Title:	Disinfection of Water used in the Poultry Industry by Combined Advanced Oxidation Processes

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Performing Department
R&D

Co-Project Directors
{NO DATA ENTERED}

Departments
{NO DATA ENTERED}

Non-Technical Summary

Global water demand has increased with population growth, industrialization and agricultural development. In the western U.S., water supplies are vulnerable to drought, groundwater depletion and water quality issues. The resulting problems of freshwater scarcity have negative financial impacts on agricultural communities and other water-intensive industries.

Food-borne illnesses, another problem facing the poultry industry, are major public health concerns. Pathogenic bacteria must be destroyed to avoid cross-contamination of poultry carcasses during meat processing. Further, the release of poultry wastewater can be harmful to the environment and bacteria must be disinfected before discharge into the sewer. Chlorine is the most common disinfectant in wastewater treatment systems and by-products of chlorine disinfection have potential detrimental effects on human health and the environment.

Contaminants such as viruses, pesticides and pharmaceuticals can also impact water quality. Decontamination methods that are environmentally friendly have become an area of interest for the U.S. government (e.g. water security, environmental protection and potential danger of chemical-warfare attacks) as well as local stakeholders and wastewater treatment facilities. To respond to these growing interests, a better understanding of decontamination, pollution prevention and water reuse is urgently needed.

This Small Business Innovation Research Phase I project involves synthetic and physical chemistry, microbiology and the design of disinfection equipment. Our multidisciplinary team intends to develop improved methods for both the remediation and reuse of wastewater with advanced technology. The technology was developed specifically for poultry processing and can be integrated into current industry practices. Micro-Tracers, Inc. has many contacts at poultry processing plants who are willing to consider the technology at least on a trial basis.

The proposed application has the potential to reduce food-borne pathogens with fewer toxic by-products and save poultry processors financially by cutting freshwater and wastewater disposal costs. A comparative economic analysis of chorine and chlorine-free water treatment techniques has identified a viable opportunity for this technology to improve wastewater disinfection practices. In addition to saving poultry processors financially by cutting freshwater, sewer and energy costs, the proposed water purification technology may provide many positive externalities to society such as greater conservation of water and lower public health risks.

Accomplishments

Major goals of the project

The overall goal of the project is to conserve freshwater by disinfecting and reusing poultry process wastewater.

During the Phase I effort, the four goals are:

1. Synthesize and evaluate fluorescent dyes with increased ability to generate singlet oxygen and high photostability
2. Investigate the recent improvements to an electro-photochemical apparatus for killing pathogens in poultry wastewater
3. Validate technical feasibility of various equipment and dyes
4. Advance commercialization efforts

The primary objective for the Phase I effort is to generate hydrogen peroxide (H_2O_2), hydroxyl radical (OH•) and singlet oxygen ($^1O_2$) during electro/photochemical treatment. The secondary objective is to determine which equipment variables and dyes have the fastest biocidal effect. The project will culminate with the submission of final reports and a Phase II market analysis.

The objectives for the Phase I effort are:

1.1 Provide experimental proof of:
- The generation of H_2O_2 and OH• during electrolysis of water containing chlorine free electrolytes using a low-voltage, alternating electric current
- The generation of $^1O_2$ from photosensitizers with improved lightfastness incorporated in solid substrate under irradiation with visible light
- Combined biocidal effects of electrochemical and photo-chemical treatment on water contaminated with pathogenic and nonpathogenic bacteria
- The presence of potential harmful by-products

1.2 Compare disinfection performance for electro/photochemical treatments and investigate relationship between disinfectant concentrations and bactericidal effect

2.1 Estimate minimum time needed for disinfection with the following variables:
- Electrode materials (copper, stainless steel and titanium)
- Electric frequencies (region from 1 to 60 Hz)
- Electrolytes (carbonate and bicarbonate of sodium, sulfates and phosphates)
- Dyes with improved lightfastness
- Sources of light
- Encapsulation of dyes in appropriate polymer carrier immersed in water

2.2 Rank equipment and dyes for optimal equipment performance

3. Update cost estimates, explore commercial viability and compile final reports

Subsequent Phase II efforts will scale-up benchtop application to a pilot-scale system within a small-scale poultry processing facility. Upon completion, a new chlorine-free approach will be available to disinfect and reuse wastewater that Micro-Tracers, Inc. intends to market to poultry processors.

