Inactivation of Influenza Virus by Ozone Gas

TANAKA Hiroshi : P. E. Jp, Chemical Engineering Department, Products Development Center, Corporate Research & Development
SAKURAI Miei : Doctor of Engineering, Senior Researcher, Chemical Engineering Department, Products Development Center, Corporate Research & Development
ISHII Kousuke : Doctor of Engineering, Senior Researcher, Chemical Engineering Department, Products Development Center, Corporate Research & Development
MATSUZAWA Yoshiaki : Doctor of Engineering, Manager, Chemical Engineering Department, Products Development Center, Corporate Research & Development

More than 99.99% of influenza A virus particles attached to a plastic carrier were inactivated by exposure to 10 ppm (V/V) - ozone gas for 210 min at 23 to 29℃ and a relative humidity of 64 to 65%. When the virus was exposed to 20 ppm (V/V) - ozone gas for 150 min, more than 99.999% was inactivated. In contrast, the virus remained active after 10 hours under similar conditions without ozone gas. These data suggest that office disinfection against influenza viruses might be accomplished by ozone gas fumigation during night-time hours.

1. Introduction

Cases of humans becoming infected with a particularly virulent type of the avian influenza (bird flu) virus have been confirmed in recent years, and a global outbreak of human infection with a new influenza virus of swine origin has occurred in 2009. Now concerns are growing over the possibility of an influenza pandemic being caused by a highly virulent virus. In order to prevent infection, various methods for inactivating such a virus (to eliminate its infectivity) have been studied. One of the most promising of these is virus inactivation using ozone gas.

The effectiveness of ozone gas has been demonstrated in much of the literature on this matter. Shinriki et al. conducted disinfection experiments using 1 400 ppm of ozone gas and reported that ozone inactivated *Bacillus* spores.[1] Nakamura et al. performed inactivation examinations with MRSA (Methicillin-Resistant *Staphylococcus aureus*), *Escherichia coli*, *Pseudomonas aeruginosa*, Tubercle bacillus, and *Aspergillus niger* by changing the concentration of ozone gas from 5 to 400 ppm.[2] In addition, Murray et al. inactivated influenza viruses, herpes virus, vaccinia virus, adenovirus, and vesicular stomatitis virus under various ozone concentrations ranging from 800 to 1 500 ppm and demonstrated with this mechanism that ozone is effective in inactivating a broad spectrum of viruses.[3]

In most of these studies, including the above-mentioned examples, ozone gas has been used at a concentration of several hundred ppm or above. However, attention needs to be paid to the kind of materials that are to be used in the examination when applying a high concentration of ozone gas to viruses because long-term exposure to ozone gas can degrade some materials.

To prevent influenza infection, measures suited to each possible route of transmission are required. One such route is droplet transmission; that is, transmission via droplets produced by sneezing or coughing. To prevent these droplets from being dispersed in the air, it is recommended that a surgical mask or other suitable face covering be worn.[4] With contact transmission that occurs via viruses which adhere to the surface of the skin of your hands and fingers or to various objects, however, very little of the virus is dispersed in the air. Consequently, it is difficult to filter them from the air or remove them from a room using an air purifier. In addition, it has been confirmed that influenza viruses that adhere to the surface of a material can retain their infectivity even 24 hours after adhesion.[5] Effective preventive measures are therefore required, especially to deal with contact transmission.

In this study, we conducted ozone fumigation experiments using an influenza virus adhered or fixed to carriers so as to verify the effectiveness of ozone gas, envisaging that fumigation could be conducted using ozone gas at a lower concentration of about tens of ppm in living spaces such as office buildings at night, for example, when no one is present.

2. Experimental method

2.1 Experimental apparatus

In order to maintain constant ozone concentration, an experimental apparatus such as that shown in **Fig. 1** was used. Ozone gas, which was generated by passing oxygen gas through an ozone generator, was introduced

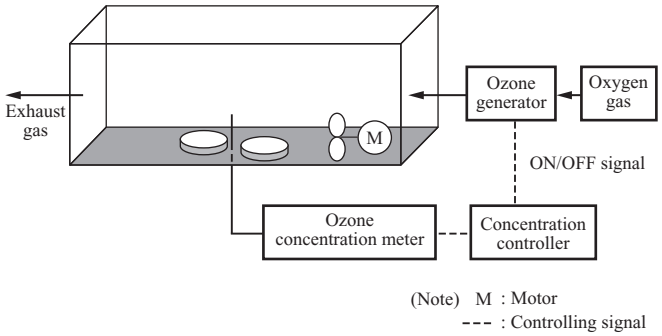

Fig. 1 Schematic of experimental apparatus

into a box-shaped experimental apparatus—which had a volume of 160 liters and was made of transparent polyvinyl chloride plates that isolate the air it contains from the outer environment—at a constant flow rate of 4*l*/min. In order to maintain a homogenous state within the experimental apparatus, a fan installed inside it was continuously rotated to circulate the gas. The concentration of ozone in the experimental apparatus was measured by an ozone concentration meter, and a constant concentration was maintained by means of a signal sent by the meter to switch the ozone generator on and off as required.

