Gaseous Ozone Efficiency for Control of Aerosolized Phages

Project Report

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EMO3

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Introduction

Nosocomial infections are hospital-acquired infections which can be spread to users, staff members and visitors. The main method to limit these infections is by isolating patients, thus reducing direct contact between people. These infections can extend the length of stay for patients on average of about 2 weeks and double hospitalization costs (Chen et al. 2009). It has been known and proven that several infections (such as flu, colds and chickenpox) can be air-transmitted through bioaerosols, which makes them difficult to control. To this day, the airborne transmission process for many diseases remain unclear. Most often, aerosol transmission have indirectly been identified due to mathematical epidemiological models (Marks et al. 2003; Yu et al. 2004).

Certain viruses, such as the Norovirus, have been shown to resist aerosolization stress and retain more than 80% of their infectious potential (Bonifait et al. 2015). It has also been demonstrated that the infectivity of bacteriophages (bacteria viruses used as human virus models) (Verreault et al. 2008) could be adjusted according to temperature and ambient relative humidity (RH) (Verreault et al. 2015). Finally, the use of several phages is necessary when demonstrating the effectiveness of germicidal treatments (Turgeon et al. 2016).

Regarding health centers, where rooms and furniture are disinfected regularly, ambient air, however, does not undergo any sanitation procedure, which does therefore not impede the transmission of infections. Moreover, our team has documented the presence of large concentrations of Norovirus in the air of health care centers during gastroenteritis outbreaks (Bonifait et al. 2015).

Objectives

The objectives of this CRSNG-EMO3 partnership engagement project are to:

- Develop an aerosolization system using a rotating chamber to expose airborne bacteriophages to ozone gas.
- Determine the effect of ozone gas on the infectivity of aerosolized bacteriophages in various contact times with three relative humidity levels.

Methodology

Four bacteriophages (or phages) models (Phi6, PhiX174, PR772 and MS2) have been selected for the project, considering they have the structure as well as genetic material (DNA and RNA) akin to human viruses. Moreover, as they do not cause any infectious risks to the researchers, this enables a safe setup of the model before transitioning to gastroenteritis (Norovirus), flu (Influenza) and cold (Rhinovirus) viruses.

Phages were simultaneously aerosolized in an environmental rotating chamber. The three RH levels for the experiments were 20%, 55% and 85%. A 10-minute period was allotted in order to stabilize the aerosols before exposing them to a 1.13 ± 0.26 ppm ozone concentration. The ozone was produced with a generator supplied by EMO3 inc.. The aerosols were collected with a sampler after exposure times of 0, 30 and 60 minutes. The counting of infectious phages was carried out on culture medium, whereas the genetic material was quantified through a molecular biology approach (qPCR).

Results

The results for each phage are presented in form of comparative graphs. Each graph pair (figures 1 to 4) illustrate the results of each phage at the three tested RH, namely 20%, 55% and 85%.

The graphs on the left show the **infectious ratios** obtained after exposure to oxygen, which is the baseline monitoring or condition. This therefore demonstrates the phages' viability after their aerosolization, maturation inside the chamber and sampling.

Infectious ratios are one way of presenting results when applying a culture method and a genetic material detecting method; the qPCR for this project. A phage is infectious if there are lysis plaques on the culture medium. On the other hand, qPCR is used to determine whether the number of genetic material copies is identical amongst the samples and replicas. This number should not vary since the composition of aerosolized liquid that contains the phages is identical for all experiments.

A ratio of 1 or 10^0 means that all detected phages by qPCR were infectious, therefore counted on culture medium. If the number of copies resulting from qPCR remains constant, although the number of lysis plaques decreases between two periods, this means that this condition influences phage infectivity.

It should be considered that several variables, including relative humidity, exposure time, or exposure to oxygen or ozone, can affect phage infectivity. For example, some phages are more resistant to a RH of 85% rather than 20%, as is the case with Phi6. The effect of these variables will be used to interpret the results.

Each point on the graphs represents a replica carried out for a tested condition. It should be noted that for several samples, the results lied below the limit of detection of the methods used. These are shown on the graphs under the dotted line.

