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Title: Treatment of Rendering Plant Wastewater with Ozone to Reduce Biochemical Oxygen Demand, Chemical Oxygen Demand, Color, Malodor Emissions and Bacterial Content

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Objective: To treat rendering plant wastewater with ozone to reduce biochemical oxygen demand, chemical oxygen demand, color, malodor emissions and bacterial content.

Background

Wastewater treatment is a costly expense for rendering plants. Excessive releases of organic laden water can result in large surcharges by municipal wastewater authorities. Ozonation was investigated as a potential treatment procedure to reduce bacterial content, BODs and COD in rendering plant wastewater, the industry would greatly benefit by decreased waste treatment costs and/or municipal surcharges. Additional benefits of ozonating wastewater should include reduction in color, malodorous emissions, and bacterial content. Reducing color and malodorous emissions will help the public image of the rendering industry by promoting good neighbor relations. Reduction in bacterial content of the wastewater will assist in the rendering industries efforts to provide biosecurity via pathogen reduction/discharge.

This research project is designed as a preliminary investigation into the use of ozone in treating rendering plant wastewater. If successful in reducing BODs, COD, color, odor and bacterial content, further studies will be needed to determine cost of scaling up to commercial size operations.

Experimental Procedures

This research was a preliminary study to determine the effect of ozone in reducing chemical oxygen demand (COD), biochemical oxygen demand (BOD), bacterial content and percent solids of animal processing plant rendering wastewater. Samples of wastewater were collected from Brown Packing Incorporated in Gaffney, South Carolina. The packing plant samples were collected from the discharge pit (6 feet x 10 feet x 10 feet) of the harvest floor (HF) and from the lagoon discharge pit (6 feet x 10 feet x 10 feet) (LDIS). Both of the packing plant samples were flowing wastewater. A bucket was used to catch approximately 20 liters of wastewater. From this sample, a 2000 mL sample was collected. Samples were transported to the laboratory for analysis and treatment.

Samples of blood stick water were provided by American Protein Products, Inc., Cummings, GA. Blood stick water was examined for COD and BOD only.

Samples were stored under refrigeration until use. Ozone was produced by a Longmark Pure Power O3\textsuperscript+ ozonator (Yreka, CA) and transported by air produced by two Tetra Luft pumps (Tetra/Second Nature, Blacksburg, VA) (max pressure 7.25psi). All feed air was passed though an Alltech\textsuperscript® gas purifier filled with Drierite\textsuperscript® [anhydrous calcium sulfate (97%CaSO\textsubscript{4}, 3%CoCl\textsubscript{2})]. Air passed from the gas purifier through the ozonator where ozone was generated. The ozone was then bubbled through the sample as follows: a 150 mL sample of wastewater was transferred into a 125 mL Ace Glass Impinger Unit (Model # 7538-27, Vineland, NJ). Subsamples (10 mL) were collected using a sterile 10 mL pipette at 0 min, 30 min, and 60 min of ozonation for the Brown Packing samples and 0, 30, 60, 120, 240 min for the blood stick water samples. Collected subsamples were stored under
refrigeration (4°C) until used for further analysis. Samples were collected and stored in sterile 50 mL polypropylene conical centrifuge tubes (VWR brand Scientific Products, Suwanee, GA).

Preliminary studies using distilled, deionized water (ddH₂O) were performed to determine ozonation levels. The amount of ozone generated was determined to be 0.3 PPM in dH₂O at 30 and 60 min using the DPD method Ozone Test Kit (Hach Company, Loveland, CO).