2.2 Experimental conditions

Because the actual inactivation of influenza viruses is expected to be carried out in a common residential environment, our experiment was conducted under controlled conditions, with the air temperature in the experimental room at between 23 and 29°C and relative humidity of between 64 and 65%. The experiment was conducted with ozone concentrations of 10 ppm and 20 ppm. Given that influenza viruses may lose their infectivity regardless of ozone fumigation, the same experiment was also performed using only oxygen gas that had not been passed through the ozone generator.

2.3 Preparation of the virus

0.1 m*l* each of a solution of influenza virus A/PR/8/34 (H1N1) with a known titer was added in drops to each carrier and then spread over its entire surface using a micropipette tip. After being left to stand for about 20 minutes, the solution dried and became fixed. **Figure 2** shows the immobilization of the influenza virus particles. Plastic petri dishes (60 mm in dia., polystyrene) and glass petri dishes (60 mm in dia.) were used as the carriers to which the influenza virus was applied.

The experiment was started after the multiple carriers of the influenza virus were carefully placed in a controlled experimental apparatus with a constant ozone concentration. When a certain period of time had elapsed after the start of the experiment, the carriers were individually removed from the experimental apparatus and the virus was recovered by immediately washing it away from their surfaces with sterilized water. The titer was measured using the recovered liquid.

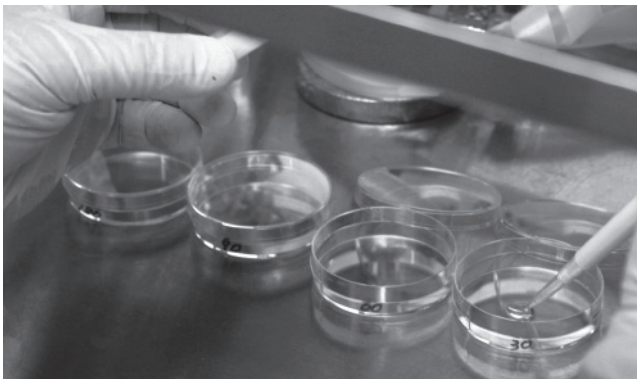

Fig. 2 Immobilization of virus particles

2.4 Measurement of the titer

The titer of the influenza virus was measured by infecting the virus solutions, which had been subjected to a ten-fold serial dilution, with MDCK cells (Madin-Darby Canine Kidney cells), incubating the solutions in multiple culture bottles and then observing the cytopathic effect with a microscope. The titer was expressed as $TCID_{50}$ (the dilution rate of the virus when the cytopathic effect is observed in half of the culture bottles), with this value obtained through the Reed-Muench method.[6), (7)]

3. Results and discussion

3.1 Changes in the titer after 10 hours of ozone fumigation

The first experiment was conducted by fumigating the experimental apparatus with ozone to a concentration of either 0 or 20 ppm for 10 hours. **Figure 3** shows how effective this proved in inactivating the influenza virus when plastic and glass petri dishes were used as the carriers. We found that not less than 99.999 9% of the virus in a dried state was inactivated, irrespective of

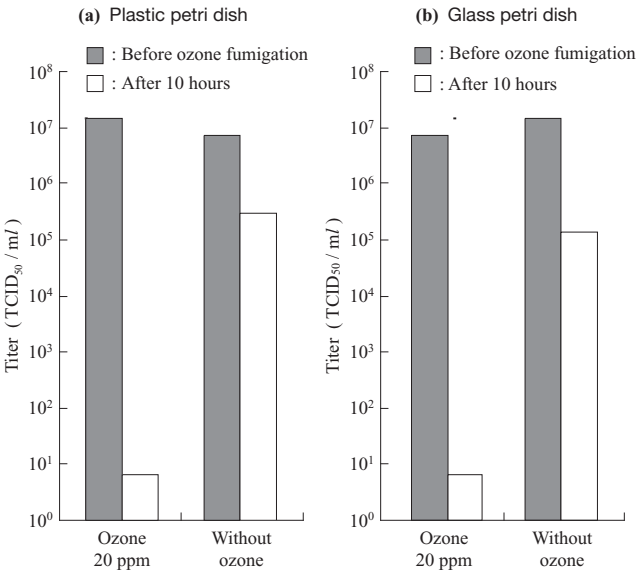

Fig. 3 Inactivation of influenza virus attached to carrier surfaces

whether the petri dishes were made of plastic or glass.

In contrast, the titer remained at values of not less than 10^5 $TCID_{50}$/m*l* even after 10 hours had passed in the experiment conducted without ozone gas.

3.2 Influence of ozone concentration and fumigation time

Changes in the titer over time while changing the ozone concentration in the experiment conducted using plastic petri dishes as the carriers for the influenza virus are shown in **Fig. 4**. Ozone gas was confirmed to result in log reductions in the titer, especially in the initial stage of the inactivation.

