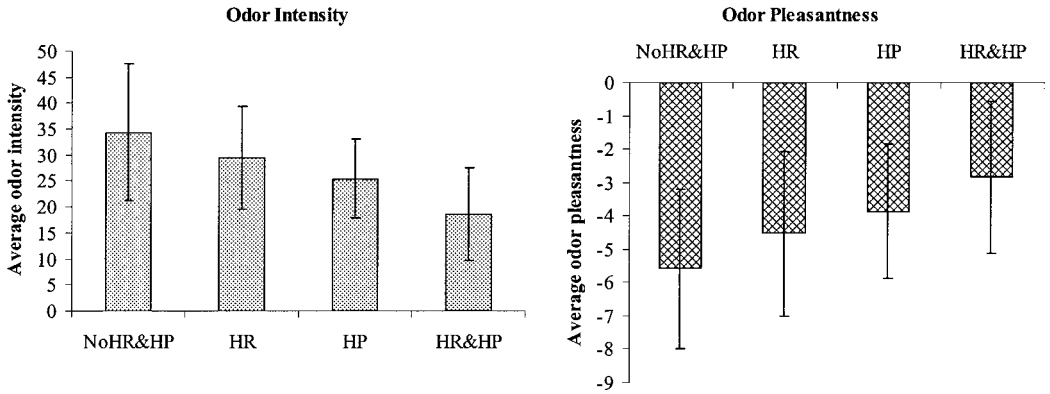

Figure 1. Odor intensity and pleasantness of swine slurry samples after 2 h treatment with minced horseradish roots (1:10 plant tissue to slurry ratio) and hydrogen peroxide (34 mM) at 25 °C: NoHR&HP = nontreated swine slurry control; HR = horseradish (control); HP = hydrogen peroxide (control); HR&HP = full treatment.

Table 4. Concentration (mg L^{-1}) of Indoles, Phenols, and Volatile Fatty Acids in Swine Slurry Treated with 10 % w/v Horseradish and Peroxides (P): 34 mM H_2O_2 or CaO_2[a]

	analysis of variance[b]		treatment mean comparisons,[c] mg L^{-1}						
compd	% of total variation due to treaments	probability of significant *F* test	NoHR&P	CP	HP	HR	HR&CP	HR&HP	LSD ($\alpha = 0.05$)[d]
				Indolic Compounds					
indole	NS	0.0753	1.01	1.06	0.98	1.77	<0.50	1.45	
skatole	NS	0.7997	1.45	2.04	1.67	1.71	1.93	2.12	
				Phenolic Compounds					
phenol	81	0.0005	2.22 A	2.01 A	1.90 A	1.36 A	<0.50 B	<0.50 B	0.97
p-cresol	96	<0.0001	3.28 A	2.95 AB	2.92 AB	2.83 AB	<0.50 C	2.71 B	0.52
p-ethylphenol	96	<0.0001	1.50 B	1.89 A	1.57 B	1.64 AB	<0.50 C	1.55 B	0.26
				Volatile Fatty Acids					
isobutyric acid	NS	0.8367	10.01	8.80	9.96	9.25	12.38	10.84	
isocaproic acid	83	0.0003	<2.00 B	<2.00 B	<2.00 B	<2.00 B	4.73 A	7.68 A	3.00
isovaleric acid	NS	0.8042	15.35	15.39	15.43	15.38	17.42	15.15	
n-butyric acid	NS	0.9104	49.54	45.07	48.52	46.52	53.79	50.82	
n-caproic acid	85	0.0003	<2.00 B	<2.00 B	2.79 B	<2.00 B	10.34 A	<2.00 B	3.80
n-valeric acid	NS	0.5515	12.61	13.16	12.28	12.06	15.47	12.48	
propionic acid	59	0.0354	69.34 A	47.48 C	67.55 AB	65.02 AB	51.37 BC	72.81 A	17.05

[a] NoHR&P = nontreated swine slurry control; CP = calcium peroxide (control for HR&CP); HP = hydrogen peroxide (control for HR&HP); HR = horseradish (control for HR&HP and HR&CP); HR&CP = full treatment using CP; HR&HP = full treatment using HP. [b] $N = 6$ treatments × 3 replicates per compound = 18 observations. [c] Mean values in a row with the same letter are not significantly different ($\alpha = 0.5$). [d] Treatment mean separation using Fisher's LSD at $\alpha = 0.5$ was done only when the *F* test was significant at the ≤5% probability level.

(from −6 to −5 or −4) occurred in swine slurry samples amended only with HR or HP.

Horseradish Treatment Experiments Involving Odorant Measurements by gas Chromatography. *Hoseradish Treatment Using 34 mM of H_2O_2 or CaO_2.* The concentration mean (mg L^{-1}) of three replicates for each compound in treated and control swine slurry samples and results of analysis of variance and mean comparisons are given in **Table 4**. All but two odorants (isocaproic and *n*-caproic acid) were present in the swine slurry samples as shown for untreated samples (**Table 4**, NoHR&P, fourth column). The concentrations of the odorants in untreated samples ranged from less than the detection limit to about 69 mg L^{-1}. Overall, the greatest amount of odorants in the untreated slurry were VFAs with a total concentration of 157 mg L^{-1}, followed by phenolic compounds (7 mg L^{-1}) and indolic compounds with (2.5 mg L^{-1}).