What was accomplished under these goals?

Wastewater reuse presents a unique opportunity to improve freshwater conservation practices in regions facing water shortages. We believe our approach can help protect water resources and is aligned with NIFA Strategic Goal 1: Science, Subgoal 1.3. In addition to water conservation, the proposed technology can lower public health risks and is aligned with NIFA Strategic Goal 1: Science, Subgoal 1.6. Our methods of electrochemical and photochemical treatment are considered environmentally friendly because end-products of the reactions are innocuous (e.g. CO_2, H_2O and inorganic salts).

During Phase I research efforts, poultry processing wastewater was treated through the in-situ generation of oxidants such as hydrogen peroxide, hydroxyl radicals and singlet oxygen. Generating these disinfectants on-site in the wastewater validated the technical feasibility and reduced handling hazards. The findings were presented to the scientific community at the 2019 National Meeting of the American Chemical Society (ACS) and a preliminary version of the proposed equipment was included in a U.S. Provisional Patent application (Provisional Patent # 62/764,267, Priority date is 07/25/18).

This technology has potential to save poultry processors financially by cutting freshwater, sewer and energy costs. Other potential energy and costs savings will result from the reduced production, shipping, handling and storage of hazardous chlorine chemicals in addition to fewer toxic by-products in wastewater. Disinfection of water contaminated with bacteria was evaluated and specific equipment parameters (e.g. copper and titanium electrodes and oligothiophene-based dyes) were identified for optimal disinfection performance and commercial profitability.

1.1. Provide experimental proof of:
The generation of $^1O_2$ from photosensitizers with improved lightfastness incorporated in solid substrate under irradiation with visible light

The photoluminescence properties of the photosensitizers were evaluated in solution, and when incorporated in the polymers. Determination of the $^1O_2$ generation efficiency was first performed through the comparative dilution method against the known standard in addition an established colorimetric method using (cysteinato-N,S)bis(ethylenediamine)cobalt-(III).
The $^1O_2$ emission efficiencies were measured and reported in Final Technical Report submitted the NIFA National Program Leader.

In conclusion, experimental evidence of $^1O_2$ generation of investigated photosensitizers has been provided and a quantitative evaluation of $^1O_2$ generation efficiency for solutions of photosensitizers in organic solvents as well for their dispersions in polymer films have been performed. In trials with poultry wastewater, generation of $^1O_2$ was confirmed however additional wastewater compounds were found to consume $^1O_2$ in competition with indicator.

The generation of H_2O_2 and $OH^{\bullet}$ during electrolysis of water containing chlorine free electrolytes using a low-voltage, alternating electric current

A colorimetric method using potassium iodide was used to determine the concentration of peroxide derivatives during the treatments with a constant 0.1% concentration of inorganic salt as the electrolyte. During the electrolysis of DI water, the in-situ generation of peroxide derivatives for various electrolytes was measured over 60 minute experimental trials. Detection of $OH^{\bullet}$ was performed using an indirect technique known as spin-trapping with p-nitrosodimethylaniline. Decoloration of RNO was measured in DI water and poultry wastewater.

In conclusion, the generation of peroxide derivatives and hydroxyl radical was measured during timed trials. The rate of RNO discoloration in poultry water was impacted by additional organic compounds in poultry wastewater that compete with colorimetric indicator to react with non-selective oxidants like $OH^{?}$.

Combined bactericidal effect of electrochemical and photochemical treatment on water contaminated with pathogenic and nonpathogenic bacteria

During timed disinfection trials, samples were collected for microbiological analysis. Samples underwent a serial dilution and a pour plate method was used to determine the colony-forming units (CFU) per ml of test solution. The bactericidal effect of various treatments was estimated with a slope equation (ratio of the change in colony forming units over the change in time; slope = $\Delta CFU/\Delta time$) and a disinfection kinetic model was used to estimated time for complete elimination of bacteria (Td).

Slope and Td values were calculated for four treatments: control, electrochemical only, photochemical only and combined electro+photo treatment. Preliminary trials with the stainless steel electrode in DI water found Td values increasing in the following order control < photochemistry only < electrochemistry only < combined electro+photo treatments. The combined treatment led to an 80% and 25% reduction of Td for photochemical only and electrochemical only treatments, respectively.

