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FULL PAPER

Effective microbial disinfection in food industry with hydroxyl radical fumigation

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Abstract

Hydrogen peroxide (H_2O_2) fumigation has recently been explored and tested to be a good fumigant replacement of formaldehyde. This technique has been proven safer, less irritating and requires shorter exposure times. Surface disinfection has long been implemented with toxic formaldehyde or 35% hydrogen peroxide (H_2O_2). The results showed that they could be replaced with a safer and stronger oxidizing agent, activated H_2O_2 in a vaporized form. Aerosolization by aerosol generators has been used to produce aerosols containing hydroxyl radicals of hydrogen peroxide. The dispersal of this highly oxidizing mist of micron-size droplets destroyed *Escherichia coli* and *Aspergillus niger* colonies that have been artificially spiked on surfaces. The experiments demonstrated efficient disinfection by integrating 1 to 5% H_2O_2 fumigation with ozone (O_3) and ultraviolet light (UV-C). Studies with *E. coli* and *A. niger* showed some disinfection with either O_3 or UV-C. Combining H_2O_2 fumigation with both O_3 and UV-C exposure considerably accelerated the microbial inactivation. This approach allowed fast disinfection with 1 to 5% H_2O_2 while offering cheaper and safer disinfection for healthcare settings.

1. Introduction

Bacterial and mold contamination in food industries provided poor hygiene practices that likely contribute to infectious disease outbreaks. High bacterial contamination on any food processing areas can signify poor sanitation practices and generate detrimental consequences on human health. Possibly a few of these microbes may act as human pathogens or instigate allergic reactions (Wu *et al*., 2016). Poor hygiene and improper food preparation practices had previously been demonstrated as contributing to many foodborne diseases and outbreaks (Erickson *et al*., 2015). At the presence of high microbial counts, cross-contamination permitted the transfer of microorganisms (bacteria, virus, parasites, or fungi) from one contaminated area to the other. Unfortunately, people in the food supply chain occasionally have little awareness of surfaces and equipment with high microbial contamination, which are unable to detect by simple visual inspection and take several hours to days to validate (Erickson *et al*., 2015). Unsanitized processes or areas post a high risk to the transmission and the occurrence of sporadic foodborne outbreaks. Thorough sanitizing of food processing surfaces and areas serve as an effective precaution to prevent the chance of cross-contamination and eliminate the risk for humans to ingest contaminated food and become ill. The highly effective antimicrobial activity but minimal toxicity of the residual chemical (e.g., organic acids, chlorine dioxide, hydrogen peroxide, and ozonated water) have been done to find alternative aqueous sanitizers. (Huang and Chen, 2011; Kingsley *et al.*, 2014). Traditionally, fumigation with formaldehyde has been used to disinfect indoor areas, but formaldehyde is toxic and harmful for humans and the environment (CDC, 2008). Alternatively, replacing formaldehyde with hydrogen peroxide is proven to be safer and require shorter contact times (Krause *et al.*, 2001; Kahnert *et al.*, 2005). For example, vaporized hydrogen peroxide (VHP) has widely been used to remove casual agents in healthcare settings. However, VHP requires a high concentration of hydrogen peroxide (typically, >35%), which must be used with extreme caution with respect to contact the skin or eyes (Kimura, 2012). Instead of VHP, this work explored fumigation at 1 to 5% H_2O_2. The H_2O_2 solution was dispersed into

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aerosols with ultrasonic transducers. Additional treatments with O_3 and/or UV-C were studied systematically. These treatments have been reported to activate the generation of hydroxyl (OH·) radicals, which are typically more oxidizing than H_2O_2 molecules (Kimura, 2012). This paper had sheds light on disinfection technology that integrates ozonation and photolysis to produce highly oxidizing agents from H_2O_2, for fast-acting effect. This allows the disinfection to take place at low concentration of H_2O_2 to reduce the material cost as well as the toxicity of condensate residues.

2. Materials and methods

2.1 Strains and culture media

E. coli DMST 4609 and *A. niger* ATCC 44310 were used to test for bacteria and mold inactivation, respectively. The stock cultures were then kept in Tryptic Soy Broth, TSB (Lab M, UK), containing 20% glycerol and stored at -18˚C. Prior to use, the bacterial and mold stocks were grown in TSB for 18-24 h at 37 ˚C and Potato Dextrose Broth, PDB (Lab M, UK) for 5 days at 30˚C, respectively.

2.2 Growth conditions and viable counts

The testing strains were aseptically transferred into the surface of corresponding agar media in the mini Petri dish format. Plate Count Agar (PCA, Difco, USA) and Potato Dextrose Agar (PDA, Lab M, UK) were used to cultivate *E. coli* and *A. niger* colonies, respectively. Each inoculation volume contained 30 µL of the strain sample at 10^4 CFU/cm^2 and spread over the agar surface with a glass rod.