Analysis of variance revealed that treating swine slurry with 10% (w/v) horseradish and 34 mM H_2O_2 or CaO_2 had no significant effect on the concentration of indolic compounds as evidenced by the *F* test at $\alpha = 0.05$ (**Table 4**). There were significant treatment effects on three VFAs: isocaproic ($p < 0.0003$), *n*-caproic ($p < 0.0003$), and propionic acid ($p < 0.0354$). However, the initial concentrations of isocaproic acid and *n*-caproic acid were too low (less than the detection limit) to be of significance. Thus, the main notable effects on VFAs were those of HR&CP and CP on propionic acid, which was significantly reduced (by 25% and 31%, respectively) as compared to the control (NoHR&P). The HR&HP was not effective in reducing any of the VFAs. Unlike indolic compounds and VFAs, over 80% of the variation in concentration of phenolic odorants was attributed to treatment effects (**Table 4**). The effect of HR&CP on phenolic compounds was very impressive in that it resulted in a complete, 100%, removal of phenol, *p*-cresol, and ethylphenol (detection limit of <0.50 mg L^{-1}). The HR&HP treatment significantly reduced the concentration of phenolic compounds and also completely removed phenol. The significant effects of specific treatments on odorant concentrations compared can be ordered as follows:

HR&CP (<0.50 mg L^{-1}) = HR&HP (<0.50 mg L^{-1}) > NoHR&P (2.22 mg L^{-1}) for phenol,

HR&CP (<0.50 mg L^{-1}) > HR&HP (2.71 mg L^{-1}) > NoHR&P (3.28 mg L^{-1}) for *p*-cresol,

HR&CP (<0.50 mg L^{-1}) > HR&HP (1.55 mg L^{-1}) = NoHR&P (1.50 mg L^{-1}) for *p*-ethylphenol,

Table 5. Concentration (mg L^{-1}) of Indoles, Phenols, and Volatile Fatty Acids in Swine Slurry Treated with 10 % Horseradish and Peroxides (P): 68 mM H_2O_2 or 34 mM CaO_2[a]

	analysis of variance[b]		treatment mean comparisons,[c] mg L^{-1}						
compd	% of total variation due to treaments	probability of significant *F* test	NoHR&P	CP	HP	HR	HR&CP	HR&HP	LSD ($\alpha = 0.05$)[d]
				Indolic Compounds					
indole	59	0.0383	1.54 AB	0.50 BC	0.97 ABC	1.75 A	0.50 BC	<0.50 C	1.13
skatole	NS	0.3105	2.26	2.04	1.44	1.65	3.99	1.34	
				Phenolic Compounds					
phenol	81	<0.0001	2.28 BC	2.77 AB	2.81 A	2.20 C	<0.50 D	<0.50 D	0.52
p-cresol	93	<0.0001	3.45 A	3.68 A	3.68 A	2.95 A	<0.50 B	<0.50 B	1.02
p-ethylphenol	95	<0.0001	1.70 B	2.25 A	1.94 AB	1.76 B	<0.50 C	<0.50 C	0.46
				Volatile Fatty Acids					
isobutyric acid	68	0.0092	16.61 AB	7.80 CD	17.66 A	10.05 BCD	5.19 D	13.26 ABC	6.70
isocaproic acid	64	0.0195	<2.00 B	2.54 B	<2.00 B	<2.00 B	2.63 B	7.86 A	4.61
isovaleric acid	NS	0.1009	16.94	15.02	18.28	15.31	13.77	16.21	
n-butyric acid	70	0.0065	80.74 AB	47.95 CD	85.78 A	57.61 BCD	37.82 D	69.02 ABC	24.26
n-caproic acid	NS	0.1466	<2.00	<2.00	<2.00	<2.00	6.00	3.14	
n-valeric acid	NS	0.1403	14.80	13.80	16.47	13.27	12.52	14.33	
propionic acid	85	<0.0001	98.07 A	42.06 C	106.60 A	71.27 B	30.29 C	85.19 AB	25.57

[a] NoHR&P = nontreated swine slurry control; CP = calcium peroxide (control for HR&CP); HP = hydrogen peroxide (control for HR&HP); HR = horseradish (control for HR&HP and HR&CP); HR&CP = full treatment using CP; HR&HP = full treatment using HP. [b] $N = 6$ treatments $\times$ 3 replicates per compound = 18 observations. [c] Mean values in a row with the same letter are not significantly different ($\alpha = 0.5$). [d] Treatment mean separation using Fisher's LSD at $\alpha = 0.5$ was done only when the *F* test was significant at the $\leq$5% probability level.

HR&CP (51.37 mg L^{-1}) > HR&HP (72.81 mg L^{-1}) = NoHR&P (69.34 mg L^{-1}) for propionic acid.

The results showed that at equal concentrations of CaO_2 and H_2O_2 (34 mM), HR&CP was more effective than HR&HP in reducing the concentration of malodor indicators in swine slurry.