The graphs on the right, exemplify the **relative infectious ratios**, which help assess an additional ozone effect. A ratio greater than or equal to 1 indicates that ozone does not provide "added value". In contrast, a ratio smaller than 1 reveals that ozone has a virucidal effect. The lower the ratio, the greater the significance of the ozone’s effect. The dotted lines represent the detection method’s threshold. A value below this line is therefore not quantifiable, but close to zero.

It should be noted that, the statistical analyses have not yet been conducted; this report thus presents mere raw data and trends.

PhiX174

For PhiX174 (figure 1A), the basic ratios at 20% and 55% RH decrease by about one order of magnitude over time. The ratios remain constant at 85% RH and are near to 1, which means that almost all phages are infectious.

When PhiX174 is exposed to ozone (figure 1B), the added effect is weak at 20% RH. At 55%, there is a small decrease as of time 0, but it does not change over time. The virucidal effect of ozone is however quite notable when PhiX174 is exposed to 85% RH from time 0. After 30 minutes of exposure, the values fall under the detection method limit.

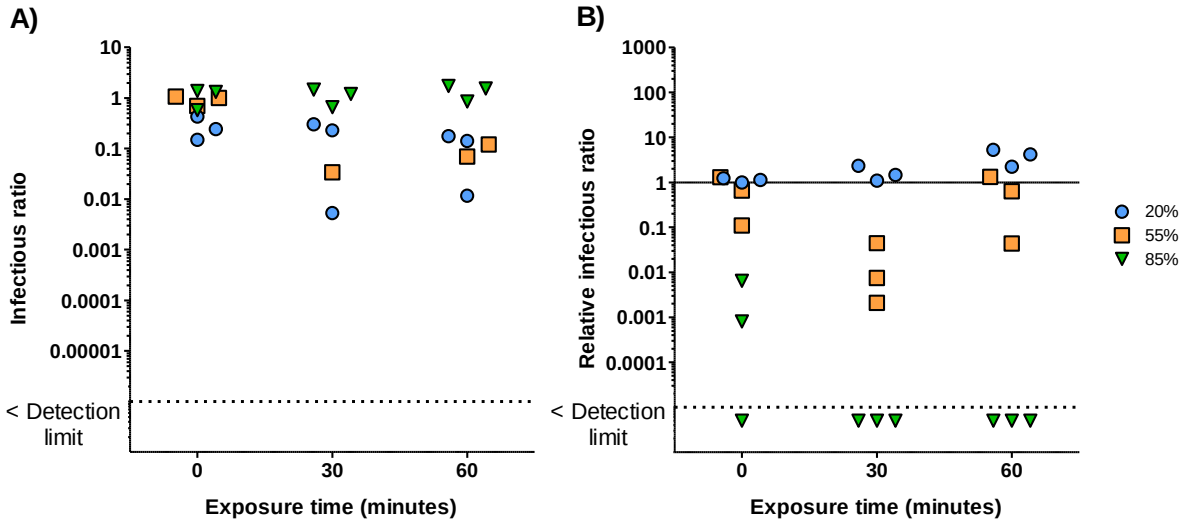

Figure 1: A) Effect of three relative humidity and three exposure times on phage PhiX174 infectivity (without ozone). **B)** Ozone effect on phage PhiX174 infectivity at three relative humidity and three exposure times. The solid line represents the reference value without ozone (Graph A).

PR772

For PR772 (figure 2A), the basic ratios at 20% RH are very low and are near the detection method's limit. These are slightly higher at 55% RH. At 85% RH, the ratios at times zero are close to 1, and decrease by an order of magnitude after 60 minutes of maturation.

When ozone is added (figure 2B), the effect of 20% RH is not quantifiable as statistical analyses have not been performed. As a matter of fact, no trend is observable as the points are scattered on the graph. At 55% RH, the effect is gradual over time and the values fall below the detection limit after 60 minutes of exposure. There is a decrease of 2 to 3 orders of magnitude at time 0 with 85% RH. The ratios then drop below the detection limit at 30 and 60 minutes of exposure.

A relative humidity of 85% is likewise more efficient for inactivating PR772.