2.3 Fumigation setup

The generation of OH· was achieved using a patent-pending technology Thailand-1701000719, initiated by the mixing of O_3 and H_2O_2 solution using a Venturi mixer (Rano Tech Co., Ltd, Thailand). O_3 was produced by feeding oxygen gas at flow rates 2 L/min through the O_3 tube (7 g/L air-cooled ceramic O_3 tubes (Arinnovation, Thailand). The circulation of OH· and H_2O_2 was photocatalyzed by 4 UV-C lamps with 15 watts per unit located after a circulation pump to further activate more free radicals in the system. The total working volume of this system was 10 L where three piezoelectric transducers (133 mL/h vaporization rate) was installed inside to create instant OH· fume. Forced convection by a 5 W fan carried the generated fume via a conduit discharging to the bottom chamber inlet. The fume distribution inside the chamber was monitored by the %RH change using two data loggers (top and bottom dataloggers). The excess fume escaped the chamber through the outlet opening. The inoculated plates were installed face-down at the top and the sides of a testing chamber (34 × 34 × 34 cm^3). In the setup (shown in Figure 1), H_2O_2 solution and O_3 were mixed at a venturi mixer to generate the hydroxyl (OH·) radicals. The mixture was flowed through a reservoir that contained a piezoelectric transducer and a fan to deliver the fume to the testing chamber. In some scenarios, the mixture also flowed through the UV-C sterilizer.

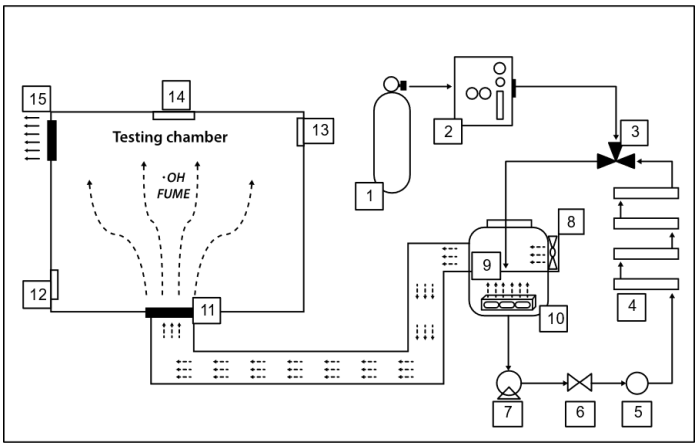

Figure 1. Schematic diagram of a 34 × 34 × 34 cm^3 testing chamber connected to the OH· aerosolization system consisting of (1) oxygen tank, (2) ozone generator, (3) venturi mixer, (4) UV-C lamps, (5) flow meter, (6) flow control valve, (7) water pump, (8) fan, (9) piezoelectric transducers, (10) holding reservoir, (11) chamber inlet, (12) bottom data-logger, (13) top data-logger, (14) inoculated plate, (15) chamber outlet.

2.4 Fumigation experiments

The inoculated agar plates at 10^4 CFU/cm^2 in each strain (*E. coli* and *A. niger*) were attached to the top and side surfaces of the chamber. There were 10 plates in each treatment for testing the disinfection efficiency. The concentration of H_2O_2 solution was varied from 1, 3, and 5%. The treatments consisted of H_2O_2 combined with O_3, UV-C, and O_3 with UV-C at different concentration of H_2O_2. The fumigation times were varied at 0, 2, 4, 6, 8, 10, 12, 14, and 16 mins in order to demonstrate any differences in their ability to kill bacteria and mold on the surface of the inoculated plates. The viable cell count after fumigation in each time was performed by incubating the inoculated plates in the oven at 37°C for overnight for *E. coli* and 5-7 days at 30°C for *A. niger*. The colonies at different fumigation times were counted as followed:

log ((number of colonies * dilution factor)/(area of petri dish plate))

2.5 Empirical model for disinfection kinetics

To compare the inactivation between the different experiments (i.e., 1% H_2O_2, 1% H_2O_2 and O_3, 1% H_2O_2 and UV-C, and 1% H_2O_2, O_3, and UV-C) as described the materials and methods above, the results were fitted to a modified Chick-Watson equation (Cho *et al.*, 2013) using nonlinear least-square solver in MATLAB® (tolerance = 10^{-6}). Log-Linear model has been widely accepted and used to describe the microbial inactivation resulted from the application of both thermal and non-thermal processes. Based on the results, it was observed that the curve of the *E. coli* reduction profiles and the treatment times both were in accordance as determined using the pseudo-first-order model. Generally, this model assumes the death rate of microorganisms, which follows the rules for first-order kinetics. Moreover, this model is widely used to assess the effect of a wide array of processing factors such as UV-C, Ozone treatments, etc., on microbial inactivation (Ochoa-Velasco *et al.*, 2020). Therefore, this model was used to predict the pseudo-first order kinetics and accounted for the tail at the end. Moreover, the first-order kinetics model is advantageous in evaluating the effect of various treatments on foodborne microbes based on its suitability and simplicity.

