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HYDROXYL RADICAL FUMIGATION AS AN ALTERNATIVE, EFFECTIVE, ECO-FRIENDLY SURFACE AND HAND SANITIZATION

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Abstract

A lot of people suffers from the sickness every year due to the diseases infected by pathogens resulting from poor hygiene and sanitation. Hospital-associated infections (HAIs) have been estimated to kill as many people as the combination of AIDS and breast cancer. The use of combined UV-C and ozonation was applied to enhance the production of •OH using hydrogen peroxide (H_2O_2) as substrate. The development of •OH fume, was varied to the two concentrations (3% and 5%). The contact time were varied at 5, 10, 15, 20 min. The use of H_2O_2 as high as 5% alone was not effective for surface and hand disinfectant when applying ultrasonic fumigation. The aerosols generated from this technique was too small and well airborned; hence, more effective oxidizing agents must be developed. Due to its short life and auto oxidation to water and oxygen, this •OH fumigation technique was an effective means for surface disinfection and hand sanitization that leaves no footprints or toxic residue after used making it as a useful alternative for such application.

Keywords: Advanced oxidation processes, Disinfection, Hydrogen peroxide, Hand sanitization, Fumigation

Introduction

Almost 2 million patients experience HAIs in the US and 19% of these patients died each year [1,2]. It has been widely accepted that the origin of these infections is strategically avoidable by improving handwashing practices and routine environmental surface sanitization [3]. A lot of improved hospital practice campaigns have been done in gearing towards the improvement of hand washing compliance and better personal hygiene in healthcare facilities proven to save millions on hospital expenses and public health savings [4].

Many contaminated surfaces in healthcare environment can harbor non- and pathogenic bacteria alike. Several authors suggested that bacteria have the ability to multiply and attach to both engineered plastic and metal surfaces (e.g., polystyrene, polypropylene and stainless steel) [5,6]. High levels of deteriorating and pathogenic microorganisms revealed poor hygienic and sanitary quality in the products analyzed. Especially the number of bacteria (i.e., *E. coli*, *S. aureus*) ranged from 4 to 8 log CFU/ml can cause food intoxication; therefore, they are indicative of consumers' health risks [7,8]. Therefore, there were constant need of improvement for an effective but eco-friendly sanitizing agent and method in reducing bacterial contamination for critical environmental surfaces, especially in healthcares, food and pharmaceutical industries [9]. There were already a few popular aqueous sanitizers, e.g., organic acids, chlorine dioxide, vaporized hydrogen peroxide (VHP), and ozonated water, exhibiting adequate antimicrobial activity with the acceptable toxicity of the residual chemicals commercially available [10,11]. Each of these chemicals has its own beneficial characteristics and drawbacks and widely applied to industrial and clinical settings.

We are investigating an affordable and more environmental-friendly application of advanced oxidation technology for surface and hand sanitation by the contact with hydroxyl radical (•OH) fume. Hence, we are focusing on low concentration application of H_2O_2 on surface sanitization and its application on hand disinfection. In this paper, H_2O_2-based solution together with UV-C photocatalysis and ozonation was explored and compared its

effectiveness in inactivating Gram-positive and Gram-negative bacteria to H_2O_2 alone. Also the potential of using •OH fume for hand sanitation was explored.

Materials and methods

Materials

Escherichia coli ATCC 25922 and *Staphylococcus aureus* ATCC 25923 stock cultures were received from the Department of Medical Sciences Thailand (DMST, Bangkok, Thailand) and kept in TSB containing 20% glycerol and stored at –80 °C. Prior to use, the bacterial stocks were grown in The Trypticase soy broth (TSB, Himedia, Mumbai, India) for 18–24 h at 37 °C.

