Microwave-irradiation-assisted HVAC Filtration for Inactivation of Viral Aerosols

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ABSTRACT

Inactivation of collected viral aerosols is important for preventing a filter medium’s serving as a fomite. The focus of this study was to evaluate the inactivation efficiency (IE) achieved through filtration coupled with microwave irradiation. MS2 aerosolized through a Collison nebulizer was fed into the system and collected onto the filter. For in-flight microwave decontamination, microwave irradiation was applied to an HVAC (heating, ventilation and air conditioning) filter supported on a SiC disk for three cycles of selected irradiation times per 10 min (i.e., 1, 2.5, 5, and 10 min/10 min) at power levels ranging from 125 W to 375 W. The survival fraction (SF) on the substrate and the IE through the entire system were investigated to determine the efficacy of this approach. SF decreased and IE increased as microwave power level was increased (p = 0.02 and p < 0.01, respectively) or the application time was extended (p = 0.03 and p < 0.01, respectively). Both measures changed sharply above a threshold temperature of around 90°C and reached 2 logs at 116 and 109°C, respectively. The log SF and IE of –2.59 and 3.62, respectively, were observed when the operating condition of 375 W for 10 min/cycle was used and the SiC disk facilitated microwave absorption. When a quartz frit was used as a support instead of the SiC disk, log inactivation efficiencies of 0.8, 1.0, and 1.3 were measured at relative humidities of 30%, 60% and 90%, respectively, under the same irradiation conditions. Relative humidity is a significant parameter from 50–80°C (p = 0.01). The results demonstrate that microwave-assisted filtration systems can be used as an effective means for inactivating viruses.

Keywords: HVAC filter; Mask; Microwave; MS2; Inactivation efficiency; Survival fraction

INTRODUCTION

Microwaves—electromagnetic waves with frequencies between 300 MHz and 300 GHz—are widely applied in food processing, wood drying, plastic and rubber treating, curing and preheating ceramics as well as in cleanup processes (Park et al., 2006). Microwaves are non-ionizing but sufficient to cause the molecules in matter to vibrate, thereby causing friction, which is subsequently transformed into heat for various applications. Among the diverse applications, the use of microwaves for decontamination was studied soon after microwaves became available. Goldblith and Wang (1967) and Fujikawa et al. (1992) compared the effect of microwave irradiation on Escherichia coli (E. coli) and Bacillus subtilis (B. subtilis). They concluded that the heat produced was a key factor for inactivating the bacteria in solid and aqueous phases. Meanwhile, there has been research demonstrating additional effects, beyond the purely thermal mode of inactivation. For example, distortion of membrane structure and function (Phelan et al., 1994), altered enzyme activity (Dreyfuss and Chipley, 1980), disruption of weak bonds (Betti et al., 2004), increased release of various substances (Woo et al., 2000; Celandroni et al., 2004; Campanha et al., 2007), and increased ionic strength due to an increased current within cells (Watanabe et al., 2000) have all been reported. However, all of the aforementioned research was conducted in the liquid, solid, or aqueous phase.

In recent years, microwave inactivation of airborne microorganisms has gained more interest because of increasing concerns about health-related issues, including outbreaks of pathogenic airborne viruses (e.g., SARS, H1N1 and swine flu). For examples, Hamid et al. (2001) measured 90% inactivation efficiency (IE) by applying microwave irradiation to airborne bacteria and fungi at 600 W for four periods of 2.5 min, each separated by 5 min from the next. Elhafi et al. (2004) demonstrated that infectious bronchitis virus, avian pneumovirus, Newcastle disease virus and avian influenza virus were inactivated on dried swabs in less than 20 s at 1250 W. In another study, Wu and Yao (2010a) reported IEs of 65% and 6% against airborne B. subtilis var niger spore and Pseudomonas fluorescens, respectively, in an air stream after exposure to microwaves at 700 W for...
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2 min. Wu and Yao (2010b) showed gene mutation through polymerase chain reaction-denaturing gradient gel electrophoresis after microwave application. Other recent studies (Heimbuch et al., 2010; Zhang et al., 2010) have focused on microwave inactivation of contaminated filters. Although filters are effective devices for capturing bioaerosols—utilized to reduce the spread of infectious viruses, both in virtually all modern heating, ventilation and air conditioning (HVAC) systems and in filtering facepiece respirators (FFRs) at healthcare facilities and by first responders—they are limited as a preventive method because they inactivate neither viruses that pass through the filter nor those that are captured. As some pathogens have a low infectious or lethal dose, viruses that penetrate or reaerosolize have the opportunity to infect people the filter was intended to protect (McCrumb, 1961). Heimbuch et al. (2010) reported that microwave-generated steam at 1250 W for 2 min induced a 5-log IE for H1N1 virus collected on FFRs. Zhang et al. (2010) demonstrated that microwave irradiation could provide an adequate method for inactivating B. subtilis endospores and E. coli via a microwave-assisted nanofibrous air filtration system.

