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Airborne disinfection using microwave-based technology: Energy efficient and distinct inactivation mechanism compared with waterborne disinfection

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ABSTRACT

Microwave has been extensively applied to inactivate microorganisms in liquids, food, and surfaces. However, energy efficiency is a limiting factor for the environmental application. The utilization pathway and energy efficiency of the microwave in different media have not been investigated. In this study, the inactivation performance, energy utilization, and bactericidal mechanisms for microwave-irradiated airborne and waterborne Escherichia coli were compared. A Beer-Lambert law-based model was also developed and validated to compare the inactivation performance in different phases. Microwave had greater inactivation effect on airborne bacteria than waterborne bacteria. The inactivation rate constant for airborne E. coli (0.29 s⁻¹) was nearly 20 times higher than that of waterborne species (0.014 s⁻¹). Most of the absorbed microwave energy (92.3%) was converted to increase water temperature instead of inactivating the waterborne bacteria, because the microwave photons were easily absorbed by water molecules. By contrast, 45.4% of the absorbed energy could disinfect the airborne bacteria. Finally, the required energies for 1-log inactivation were calculated as 2.3 J and 116.9 J per log-inactivation for airborne and waterborne E. coli, respectively. The airborne and waterborne E. coli samples showed distinct microwave inactivation mechanisms. Waterborne E. coli disinfection was primarily due to thermal effect, while the non-thermal effect was the major mechanism for airborne E. coli inactivation.

1. Introduction

Airborne particles contain living microorganism, such as virus, bacteria, and fungal spores. These small particles, with sizes ranging from less than 1 μm–100 μm, can affect human health by causing infectious diseases, acute toxic reactions, and allergies (Gergen, 2001; Liu, Zhang, Wen, & Wang, 2017). Outbreaks of severe acute respiratory syndrome (SARS) and influenza H1N1 viral infections across the globe have attracted worldwide attention for airborne microbial prevention and control measures (Liang et al., 2012; Zhang, Damit, Welch & Park, 2010).

Some technologies were developed for airborne microorganism disinfection. Traditionally, particulate filters inside heating, ventilation and air-conditioning (HVAC) systems have been utilized to control airborne microorganism. However, not all the collected biological agents are killed, sometimes they may accumulate on the filter surface and become a potential source of diseases.

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(Maus, Goppelsroder & Umhauer, 2001; Moritz, Peters, Nipko & Ruden, 2001). Ultraviolet (UV) light has been successfully used as another alternative for airborne microorganism disinfection (Linnes, Rudnick, Hunt, Mcdevitt, & Nardell, 2014; Wang, Xi, Hu, & Yao, 2009), but the efficacy can be limited by environmental factors (irradiation distance etc.) and the phenomena of bacterial re-activation (Guo, Hu, & Liu, 2009).

Irradiation-based disinfection technologies are becoming popular because of advances in equipment reliability and reduction of undesirable disinfection by-products (Plazas-Tuttle, Das, Sabaraya, & Saleh, 2017). Microwaves (MWs) are electromagnetic waves with frequency ranging from 300 GHz to 300 MHz and have been proven to exhibit germicidal activity. Rapid development in MW technology for industrial and household applications has prompted significant interest for environmental concerns. Many studies have utilized MWs to inactivate microorganisms in solid, liquid, water phases, and a few studies on airborne microbes (Awuah, Ramaswamy, Economides, & Mallikarjun, 2005; Mainelis et al., 2002; Mawioo, Rwemamu, Garcia, Hooijmans, & Brdjanovic, 2016; Takushima, Miyakawa, & Kanno, 2007; Wang, Zhang, & Liu, 2018). The application of MW technology for environmental concerns is limited by energy efficiency, because MW energy is primarily converted to heat the media instead of inactivating the microbial cells (Wu & Yao, 2013). However, the comprehensive investigation on the utilization pathway and energy efficiency during MW irradiation has not been conducted yet.

MW-induced reactions have been studied since the mid-1980s (Zhang, Damit, Welch & Park, 2010). Studies, especially the earlier ones on food and liquid sterilization, have shown that microbes are inactivated by MW solely through thermal effects (Fujikawa, Ushioda, & Kudo, 1992; Jung, Lee, & Kim, 2009). However, some recent studies have reported non-thermal effects during MW disinfection of microorganisms (Celandroni, Longo, Tosoratti, Giannessi, & Senesi, 2004; Yu & Yao, 2010). These conflicting results were determined from different media, suggesting that the inactivation mechanism for microbes might change with various media considering the distinct interaction of MW photons with the media.

This study presents the inactivation of a common environmental microorganism in which it’s a typical bioaerosol that exists in the air (Hurtado et al., 2014), *Escherichia coli*, after airborne and waterborne exposures to MW irradiation. A Beer-Lambert law-based model was developed and validated to compare the inactivation performance in the different phases. The antimicrobial performance, energy utilization, and inactivation mechanisms under the two phases were investigated. The feasibility and advantages of MW antimicrobial technology for airborne bacteria will demonstrate a promising application of the widely available MW technology as a potential disinfection device.

