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United States Department of Agriculture
Project Initiation

Project Data for Accession Number 1034541

Project Title

Mitigating Antimicrobial Resistance in Agricultural Wastewater Using Nano-Ozone.

Project Details

Sponsoring Institution National Institute of Food and Agriculture	**Project Status** ACTIVE
Funding Source NIFA Non Formula	**Grant Year** 2026
Grant No. 2026-67022-46023	**Cumulative Award Amt.** $299,996.00
Proposal No. 2024-11635	**Multistate No.** (N/A)
Project Start Date Apr 01, 2026	**Project End Date** Mar 31, 2028
Program Code [A1511] Agriculture Systems and Technology: Nanotechnology for Agricultural and Food Systems	

Project Director

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Performing Department

(N/A)

Non Technical Summary

Antibiotics are widely used in animal agriculture to maintain livestock health, but residues of these pharmaceuticals and the genes that make bacteria resistant to them often end up in wastewater from farms. These contaminants can persist in waste and lagoon systems that are used to store and treat manure, and these contaminants can eventually reach the environment. Their spread contributes to antimicrobial resistance, a serious public health challenge that affects communities, food systems, and environmental quality. As antibiotic-resistant bacteria become harder to treat, the economic and health impacts expand beyond farms to hospitals, families, and communities. This project addresses the urgent need for safer and more effective ways to remove antibiotics and resistance genes from agricultural wastewater.To address this challenge, the project will test a new wastewater treatment approach known as ozone nanobubble technology. This method uses extremely small but highly stable bubbles containing ozone, a strong disinfectant, to help break down antibiotics and deactivate resistance-related genes. The research team will

generate these nanobubbles, test them in small laboratory systems, and then evaluate the technology in a larger 50-liter setup using real wastewater from swine farms. Throughout the project, the team will measure how quickly the nanobubbles of ozone remove antibiotics and reduce the genetic material linked to antimicrobial resistance. The new technology will be compared to existing methods of ozonation for comparison. The work will also involve hands-on training for students, collaboration across engineering and microbiology teams, and engagement with agricultural producers and Extension specialists who may one day use this technologyIf successful, this project will provide scientific evidence for a new, efficient, and potentially low-cost treatment method that could help farms reduce the spread of antimicrobial resistance. Cleaner wastewater means safer reuse of water on farms, lower risks to rural workers and nearby communities, and reduced environmental contamination. The project also lays the foundation for future large-scale systems that could help protect public health, support sustainable agricultural practices, and strengthen rural economies. By improving how we manage antibiotics in farm wastewater, this project contributes to long-term goals of safer food production, healthier communities, and more environmentally responsible agriculture.

Animal Health Component

Animal Health Component: 5%

Research Effort Categories

Basic	30%
Applied	70%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
403	0210	2020	50%
133	0210	2000	20%
402	5370	2020	10%
133	0210	1000	20%

Knowledge Area

[403] Waste Disposal, Recycling, and Reuse

[133] Pollution Prevention and Mitigation

[402] Engineering Systems and Equipment

Subject Of Investigation

[0210] Water resources

[5370] Sewage and waste disposal facilities and systems

Field Of Science

[2020] Engineering

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[2000] Chemistry

[1000] Biochemistry and biophysics

Keywords

antibiotics, emerging contaminants, oxidation, ozone, nanobubble, antibiotic resistant genes