The presence or lack of potential harmful by-products

Before and after electro/photochemical treatment, poultry process wastewater was sent off-site for analysis of total dissolved solids (SM2540 C), total suspended solids (SM2540 D), chemical oxygen demand (SM5220 D) and biochemical oxygen demand (SM 5210 B). The presence copper in wastewater was determined by a colorimetric method involving cuprizone and the lack of leaching of thiophene-based photosensitizers from their dispersions in polymer films has been established using absorption and photo luminescence spectroscopy.

1.2. Comparison of the bactericidal effect of electro/photochemical treatments and investigate relationship between bacterial population and $OH^{\bullet}$ generation.

To compare the bactericidal effect of the different treatments conditions (e.g. electrochemistry, photochemistry and combined electro + photochemistry) over a 60 minute period, a linear model and tukey-adjusted post-hoc comparisons were used. For post-hoc comparisons, if one level has a significantly steeper slope than others, its bacteria populations are being killed at a faster rate.

In conclusion, a comparison electrochemical only, photochemical only and combined electro+photo treatment confirmed the combo treatment caused significantly faster mortality in bacteria than other treatments. A strong inverse relationship between bacterial count and RNO absorbance was observed for all metal types. All three metal electrodes generated $OH^{\bullet}$ however the antimicrobial properties of copper likely contributed to an enhanced bactericidal effect relative to non-copper electrochemical processes.

2.1 Investigate the following variables:
Electrode materials
Time for complete elimination of bacteria (Td) ranged from 58 minutes to 113 for electrode material trials in poultry wastewater. The specific metals increased in Td estimates in the following order: Control < Stainless Steel < Copper < Titanium.

Electrolytes
For the investigated electrolytes, the rate of formation of peroxide derivatives increased in the following order: carbonate < bicarbonate < phosphate buffer < ammonium sulfate.

Photosensitizers, polymer carriers, sources of light and electric frequencies
The best results were obtained using bitheophine photosensitizers incorperated into polymer films of ethocel plus shellac with radiation provided by a conventaional halogen lamp. Best results were also obtained using an electric frequency of 60 Hz.

3. Continue to update cost estimates and explore commercial viability

Micro-Tracers, Inc. is participating in the Commercialization Assistance Program (CAP) and a collaboration with the Larta Institute is currently ongoing. The product of this collaboration, a Commercialization Plan, will be included in a Phase II application in Spring 2020. If funded, the proposed technology could save poultry processors financially by cutting freshwater, sewer and energy costs.

What opportunities for training and professional development has the project provided?
{NO DATA ENTERED}

How have the results been disseminated to communities of interest?
{NO DATA ENTERED}

What do you plan to do during the next reporting period to accomplish the goals?
{Nothing to report}

Participants
Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Scientist	0.6	0	0	0.4	1
Professional	0.1	0	0	0	0.1
Technical	0.1	0	0	0	0.1

Actual FTE's for this Reporting Period

Role	Non-Students or faculty	Students with Staffing Roles			Computed Total by Role
		Undergraduate	Graduate	Post-Doctorate	
Administrative	0	0	0	0	0
Other	0.8	0	0	0	0.8
Computed Total	1.6	0	0	0.4	2.0

Student Count by Classification of Instructional Programs (CIP) Code

Undergraduate	Graduate	Post-Doctorate	CIP Code
		1	40.05 Chemistry.

Target Audience

Poultry processors are the target audience that will be the primary focus of effort for the duration of the project. However, additional audiences that will receive secondary focus include: the scientific community, public health and natural resource managers and government regulators. The connections between the project and the mentioned groups are described below.

Poultry Processors

Poultry producers face rapidly rising costs of freshwater and wastewater disposal. Wastewater reuse is a promising method to decrease the amount of water needed for industrial food processes, such as poultry immersion chilling. In 2004, water recycling was reported at about half of the large U.S. poultry processing facilities. Reused poultry chiller water is allowed after measures have been taken to reduce microbiological contamination. We propose reducing discharged chiller-bath overflow water by 50% with potential cost savings of $4.58 /1000 gal.