Where C/C_0 = the reduction in the bacterial or mold concentration at time (t), k_1 = the inactivation rate constant (min^{-1}) and k_2 = the first order decay constant (min^{-1}).

$$\mathrm{Log}\frac{C}{C_o} = -k_1[1-\exp(-k_2 t)]$$

The empirical kinetic parameters were summarized in Table 1. These parameters were used to plot model lines in Figure 2.

2.6 Statistical Analysis

All statistical analysis was performed with SPSS 20.0 (SPSS Inc., Chicago, lL). Differences between treatments were determined by analysis of variance (ANOVA). The significant difference was designed at $P < 0.05$.

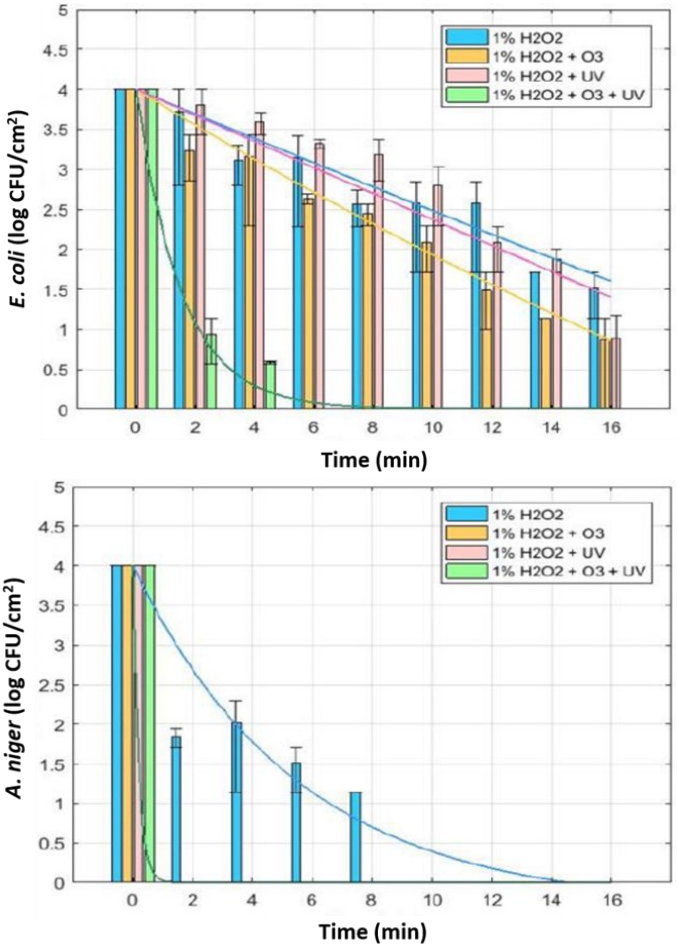

Figure 2. Microbial counts at different times upon exposure to different disinfection scenarios. (Top) *E. coli*, (Bottom) *A. niger*. Columns are experimental results. The lines are the fitted models. For *A. niger*, the lines for [1% H_2O_2 + O_3], [1% H_2O_2 + UV-C], and [1% H_2O_2 + O_3 + UV-C] overlap.

3. Results

3.1 Primary activity of H_2O_2 fumigation

The bactericidal property of liquid H_2O_2 has been well documented to increase with its higher concentration. In this work, an alternative concept of H_2O_2 fumigation (i.e., ultrasonic fumigation using low concentration of H_2O_2) was demonstrated to inactivate microbial contamination on spiked surfaces using two models of microorganisms including bacteria (*E. coli* and *A. niger*) on spiked surfaces. Figures 3-4 show different degrees of inactivation comparing the effectiveness of this H_2O_2 fumigation on bacterial and mold samples using different concentrations of H_2O_2 fume (i.e., 1, 3,

Table 1. Rate constants for the inactivation of *E. coli* and *A. niger* under different conditions obtained using the modified Chick-Watson model. These parameters were used to plot model lines in Figure 2.

Treatments	*E. coli*			*A. niger*		
	$k_1(min^{-1})$	$k_2(min^{-1})$	R^2	$k_1(min^{-1})$	$k_2(min^{-1})$	R^2
1% H_2O_2	35.20 ± 0.18^a	0.0004 ± 0.0120^c	0.95	4.31 ± 0.24^a	0.1810 ± 0.0140^b	0.95
1% H_2O_2 + O_3	12.20 ± 0.10^c	0.0190 ± 0.0080^b	0.96	4.00 ± 0.10^b	5.0700 ± 0.1190^a	-
1% H_2O_2 + UV-C	23.30 ± 0.22^b	0.0070 ± 0.0200^c	0.94	4.00 ± 0.10^b	5.0700 ± 0.1210^a	-
1% H_2O_2 + O_3 + UV-C	3.99 ± 0.16^d	0.6600 ± 0.1120^a	0.90	4.00 ± 0.12^b	5.0700 ± 0.0912^a	-

Values in the columns with different superscripts mean that the values are significantly different ($p<0.05$)