Horseradish Treatment with 68 mM H_2O_2 and 34 mM CaO_2. **Table 5** shows the results of an experiment in which the conditions were the same as in the experiment described in the previous section except that swine slurry samples were from a different batch and H_2O_2 concentration was doubled (i.e., increased from 34 to 68 mM) to see if odorant removal would be comparable to that achieved using 34 mM CaO_2. As in the previous experiment, isocaproic and *n*-caproic acid were not detected in the untreated samples (**Table 5**). The initial concentrations of the odorants in untreated samples of this batch of swine slurry were higher than in the previous experiment. They ranged from less than the detection limit to about 100 mg L^{-1}. The difference was mainly due to increased concentrations of VFAs: from a total of 157 mg L^{-1} in the previous experiment to a total of 230 mg L^{-1} in this one. The concentrations of indolic and phenolic compounds were essentially the same in both experiments.

Analysis of variance in this experiment showed that deodorization treatments had significant effects on the concentration of 7 (out of 12) odorants as indicated by the *F* test. The three compounds whose concentrations were significantly reduced in this experiment but not in the previous one were indole ($p < 0.0383$), isobutyric acid ($p < 0.0092$) and *n*-butyric acid ($p < 0.0065$). Increasing H_2O_2 concentration in HR&HP treatment from 34 to 68 mM seemed to have had the greatest effect on the concentration of indole, phenol, *p*-cresol, and *p*-ethylphenol because they were all reduced to below the detection limit (**Table 5**). It appears that doubling the concentration of H_2O_2 in HR&HP treatment not only enhanced the removal of indole but also made the treatment as effective as HR&CP in removing phenolic compounds from swine slurry. This study also showed an increased effectiveness of HR&CP in reducing propionic acid. In the previous experiment it reduced the odorant concentration by 25% compared to the NoHR&P, whereas in this experiment it reduced the concentration by about 70% (from 98 to 30 mg L^{-1}). The significant effects of specific treatments on odorant concentrations can be ordered as follows:

HR&HP (<0.50 mg L^{-1}) = HR&CP (0.50 mg L^{-1}) > NoHR&P (1.54 mg L^{-1}) for indole,

HR&CP (<0.50 mg L^{-1}) = HR&HP (<0.50 mg L^{-1}) > NoHR&P (2.2 mg L^{-1}) for phenol,

HR&CP (<0.50 mg L^{-1}) = HR&HP (<0.50 mg L^{-1}) > NoHR&P (3.28 mg L^{-1}) for *p*-cresol,

HR&CP (<0.50 mg L^{-1}) = HR&HP (<0.50 mg L^{-1}) > NoHR&P(1.50 mg L^{-1}) for *p*-ethylphenol,

HR&CP (5.19 mg L^{-1}) > HR&HP (13.26 mg L^{-1}) = NoHR&P (69.34 mg L^{-1}) for isobutyric acid,

HR&CP (37.82 mg L^{-1}) > HR&HP (69.02 mg L^{-1}) = NoHR&P (80.74 mg L^{-1}) for *n*-butyric acid,

HR&CP (30.29 mg L^{-1}) > HR&HP (85.19 mg L^{-1}) = NoHR&P (98.07 mg L^{-1}) for propionic acid.

Overall Effects of Horseradish Treatment. The overall effects of horseradish treatment on total concentrations of indolic, phenolic, and VFAs odorants in the two experiments using 34 or 68 mM H_2O_2 are presented in **Figure 2**. There were no significant decreases in total indolic compounds as shown in parts **a** and **b** of **Figure 2**, although the effect on indolic odorants by HR&HP treatment at the 68 mM H_2O_2 level is notable in **Figure 2b**. For total phenols, HR&HP at 68 mM H_2O_2 was 100% effective (parts **c** and **d** of **Figure 2**). The graphs showing total VFAs (parts **e** and **f** of **Figure 2**) reveal that HR&HP had no effect, but HR&CP had the potential to reduce the concentrations of VFAs. This was mostly due to the effectiveness of HR&CP in reducing propionic acid that accounted for about 43% of total VFAs in the swine slurry used in each of the two experiments (**Tables 4** and **5**). To summarize, the analysis of the concentrations of individual or total indolic, phenolic. and VFAs odorants clearly revealed that doubling the H_2O_2 in HR&HP from 34 to 68 mM made the treatment just as effective as HR&CP (with 34 mM CaO_2) in reducing phenolic compounds in swine slurry. Furthermore, the HR&CP and CP significantly reduced the concentration of propionic acid.