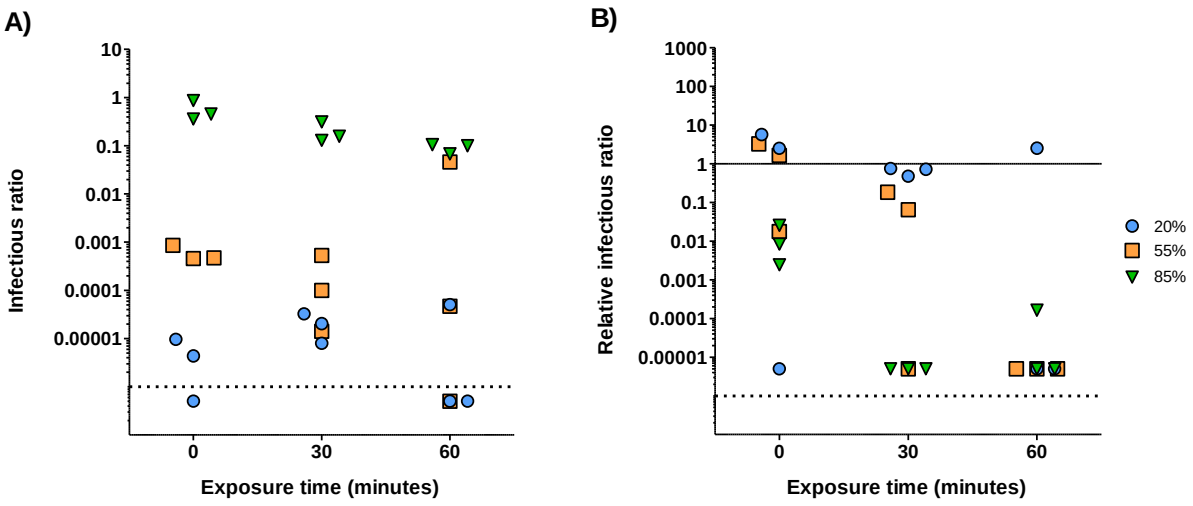

Figure 2: A) Effect of three relative humidity and three exposure times on phage PR772 infectivity (without ozone). **B)** Ozone effect on phage PR772 infectivity at three relative humidity and three exposure times. The solid line represents the reference value without ozone (Graph A).

MS2

In figure 3A, we see that for MS2, the basic level at 55% RH decreases over time. This scenario is not observed at 20% nor 85% RH.

At 20% RH, the relative infectious ratios are almost equivalent to the basic ones (figure 3B) and these gradually decrease with time to 55% RH. The ratios decrease drastically at a 85% RH by 4 orders of magnitude at time 0 and falling below the detection limit after 30 minutes.

A 85% RH supplemented with 1.13 ± 0.26 ppm of ozone is therefore the best situation for eliminating the infectivity of MS2. This outcome is very promising since this phage constitutes a Norovirus model; a virus known to be very resistant.