Indoor air quality is strongly dependent on the HVAC system. If infectious viruses can be inactivated while they circulate through the HVAC system, the risk of spreading viruses can be reduced. However, no research has been conducted to evaluate the applicability of the microwave inactivation technology to commercial HVAC filters even though these filters are commonly used in hospitals and residential buildings for collective protection. Therefore, the objective of this study was to evaluate the inactivation performance of microwave-irradiation assistance to HVAC filtration systems during in-flight filtration against MS2 bacteriophage (MS2). Key parameters examined were microwave power level, microwave application time, and relative humidity. The thermal stability of the filter media was also investigated.

**MATERIALS AND METHOD**

**Test Filters and Agent**

Two commercial HVAC filters made of polyethylene (PE) and polypropylene (PP) (Filter 1; 3M) and synthetic polymer (Filter 2; True Blue) were selected as test filters, and glass microfiber LydAir MG (Filter 3; Lydall) was used for comparison. MS2 (ATCC® 15597-B1™) was applied as a test agent. It is a surrogate for enteroviruses because of their similar structural properties and resistance to heat and chemicals (Brion et al., 1999; Prescott et al., 2006). Freeze-dried MS2 was suspended in DI water with a titer of around $10^6–10^9$ plaque-forming units (PFU)/mL as the virus stock suspension.

**Experimental System**

A microwave oven (Panasonic, NN-T945SF, 2.45 GHz, continuous irradiation) with two one-inch holes in the back was used in this study. Because common filter holders could not survive in the microwave, a custom-made quartz filter holder was placed inside the microwave. To support the filter material and to enhance heat transfer, a SiC disk was employed inside the quartz reactor.

The experimental set-up for testing the inactivation of the virus is shown in Fig. 1. Six L/min of dry air was passed through a six-jet Collison nebulizer (Model CN25, BGI Inc., MA) to aerosolize the viruses. A second air stream passed through the humidifier and then rejoined the flow. After the combined flow passed through the mixing chamber, it was split three equal ways, whence each stream proceeded toward the filtration unit at 4 L/min, corresponding to a face velocity of 5.3 cm/s, which is a standard face velocity for ventilation system testing (U.S. Army, 1998). Of the three flows, two were directed to filter holders outside the microwave, one with and one without an HVAC filter, as controls. The third was equipped with an HVAC filter 47 mm in diameter (effective diameter 40 mm for the quartz reactor used) inside the microwave.

Fig. 1. Experimental set up for microwave-irradiation-assisted filtration.
the microwave oven. The filters inside and outside the microwave oven were labeled A and B, respectively. The BioSamplers downstream of the microwave/filtration system and non-irradiated filter were labeled C and D, respectively. The BioSampler downstream of the empty filter holder (control) was labeled E.