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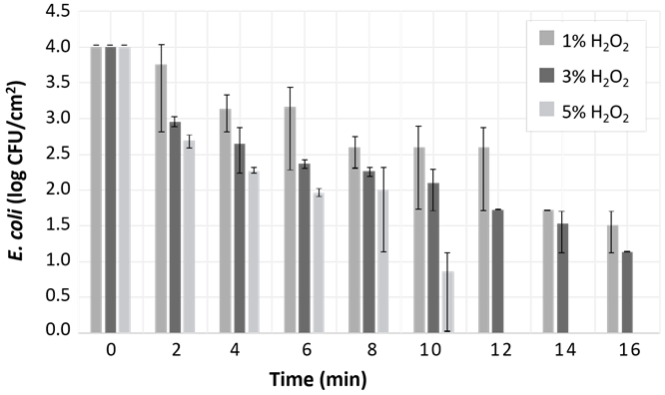

Figure 3. *E. coli* colony counts after treating with H_2O_2 fumes at different times by using different H_2O_2 concentrations (1%, 3%, and 5%). Each bar represents mean ± SEM, n = 10

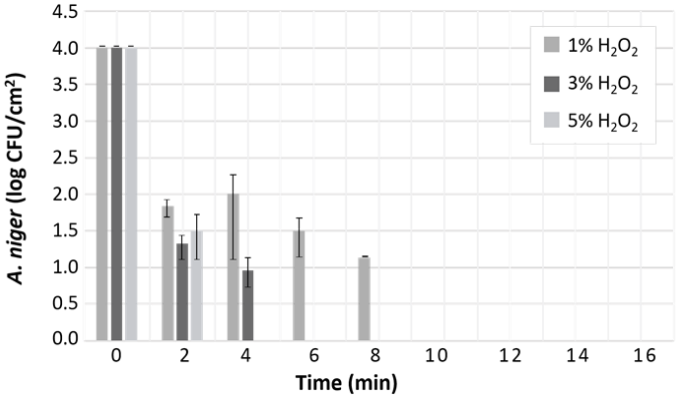

Figure 4. *A. niger* colony counts after treating with H_2O_2 fumes at different times by using different H_2O_2 concentrations (1%, 3%, and 5%). Each bar represents mean ± SEM, n = 10

and 5% H_2O_2). The ultrasonic transducer was able to energize H_2O_2 solution and successfully create stream of H_2O_2 aerosols producing substantial microbial reduction within 15 mins. Increasing the antimicrobial agent concentration generally improved the effectiveness of the treatment. With the 4 log CFU/cm^2 of the initial *E. coli* contamination, only 5% H_2O_2 fume enabled total disinfection of the spiked surfaces within 12 mins whereas the lower concentrations (i.e., 1% and 3% H_2O_2) were unable to produce complete sterility. From the results of *A. niger* survivability, this H_2O_2 fumigation was more effective towards mold inactivation than the bacteria. Because it has been reported that, the commercial vaporized H_2O_2 fume system is highly effective to rapidly oxidize fungal vegetative forms and spores than bacterial spores (Sandle, 2006). Often time, fungal spores are less resistant than bacterial spores. For mold, the H_2O_2 fume as low as 1% H_2O_2 was able to eliminate artificial *A. niger* contamination within 10 mins where the same H_2O_2 fume only produced 20-25% inactivation of *E. coli* on the normalized scale. A higher concentration of H_2O_2 fume (i.e., 5% H_2O_2) shortened the treatment time to 4 mins. Seemingly, mold was more susceptible to this low-concentration H_2O_2 fumigation than bacteria. The inactivation kinetics was studied using different H_2O_2 concentrations, without O_3 or UV-C treatment. Under this condition, the result found that the bacteria were inactivated less effectively than the mold. With 5% H_2O_2, the total disinfection time for the bacteria and the mold were 12 and 4 mins, respectively. In line with previous work (Raffellini, 2008), at higher concentrations of H_2O_2 observed greater bacterial inactivation.

3.2 Effect of ozonation on the activity of H_2O_2 fumigation

In this experiment, the efficacy of H_2O_2 fumigation was enhanced by applying ozonation to excite the formation of free radicals. Figures 5–7 show that *E. coli* destruction was improved by coupling H_2O_2 fumigation with ozonation. The total disinfection of *E. coli* with O_3 happened earlier than the ones without O_3. This effect was even more prominent in the experiment with *A. niger*. It required less than 2 mins for total disinfection of *A. niger* at all H_2O_2 concentrations (Figure 8-10). When the ozonation application, the synergy between the H_2O_2 fumigation with ozonation produced significant cells reduction via the generation of •OH and enabled the reduction of both *E. coli* and *A. niger*. Presumably, the mechanism for the photolysis of H_2O_2 is the cleavage of the molecule into two OH•. Also, O_3 can combine with

