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Odor Control in Waste Management Lagoons via Reduction of p-Cresol using Horseradish Peroxidase

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Abstract. *Para-cresol, p-ethyl phenol and isovaleric acid have been identified as the most persistent and most significant contributors to odor 1.6 km downwind from waste lagoons in concentrated animal feeding operations (CAFOs), even though more than 200 volatile compounds contribute to odor from these lagoons. Previous studies have indicated that horseradish peroxidase (HRP) coupling with p-cresol generates a high yield of a unique product, Plummerer's ketone. Our preliminary laboratory studies with synthetic p-cresol, hydrogen peroxide, and HRP demonstrated that p-cresol can be reduced significantly in a hydrogen peroxide catalyzed reaction with HRP. Using GC/MS and SPME technologies, a 75% reduction was observed in relative concentrations of 100 ml 1% p-cresol after treatment with 1 ml of 170 units of HRP (prepared at 170 units/mg and 1 mg/ml) in the presence of 3% hydrogen peroxide. Further analyses using semi- volatile organic analyses-GC/MS shows a 41% reduction in the amount of p-cresol in the samples treated with HRP in the presence of hydrogen peroxide. Data obtained from these preliminary studies demonstrate that HRP has the potential to reduce the p-cresol content in CAFO effluent and has been a basis for further studies.*

Keywords. p-cresol, p-ethyl phenol, horseradish peroxidase, odor, feedyard, CAFO

Introduction

Odor emission from waste lagoons at concentrated animal feeding operations (CAFOs) has been a source of concern for some time. Though more than 200 volatile compounds contribute to odor from these CAFOs, p-cresol, p-ethyl phenol and isovaleric acid have been identified as the most persistent and biggest contributors to odor more than 1.6 km downwind of the source (Auvermann et al., 2001; Wright et al., 2004).

Para-cresol (p-cresol or 4-methyl phenol) is a naturally occurring metabolic product formed by bacteria under anaerobic conditions. Such conditions exist in the rumen or in the digestive tract of non-ruminants and substantial amounts of p-cresol are excreted by animals (Kerina et al., 1993).

Horseradish peroxidase (HRP) is widely used as a reporter enzyme in colorimetric and flourimetric assays (Ryan et al., 1994). HRP has been used extensively in studies of the peroxidase-catalyzed oxidation of a variety of electron donors with hydrogen peroxide. It also catalyzes the oxidative coupling and further polymerization of phenols (Dordick et al., 1987; Joo et al., 1997). Whereas the phenols generate a complex mixture of products by random coupling of resonance tautomers, HRP coupling of p-cresol generates a high yield of 2,2 biphenol derivative (Holland, 1992). Our preliminary studies examine the effect of horseradish peroxidase on the polymerization of p-cresol. While some experiments have been carried out using horseradish roots to reduce odor in swine feedlots (Govere et al., 2005), we are of the opinion that the use of horseradish peroxidase will be a more economical, environmental friendly, and applicable approach to the odor problem.

Para-cresol

Para-cresol is an organic molecule which is a naturally occurring metabolic product being formed by bacteria under anaerobic conditions. Para-cresol, p-ethyl phenol, isovaleric acid, 2-amino acetophenone, indole and skatole have been recognized as compounds characterized by relatively low volatility, high polarity and extreme high potency in odor assays (Wright et al., 2004). Phenol and p-cresol are frequently used as odor indicators in odor studies (Bourgue et al., 1987). However, p-cresol may be preferred to phenol as an odor indicator for two main reasons. First, p-cresol is foul smelling, whereas phenol has a sweet and rather pleasant odor (Yu et al., 1990). Second, the odor recognition level of phenol is 0.059 ppm (59 ppb), whereas p-cresol can be recognized at concentrations of 0.0019 ppm (1.9 ppb) (Yasuhara et al., 1984).