For in-flight microwave decontamination, microwave irradiation was applied for three 10-min cycles that included selected periods of irradiation—1, 2.5, 5 and 10 min/10 min—at three different microwave power levels, 125, 250 and 375 W. To select the microwave application conditions, the thermal stability of three test filters was analyzed by thermogravimetric analysis and simultaneous differential thermal analysis (TGA/SDTA) (851E, Mettler–Toledo Inc., OH), and the temperature of filters on the SiC disk under different applied conditions was measured with an IR pyrometer (OS533E, Omega Engineering Inc., CT). After irradiation, the test filter was taken off the filter holder in the experimental system and subjected to wrist-action shaking (Model 75, Burrell Scientific, PA) with a shaking angle of 20° for 15 min to extract the viruses (Woo et al., 2010).

The extracted MS2 was assayed with E. coli as a host by the single-layer method (EPA, 1984). For enumeration of MS2 viruses within an adequate count range of 30–300 PFU/mL, 1 mL of diluted MS2 was mixed with 9 mL of #271 agar and 1 mL of #271 medium with log phage E. coli and poured into the petri dish. After the mixture solidified the plate was stored in the incubator at 37°C for one night before counting.

The effectiveness of the inactivation process was evaluated by using two parameters: survival fraction (SF) and IE. The SF under microwave irradiation was calculated by comparing the viable MS2 in the two filters:

$$\text{SF} = \frac{C_A}{C_B}$$  \hspace{1cm} (1)

where $C_A$ and $C_B$ are the viral concentrations collected by filters A and B, respectively.

Viral aerosols penetrating the test filters under microwave irradiation were collected in BioSamplers containing 15 mL of DI water. The IE through the microwave/filtration system was obtained by comparing the viable MS2 concentration in the two BioSamplers:

$$\text{IE} = \frac{C_C}{C_D}$$  \hspace{1cm} (2)

where $C_C$ and $C_E$ are the concentrations of viable viruses collected in the BioSamplers C and E, respectively.

The filtration efficiency of the filter itself $(1-C_D/C_A)$ was used to confirm the stability of this system after each test. The pressure drop of the filter was measured by a Magnehelic gauge to evaluate the degradation or change of filters after decontamination test. Triplicate experiments and duplicate assays were carried out, and 1-way ANOVA was used for statistical analysis after confirming over 90% of normality (Design-Expert® 8.0).

The scanning electron microscopy (SEM, JEOL JSM-6330F, JEOL Inc., MA) images of virus-contaminated filters were taken after conventional oven heating and after microwave irradiation heating to investigate non-thermal effects of microwave irradiation. A conventional oven (ISOTEMP® oven 230G, Fisher Scientific, PA) was used to provide purely thermal effects. Filters contaminated with a virus suspension of $10^{10}$ PFU/mL of DI water were either microwaved or inserted into a conventional oven for 30 mins.

**RESULTS AND DISCUSSION**

**Temperature Measurement of Test Filters**

TGA/DTA was used to determine the appropriate temperature range for microwave-assisted HVAC filtration because of the concern that the polymer fiber of the filter might experience melting or other mutations during the thermal process. As displayed in Fig. 2(a), no residual moisture loss around 100°C was observed in all three filters,
physical filtration efficiency (1–log removal efficiency of 0.53 (i.e., 71%) coming from the additional disinfection was observed beyond the inherent flight. At the lowest setting—125 W for 1 min/cycle—no IE is attributed to two factors: 1) physical capture by the air stream. At 375 W for 10 min/cycle, the max temperature was around 120°C, whereas it reached 165°C without airflow, illustrating the cooling effect by the air stream. Based on the temperature measurement, a maximum power level of 375 W was selected to investigate the IE and SF in this study for Filters 1 and 2. Higher power levels of 500 W and 750 W were selected only for Filter 3 because of its high thermal stability as mentioned previously.