Goals / Objectives

The overall goal of this project is to establish the viability and effectiveness of ozone-based nanobubble technology (nano-ozone) for mitigating antibiotics and antimicrobial resistance genes (ARGs) in agricultural wastewater, specifically lagoon effluent from animal feeding operations. By improving the removal of priority contaminants of emerging concern, this work aims to support safer waste reuse within agricultural systems, reduce the spread of antimicrobial resistance, and strengthen environmental and public health protections in rural communities. This goal aligns with USDA priorities related to improving waste management practices, supporting circular bioeconomy principles, safeguarding agricultural productivity, and enhancing rural health outcomes. The major objectives of the project include the following: (i) Development of a foundational understanding of ozone nanobubble behavior and stability in agriculturally relevant, high-organic matter wastewaters. The project will determine whether nano-ozone will remain stable long enough within real lagoon matrices to serve as an effective oxidative treatment, in comparison with conventional ozonation systems. (ii) Demonstration of the ability of nano-ozone to degrade antibiotics and deactivate ARGs under realistic bench-scale and pilot-scale treatment conditions. This goal includes generating mechanistic knowledge and quantitative degradation kinetics for priority antibiotics and corresponding ARGs identified in agricultural waste streams. (iii) Establishment of the technological and scientific foundation necessary for scaling nano-ozone treatment toward full-scale agricultural wastewater management. The project outcomes will support future integrated USDA proposals and provide actionable evidence for the adoption of nano-ozone technology in animal feeding operations (AFOs).Specific, MeasurableObjectives:Objective 1: Assess the stability and behavior of ozone nanobubbles in agriculturally relevant liquid waste systems. This objective evaluates whether nano-ozone provides improved ozone persistence compared to conventional ozonation in high organic mqatter environments. We will (i) characterize nano-bubble size and number distributions generated under varying gas flow rates using particle size analysis instrumentation; (ii) determine ozone stability and decay kinetics in nano-ozone solutions prepared in deionized water, synthetic wastewater, and swine lagoon effluent over a three-week period; and (iii) compare nano-ozone stability with conventional macrobubble ozonation under parallel experimental conditions. Measurable outputs for this objective include time-series ozone concentration profiles, nano-bubble size and number distributions, and comparative decay curves for nano-ozone versus conventional ozonation.Objective 2: Evaluate the degradation of antibiotics and the deactivation of ARGs using nano-ozone at bench-scale and pilot-scale. This objective establishes treatment performance and mechanistic understanding of nano-ozone under realistic use scenarios. Activities under this objective include (i) quantifying degradation kinetics for four priority antibiotics (oxytetracycline, chlortetracycline, sulfamethoxazole, and sulfamethazine) across deionized water, synthetic wastewater, and real lagoon wastewater; (ii) determining deactivation kinetics for extracellular and intracellular ARGs (e.g., tet, sul, betalactam, macrolide, and fluoroquinolone resistance genes) using qPCR following nanoo-ozonation; (iii) comparing nano-ozone treatment performance to conventional ozonation by evaluating differences in contaminant treatment efficiency and CT (concentration × time)-based degradation behavior; and (iv) conducting pilot-scale nano-ozone trials (50-L system) in real swine lagoon effluent to validate treatment performance at operationally relevant scales. Measurable outputs include first-order degradation rate constants for each antibiotic, ARG log-reduction values, and CT-based performance assessments, bench-to-pilot scale comparison datasets, and quantitative evidence supporting the scalability of nano-ozone.Project Impact and Alignment with USDA Priorities:Completion of these objectives will provide fundamental and applied knowledge critical to the development of cost-effective, energy-efficient, and scalable treatment systems for agricultural waste streams. This work will also support circular bioeconomy principles by enabling safer reuse of lagoon effluent in AFOs and will address USDA AMR Action Plan Objective 3--identifying feasible practices to reduce antimicrobial resistance in food & animal production environments. Finally, this project will

build the scientific basis for futureUSDA and other federal and industry-funded grants aimed at developing full-scale demonstration systems.