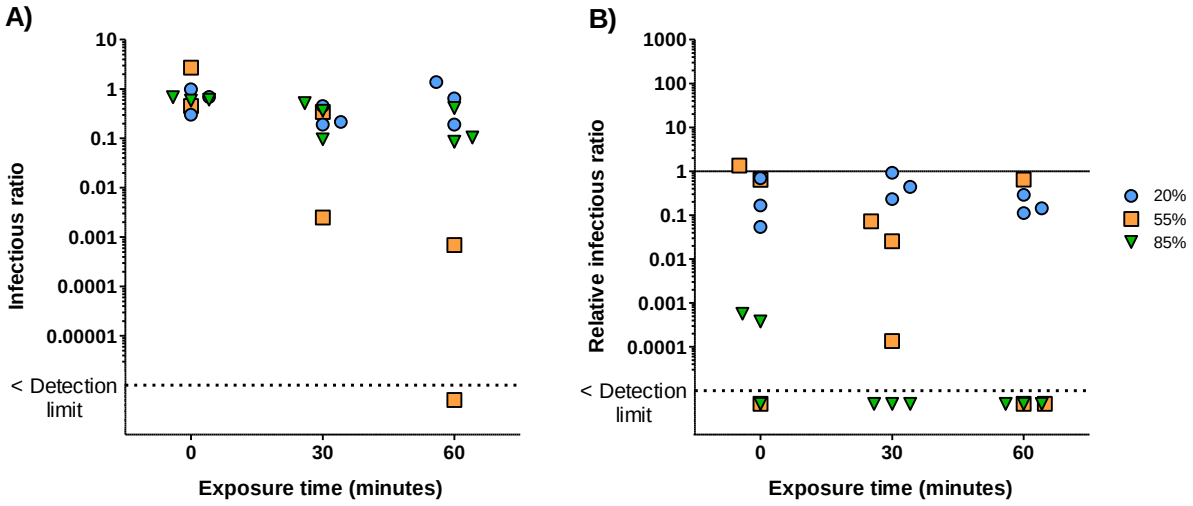

Figure 3: A) Effect of three relative humidity and three exposure times on phage MS2 infectivity (without ozone). **B)** Ozone effect on phage MS2 infectivity at three relative humidity and three exposure times. The solid line represents the reference value without ozone (Graph A).

Phi 6

Basic infectious ratios (figure 4A) at a 20% RH are almost all beneath the detection limit. Given this result, relative infectious ratios are not displayed in figure 4B. At 55% RH, infectious ratios are near the detection limit (one value below the limit at each time). Phi6 prefers a 85% RH, the ratios being the highest among the three tested humidity.

The relative infectious ratios (figure 4B) are near 1 to 55% RH, although one of the three replicas is below the detection limit for each time. At 85% RH, the ratio at time 0 is close to 1 and drops considerably below the detection limit after 30 minutes of exposure. However, it must be considered that the basic ratios are very low, between 10^{-6} and 10^{-3}. Consequently, few phages remain infectious (between 1 / 1,000,000 and 1 / 1,000). The additional effect of ozone is then rather limited.

An 85% RH seems more effective for Phi6, but the ozone effect remains questionable.

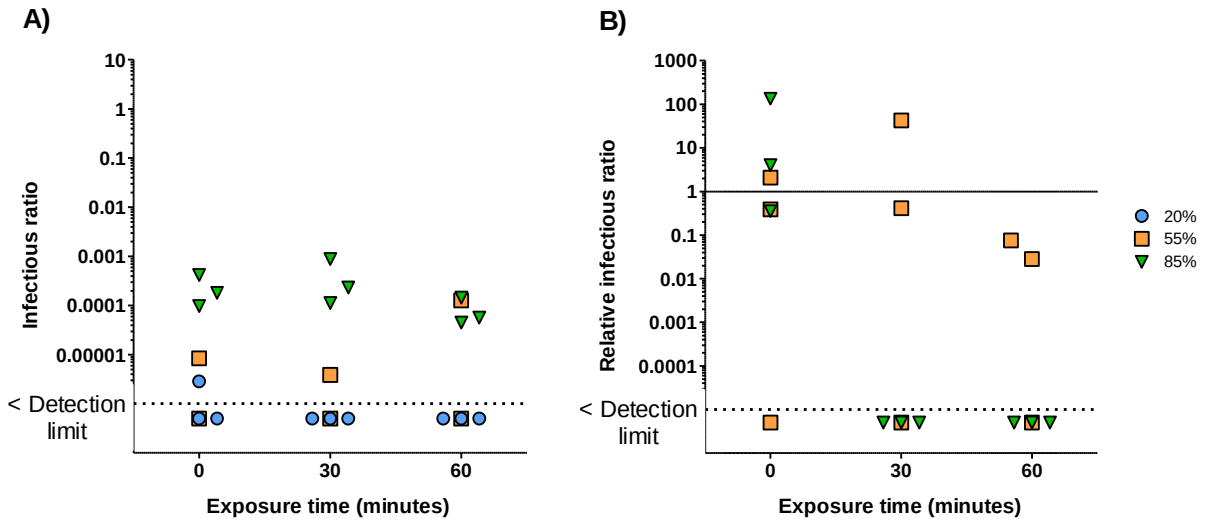

Figure 4: A) Effect of three relative humidity and three exposure times on phage Phi6 infectivity (without ozone). **B)** Ozone effect on phage Phi6 infectivity at three relative humidity and three exposure times. The solid line represents the reference value without ozone (Graph A).

Conclusion

In conclusion, the start of this study made it possible to confirm the effect of various exposure conditions on the viability of three aerosolized phages. Of all phages combined, the best tested condition is the exposure to 1.13 ± 0.26 ppm ozone combined with a 85% RH. The findings are encouraging, especially in the case of phage MS2 since it is known to be very resistant, as is the Norovirus.