With an ozone concentration of 20 ppm, the initial titer was 1.4×10^6 $TCID_{50}$/m*l*, while after 150 minutes the titer was 6.3×10^0 $TCID_{50}$/m*l*, which corresponds to a reduction of 99.999%. With an ozone concentration of 10 ppm, the initial titer was 2.0×10^6 $TCID_{50}$/m*l*, while after 210 minutes the titer was 7.2×10^1 $TCID_{50}$/m*l*, which again corresponds to a reduction of 99.996%. In both cases, not less than 99.99% of the influenza virus was inactivated.

This shows that by introducing ozone gas into an area at lower concentrations over several hours, the titer of the influenza virus can be dramatically reduced.

3.3 Relationship between the CT value and inactivation effect

The effectiveness of ozone in inactivating microorganisms or viruses is generally evaluated by means of the CT value (concentration-time value).[8] A product of the concentration of the inactivating agent and the fumigation time, the CT value is used as an index of a substance's inactivation effect based on the premise that when the CT value is constant, the degree of inactivation is the same. **Figure 5** shows the relationship between the titer and the CT value during a time period where changes in titer were observed using some of the results shown in **Fig. 4**.

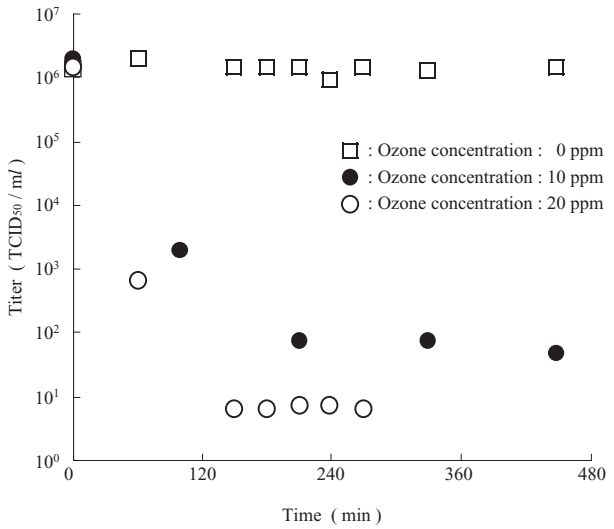

Fig. 4 Time course of influenza virus inactivation at different concentrations of ozone gas

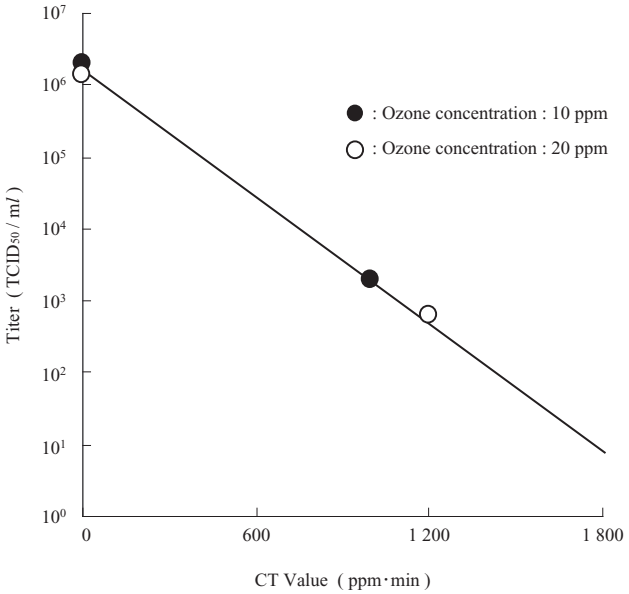

Fig. 5 Relationship between inactivation efficiency and CT value of ozone gas

The titer that was measured at up to 60 minutes after fumigation with ozone gas to an ozone concentration of 20 ppm and that at up to 100 minutes after fumigation with ozone gas to an ozone concentration of 10 ppm were plotted on the same line. The results suggest that it might be possible to use the CT value as an index of inactivation efficiency for influenza virus inactivation using ozone.

The results of this experiment prove that ozone gas can be used to almost completely inactivate influenza viruses on plastic and glass surfaces.

4. Conclusion

In order to demonstrate the inactivation efficiency of relatively low concentrations of ozone gas on an influenza virus that has adhered to the walls or floor of a room and dried, a virus was applied to plastic and glass carriers, immobilized, and then subjected to ozone gas fumigation experiments in an experimental apparatus with a volume of 160 liters. Measuring the effectiveness in reducing the titer of the influenza virus under a constant ozone concentration demonstrated the following points.

(1) When the ozone concentration was 0 ppm, the influenza virus that has dried on the carriers maintained a high infectivity even after 10 hours.

(2) When the ozone concentration was between 10 and 20 ppm, the infectivity of the influenza virus decreased logarithmically over time. When the ozone gas concentration was 20 ppm, 99.999% of the influenza virus was inactivated after 2.5 hours of fumigation, and when the ozone concentration was 10 ppm, not less than 99.99% of virus was inactivated after 3.5 hours of fumigation.

(3) It was suggested that the CT value could be

utilized as an index of inactivation efficiency for influenza virus inactivation using ozone.

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