Poultry processors are dependent on water disinfection to maintain food safety and avoid the economic impacts associated with foodborne illness outbreaks. The USDA Economic Research Service (ERS) estimates that foodborne illness costs the U.S. economy over $15 billion annually.. In addition to saving poultry processors financially by cutting freshwater and sewer costs, the proposed research can help ensure that food supply is safe for consumers and meets foreign and domestic regulatory requirements. The most common disinfection method involves adding chlorine; however, consumers have raised concerns about disinfection by-products such as trihalomethane. Other potential energy and costs savings will result from the reduced production, shipping, handling and storage of hazardous chlorine chemicals.

The Scientific Community

A number of studies have investigated the potential benefit of combined Advanced Oxidation Processes (AOPs) to disinfect poultry wastewater and eggshells. While authors have identified additive effects when killing bacteria, to the best of our knowledge, none have investigated the proposed combination of treatments.

Further, prior to our presentation in 2019, the rate of hydrogen peroxide and hydroxyl radical generation during electrolysis of water containing chlorine-free electrolytes using alternating electric current and electrodes made of stainless steel, copper and titanium was unreported. Our findings were presented at the 2019 National Meeting of the American Chemical Society (ACS).

Terthiophene and other thiophene-based molecules are naturally occurring secondary metabolites in plants, such as in the plant family Asteraceae. Their insecticidal, nematocidal and larvicidal properties have been extensively studied and are attributed to their ability to generate $^1O_2$. Depending on the oligothiophene, $^1O_2$ generation efficiencies of up to 0.95 have been reported. To date, use of compounds of the oligothiophene family for poultry wastewater treatment has not been reported.

Public Health and Natural Resource Managers

This technology can lower public health risks and protect natural resources. The proposed approach uses AOPs which are considered environmentally friendly because end-products of the reactions are innocuous (e.g. CO_2, H_2O and inorganic salts). Water recycling has the added public benefit of reducing the environmental impacts associated with water

consumption, especially in regions facing water restrictions.

Government Regulators

To prevent microbial contamination, the USDA has developed Hazard Analysis Critical Control Point (HACCP) programs and recently increased the stringency of pathogen standards for broiler chickens. While these methods improve food safety, they also create financial and logistical burdens for the poultry industry. Most poultry processing plants utilize chlorine to disinfect their processing water for less than $1 per 1,000 gal. Others, including those that export to Russia or the European Union, have converted to more costly alternative water treatments such as ozonation and Ultraviolet radiation (UV).

In response to public health concerns about chlorine disinfection and microbial cross-contamination, the European Union and Russia have restricted the importation of chemically treated chicken. Prior to the European ban in 1997, annual U.S. exports were valued over $50 million and prior to the Russian ban in 2013, annual U.S. exports were valued at $310 million. Reducing the chemical and biological hazards associated with chlorine use in poultry processing could help reopen these markets for U.S. producers.

While the current application focuses on food manufacturing and specifically poultry processing, additional opportunities have been identified. This technology has additional potential uses to decontaminate wastewater containing pesticides, viruses, pharmaceuticals organic pollutants and chemical warfare agents. Decontamination methods that are environmentally friendly have become an area of interest for the local stakeholders, wastewater treatment facilities as well as the U.S. government. To respond to these growing interests, a better understanding of decontamination, pollution prevention and water reuse is urgently needed.

Products

Type	Status	Year Published	NIFA Support Acknowledged
Conference Papers and	Submitted	2019	YES

Citation

Carlson, M., Barashkov, N., Lam, L., and Z. Eisenberg. (2019). "Advanced oxidation processes used to disinfect wastewater: Role of electrode material" General Poster Session at the Spring 2019 National Meeting of the American Chemical Society. Orlando, Florida. April, 2019. Abstract ID: 3106700.

Patent(s) and Plant Variety Protection(s)

Application Number	Application Filing Date	Title
62/764,267	07/25/2018	Chlorine-free electrolytic and photochemical disinfection method for sterilization of contaminated water

Other Products

Product Type

Instruments or Equipment

Description

A benchtop dynamic flow system was designed to circulate 2 liters of contaminated wastewater through two reaction cells then back to a small positive displacement pump. The proposed Phase II technology will be upscaled from the benchtop system and incorporated into a poultry wastewater recirculation tank.

Changes/Problems

{Nothing to report}