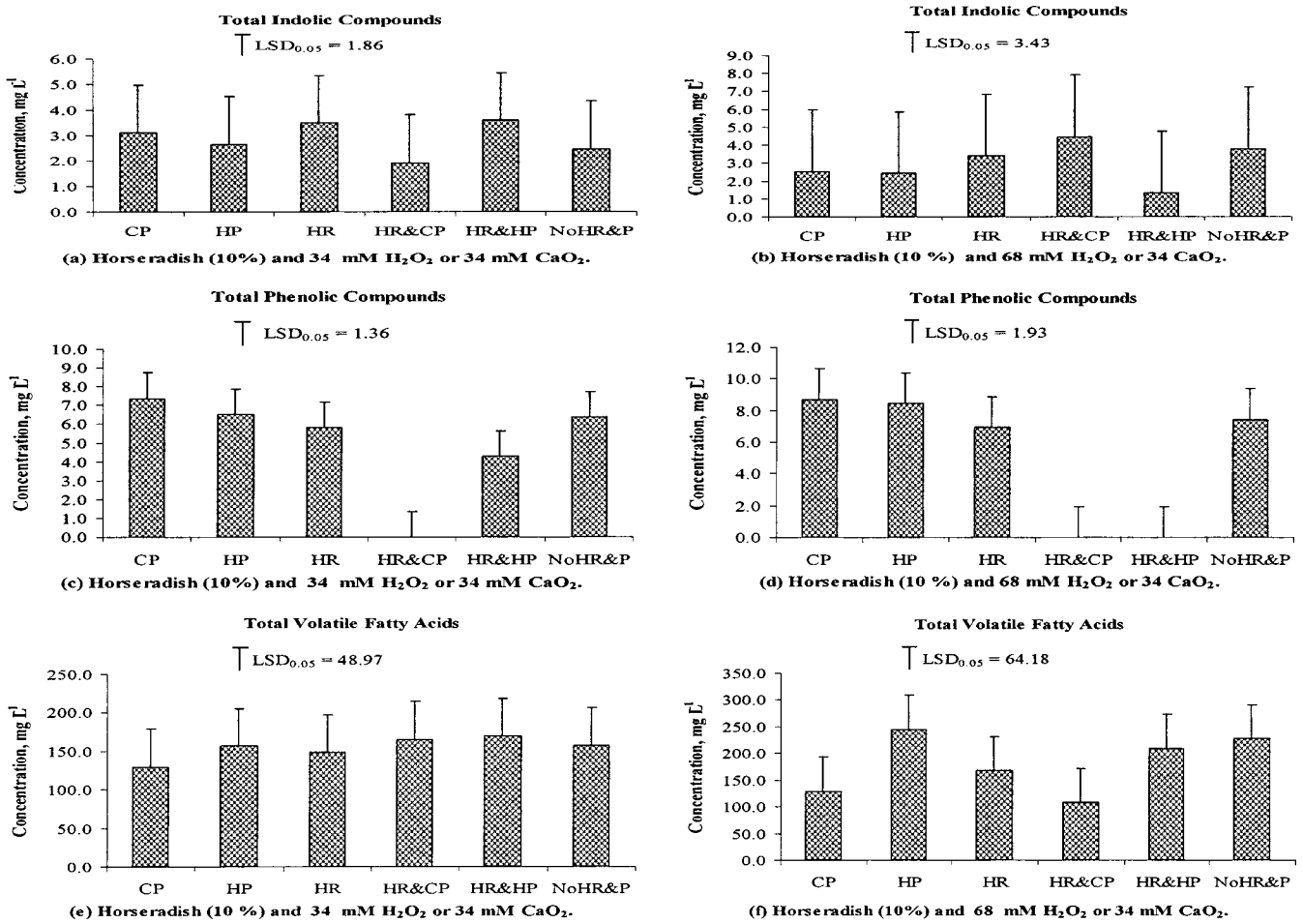

Figure 2. Concentration (mg L^{-1}) of indolic and phenolic compounds and volatile fatty acids in swine slurry (30 mL) treated with horseradish (3 g) and/or peroxides (P): CP = calcium peroxide (control for HR&CP); HP = hydrogen peroxide (control for HR&HP); HR = horseradish (control for HR&HP and HR&CP); HR&CP = full treatment using CP; HR&HP = full treatment using HP; NoHR&P = nontreated swine slurry control

Effects of Post-Treatment Time on Odorant Concentrations after Horseradish Treatment. This experiment investigated the effect of post-treatment time on the concentration of odorants after the slurry samples were incubated with horseradish and peroxides for 2, 24, and 72 h at 25 °C and then analyzed for odorant concentrations. At the same time, we tested the effect of reducing peroxide concentration to 26 mM CaO_2 and 52 mM H_2O_2 (one-third less than in the second of previous experiments).

The concentration range of the odorants in the swine slurry used in this experiment was similar to the previous experiment (below the detection limit to 100 mg L^{-1}). However, this batch contained *n*-caproic acid, unlike previous batches. The analysis of variance revealed that the post-treatment time significantly affected the concentrations of all odorants as indicated by the significant *F* test for odorants that were present in the swine slurry samples (**Table 6**). The treatments (CP, HP, HR HR&CP, HR&HP, NoHR&P) showed significant effects on 6 of the 10 odorants that were present in swine slurry. As shown by mean comparisons of concentrations (**Table 6**), reducing the peroxides in the HR&HP and HR&CP caused them to be less effective. For example, phenolic compounds were not completely removed as in the previous experiment and HP&CP was ineffective in decreasing propionic acid.