2. Materials and methods

2.1. Preparation and generation of waterborne and airborne E. coli

*E. coli* bacteria (CMCC1.3373) inoculated into nutrient broth medium (Beijing Sanyao Science Technology, Inc. China) were cultured in a constant temperature oscillation incubator (IS-RSDA, Crystal Technology & Industries, Inc. USA) at 37 °C for 24 h at 170 r/min (revolutions per minute). The suspension was centrifuged at 9500 r/min for 10 min, and then used the 0.9% NaCl solution (weight percentage) to wash the suspension for three times to remove the medium and diluted to a certain concentration (about $1.0 \times 10^8$ CFU/L). The waterborne *E. coli* bacteria was added into the aerosol generator (Topas, ATM226, Germany) to form airborne *E. coli* with particle size of 1–5 μm (Support Information, Fig. S1)(Wang, Lu & Zhang, 2018).

2.2. Experimental setup of MW irradiation reactor

For airborne *E. coli*, the schematic of the experimental setup for inactivating *E. coli* is shown in Fig. 1. The *E. coli* suspension was aerosolized using an aerosol generator, diluted with a certain continuous flow of sterile air and transfused into the MW irradiation tube inside the MW device (M1-L213B, China). The low and high power levels of the MW device were 119 W and 700 W, respectively. The tube diameter and wall thickness of the tube were 100 mm and 1 mm, respectively. After MW irradiation, the airborne samples
were discharged from the outlet of the MW device and collected by the six-stage Andersen sampler for further analysis. For waterborne E. coli tests, the 15 mL of water samples containing E. coli were pumped into the MW irradiation tube directly (Dashed box part in Fig. 1). After MW irradiation, 0.1 mL samples were discharged and collected for further analysis.

2.3. Sampling and analysis of waterborne and airborne E. coli

For airborne E. coli, a six-stage Andersen sampler (ZLKZR-B01, Beijing Jolyc Technology Co., Ltd, China) was used to collect bioaerosol samples from both the inlet and outlet of MW irradiation reactor. A glass petri dish containing 25 mL of nutrient agar medium (Beijing Sanyao Science Technology, Inc. China) was placed on each stage of the device, and samples were obtained at a flow rate of 28.3 L/min for 5 min (Wang, Lu & Zhang, 2018). For waterborne E. coli, 0.1 mL sample was directly added into a glass petri dish containing 25 mL nutrient agar medium and spread evenly. After sampling, the nutrient agar plates for both waterborne and airborne samples were incubated for 24 h in a constant-temperature incubator at 37 °C. The number of colony-forming units (CFUs) on each nutrient agar plate was manually counted. The tests were carried out in triplicate for each sample. (Wang, Lu & Zhang, 2018).

2.4. Energy calculation

The absorbed energies of MW in water \( E_{1,w} \) and air \( E_{1,a} \) can be calculated as follows (Farag, Sobhy, Akyel, Doucet, & Chaouki, 2012).

\[
E_1 = I_V V \cdot t = \frac{2 \pi e_0 \varepsilon_r \beta^2 \tan \delta \cdot V \cdot t}{2 \pi}
\]

where \( I_V (J \cdot s^{-1} \cdot m^{-3}) \) is the absorbed volume energy intensity by the medium (air or water), \( V (m^3) \) is the sample volume, \( t (s) \) is the irradiation time, \( f (Hz) \) is the frequency of the microwave, \( e_0 (F/m) \) is the vacuum permittivity, \( \varepsilon_r (F/m) \) is the relative dielectric constant, \( \beta (V/m) \) is the internal electric field strength, and \( \delta \) is the dielectric loss angle.

The curves for the temperature variation in the airborne and waterborne samples were monitored using a k-type beaded wire stainless steel thermocouple (SC-K 36, Beijing Boyikang Laboratory Instrument Co., Ltd, China) during MW irradiation. Therefore, the energy used to increase the temperatures of water \( E_{2,w} \) and air \( E_{2,a} \) can be calculated as follows.

\[
E_2 = C m \Delta T
\]

where \( C (J/g/K) \) is the specific heat capacity of media, \( m (g) \) is the mass of medium, and \( T (K) \) is the absolute temperature.

The energy utilized for bacteria inactivation \( E_3 \) can be deduced as follows (Wang, Chen et al., 2019; Wang, Lu, et al., 2019).

\[
E_3 = \left( E_1 - E_2 \right) \frac{1}{\log(c_0/c_f)}
\]

All the values for the parameters can be found in SI 4 (Support Information).