Project Methods

This project tests ozone-based nanobubble (nano-ozone) technology for mitigating antibiotics and antimicrobial resistance genes (ARGs) in agricultural wastewater, with side-by-side comparisons to conventional ozonation and validation in bench-and pilot-scale systems using real swine lagoon effluent.Bench-scale generation and characterization of nano-ozoneGas and nanobubble generation: Ozone will be produced with a bench-top corona-discharge generator and introduced into water via a nanobubble generator capable of producing 20-1000 nm bubbles using a compressor as needed.For these experiments, the working volumes are up to 15 L.Matrices and operating conditions: Gas flow rates (e.g., 2-4 L/min) will be varied to study bubble formation and ozone loading. Deionized water, synthetic wastewater, and real swine lagoon effluent will be used to test the impact of wastewater quality. Nanobubble sizing/counts: Size and number distributions will be determined using zeta potential and a particle size analyzer (e.g., NanoSight NS300).Ozone tracking: Ozone concentration in bulk/nanobubbles is quantified using the indigo method (A690) over a multi-week period to generate decay profiles and estimate persistence.Contaminant degradation and ARG deactivationexperimentsTarget contaminants: Four agriculturally relevant antibiotics (oxytetracycline, chlortetracycline, sulfamethoxazole, and sulfamethazine) will be tested in three water types. ARGs and molecular methods: Extracellular and intracellular ARGs (e.g., tet, sul,ampicillin-resistance on plasmids) are generated via molecular cloning and dosed to experiments. Total DNA is captured via filtration and ethanol precipitation, followed by extraction and qPCR assays designed to track both native and spiked ARGs. Reactive species will utilize chemical probe-based methds using use of para chlorobenzoic acid (pCBA) as a hydroxyl radical probe. Competition kinetics will be usednto estimate second order rate constants as needed.Kinetic degradation setup: Nano-ozone will be introduced at multiple ozone levels (nominally ~1, 5, 10 mg/L), in water containing antibiotics are spiked (~1 mg/L) and ARGS, and time-series samples are collected up to several hours; reactions are quenched prior to analysis. Conventional ozonation is run in parallel as the control.Pilot-scale validationConfiguration and matrix: A ~50 L pilot tank will replace the 15-L bench vessel for trials using real lagoon effluent from OSU's Swine Research and Education Center and/or other local swine lagoons. Physicochemical characterization (e.g., solids, DOC /DON, pH, alkalinity, conductivity) precedes treatment tests.Measurement endpoints: Time?series changes in antibiotic concentrations (LC-MS/MS), ARG abundance (qPCR), and ozone CT are recorded over typically up to ~3 hours.Data analysis, evaluation, and interpretationKinetics modeling: Fit antibiotic decay and ARG reductions to first-order or pseudo-first-order models; compute rate constants (k), half-lives (t1/2), and 95% confidence intervals. Evaluate ARG response vs CT (Chick-Watson framework).Mechanism diagnostics: Compare outcomes across matrices (DI, synthetic, lagoon) to assess organic matter effects; use pCBA loss to infer hydroxyl radical contributions versus direct ozone attack.Comparative performance: Quantitatively compare nano-ozone to conventional ozonation for (a) ozone persistence (e.g., apparent half-life), (b) antibiotic k values, and (c) ARG log-reductions at matched CT. Use two-way ANOVA or mixed-effects models for factors such as matrix and treatment type; apply multiple-comparison corrections; report effect sizes and uncertainty.Quality control: LC-MS/MS method validation (calibration, recovery, surrogates, blanks) and qPCR MIQE adherence (standards, efficiencies, inhibition checks, negative controls).Scientific/technical performance indicatorsOzone persistence: Time-series decay curves and derived half-lives across matrices; evidence that nano-ozone sustains higher aqueous ozone than conventional ozonation in lagoon effluent.Antibiotic degradation: Estimated first-order rate constants, half-lives, and comparative k values (nano-ozone vs. conventional) for each antibiotic in each matrix; goodness of fit (R^2), uncertainty intervals.ARG deactivation: qPCR-based log-reductions of extracellular and intracellular ARGs as a function of CT; evidence for improved deactivation with nano-ozone at equivalent CT.Pilot translation: Replication of bench-scale trends at 50-L scale with real lagoon effluent, demonstrating operational feasibility (e.g., treatment time to achieve target log-reduction).Student competency gains: Pre/post self-assessment rubrics on nano-ozonation operation, LC-MS/MS sample prep /quantitation, qPCR setup/interpretation, and data analysis; advisor evaluations tied to task checklists. Individual development plans and semester-goals.Training outputs: Number of students trained; student-led abstracts, posters, and manuscripts; completion of thesis/dissertation milestones aligned with project activities.Publications and

presentations: Submissions to Environmental Science & Technology, Water Research,and ASABE; conference abstracts and talks delivered.Data and protocols: Curated datasets (ozone stability, antibiotic kinetics, ARG qPCR) and step-wise protocols for nano-ozone generation/measurement and molecular assays; requests for methods from external groups.