Time by horseradish treatment interactions had significant effects on 9 of the 10 odorants (**Table 6**). The interactions accounted for the greatest percentage of total variation for phenol (32%), *n*-butyric acid (32%), *n*-valeric acid (43%), and propionic acid (34%) (**Table 6**, column 2). The interaction results are illustrated in **Figures 3** and **4** showing concentrations for each odorant by treatment and by post-treatment time. From **Figures 3** and **4**, it can be seen that concentrations of odorants after the 72 h period were the lowest for all compounds (indole and isocaproic acid were not present in the slurry) in the nontreated (NoHR&P) samples. **Figures 3** and **4** also show that odorant concentration results after 2 and 24 h incubation periods were not significantly different within each horseradish treatment. Post-treatment time did not have an effect on phenolic compounds (*p*-cresol, *p*-ethylphenol, and phenol) treated with HR&CP.

To summarize, there were two most significant findings in this experiment. First, reducing the concentration of CaO_2 to 26 mM (from 34 mM in the previous two experiments) and that of H_2O_2 to 52 mM (from 68 mM) reduced the effectiveness of the HR&HP and HR&CP treatments. Second, there were no reoccurrences of phenolic compounds after they were removed in the initial 2 h in samples treated with HR&CP.

DISCUSSION

The human panel results indicated a 50% reduction in odor intensity and a 3-unit reduction in unpleasantness when swine slurry samples were treated with HR&HP at the 34 mM H_2O_2 level (**Figure 1**). The next three laboratory experiments showed that HR&HP treatment was only effective in reducing the concentration of phenolic compounds. Therefore, the results of malodor assessment of swine slurry treated with HR&HP by a

Table 6. Effect of Post-Treatment Time on the Concentration (mg L^{-1}) of Odorant Indicators in Swine Slurry Treated with 10 % w/v Horseradish and Peroxides (P): 52 mM H_2O_2 or 26 mM CaO_2[a]

	analysis of variance[b]		treatment mean comparisons,[c] mg L^{-1}									
source	% of total variation	probability of significant *F* test	2 h	24 h	74 h	CP	HP	HR	HR&CP	HR&HP	NoHR&P	LSD ($\alpha = 0.05$)[d]
indole												
time	NS	0.5386	<0.50	<0.50	<0.50							
HRTs[e]	NS	0.3283				<0.50	<0.50	<0.50	<0.50	<0.50	<0.50	
time × HRTs	NS	0.5823										
skatole												
time	8	0.0291	1.46 A	1.66 A	0.82 B							0.51
HRTs	36	<0.0001				1.94 BA	1.54 BC	0.83 DC	<0.50 D	0.72 DC	2.57 A	0.72
time × HRTs	21	0.0505										
p-cresol												
time	27	<0.0001	5.30 A	3.18 B	1.61 C							1.19
HRTs	32	<0.0001				4.20 A	3.92 A	4.19 A	<0.50 C	2.21 B	5.40 A	1.67
time × HRTs	17	0.0166										
p-ethylphenol												
time	13	0.0024	2.67 A	2.47 A	1.18 B							0.87
HRTs	37	<0.0001				3.13 A	2.53 AB	1.90 BC	<0.50 D	1.26 DC	3.60 A	1.23
time × HRTs	NS	0.0754										
phenol												
time	15	0.0004	1.23 B	1.94 A	0.45 C							0.68
HRTs	25	0.0003				2.51 A	0.91 BCD	1.11 BC	<0.50 D	0.80 DC	1.82 AB	0.97
time × HRTs	32	0.0009										
isobutyric acid												
time	31	<0.0001	21.50 A	20.19 A	9.53 B							4.41
HRTs	NS	0.0616				15.40	14.32	19.64	13.12	18.32	21.70	
time × HRTs	28	0.004										
isocaproic acid												
time	NS	0.2701	<2.00	<2.00	<2.00							
HRTs	NS	0.4301				<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	
time × HRTs	NS	0.4701										
isovaleric acid												
time	30	<0.0001	18.63 A	17.53 A	7.98 B							4.16
HRTs	12	0.0425				15.69 AB	13.11 B	14.29 B	11.19 B	13.28 B	20.73 A	5.88
time × HRTs	26	0.0105										
n-butyric acid												
time	10	0.008	94.44 A	82.66 A	58.60 B							22.27
HRTs	23	0.0015				56.47 C	57.76 C	110.39 A	62.57 C	106.81 AB	77.39 BC	31.50
time × HRTs	32	0.0035										
n-caproic acid												
time	41	<0.0001	8.75 A	6.17 B	1.97 C							1.94
HRTs	NS	0.0775				6.85	4.65	6.16	3.90	4.71	7.50	
time × HRTs	22	0.0142										
n-valeric acid												
time	13	0.0028	18.74 A	14.79 AB	11.05 B							4.21
HRTs	NS	0.0775				14.15	10.78	19.24	12.30	16.46	16.16	
time × HRTs	43	0.0003										
propionic acid												
time	14	0.0001	100.58 A	89.10 A	53.41 B							20.26
HRTs	30	<0.0001				39.18 D	62.94 CD	131.38 A	75.3 BC	96.75 B	74.65 BC	28.66
time × HRTs	34	<0.0001										

