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Department: Biology

Research Project Title: Ozone as a disinfectant in confined spaces in short time frames

Research Project Summary (Please provide an overview of your project -- this will be shared with students as a project description; maximum 500 words):

Since the rise of COVID-19 there has been an increased interest in disinfecting agents that are fast, effective, inexpensive and environmentally friendly. It is well established that ozone, an extremely powerful oxidant, is effective at inactivating a large array of pathogens ranging from viruses, and bacteria to fungi. Although extremely effective at disinfecting, ozone is also unstable and highly reactive with a half-life for approximately 20 min. Ozone at high concentrations is a respiratory irritant and can cause respiratory damage and distress. Therefore, it is important, when using ozone to disinfect, that the lowest effective concentration be used and that people and pets do not enter and area until the ozone has degraded below safe exposure levels. According to OSHA and the EPA, safe concentrations for light work are 0.1ppm (0.2mg/m$^3$) for eight hours. To address these issues, we developed a novel catalyst and air flow system to disinfect aircraft and an additional system to disinfect clothing and PPE in short time periods effectively killing pathogens and returning ozone to safe levels within 20 min (patent pending) without environmental release. The focus of this project is to further refine the synthesis and application of the catalyst and confirm the effectiveness of ozone treatment using different fabrics, materials, and locations within an aircraft and the sterilization chamber. The project is a collaboration between chemistry, biology and aviation.
In the space below, provide a **Project Description** of your research project in 6 pages or less that includes…

**Introduction:**
In 2020, as COVID-19 rapidly spread around the globe, there was a push to increase the availability of disinfectants. However, little regard was paid to the damage known and novel disinfectants could have on materials when used in new applications. Ozone (O₃) has been used regularly as a disinfectant since the 1970’s (Burleson et. al, 1975). However, commercially available ozone generators produce ozone at an extremely high level without significant air flow. Therefore, materials closest to the generator have high exposure, while those further away have significantly less. In addition, ozone is corrosive and the higher the concentration materials are exposed to, the greater the corrosive potential (Lee, 1996). When used in general disinfecting applications ozone is released into the atmosphere and can increase the concentration of ground level ozone. High ozone concentrations can cause asthma attacks and respiratory distress (Filippidou and Koukouliata, 2011). Therefore, it is important to disinfect with ozone using the lowest effective concentration and prevent environmental release.

Ozone is an effective disinfection agent through mechanisms that reply on the reactivity of O₃ with elements of pathogens necessary for their function. Ozone destroys viruses by diffusing through the protein coat into the nucleic acid core, where it damages viral RNA (Jiang, 2019 and Roy, 1981). At higher concentrations, ozone destroys the virus’ exterior protein shell so that the virus is unable to enter cells (Roy, 1981). Ozone interferes with bacterial cell metabolism, likely through inhibition of the enzymatic control system (Lezcano, 2001). It is believed that ozone destroys fungi and mold by diffusing through the fungal wall and into the cytoplasm, disrupting the organelles that direct cellular functions (Lezcano, 2001). Given these mechanisms, ozone likely has a similar effect on mammalian cells.

Our work aims to generate and sustain ozone at low, but effective concentrations for only the amount of time required to achieve >99% pathogen inactivation, then degrade it quickly back to oxygen within an enclosed system. Based on Tseng and Li (2008) effective ozone concentrations for sterilization can be calculated as a factor of exposure time using the following equation:

\[ [O_3] \text{mg/m}^3 \times \text{Time (min)} = 50 \]

We have reduced disinfecting time and eliminated environmental and human exposure using our novel catalyst*. We have effectively reduced the half-life of untreated ozone from 20 min to approximately 30 seconds. In addition, activated carbon scrubbers have been used to eliminate ozone, but our catalyst and airflow system is 16.2X (Figure 1) times faster than activated carbon alone. Although we have made significant strides with this work we hypothesize that additional refinement of our process and system, validation with additional materials and aircraft trials will establish our methodology is safe, efficient and a cost-effective way to reduce pathogens.

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*The novel catalyst is based on a metal organic framework. We are intentionally vague about its properties in this application as we are still in the patent process.*
The work described here will focus on three aims to support our hypothesis. **AIM 1:** We will refine catalyst incorporation into disinfecting systems in order to result in faster and more effective ozone degradation. **AIM 2:** We will verify the effectiveness of our treatment protocol on various materials such as: plastics, metals, wood, natural fibers, and synthetic fibers. **AIM 3:** We will test our refined system within an aircraft to determine how effective our modifications are in a large, sealed space.