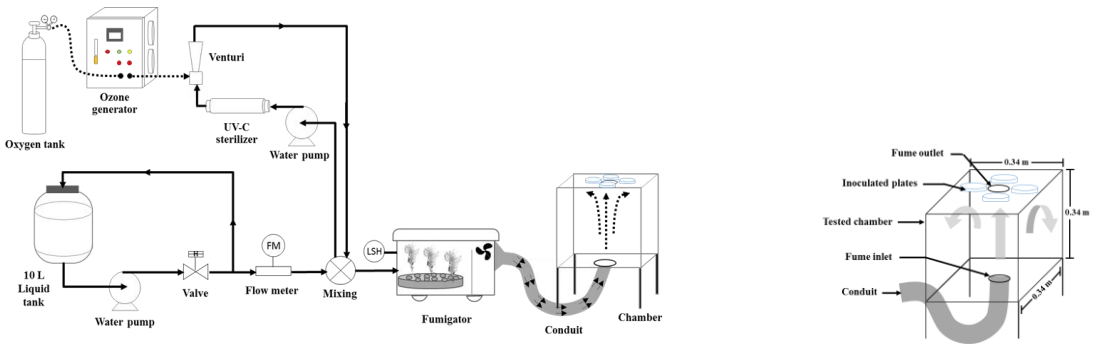
The disinfectants (i.e., hydrogen peroxide (H_2O_2, Merck, Germany) was adjusted to two concentrations (3% and 5%). The treatment times were varied to 5, 10, 15, 20 minutes. After each treatment, the disinfected plates were inoculated at 37 °C for 18-24 h and evaluated the cell culture dishes on the top of chamber were fumigated already and evaluated visually by observing density of colonies comparing to the control treatments.

Methods

Production of hydroxyl radical aerosols and p**reparation of contaminated surfaces**

A prototype of •OH fumigator was constructed as shown in Figure 1a. The system which consists of an ozone generator, UV-C unit, and ultrasonic fumigator [12]. The reservoir contained 10 L liquid of each disinfection reagents. An oxygen tank, flow rates 2 L/min, was connected to the ozone generator. The ozone gas was subsequently forced into a venturi in which the ozone gas was mixed with circulating water before transferring into a fumigator. The fumigator (Meiyan, China) contained twelve ultrasonic mist maker produces aerosols. The aerosols were further dispersed by a fan in the fumigator. The system also has 15W UV-C lamps installed in the circulation line to activate more •OH production.

Artificially-contaminated agar plates of *E. coli* and *S. aureus* were fabricated by serially diluted the culture stock and varying the cell concentration between 3 to 8 $\log_{10}$ CFU/mL using saline water. 100 µL of inoculum was introduced Petri dish (Citotest, Haimen, China) containing Plate Count Agar (PCA, Difco, USA). The final cell densities achieved in each inoculated plated were approximately varied from 1 to 7 $\log_{10}$ CFU/cm^2. The plates were installed on the top side inside the fumigated chamber as shown in Figure 1b.The total volume of the cube is 0.04 m^3 on each side.



a) **b)**

Figure 1 a) The schematic diagram of AOPs aerosolization and b) Diagram of the testing chamber made of transparent walls. Disinfectant fume is introduced from the bottom and exited at the outlet on top.

Effectiveness of hand fumigation for sanitation

Preparation of hand contamination and glove juice testing

A 1.5 mL aliquot of *E. coli* suspension at two cell densities (i.e., 6 and 8 $\log_{10}$ CFU/mL) was applied onto each volunteer's cupped hands. The aliquot was rubbed thoroughly over the hand's surfaces for 1 min and left to be air dried for 20 s. This procedure (i.e., dispensing, rubbing, and drying steps) was repeated three time to apply the total aliquot volume of 4.5 mL. During the disinfectant fume, the hands were rubbed each other thoroughly and vigorously. Exposure times were varied for every 5 sec up to 60 sec by using the procedure described in E1174 [13]. The background colony count on the tested hands was first determined by massaging the fingertips in sterile plastic bag containing 100 mL distilled water for 1 min [14]. The contaminated sample was kept in eppendorf tube and then 0.1 mL diluted samples were plated onto PCA agar plates and incubated at 37 °C for 24 h. Total numbers of CFUs were counted for each plate.