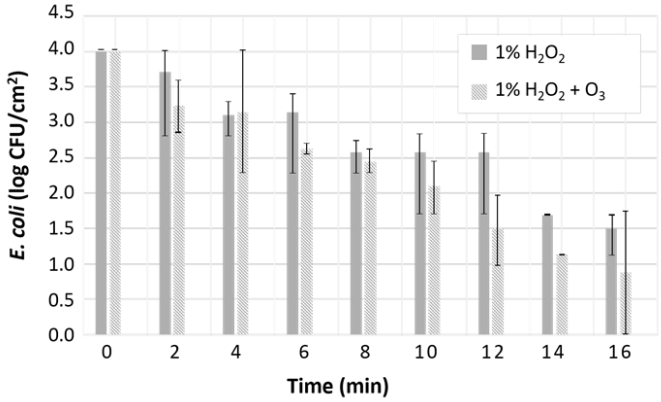

Figure 5. *E. coli* colony counts after treating with two different treatments at different times by using 1% H_2O_2 versus 1% H_2O_2 + O_3. Each bar represents mean ± SEM, n = 10

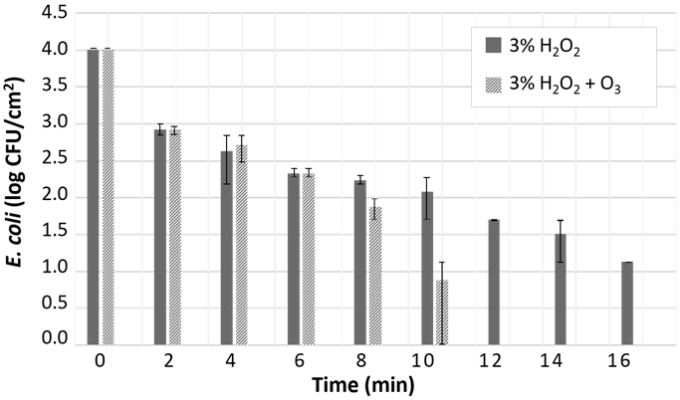

Figure 6. *E. coli* colony counts after treating with two different treatments at different times by using 3% H_2O_2 versus 3% H_2O_2 + O_3. Each bar represents mean ± SEM, n = 10

H_2O_2 solution to enhance the transformation of O_3 and H_2O_2 to OH• in OH• mist. Even in 1% H_2O_2, the combined effects of H_2O_2 and ozonation were able to harness enough •OH. Then H_2O_2 at 5% was added to increase the substrate to generate •OH (Hoigne, 1998) and a significant improvement in the efficacy of bacterial reduction was achieved. The addition of H_2O_2 to the O_3 process accelerates the decomposition of O_3, which results in an increased rate of OH• generation.

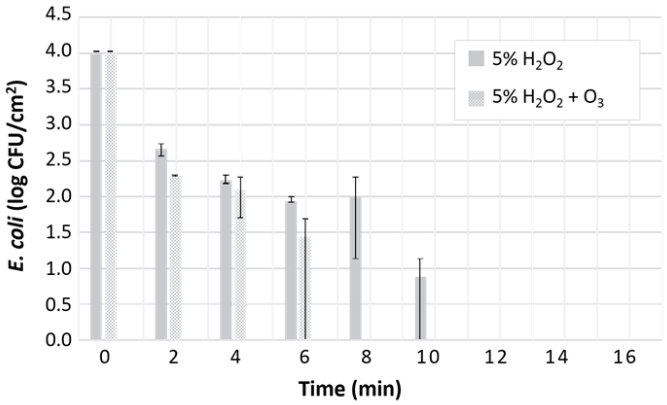

Figure 7. *E. coli* colony counts after treating with two different treatments at different times by using 5% H_2O_2 versus 5% H_2O_2 + O_3. Each bar represents mean ± SEM, n = 10

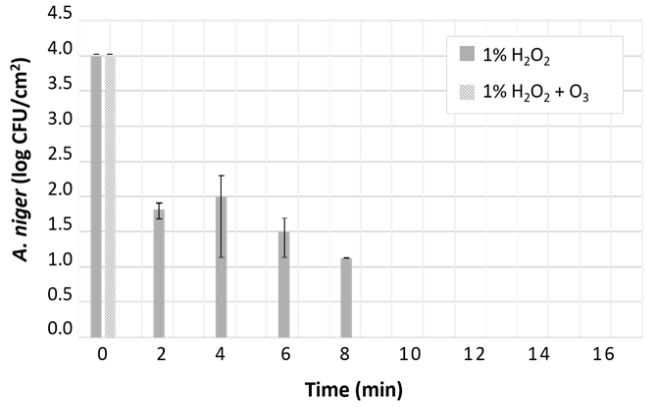

Figure 8. *A. niger* colony counts after treating with two different treatments at different times by using 1% H_2O_2 versus 1% H_2O_2+O_3. Each bar represents mean ± SEM, n = 10