Horseradish Peroxidase

Horseradish peroxidase (HRP) is isolated from horseradish roots (Amoracia rusticana) and belongs to the ferroprotoporphyrin group of peroxidases. It consist of a heme prosthetic group, 2 Ca^{2+} and 308 amino acid residues, four disulphide bridges, and 8 neutral carbohydrate side-chain (Welinder, 1979). The carbohydrate composition consists of galactose, arabinose, xylose, fucose, mannose, mannosamine and galactosamine depending on the specific isoenzyme and organism producing the enzyme(Shannon et al., 1966).

The molecular weight of HRP is approximately 44000 Daltons, which includes the polypeptide chain of 33,890 Daltons, heme plus Ca^{2+} (approximately 700 Daltons), and carbohydrate (9400 Daltons) (Welinder, 1978). HRP has at least seven isoenzymes, with an isolectric point ranging from 3.0-9.0 (Shannon et al. 1966, Welinder, 1979). The optimum pH of HRP is in the range of 6.0 to 6.5; activity at 7.5 is 84% of the maximum. The enzyme is most stable in the pH range of 5.0 to 9.0 (Schomberg et al, 1996). The following are enzyme inhibitors of HRP, including

sodium azide, cyanide, L-cystine, dichromate, ethylenethiourea, hydroxylamine, sulfide, vanadate, p-aminobenzoic acid, Cd^{+}, CO^{2+}, Cu^{2+}, Fe^{3+}, Mn^{3+}, Ni^{2+}, Pb^{2+} (Zollner, 1993).

HRP has been used extensively in studies of the peroxidase catalyzed oxidation of a variety of electron donors with hydrogen peroxide (Joo et al., 1998). It also catalyzes the oxidative coupling and further polymerization of phenols (Dordick et al.; 1987, Joo et al., 1997).

Materials and Methods

Horseradish peroxidase isolated from horseradish roots (*Amoracia rusticana*) along with synthetic p-cresol and 3% hydrogen peroxide were used in these studies, based on knowledge acquired on the properties of these compounds.

Experiment No. 1

In the first experiment, 100 ml of 1 mM p-cresol was poured into a 500 ml volumetric flask, 1 ml of 3% hydrogen peroxide was applied, and then 1.2 ml of 1 mg/ml HRP was added. The volumetric flask was covered with a thin plastic film to create a headspace, and this was left for an hour before samples were collected from the headspace. Two other volumetric flasks were also prepared with the same concentration and volume of p-cresol, one was treated with only hydrogen peroxide, while the other was treated with only HRP. A control set up containing only p-cresol was also prepared, this sample received no treatment. After an hour, the relative concentrations of p-cresol in the headspace were measured by inserting a solid phase microextraction (SPME) fiber through the plastic film. The SPME fiber was analyzed using gas chromatography/mass spectrometry (GC/MS). This technology measures the amount of p-cresol in the gas phase.

Experiment No. 2

Further analysis was done using the semivolatile organic analyses by GC/MS in a commercial laboratory (ASK Laboratories, Amarillo, TX). Four 100 ml samples of 10 mM p-cresol were prepared. Sample 1 was treated with 1 ml hydrogen peroxide, sample 2 was treated with 1.2 ml of 1mg/ml HRP, sample 3 was treated with 1 ml hydrogen peroxide and 1.2 ml HRP, and the fourth sample received no treatment and served as a control. This analysis was carried out using the solvent extraction method with methylene chloride. After the extraction, the samples were analyzed by the GC/MS spectrophotometer to determine the quantity of p-cresol in each sample.

Results and Discussion

Experiment No. 1

Results obtained from the analysis using a GC/MS and SPME technologies showed a 75% reduction in p-cresol content of samples treated with HRP and hydrogen peroxide. Samples treated with only HRP or only hydrogen peroxide showed no significant difference from the control, which illustrates that HRP reduces p-cresol in the presence of hydrogen peroxide.