[a] CP = calcium peroxide (control for HR&CP); HP = hydrogen peroxide (control for HR&HP); HR = horseradish (control for HR&HP and HR&CP); HR&CP = full treatment using CP; HR&HP = full treatment using HP; NoHR&P = nontreated swine slurry control. [b] $N = 6$ treatments × 3 replicates per time interval = 18 observations. [c] Mean values in a row with the same letter are not significantly different ($\alpha = 0.5$). [d] Treatment mean separation using Fisher's LSD at $\alpha = 0.5$ was done only when the *F* test was significant at the ≤5% probability level. [e] HRTs = horseradish-based treatments [CP, HP, HR, HR&CP, HR&HP, NoHR&P].

human panel implied that a significant decrease in the concentration of phenolic compounds (*p*-cresol, *p*-ethylphenol, and phenol) was directly related to the decrease of odor intensity and unpleasantness. Schaefer (*17*) found that odor intensity in air of swine facilities correlated best with *p*-cresol. Spoelstra (*18*) considered *p*-cresol and VFAs to be the most suitable indicators of odor emitted from swine manure. Williams (*19*) found that phenols, indoles, and VFAs correlated well with odor offensiveness of aerobically stored swine manure. Studies by the group at the National Swine Center (*8*, *20*) suggested that C2 through C9 organic acids, such as VFAs and phenolic compounds, represented a large proportion of the malodor associated with swine slurry odor released into the atmosphere. Phenolic compounds were found to be correlated with odor intensity in dairy manure (*21*) or as possible indicators of microbial community changes in swine manure storage systems (*22*). Therefore, the 100% removal of phenolic compounds at 68 mM H_2O_2 and 34 mM CaO_2 represents a technology that could make people notice a reduction in offensive odors from swine slurry treated with horseradish and peroxides. The results from the human panel also confirmed what the GC-based results showed, that the removal of phenolic odorants can be achieved within 2 h of swine slurry treatment. This is particularly important for practical purposes. The swine slurry could be treated just immediately (2 h) before land application.

The results showed that the concentration of indolic compounds and VFAs in treated swine slurry was not as successfully reduced as was that of phenolic compounds. It was also found that at equal concentrations (34 mM), HR&CP was more effective than HR&HP in reducing the concentration of phenolic compounds (**Table 4**). Increasing the concentration of H_2O_2 to 68 mM made the HR&HP treatment as effective as HR&CP

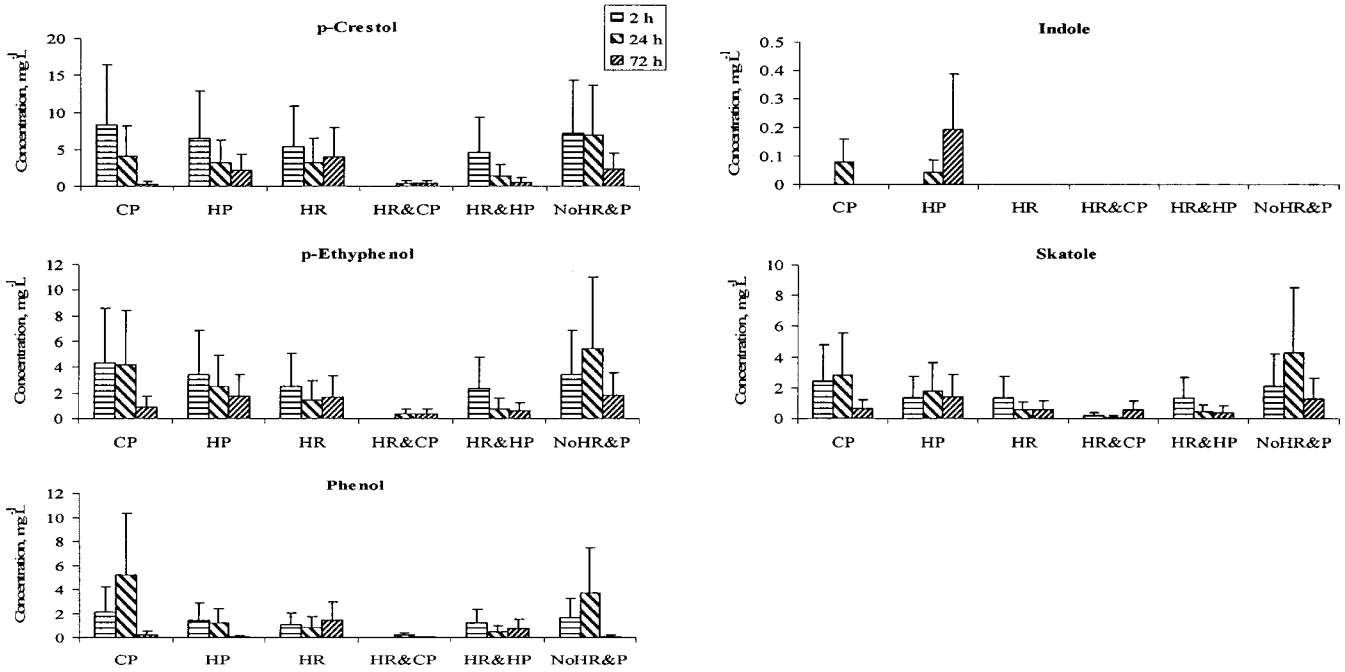

Figure 3. Concentration (mg L^{-1}) of indolic (indole and skatole) and phenolic (*p*-crestol, *p*-ethylphenol, and phenol) compounds in swine slurry (30 mL) 2, 24, and 72 h after treatment with horseradish (3 g) and/or peroxides (P): CP = calcium peroxide (26 mM) (control for HR&CP); HP = hydrogen peroxide (52 mM) (control for HR&HP); HR = horseradish (control for HR&HP and HR&CP); HR&CP = full treatment using CP; HR&HP = full treatment using HP; NoHR&P = nontreated swine slurry control.