**Inactivation Efficiency and Survival Fraction**

For Filter 1, the IE of the microwave-irradiation-assisted filtration system and the SF on the filter surface as a function of microwave power at four different microwave application times are displayed in Figs. 4(a) and (b), respectively. As shown, IE increased and SF decreased as microwave power was increased and as the application time was extended. For the IE, changes to both microwave power level (\(p < 0.01\)) and application times (\(p < 0.01\)) were significant. The IE is attributed to two factors: 1) physical capture by the filtration mechanism and 2) inactivation of viruses during flight. At the lowest setting—125 W for 1 min/cycle—no additional disinfection was observed beyond the inherent log removal efficiency of 0.53 (i.e., 71%) coming from the physical filtration efficiency (1–\(C_D/C_0\)) of filter 1 (~73%). At a power level of 375 W, 3.0 and 3.5 logs of the viable MS2 were disinfected when microwave irradiation was applied for 5 and 10 min/cycle, respectively. This suggests that an application time of 5 min/cycle is sufficient to disinfect MS2. Significant influences of both microwave irradiation time (\(p = 0.02\)) and power level (\(p = 0.03\)) upon SF were seen. The trends of SF were similar to those of IE, although a much lower SF was expected at higher microwave power levels because of the longer exposure time of 30 mins for the SF as compared to the shorter flight time of less than 5 s for the IE. However, at 375 W applied for 5 and 10 mins/cycle, a higher value of log IE was observed than the absolute value of log SF.

Physical capture is one possible reason for the higher log IE. However, the log IEs after deducting the inherent removal efficiency were still higher than the absolute values of the log SF (2.5 and 2.9 vs. 1.8 and 2.5). This may be explained by the high temperature of the SiC disk. Damit *et al.* (2010) reported that the exposure of MS2 to 250°C for 1 s resulted in 4-log SF. The temperatures of the SiC disk at 375 W immediately after irradiation at 5 and 10 min/cycle were 172°C and 203°C, respectively, whereas the temperatures of the filters on the SiC disk were 107°C and 117°C. The thickness of the SiC disk was 2.54 cm, and viruses flying through the disk were exposed to these high temperatures for 0.5 s. This exposure during flight could attribute to the higher IE. For Filter 2, similar results were seen, as shown in Figs. 4(c) and (d), although the inherent filtration efficiency was slightly higher than that of Filter 1. For Filter 3, IE and SF at 375 W, 500 W, and 750 W are displayed in Figs. 4(e) and (f). As shown, the IE and SF at 375 W are similar to those for Filters 1 and 2, although the higher inherent filtration efficiency was around 95%. At 500 W and 750 W, log SFs of ~3.47 and ~4.23 were seen, respectively. The temperatures of Filter 3 on the SiC disk at 500 W and 750 W for 10 min/cycle were 143°C and 171°C, respectively. This result suggests that the thermal stability of filter material is an important factor for microwave disinfection applications.

**Effective Temperature**

Comparing the microwave irradiation power level and application time data revealed that filter disinfection can be characterized by a threshold temperature, i.e., the temperature above which inactivation starts to increase sharply. Similarly an effective temperature, defined as the minimum temperature that must be reached for effective disinfection (2-log or greater), can also be identified. The threshold and effective temperatures can be estimated in Fig. 5, which displays log IE and log SF as a function of the temperature reached after microwave irradiation application. The data pattern greatly resembles a two-stage process—an initial accumulation of energy, and then a catastrophic release, simply indicating a threshold temperature has been reached. The IE remains unsatisfactory until the threshold temperature of around 90°C, and it reaches 2 logs at 109°C. The SF also starts to rise around 90°C and reaches 2 logs at 116°C. Once the filter reaches this temperature, effective disinfection of the virus can be assumed. IE and SF of each filter against MS2 can also be expressed as a function of temperature (\(T\)) via a log-linear relationship above the
threshold temperature, as displayed in Table 1. Although different intercepts and slopes were expected because of different inherent physical removal efficiency and thermal properties of filter, the difference was not significant ($p < 0.05$). Furthermore, when the intercepts of log IE were corrected by deducting the inherent filtration efficiency, the results showed no difference among all three filters ($p = 0.02$). Therefore, in and near the temperature region studied,

Fig. 4. Log inactivation efficiency by microwave irradiation assisted filtration system for (a) a PE-PP meltblown (Filter 1), (c) a synthetic polymer meltblown (Filter 2), and (e) a glass fiber medium (Filter 3), and log survival fraction on filter surface with a SiC disk for (b) a PE-PP meltblown (Filter 1), (d) a synthetic polymer meltblown (Filter 2), and (f) a glass fiber medium (Filter 3), as a function of microwave application time at three different microwave power levels. The error bar represents one standard deviation.
Fig. 5. (a) Log inactivation efficiency and (b) log survival fraction as a function of the temperature reached during microwave irradiation of a PE-PP meltblown (Filter 1) at 125, 250 and 375 W and a glass fiber medium (Filter 3) at 500 and 750 W. The error bar represents one standard deviation.