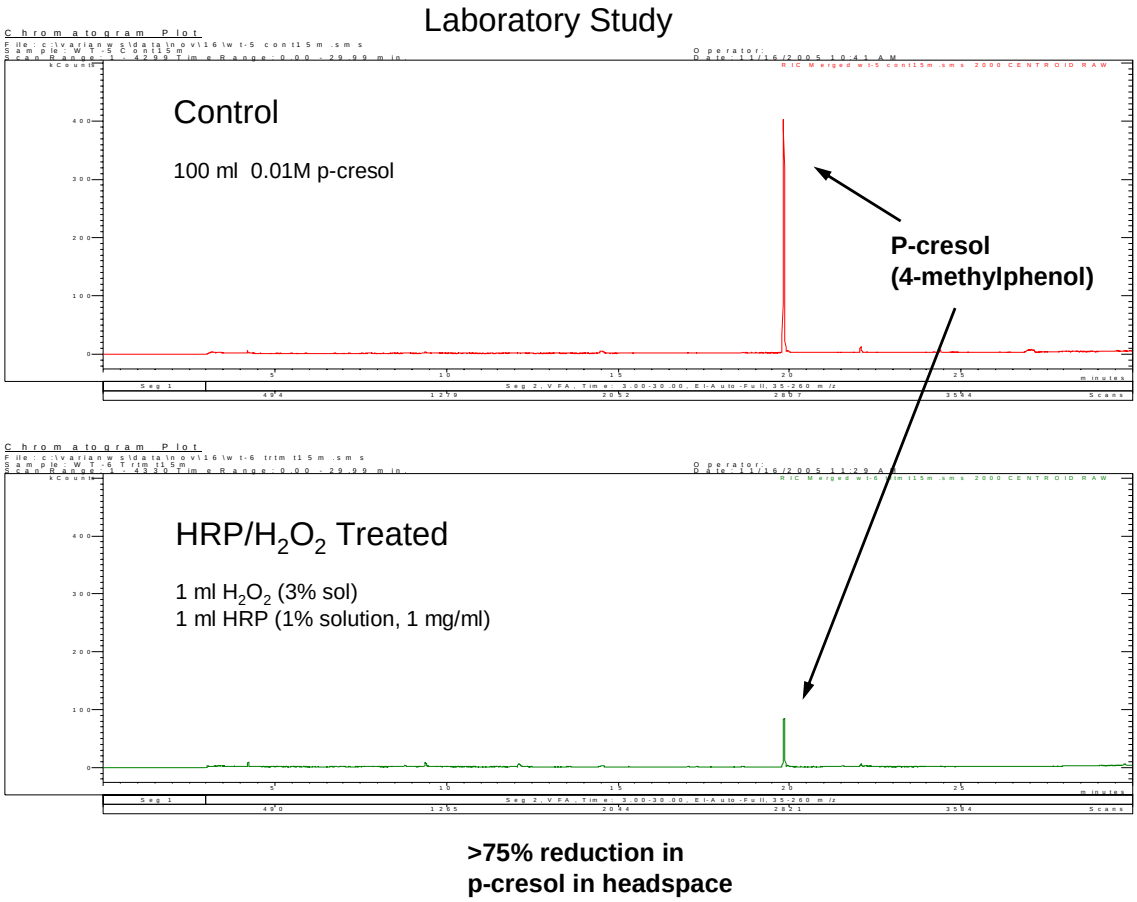

Figure 1. Chromatographs from Experiment No. 1 comparing the relative p-cresol concentrations in the sample treated with HRP/hydrogen peroxide to the control sample. A 75% reduction in the p-cresol concentration in the headspace was observed with the HRP treatment.

Experiment No. 2

Results from the extraction method showed a 41% reduction in p-cresol in samples treated with HRP and hydrogen peroxide while sample treated with only HRP and sample treated with only hydrogen peroxide showed no significant difference from the control (Table 1, Figure 2). This result supports our initial experiments using the GC/MS and SPME technologies which shows that the reaction between HRP and p-cresol occur in the presence of hydrogen peroxide.