2.5. Determination of inactivation mechanisms

Control tests (without MW) were conducted under the same temperatures when exposed to MW irradiation, which were controlled by a heater (electric heating coils). The inactivation performances for the control airborne and waterborne samples were compared with those under MW irradiation. The difference between the control and MW-irradiated samples can demonstrate the inactivation mechanisms in the air and water phases.

3. Results and discussion

3.1. Inactivation performance comparison

Fig. 2 shows the survived airborne and waterborne E. coli concentrations during MW irradiation. A control experiment without MW irradiation showed quite low antibacterial effects (Support Information, Fig. S2), indicating that MW has a role in disinfection. MW irradiation of the airborne E. coli exhibited a more rapid reduction in colony compared with that of the waterborne E. coli. The inactivation of airborne E. coli reached 2.6-log reduction (> 99% removal) in 20 s, but similar activity on waterborne E. coli was observed at 5 min. Previous studies on MW application in waterborne microbial disinfection showed that several minutes were often required to achieve more than 2-log inactivation performance (Kim, Jo, Kim, Bai, & Park, 2010; Park, Yang, Choi, & Kim, 2017). Therefore, this study presents inactivation results on airborne microbes and the comparison of these results with other reported data on waterborne microbes.

For model fits, the genetic algorithm (GA) was used to minimize the objective function (OF) and determine the rate constants.

\[
OF = \sqrt{\frac{1}{n-1} \sum (C_{cal} - C_{exp})^2}
\]

where \( n \) is the number of data points, \( C_{exp} \) and \( C_{cal} \) are the experimental and calculated concentrations of E. coli in water and air, respectively (Crittenden, 2012).

The experimental data in Fig. 2 and Fig. S3 (Support Information) demonstrated a linear decrease in survival logarithmic E. coli
concentrations with irradiation time. These results suggested that the inactivation reactions of airborne and waterborne *E. coli* followed the first-order kinetics. However, the inactivation rate constant of airborne *E. coli* was calculated as $0.29 \text{ s}^{-1}$, which was nearly 20 times higher than that of the waterborne *E. coli* ($0.014 \text{ s}^{-1}$). In this study, the fast inactivation performance under short exposure time (20 s) and expended energy compared with previous reports on waterborne cases, add to the data on the potential suitability of MW as an anti-bioaerosol technology (Hong, Park, & Lee, 2004; Woo, Rhee, & Park, 2000).

3.2. Model for *E. coli* inactivation performance

In the electromagnetic field, photons carrying electromagnetic radiant energy are absorbed to inactivate the microorganism (Wu & Yao, 2013; Wu & Yao, 2014). Herein, the detailed derivation process of the relationship between the microbial inactivation and absorbed energy is shown in SI 4 (Support Information).

Fig. 3 shows both the experimental data and model simulation results for the waterborne (Fig. 3A) and airborne *E. coli* disinfection (Fig. 3B) using MW irradiation. The model accurately predicted the survival *E. coli* concentrations under the high (700 W) and low (119 W) power levels when given a certain irradiation time.

The results in Fig. 3 indicated that MW irradiation of the airborne *E. coli* exhibited a more rapid reduction compared with that of the waterborne *E. coli*. The model simulation also clearly demonstrated sharp declines in airborne *E. coli* concentrations (Fig. 3B), but gradual declines in waterborne *E. coli* concentrations (Fig. 3A). Given the same irradiation time, the high power level presented better inactivation performance than those under the low power level. These findings were consistent with predicted results, which suggested that the model prediction was in good agreement with experimental data.

3.3. Energy efficiency and utilization pathway

The variation in inactivation performance should be related to the transformative nature of MW in different media (water and air). Although MW energy can be strongly absorbed by water molecules, a considerable part of the energy can be used to increase water temperature instead of inactivating the waterborne bacteria. However, the efficiency and pathway of energy utilization during the MW irradiation have not been analyzed. Therefore, a comprehensive investigation in air and water phases is essential.

Fig. 4 shows the temperature curves for the different media during MW irradiation. A sharp increase in temperature was observed in water, while gentle and smooth temperature changes occurred in air. Given the similar inactivation performance, 20 s (point A: 2.6-log reduction) and 5 min (point B: 2.48-log reduction) irradiation time were required for airborne and waterborne exposure, respectively. Under these conditions, the energies absorbed by the media, for heating the media, and for bacteria inactivation were...
calculated.

Water is a polar molecule with a high relative dielectric constant (78.5). Therefore, a huge amount of energy was absorbed by water (corresponding to 3.75 kJ). However, most of the energy (92.3%) was converted to increase water temperature, while only 7.7% of the absorbed energy was utilized to inactivate waterborne bacteria. By contrast, the MW photons were not easily absorbed by oxygen and nitrogen molecules in air (the relative dielectric constant is approximately 1.0). In addition, 45.4% of the absorbed energy could be used to disinfect the bacteria. Finally, the required energies for 1-log inactivation were calculated as 2.3 J per log-inactivation and 116.9 J per log-inactivation for airborne and waterborne bacteria, respectively. Therefore, the results demonstrated the superior energy efficacy for airborne microbes inactivation compared with waterborne microbes (Plazas-Tuttle et al., 2017; Wu & Yao, 2013).