**Determination of Chemical Oxygen Demand (COD)**

The chemical oxygen demand (COD) procedure was performed on the ozonated and non-ozonated samples using closed reflux, colorimetric method (Method 5220D) (Eaton et al., 1995). Samples were diluted 1 to 10 using distilled, deionized water. Two mL of each diluted sample was added to a COD Digestion Reagent Vial (High Range, 0 – 1500 mg/L COD, Catalog 21259-15, Hach Company, Loveland, CO). A blank was prepared by adding 2 mL of dH₂O to a reagent vial. Tubes were heated in a heating block (COD Reactor Model 45600, Hach Company, Loveland, CO) and allowed to react at 150°C for 2 hr. The tubes were cooled to room temperature. The absorbance of each sample was measured at 620 nm using a spectrophotometer (Spec 20, Bausch and Lomb Optical Company, Rochester, NY) fitted with a Hach® COD reagent vial adapter. The calibration of the spectrophotometer was accomplished with a blank reagent vial. The calibration was completed prior to measurement of the absorbance values. Preparation of the blanks was accomplished by adding two mL of distilled deionized water (dd H₂O) into a COD Digestion Reagent Vial (High Range, 0 – 1500 mg/L COD, Catalog 21259-15, Hach Company, Loveland, CO) and heating at 150°C for two hours. During the absorbance measurement of the samples the baseline of the spectrophotometer was frequently rechecked with the blank to ensure there was no drift. A standard curve was calculated using dilutions of a solution of potassium hydrogen phthalate in dH₂O. Concentration of each standard solution was plotted against absorbance to determine a linear model. In order to estimate mg O₂/L concentration in test samples, reverse calculation was used whereby the intercept (β₀) was forced through zero. This determined the model to be:

\[
\text{absorbance} / \beta₁ = \text{concentration} O₂ / \text{mL}, \text{or}
\]

\[
\text{Absorbance} / 0.0004678678 = \text{concentration} O₂ / \text{mL}
\]

(Eaton et al., 1995).

**Determination of Biochemical Oxygen Demand (BOD₅)**

The Biochemical Oxygen Demand (BOD₅) procedure was performed on the ozonated and non-ozonated samples using the five day biochemical oxygen demand method (Method 5210B) of Standard Methods for the Examination of Water and Wastewater (Eaton et al., 1995). Hach® BOD pillows (Catalog 14862-98, Hach Company, Loveland, CO) and Polyseed® BOD Seed Inoculum P-110 (Polybac Corp, Bethlehem, PA) were used. Preliminary tests indicated that dilutions were necessary to have results within the limits of the test. Therefore, a 0.1/mL and 0.01/mL samples were added to BOD bottles and the test procedure performed. All samples were tested in duplicate. The quality of the dilution water was checked by running the BOD₅ method in quadruplicate using deionized, distilled H₂O as the added sample. Controls were examined on day zero and day five for dissolved oxygen (DO). The YSI Model 51B oxygen meter in conjunction with a YSI 5905 BOD probe (YSI Inc, Yellow Springs, OH) was used to measure Dissolved Oxygen (DO) in samples. Calibration was accomplish by following the procedure listed in the YSI Oxygen Meter’s Owner’s Manual. Calibration was accomplished prior to the initial series and periodically throughout the sample readings. To calculate the final DO, the following formula was used (Eaton et al., 1995):

When dilution water is seeded:

\[
\text{BOD}_5, \text{mg/l} = (D₁ - D₂) - (B₁ - B₂) / P
\]

Where:

\[
D₁ = \text{Do of diluted sample immediately after preparation, mg/l}
\]

\[
D₂ = \text{Do of diluted sample immediately after preparation, after 5 days of incubation @ 20°C, mg/l}
\]

\[
P = \text{Decimal volumetric fraction of sample used}
\]

\[
B₁ = \text{DO of seed control prior to incubation mg/l}
\]

\[
B₂ = \text{DO of seed control after 5 day incubation, mg/l}
\]

\[
f = \text{ratio of seed in diluted sample to seed in seed control}
\]
Percent Solids

Twenty-five mL samples were transferred into an aluminum weighing dish (Fisher Brand®, catalog no. 08-732, Fisher Scientific, Fair Lawn, NJ) and dried at 60°C for 24 hr. After weighing, percent solids of samples were calculated using the following formula:

(wet sample + pan weight) – pan weight = wet sample weight
(dry sample + pan weight) – pan weight = dry sample weight

percent solids = \text{Dry sample weight} \times 100
\text{Wet sample weight}

Coliform Bacterial Counts

The coliform bacterial content of untreated and treated samples was conducted using serial dilutions in phosphate buffer. Samples were plated on 3M Petrifilm® coliform count plates (3M Health Care, St Paul, MN). Plates were incubated for 24 hours at 32°C in a Model 3212 incubator (National Appliance, Portland OR). All storage and incubation guidelines provided in the 3M Petrifilm® instructions were followed. After incubation, plates were enumerated using a Gallenknap colony counter (England).