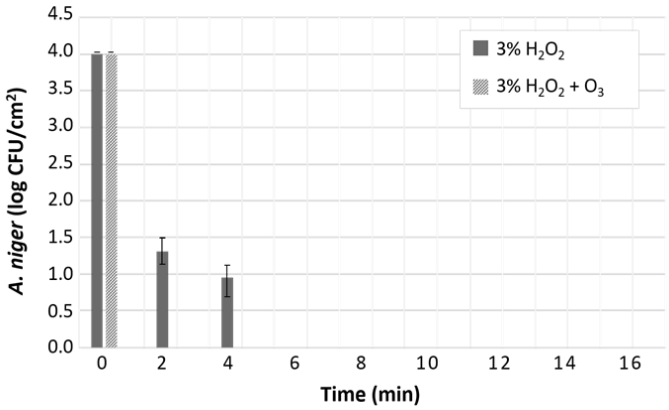

Figure 9. *A. niger* colony counts after treating with two different treatments at different times by using 3% H_2O_2 versus 3% H_2O_2+O_3. Each bar represents mean ± SEM, n = 10

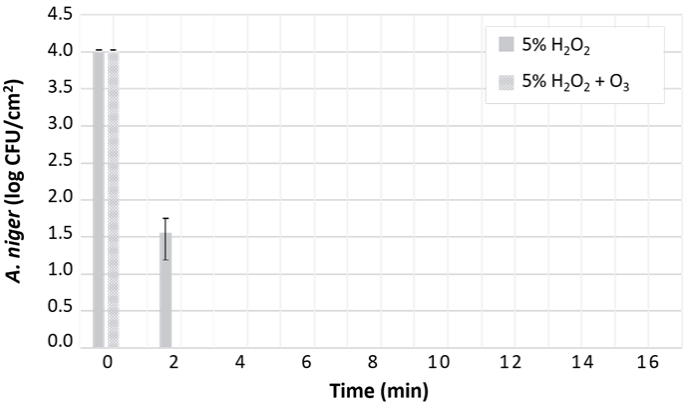

Figure 10. *A. niger* colony counts after treating with two different treatments at different times by using 5% H_2O_2 versus 5% H_2O_2+O_3. Each bar represents mean ± SEM, n = 10

3.3 Effect of photolysis on the activity of H_2O_2 fumigation

The H_2O_2 solution was exposed to UV-C light. For *E. coli*, the UV-C treatment does not significantly affect the inactivation kinetics of the H_2O_2 fumes. As shown in Figure 11–13, the *E. coli* concentration profile over time was similar to the H_2O_2 fumigation without UV-C. For *A. niger*, on the other hand, the disinfection of *A. niger* was improved by implementing the UV-C effect. Total disinfection of *A. niger* was somewhat instant (< 2 mins) at all concentrations of H_2O_2 as shown in Figure 14–16.

3.4 Synergic effects of photolytic ozonation at 3% and 5% H_2O_2 fumigation

By combining the effects of O_3 and UV-C, the activity of H_2O_2 fumigation was greatly improved. Total disinfection of *E. coli* was possible within a short time as shown in Figure 17. This synergic effect was more difficult to observe in the *A. niger* experiment because individual treatment of O_3 or UV-C was already sufficient to completely inactivate the *A. niger* (Figure 18).

3.5 Individual effects of ozonation and photolysis

The H_2O_2 fume was then subject to either O_3 or UV-C exposure in order to investigate how each influenced the activity of the H_2O_2 fumigation. Previous works provide evidence that these two treatments could initiate the formation of highly reactive hydroxyl radicals (OH·). With 1% H_2O_2, the *E. coli* disinfection was found to be slightly affected by the UV-C exposure but significantly enhanced by O_3. However, each treatment was still not effective enough to enable the total disinfection within the time studied (16 mins). In contrast, for the *A. niger* sample, each treatment (O_3 or UV-C) greatly improved the disinfection rate. The total disinfection time was reduced from 10 mins to 2 mins by treating with either

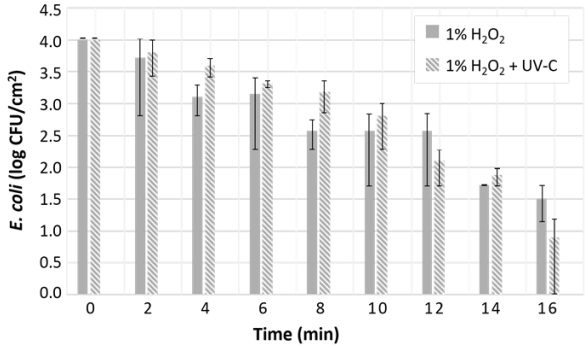

Figure 11. *E. coli* colony counts after treating with two different treatments at different times by using 1% H_2O_2 versus 1% H_2O_2 + UV-C. Each bar represents mean ± SEM, n = 10