Table 1. Linear relationship of the IE and SF of MS2 with temperature (T).

<table>
<thead>
<tr>
<th>Filter</th>
<th>Log IE</th>
<th>Log SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter 1</td>
<td>$-7.14 (-7.65)$ + 0.087$T$ ($p = 0.04$)</td>
<td>$4.67 - 0.057T$ ($p &lt; 0.01$)</td>
</tr>
<tr>
<td>Filter 2</td>
<td>$-6.69 (-7.46)$ + 0.077$T$ ($p = 0.02$)</td>
<td>$4.81 - 0.060T$ ($p &lt; 0.01$)</td>
</tr>
<tr>
<td>Filter 3</td>
<td>$-6.57 (-7.60)$ + 0.078$T$ ($p &lt; 0.01$)</td>
<td>$5.05 - 0.061T$ ($p &lt; 0.01$)</td>
</tr>
<tr>
<td>All filters $^b$</td>
<td>$-6.83 (-7.57)$ + 0.080$T$ ($p = 0.02$)</td>
<td>$5.01 - 0.060T$ ($p &lt; 0.01$)</td>
</tr>
</tbody>
</table>

$^a$The values in parentheses in log IEs are the intercepts calculated with inherent filtration efficiency deducted.

$^b$The relationships were obtained from all IEs and SFs above threshold temperature of three filters.

Thus, for an HVAC filter having 99.9% filtration efficiency to achieve a 6-log IE for MS2, the necessary temperature is $\sim 132^\circ$C.

Although thermal effect was a major factor for microwave inactivation, Khalil and Villota (1989) compared the distortion of RNA subunits in *Staphylococcus aureus* after microwave and conventional heat treatments, and found destruction of the 23S RNA by only microwave treatment, indicating the possibility of non-thermal effect. In addition, Betti (2004) reported a non-thermal effect of microwaves against plants and viruses at a sublethal temperature, and Wu and Yao (2010) confirmed visible changes of bacteria and fungi after microwave heat treatment by ESEM. Hence, in this study non-thermal effects were investigated by studying the morphological changes and SFs with and without microwaves at the same temperature. Fig. 6 displays the temperature profiles of the conventional and microwave ovens. Temperatures of the conventional oven were selected based on the temperature profiles of the microwave oven at 250 W and 375 W. The conventional oven’s temperature was stable for 30 min of test time. For the microwave oven operated at 250 W, a steady-state temperature profile was observed after 10 min. However, temperatures around 90°C at 250 W might be insufficient to inactivate MS2. Therefore, 375 W was selected for observations of morphological changes through SEM.

Fig. 7 displays SEM images of untreated, conventional-oven-treated, and microwave-treated virus-contaminated filters. As shown in Figs. 7(b) and (c), the heat of both the conventional oven and the microwave oven made the water evaporate (or removed it in some other way), and then
aggregation was observed. However, no significant difference in morphology was seen, even though the concentration of microwave-treated viruses was lower than that of conventional-oven-treated viruses. SFs of viruses on the substrates after heat treatment by microwave oven and conventional oven were also compared but no significant difference was shown, indicating that no non-thermal effect of microwaves can be elucidated in this study.