Table 1. GC/MS spectrophotometer results showing amount of p-cresol (mg/ml) in the liquid phase of each of the samples analyzed.			
Test Description	Result	Units	Date Analyzed
p-cresol	852	mg/l	05/25/06
p-cresol + H_2O_2	873	mg/l	05/25/06
p-cresol + HRP	863	mg/l	05/25/06
p-cresol + HRP + H_2O_2	510	mg/l	05/25/06

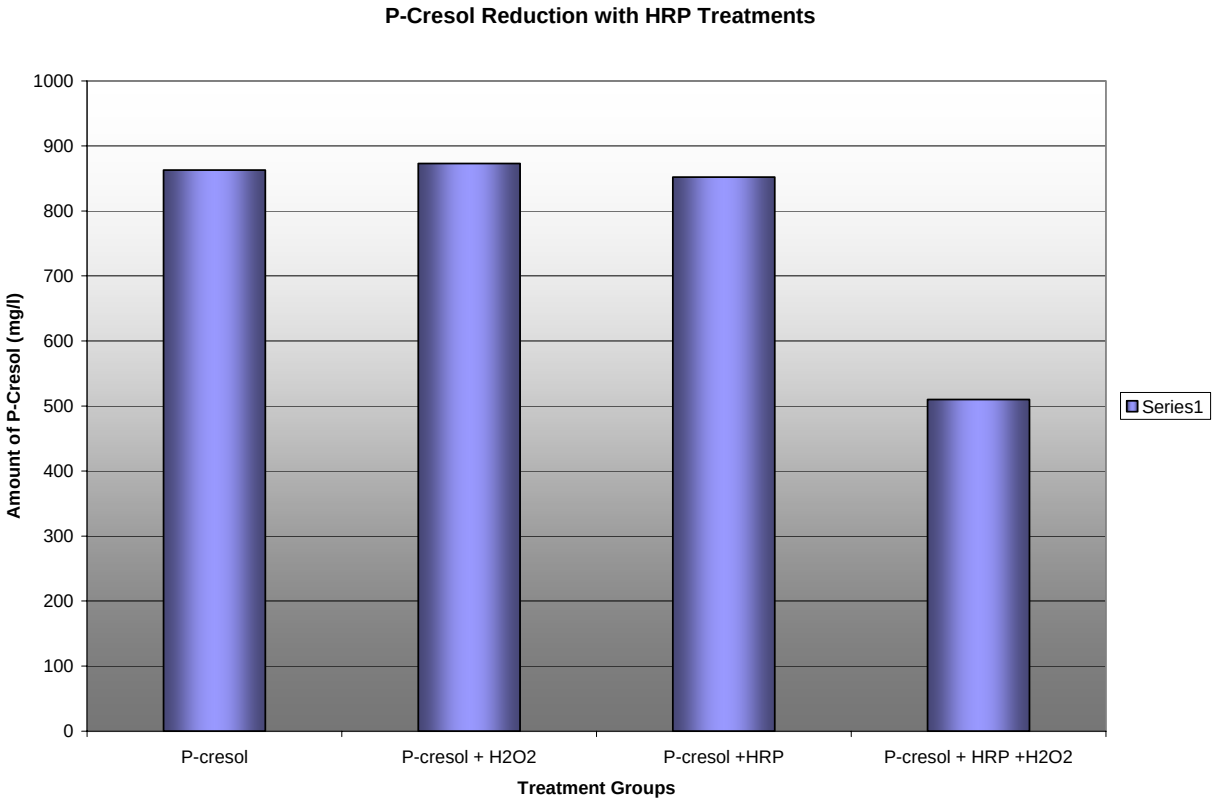

Figure 2. Graph comparing the concentrations of p-cresol (mg/L) in the liquid phase for the different treatment groups.

Conclusions

Para-cresol reacts readily with horseradish peroxidase in the presence of hydrogen peroxide which acts as a catalyst in this reaction. The reaction occurs resulting in a significant reduction in p-cresol levels and consequently odor levels. The difference in odor between p-cresol and p-cresol treated with HRP in the presence of hydrogen peroxide is obvious by human perception. On a scale of 1 to 10, where 10 stands for no odor at all, p-cresol treated with HRP in the presence of hydrogen peroxide was given a score of 9. This result has been a basis for further research employing the use of DNA recombinant technologies.

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