(**Table 5**). Flanders et al. (*6*), who investigated horseradish-mediated binding of ^{14}C-labeled 2,4-dichlorophenol to soil based on residual radioactivity in soil after sequential methanol extraction, found that horseradish-mediated binding was enhanced by a factor of 2 when CaO_2 was used instead of H_2O_2. One of the proposed explanations for this enhancement was a slow release of H_2O_2 (from CaO_2 powder) to the aqueous phase of the soil. This may explain the better performance of horseradish treatment with CaO_2 than with H_2O_2 when peroxides were used at the lower of two concentrations (34 vs 68 mM). Hydrogen peroxide is known to decompose quickly once in contact with dissolved or particulate matter, including metals and dust.

It is not surprising that the concentration of VFA and indolic odorants (**Tables 4** and **5**, **Figure 2**) was relatively unaffected, unlike that of phenolic odorants. Phenolic compounds are generally preferred substrates over indolic compounds and VFAs for horseradish peroxidase. Previous research indicated that more reactive horseradish peroxidase substrates should enhance the transformation of less reactive compounds (*23*, *24*). According to this idea, when more reactive phenolic compounds are oxidized by horseradish peroxidase, the resulting phenoxyl radicals, in turn, would react with less reactive indolic compounds and VFAs. However, even if phenoxyl free radicals are produced by the oxidation of phenolic compounds, swine manure may contain large amounts of organic matter that could intercept the free radicals or inhibit radical reaction with indolic compounds and VFAs. It is well-known that natural organic matter could serve as the major sink for hydroxyl free radicals (*25*), and the same may apply to phenoxyl radicals. Also, Lindsey and Tarr (*26*) showed that humic and fulvic acids inhibited hydroxyl radical degradation of aromatic compounds such as phenol and *o*-cresol; however, they suggested that the most likely reason for the observed inhibition was not due to scavenging of the hydroxyl radical by natural organic matter but due to decreased reactivity of aromatic compounds through the partitioning to natural organic matter. When Wu et al. (*27*) determined (after treatment of swine manure by ozone) the concentration of the same odorants as those monitored in our study, they found that chemical oxygen demand (COD), as a gross measure of organic matter content, was not affected by ozone and that ozone could significantly remove phenolic and indolic odorants but not VFAs.

A post-treatment storage of swine slurry treated with horseradish would be a necessity in the highly congested livestock operations. Therefore, it was essential to find out whether delays in the disposal of treated slurry may or may not reverse the deodorization effect caused by horseradish/peroxide treatment. In view of the results obtained, horseradish treatment with HR&CP and HR&HP reduced the concentration of phenolic odorants within 2 h, and this deodorizing effect lasted for up to 72 h (**Figure 3**). The VFAs concentration was not as effectively reduced by horseradish treatments (HR&HP and HR&CP) as that of phenolic odorants; however, on average, their concentrations did not significantly increase with the time of storage (**Figure 4**). This stability of the deodorization effect may give the farm operators a more flexible time frame to dispose the treated slurry by collecting manure from storage tanks, transporting it, and applying it to the field.

Almost 50% of all odor complaints are traced back to land application of manure, and about 45% are from animal facilities and manure storage units (*2*). Especially in states such as Pennsylvania, water regulations governing minimum setback distances for manure land application and storage facilities recommend that the manure be mechanically incorporated within 24 h of application to protect water sources from contamination through erosion (*28*). Furthermore, incorporation of manure within 24 h is a recommended soil fertility practice to reduce nitrogen loss through volatilization.

The fact that even VFAs decreased with time irrespective of the ineffectiveness of deodorization treatment is consistent with what Powers et al. (*21*) found in their study. With an experimental setup somewhat similar to that used in this study, Powers et al. (*21*) incubated 100 mL of dairy manure waste-