**Effect of Relative Humidity on Inactivation Performance**

Relative humidity is another key parameter affecting the inactivation of viruses. However, when a SiC disk is used, it is difficult to determine the effect of relative humidity because of the overwhelming thermal effect of the SiC disk as compared to relative humidity. Therefore, to investigate the effects of relative humidity, a quartz frit was used as a support instead of a SiC disk. IE through the system and SF on the filter surface as a function of microwave power level applied to Filter 1 for 5 min/cycle under three relative humidities are displayed in Figs. 8(a) and (b), respectively. IE \((p = 0.01)\) significantly increased and SF \((p < 0.01)\) significantly decreased as the application time increased. By design the quartz frit could not absorb microwave irradiation, which resulted in a lower filter temperature and less pronounced viral inactivation capacity compared to those with the SiC disk. Log IEs of 0.8, 0.9, and 1.3 were obtained at relative humidities of 30%, 60%, and 90%, respectively, at 500 W \((p < 0.01)\). Unlike the results with a SiC disk, log IE corrected for filtration efficiency of the filter itself was lower than the absolute value of log SF, indicating that the lower temperature of the support is insufficient to inactivate MS2. At the higher power level, a high IE and lower SF were seen under high relative humidity, which may be explained by the mechanism of microwave irradiation. The higher water content can contribute to more efficient heating induced by water’s molecular vibrations (Fisher et al., 2011). However, the relative humidity effect was not observed at 500 W for 10 min/cycle in Figs. 8(c) and (d). The different phenomenon can be explained by the increased concentration of water vapor at higher temperature and the higher temperature itself. At high relative humidity, final temperatures were 27°C, 43°C, 66°C, and 81°C after 5 min/cycle, and 49°C, 62°C, 78°C, and 89°C after 10 min/cycle at 125, 250, 375, and 500 W, respectively. The results suggest that relative humidity is a significant parameter from 50–80°C and that it ceases to be significant above 90°C.

**Effect of Microwave Treatment on Pressure Drop**

Pressure drops of the test filters after microwave treatment were measured to inspect for degradation of the filter. Under the operating condition, the initial pressure drops (at 5.3 cm/s) of 0.45, 0.62, and 1.20 inches H2O for Filters 1, 2 and 3, respectively, were maintained throughout several microwave irradiation tests at 375 W for 10 min/cycle. There was no significant difference in pressure drop between control and treated filters, indicating no melting or degradation. SEM images also showed no visible morphological changes.

**Comparison to Other Disinfection Technologies**

Numerous disinfection technologies, including energetic techniques and chemical treatments, and with or without filtration systems, have been studied. A study investigating bleach disinfection with 0.1% sodium hypochlorite aerosol and UV germicidal irradiation (UVGI) at a wavelength of 254 nm achieved 2-log SF of MS2; however, bleach and UVGI have limitations of chemical release and low penetration, respectively (Vo et al., 2009). Rengasamy et al. (2010) and Woo et al. (2011) confirmed the inactivation effect of biocidal filters incorporated antimicrobial agents, e.g., silver copper, oxygen species, titanium oxide and dialdehyde, but these filters did not reach 2-log SF within 30 mins. Compared to other filter disinfection technologies, the microwave-assisted filtration system was as or more effective without causing any filter damage and chemical formation. As a direct disinfection technology without filters, an electrostatic precipitator (ESP) at ± 6 kV was reported by Kettleson et al. (2009) to exhibit 2-log IE of MS2 and at –10 kV it could reach above 6-log IE. However, using negative corona in ESP disinfection should be cautioned because of the formation of ozone. Grinshpun et al. (2010) demonstrated 2-log IE of MS2 by dry heat treatment at 125°C for 0.24 s. This value is similar to that obtained in this study after deducting the inherent filtration efficiency, and it confirms the thermal effect of microwave

**CONCLUSIONS**

This study demonstrates that microwave-assisted-filtration...
is an efficient approach for inactivating viral aerosols. Microwave power and application time are key operating parameters for controlling the disinfection effectiveness of viral agents. Both factors combine to yield a threshold temperature of ~90°C. Relative humidity is another pivotal parameter for viability of viruses at warm-to-hot-water temperatures, but it becomes insignificant at temperatures above 90°C. When sufficient microwave power is applied across a thermally stable filter material, a high inactivation efficiency of around 5 logs through the system can be reached at temperatures lower than those of other dry heat treatments.

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