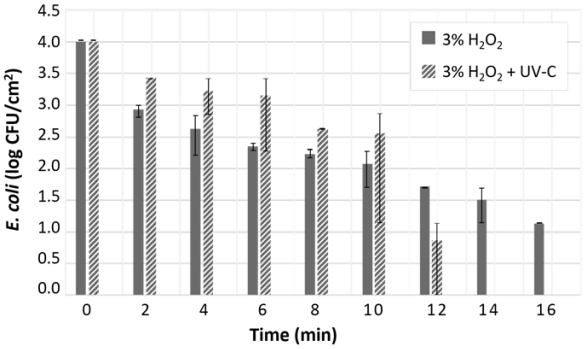

Figure 12. *E. coli* colony counts after treating with two different treatments at different times by using 3% H_2O_2 versus 3% H_2O_2 + UV-C. Each bar represents mean ± SEM, n = 10

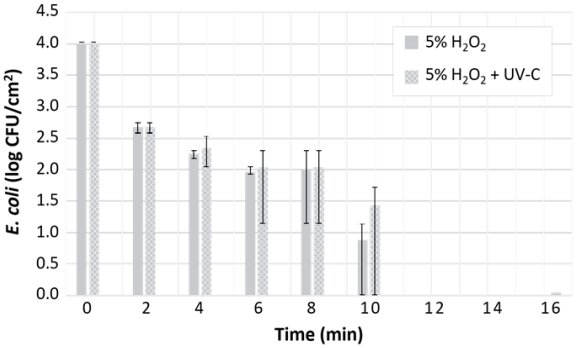

Figure 13. *E. coli* colony counts after treating with two different treatments at different times by using 5% H_2O_2 versus 5% H_2O_2 + UV-C. Each bar represents mean ± SEM, n = 10

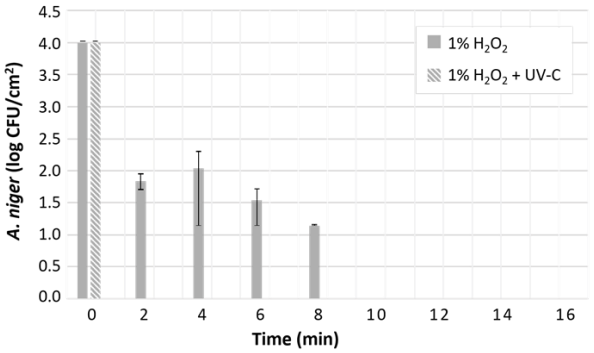

Figure 14. *A. niger* colony counts after treating with two different treatments at different times by using 1% H_2O_2 versus 1% H_2O_2 + UV-C. Each bar represents mean ± SEM, n = 10

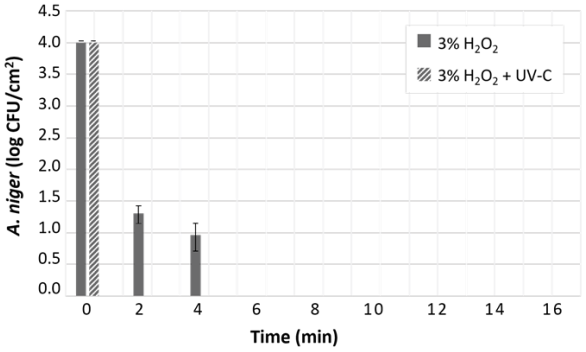

Figure 15. *A. niger* colony counts after treating with two different treatments at different times by using 3% H_2O_2 versus 3% H_2O_2 + UV-C. Each bar represents mean ± SEM, n = 10

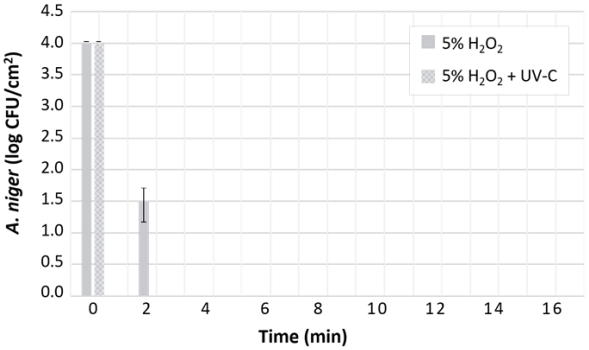

Figure 16. *A. niger* colony counts after treating with two different treatments at different times by using 5% H_2O_2 versus 5% H_2O_2 + UV-C. Each bar represents mean ± SEM, n = 10

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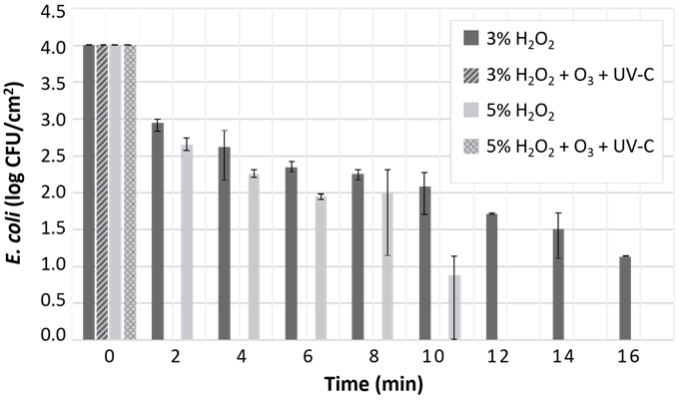