Fig. 3. Experimental data and model simulation results for waterborne (A) and airborne (B) Escherichia coli using MW irradiation. The blue and green solid lines show model curves under low power (119 W) and high power (700 W), respectively. The blue triangular and green square dots represent experimental data under low power (119 W) and high power (700 W), respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. Energy utilization during MW irradiation on airborne and waterborne Escherichia coli. The blue and red curves represent the temperatures changes during MW irradiation in waterborne and airborne tests, respectively. Point A and B represent the conditions achieving similar inactivation performance for airborne and waterborne tests, respectively. The calculation formula we have mentioned in Section 2.4. Energy calculation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
3.4. Inactivation mechanisms investigation

3.4.1. Thermal and non-thermal effects of MW inactivation

An increase in temperature over time can result in denaturation and damage to the cell membrane inside the bacteria, affecting cell viability. The survival of waterborne *E. coli* cultures presented similar inactivation under MW irradiation and sole thermal heating conditions (Fig. 5A).

This phenomenon suggested that thermal effect is likely to be the dominant mechanism for waterborne bacteria inactivation. However, different conditions were observed for airborne bacteria. The recorded temperature change was 1.10 ± 0.20 °C from room temperature (22 °C) when the airborne samples were irradiated by MW for 20 s. Fig. 5B illustrates a significant difference between MW irradiation and sole thermal treatment. These results indicated that the two kinds of mechanisms played certain roles in airborne sterilization, but non-thermal effects should be the primary inactivation mechanism for airborne bacteria.

3.4.2. Distinct inactivation mechanisms of airborne and waterborne *E. coli*

The distinct inactivation mechanisms should be attributed to the different characteristics of electromagnetic fields in air and water phases. The MW photons can be easily absorbed by water molecules to increase water temperature (Hitchcock, 2001; Jones, Lelyveld, Mavrofdis, Kingman, & Miles, 2002). Therefore, the thermal effect plays a major role in waterborne bacteria inactivation (Cara et al., 1999). However, the MW photons are not easily eliminated by oxygen and nitrogen molecules in air (Hitchcock, 2001). Instead, most MW photons can directly react with the target microbial cells, and this process represents the non-thermal effects (Betti et al., 2004; Hong et al., 2004; Woo et al., 2000).

Previous researches showed that MW can alter enzymatic activities by disrupting weak bonds in active protein forms (Dreyfuss & Chipley, 1980), and produce reactive oxygen species (such as ·OH) (Betti et al., 2004), which was used to inactivate microorganisms (Watanabe, Kakita, Kashihe, Mika, & Tsukiji, 2000). Moreover, MW irradiation has been proven to present non-thermal effects on cell viability by inducing variations in ionic homeostasis within cells. Kesari et al., reported one possible way in which MW irradiation induced changes in the apoptotic process in cells is that microwave radiation can cause membrane permeability changes and DNA/RNA damages (Kesari, 2017).

Fig. 6 shows the leakages of intracellular ions (Ca^{2+} and K⁺), DNA, and proteins from cells exposed to MW irradiation (detailed information can be found in Support Information, SI 4). The result was in accordance with the previous study in which increased protein levels *E. coli* cell solutions were observed after MW irradiation (Dreyfuss & Chipley, 1980). Protein and DNA molecules are generally very large to pass through the undamaged cell membrane. It was suggested that MW might interact with the cell membrane, resulting in irreversible changes in its permeability. The membrane provides a permeability barrier to the passage of small ions such as K⁺ and Ca^{2+} (Campanha, Ana Clara, Ligny, Lourenco, Vergani, & Spolidorio, 2007). The leakages of K⁺ and Ca^{2+} observed in the present investigation indicated a disruption of the permeability barrier.

4. Conclusions

In summary, the inactivation performance, energy utilization, and bacterial inactivation mechanisms were compared between MW-irradiated airborne and waterborne *E. coli*. The results demonstrate that MW is an efficient and a high-energy utilization technology for inactivating airborne *E. coli* compared with waterborne *E. coli*. The mechanisms of MW inactivation of microbes differ in airborne and waterborne samples. The thermal effect was the main factor in waterborne *E. coli* disinfection by MW. By contrast, the mechanism of MW inactivation of airborne *E. coli* was mainly attributed to non-thermal effects.
Fig. 6. Leakages of intracellular ions (Ca$^{2+}$ and K$^+$), DNA, and proteins from cells exposed to MW irradiation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaerosci.2019.105437.

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