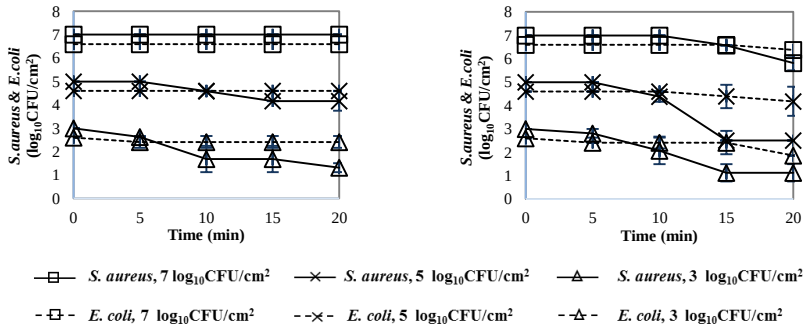
Results and discussion

Results

Hydrogen peroxide fumigation

Figure 2 shows the effectiveness of H_2O_2 fumigation generated from our prototype equipment on contaminated surfaces with *S. aureus and E. coli* at the initial cell loadings of 3, 5 and 7 $\log_{10}$ CFU/cm^2 over the course of 20 min. The 5% H_2O_2 treatment produced slightly better bacterial kill comparing to the 3% H_2O_2 treatment. At the same strength of H_2O_2, longer treatment reduced the bacteria present on the contaminated agar surface and *S. aureus* in general was more vulnerable than *E. coli*. At high initial cell contamination (i.e, 7 $\log_{10}$ CFU/cm^2), only 5% H_2O_2 returned 1 log reduction after 20 minute treatment. At lower cell density (e.g., 3 $\log_{10}$ CFU/cm^2), as high as 3 log reduction can be observed on *S. aureus* contaminated plates.

Huang, Ye and Chen (2012) used 3% of H_2O_2 to wash and decontaminate baby spinach leaves for 5 min and achieved 1.6 $\log_{10}$ CFU/g reduction of *E. coli* O157:H7 [15]. Ukuku and Fett (2002) used 5% H_2O_2 solution to disinfect melon surface for 2 min and obtained 2.0–3.5 $\log_{10}$ CFU/cm^2 reduction of *L. monocytogenes* [16]. In the aerosolized form, 10 min treatment of hydrogen peroxide vapor treatment at 1, 3, and 5% was able to provide significant bacterial decontamination of *S. Typhimurium* (1.48, 2.09, and 2.63 $\log_{10}$ CFU/g reduction, respectively) and *E. coli* O157:H7 (1.62, 2.14, and 2.94 $\log_{10}$ CFU/g reduction) on lettuce leaves [17]. Similarly, our experiments showed 2-3 log reduction at lower cell contamination on the agar plates. But the H_2O_2 fumigation was less effective when initial contamination was high (i.e., 7 $\log_{10}$ CFU/cm^2).



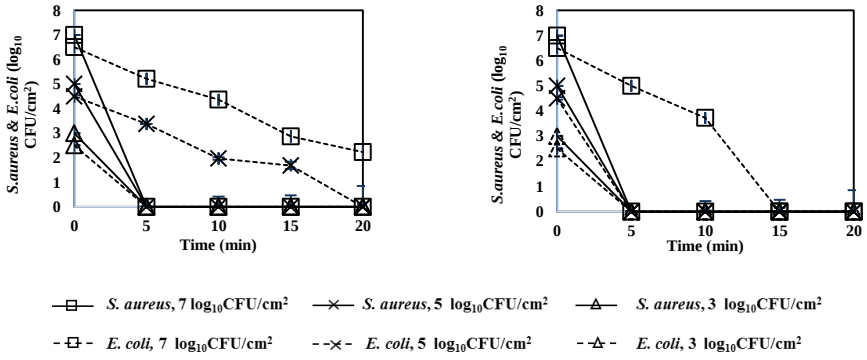
a) 3% H_2O_2 **b)** 5% H_2O_2

Figure 2 Effect of hydrogen peroxide concentration fumigation

Improvement of H_2O_2 treatment by AOPs

In this experiment, ozonation and UV-C photocatalysis are combined with H_2O_2 fumigation to generate •OH fume for bacterial decontamination. In the AOPs scheme (see Figure 1a), H_2O_2 serves as a substrate to excessively produce reactive oxygen derivatives (e.g., hydroxyl radicals, superoxide anions), which are able to non-selectively