Aerobic Plate Count

The aerobic bacterial content of untreated and treated samples was conducted using serial dilutions in phosphate buffer. Samples were plated on 3M Petrifilm® Aerobic Count Plates (3M Health Care, St Paul, MN). Plates were incubated for 24 hours at 32°C in a Model 3212 incubator (National Appliance, Portland OR). All storage and incubation guidelines provided in the 3M Petrifilm® instructions were followed. After incubation, plates were enumerated using a Gallenknap colony counter (England).

Odor Components

Wastewater samples from the harvest floor and lagoon discharge pit were analyzed for malodorous odor components by high pressure liquid chromatography (HPLC) for different classes of malodor compounds: (a) volatile amines including ammonia; and (b) aromatics including skatole and cresols. Samples were analyzed for ammonia by NH₃ electrode (Lazar Industries). Aromatics and the 3,5-dinitro benzylthioesters were analyzed by HPLC using a Rainin gradient Dynamax™ system with Macintosh computer control and Method Manager™ software (Rainin Instrument Co., Woburn, MA. and using a water/ acetonitrile gradient and a C5 reverse phase column (Phenomenex, Torrence, CA) with detection at 280 nm.

Experimental Design and Statistical Analyses

A Randomized Complete Block Design (RCBD) was used to analyze all raw data. The design allowed analysis over time and treatment effect. At confidence levels of 99% and 95% Fisher’s LSD, analysis of variance (ANOVA) and pair-wise testing were performed. LSD analyses were performed on sample treatment by time. T-tests also were used to determine if there was a significant difference between ozonation times. For COD, a standard curve was obtained using Proc GLM (SAS/STAT® User’s Guide, 1989).

For bacterial counts, raw data was transformed to log units. The transformed data was analyzed using a Randomized Complete Block Design (RCBD) to test for analysis of variance. Fisher’s LSD was performed to determine if a significant difference existed between treatment, time and mean (α = 0.05).

Results and Discussion

Observations

Ozonation caused noticeable differences in color and odor of all samples with increasingly evident changes with longer treatment times. All samples foamed as the ozone was bubbled through the liquid. This observation has been noted in other experiments whereby ozone is bubbled through a column of liquid and especially liquids with a high protein content. This bubbling can be reduced by using a venturi system for injecting ozone; however, it still remains a problem to be resolved with ozonation of high organic and particularly high protein liquids. Ozonation of blood stick water yielded copious amounts of foam. After six hours of ozonation, blood stick water was perfectly clear (Figure 1). Such a long ozonation time is prohibitive; however, increasing ozone concentration can lead to
shorter ozonation treatment times. Additionally, complete ozonation to clarity is likely not necessary for an industry that employs other wastewater treatment systems.

Variability in samples was great and therefore the standard error for all tests was high.

Figure 1. Blood stick water before (left) and after (right) 240 min ozonation.

Chemical Oxygen Demand (COD)
COD levels for the discharge of the packing plant harvest floor (HF) were considerably lower than COD values for samples the packing plant anaerobic lagoon discharge (LDIS) although these parameters were not statistically analyzed across different samples (Figure 2). There was no significant difference between 0 and 30 samples (P > .1) for HF COD values. Between 0 min and 60 min, there was a significant reduction in COD levels (P < .03) for HF samples. COD levels for LDIS significantly increased between 0 min and 30 min (P < .0001) and then decreased between 30 min and 60 min with an overall significant decrease (P < .0001) between 0 min and 60 min of 21.1% (Figure 2). It is unclear as to why the COD levels of the LDIS increased between 0 min and 30 min.

Measurements of COD for blood stick water revealed significant reductions (P < .01) between each treatment time (Figure 3).

Biochemical Oxygen Demand BOD₅
There was no significant difference in HF Floor samples between 0 min to 30 min ozonation (P > .30) and between 0 min and 60 min ozonation (P > .30) (Figure 4). BOD₅ levels for LDIS significantly increased by 50% at 30 min ozonation (P < .03) and maintained the same level at 60 min ozonation. Similar results have been reported
by other researchers for ozonated wastewater samples. This may be due to high nitrogen content of samples interfering with the growth of microorganisms during the BOD$_3$ test (Knight, 2000; Eaton et al., 1995). Another possible hypothesis proposed to explain increases in BOD levels after ozonation is due to the physical size of the particles. It is possible that recalcitrant organic material on the particle surface are systematically degraded into smaller particles that are capable of use as nutrient sources by the BOD Polyseed bacteria. During their growth, the bacteria use the dissolved oxygen (DO). With continued ozonation of a sample, the smaller particles are completely oxidized and, therefore, are not available for BOD bacteria to use. Continued ozonation will degrade further layers from the larger particles similar to peeling layers off of an onion (Greene, 2001).