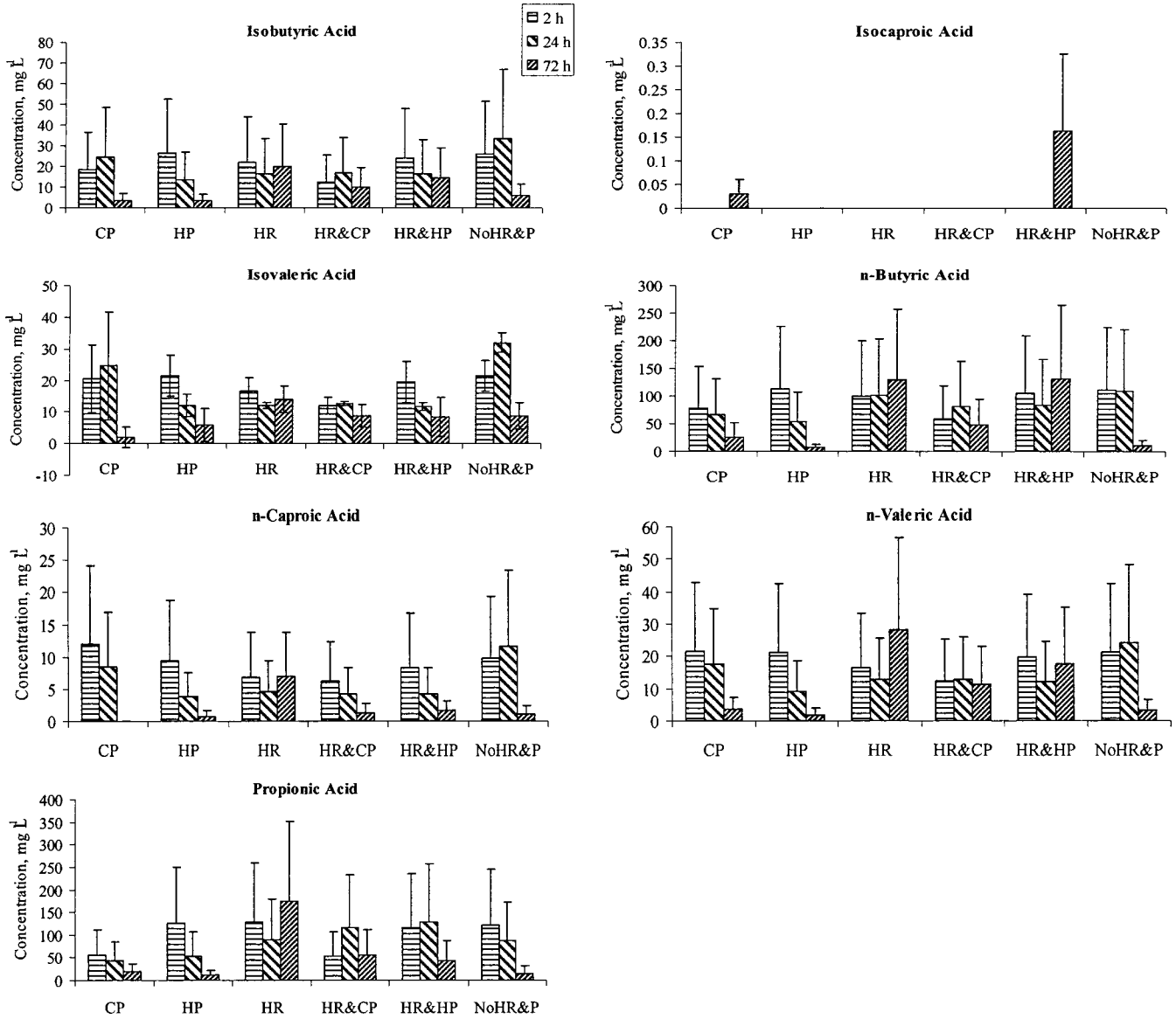

Figure 4. Concentration (mg L^{-1}) of volatile fatty acids in swine slurry (30 mL) 2, 24, and 72 h after treatment with horseradish (3 g) and/or peroxides (P): CP = calcium peroxide (26 mM) (control for HR&CP); HP = hydrogen peroxide (52 mM) (control for HR&HP); HR = horseradish (control for HR&HP and HR&CP); HR&CP = full treatment using CP; HR&HP = full treatment using HP; NoHR&P = nontreated swine slurry control.

waters in an Erlenmeyer flask for 3 days at room temperature (22−24 °C) without any amendments. When they evaluated the odor intensity and odorant concentrations of manure after 1, 2, and 3 days of storage, the odor intensity and the concentration of phenol and total VFAs decreased over time, which is consistent with our results.

The use of horseradish with small amounts of either H_2O_2 or CaO_2 serving as electron acceptors is a novel technique for reducing the concentration of odorants in swine slurry. The results of this study demonstrated that horseradish treatment is particularly effective in removing phenolic odorants. At equal concentrations in swine slurry, CaO_2 is more effective than H_2O_2 in reducing phenolic compounds. The deodorization effect is long-lasting, and that may give farm operators a more flexible time frame for the disposal of treated slurry by collecting manure from storage tanks, transporting it, and applying it to the field. In addition to a 100% deodorization effect on phenolic odorants, horseradish plus CaO_2 shows potential to reduce concentration of volatile fatty acids. Use of horseradish with small amounts of CaO_2 as a deodorization technique has added benefits, such as liming effect and supply of calcium for plant production. Studies are underway to apply this technique on a larger scale. More work is required to find ways to increase the removal of indolic odorants and volatile fatty acids from swine slurry.

We thank David Hosterman and his team at the Penn State Swine Center for assistance in collecting the swine slurry samples.

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Received for review October 14, 2004. Revised manuscript received April 8, 2005. Accepted April 9, 2005. Financial support for this research from The Commonwealth of Pennsylvania Department of Agriculture (Grant PDA ME 441830, Enzymatic Deodorization of Swine Manure) is greatly appreciated.

JF0404290