Figure 17. *E. coli* colony counts at different times after using two different treatments (H_2O_2 versus H_2O_2 + O_3 + UV-C) for two concentrations of H_2O_2 (3% and 5%). Each bar represents mean ± SEM, n = 10

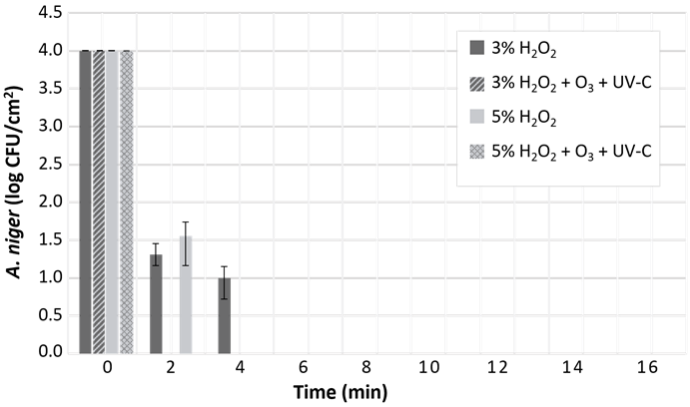

Figure 18. *A. niger* colony counts at different times after using two different treatments (H_2O_2 versus H_2O_2 + O_3 + UV-C) for two concentrations of H_2O_2 (3% and 5%). Each bar represents mean ± SEM, n = 10

O_3 or UV-C alone.

3.6 Synergic effect of photolytic ozonation

In this subsequent experiment, both ozonation and photolysis were simultaneously applied to the H_2O_2 fumigation. Upon these conditions, the k_1 and k_2 values (Table 1) of *E. coli* were significantly different at any given treatments ($p<0.05$). The empirical parameters model in Table 1 (lines in Figure 2) was fitted to the results to demonstrate this effect. The linear model predicted a lower k_1 and higher k_2 values; it meant that *E. coli* was the least resistant strain to 1% H_2O_2 combined with O_3 and UV-C. At 1% H_2O_2 combining the effects of O_3 and UV-C, the total *E. coli* disinfection was completed within 6 mins as shown in Figure 2. Such synergic effect was not obvious in the *A. niger* experiment and treatments were not significantly affect the inactivation kinetics of the H_2O_2 fumes. Because any individual treatment, either O_3 or UV-C, was already sufficient to disinfect (<2 mins for *A. niger*).

4. Discussion

The disinfection with hydrogen peroxide alone resulted in slow disinfection since the H_2O_2 molecule is only mildly oxidizing with a low potential (1.78 eV) (Zhou and Smith, 2002). One way to increase the inactivation kinetics is to decompose H_2O_2 into other more active oxidants such as OH· radicals (2.80 eV) (Zhou and Smith, 2002). Our results suggested that the use of O_3 was more effective in doing this than UV-C photolysis. The mechanism for the photolysis of H_2O_2 is the cleavage of the H_2O_2 into two OH· while the mechanism for the ozonation of H_2O_2 is less direct. In a weak acid solution, H_2O_2 partially dissociates into hydroperoxide ions (HO_{2-}). Ozonation can then rapidly convert HO_{2-} to OH· (Hoigné, 1998). However, when both O_3 and UV-C treatments were combined, the disinfection rate was considerably increased. The UV-C light tends to activate several elementary reactions that generate more OH· radicals (Munter, 2001). For example, under the UV-C light, the reaction between O_3 and H_2O_2 to produce OH· and O_2 occurs. Therefore, when both O_3 and UV-C were combined for the H_2O_2 fumigation, the total disinfection of bacteria and mold can be achieved at low H_2O_2 concentration within a short time. Upon total mineralization, the end products of complete oxidation are simply carbon dioxide and water. Similar to VHP, the residues after the fumigation have been reported to be safe (Krause *et al.*, 2001). Therefore, this approach offers a promising alternative for disinfection in the food industry, hospitals, clinics, and other enclosed areas; it is fast, inexpensive, and safer.

5. Conclusion

In this research, the results presented the successful methodology for surface disinfection using hydrogen peroxide (H_2O_2) fumigation in couple with ozonation and UV photolysis. Oxidizing agents have been widely used in food industry, hospitals and clinics for cleaning, yet existing methods have some disadvantages. For example, vaporized hydrogen peroxide (VHP) requires high concentration of H_2O_2. In this work, a system that produces aerosols of H_2O_2 solution to inactivate the microorganisms was developed. Exposure of the fumes to either ozone or UV light has found to enhance the rate of disinfection. When combining both ozone and UV effects to the fumigation system, the disinfection was the most efficient, making it possible to clean the surface totally within a very short time and with a low concentration of H_2O_2.

Conflict of interest

There is no conflict of interest declared

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