BOD levels of blood stick water declined steadily over treatment time with significantly different ($P < 0.01$) decreases with each successive treatment time (Figure 5).

**Percent Solids**

HF total percent solids decreased by 88.8% and 89.6%, from 0 min to 30 min ozonation and 0 min to 60 min ozonation, respectively (Figure 6). LDIS total percent solids decreased by 89.9% and 20.0% from 0 min to 30 min ozonation and 0 min to 60 min ozonation, respectively. Ozonation causes progressive oxidation and degradation of organic compounds. Decreases in total percent solids in wastewater are consistent with results obtained by other researchers (Knight, 2000).

**Coliform Bacterial Counts**

The coliform bacterial count of HF and LDIS samples decreased by 48.7% and 65.4% from 0 min to 30 min ozonation and from 0 min to 60 min ozonation, respectively ($P < .0001$) (Figure 7).

**Aerobic Bacterial Counts**

The aerobic bacterial content of HF samples significantly decreased by 44.5% between 0 min and 30 min ozonation and by 65.6% between 0 min and 60 min ozonation ($P < .0001$). LDIS samples had a significant decrease ($P < .0001$) of 54.5% in aerobic bacterial content between 0 min and 60 min ozonation. The same trend was noted between 0 min and 60 min ozonation where the aerobic bacterial plate count was significantly decreased by 72.6%, ($P < .0001$) (Figure 8). Decreases in coliform bacterial counts and aerobic bacterial plate counts with increasing ozonation treatment were expected since ozone is a potent oxidizing agent that readily destroys a wide variety of microorganisms. Ozone causes oxidation of the bacterial cell wall and disrupts enzymes that are necessary for growth, metabolism and respiration.

**Odor Components**

Ammonia in lagoon pit samples was reduced by 31 to 49% between 0 and 60 minute ozonation. Skatole, indole and cresol levels were all decreased by at least 50% after 60 min ozonation.

**Conclusions**

Ozone holds promise as a pre-treatment method for wastewater remediation. Ozone treatment causes progressive oxidation of organic compounds until ultimately carbon dioxide. As the second most powerful oxidizing agent known, ozone has the potential to oxidize any compound to carbon dioxide given sufficient concentration and time. Ozone is generated on-site with ozonator devices. There are no hazardous storage concerns. However, ozone is toxic upon extended exposure. But just as we have learned in the food industry to manage toxic chlorine sanitizing compounds, so can we learn to safely manage ozone. Ozone destrukt units (ultraviolet light or heat) are available. Another more simplistic approach to ozone management involves proper ventilation.

Future issues to be addressed include cost efficiency, how far to ozonate and how to prevent foam formation. It is hypothesized that rendering plants would not want to ozonate wastewater to complete clarity due to time and energy concerns. However, a partial treatment with ozone can degrade many of more recalcitrant molecules in wastewater thereby allowing quicker degradation by other chemical or microbiological means. Our experience with ozonation of wastewater indicates that there can be great batch-to-batch variability. This variability can be attributed to variability in the concentration and composition of the waste stream. Additionally, depending on the ozonator design, ozone output may decrease with time. Ozone detection meters (ORP meters) are useful in monitoring ozone output. In the experience of this laboratory, ozonator manufacturing companies vary greatly in knowledge and quality of equipment. The industry has undergone a rapid transformation in the past few years. A
number of companies have come and gone (including the manufacturer of the ozonator used in this experiment). A list of companies with which we have had success is available upon request.

LITERATURE CITED


Figure 2. Least square mean chemical oxygen demand (COD) of samples from Brown Packing Plant, Gaffney, SC

mg/L

Ozonation treatment time (min)
Figure 3. Least square mean COD for blood stick water
Figure 4. Least square mean biochemical oxygen demand (BOD) of samples from Brown Packing Plant, Gaffney, SC
Figure 5. Least square mean BOD for blood stick water
Figure 6. Least square mean total solids of samples from Brown Packing Plant, Gaffney, SC
Figure 7. Least square mean coliform bacterial content per mL of samples from Brown Packing Plant, Gaffney, SC.
Figure 8. Least square mean total aerobic bacterial content per mL of samples from Brown Packing Plant, Gaffney, SC.