Methodology:

**AIM 1:** Refinement of catalyst incorporation into disinfecting systems.

We have made significant strides in determining how to generate the proper airflow to distribute ozone evenly around an aircraft or within our small disinfecting chamber (Figures 2 and 3). We have further tested our catalyst in our small chamber incorporated into numerous filters and materials. In this aim we plan to test the incorporation of our catalyst into carbon fiber filters. We will determine the ideal number of filters and the ideal placement of the filters relative to the centrifugal fans we are using to distribute air. Filter placement will vary based on distance between filters, horizontal or vertical air flow and space between filters and fans.

**AIM 2:** Verify the effectiveness of our treatment protocol on various materials such as: plastics, metals, wood, natural fibers, and synthetic fibers.

We have confirmed the well-known ability of ozone to kill *E. coli*, our surrogate organism. We chose a bacterial species because, unlike viruses, they have defense mechanisms to survive exposure to reactive oxygen species. Therefore, they are harder to neutralize with ozone. To date, we have only used ozone to neutralize *E. coli* on petri dishes and on agar. However, there is a large body of evidence demonstrating ozone’s ability to inactivate viruses, fungi, and numerous bacterial species. In this aim we intend to place *E. coli* on a variety of materials: plastics, metals, wood, natural fibers, and synthetic fibers. We will determine the ability of ozone to neutralize pathogens on these varied materials. If we encounter difficulty we will adjust ozone exposure time, concentration and/or distribution.
Figure 2: Ozone distribution throughout an MD80 aircraft resulted in average *E. coli* kill rates of ~98%. A) Demonstrates the ability of our prototype to distribute air throughout the fuselage. B) Demonstrated safe and effective monitoring, and remote operation of our prototype. C) Shows placement of *E. coli* samples throughout the aircraft and *E. coli* kill rates after ozone exposure.

**AIM 3:** Test our refined system within an aircraft to determine how effective our modifications are in a large, sealed space. Once we have completed Aims 1 and 2 we will incorporate our refined filters and methodology into our aircraft disinfecting unit. We will then treat the MD-80 using our unit (Figure 3A and B) and determine if we effectively killed the *E. coli* we strategically placed throughout the fuselage, cockpit and lavatories. We have previously demonstrated a >97% kill rate on aircraft (Figure 2) but we are aiming for 100% effectiveness. In these trials we will continue to place *E. coli* in the positions indicated in Figure 2C and treat with our modified units. If we encounter difficulties completely neutralizing ozone, we will increase exposure time or concentration accordingly.
Figure 3: The internal placement of our prototype components. A) Is our suitcase sized prototype for smaller spaces and aircraft. B) Is the design of our prototype for large spaces and aircraft such as the MD 80. C) and D) Is our small chamber used to sterilize items such as PPE.

Works Cited:


- **Mentorship Plan**
  - **Student Role**
    - The student in this investigation will be responsible for carrying out all experiments listed above in the methods section. This will involve solution preparation, catalyst synthesis, *E. coli* trials and preparation etc. The student will also be responsible for collecting and analyzing all of the data in collaboration with the faculty mentor.

- **Engagement with Student**
  - I will directly train the student on the benchwork techniques. I will be present on campus to help the student carry out the project and engage in the research.
  - In addition to these experimental responsibilities, the student will be responsible for weekly correspondence with the faculty mentors through zoom or google drive/meets and is required to attend group research meetings online.

- **Accountability**
  - The student will be expected to engage in all SURE events and carry out AT LEAST 25 hours of research per week. If the student fails to engage in lab meetings or SURE events pay may be withheld.

- **Specific Skills Development**
  - The student will gain skills in regards to specific lab techniques.
  - The student will also develop career and interpersonal skills, presentation skills, time management skills and the ability to collaborate.

- **Proposed Timeline that includes Aims and/or Goals**
  - **Weeks 1-4** we will address aim 1
  - **Weeks 5-8** we will address aim 2 and work on modifying the prototypes
  - **Week 9** we will conduct aircraft trials.
- **Week 10** the student will work on their presentation and writing up results

- **Budget up to $500** (not included in the 6 page limit)
  
  *We are applying for a Doherty for this work, but already have supplies to start as this is an ongoing project.*
Criteria for Student Applicants (Please report minimum criteria you will expect from student applicants, such as coursework that must be completed prior to starting work on this project):

Student must have complete general chemistry and general biology. Completion of microbiology is preferred but not required.