attack essential cell components such as DNA, lipids, and proteins [18]. Figure 3 demonstrates the significant improvement of our patent-pending technology of •OH fumigator. At same 3% H_2O_2 as in Figure 2a, the conversion of H_2O_2 to •OH in Figure 3a was able to produce substantial reduction of both *S. aureus and E. coli*; although, *E. coli* was much more resilient than *S. aureus*. All *S. aureus* contamination levels were brought down to complete sterility within 5 min of treatment time but the *E. coli* contamination higher than 5 $\log_{10}$ CFU/cm^2 requires more than 20 min to total sterile condition. Nevertheless, the stronger H_2O_2 concentration (i.e., 5% H_2O_2 in Figure 3b) was able to inactive *E. coli* contamination as high as of 7 $\log_{10}$ CFU/cm^2 within 15 min. The synergy of H_2O_2/Ozonation/UV-C water maintained high level of reactive •OH in the fume facilitating instant oxidation and fast microbial inactivation at the point of contact similar to other research works [19,20].



a) 3% H_2O_2/Ozonation/UV-C **b)** 5% H_2O_2/Ozonation/UV-C

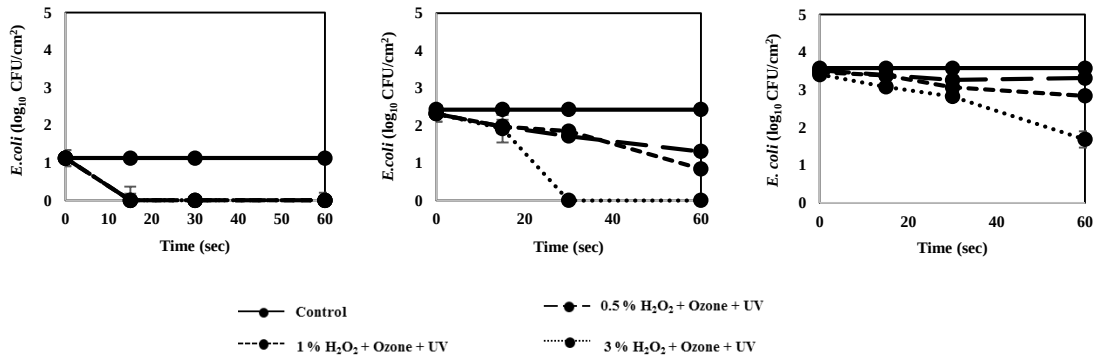
Figure 3 Effect of H_2O_2 concentrations combined with UV-C photocatalysis and ozonation. H_2O_2 treatment on the initial cell 3, 5 and 7 $\log_{10}$ CFU/cm^2 of *E. coli* and *S. aureus*

Application of •OH fume for hand sanitation

The normal human skin usually has the total aerobic bacterial counts ranging from more than 1 x 10^6 CFU/cm^2 (e.g., scalp and axilla) to 1 x 10^4 CFU/cm^2 (e.g., forearm) [21]. Statistics showed that healthcare workers can on average have the total bacterial counts from 3.9 x 10^4 to 4.6 x 10^6 CFU/cm^2 [22] and normal fingertip areas can harbor as many as 300 CFU if counted by agar contact methods [23].

Many infectious diseases can be spread from one person to another by contacting to these contaminated body surfaces. The use of H_2O_2/Ozonation/UV-C fume was proposed as an alternative to common hand-washing products to effectively sanitize hands and contaminated surfaces.

To apply this fumigation concept for surface disinfection and hand sanitization, rubbing of both hands in the H_2O_2/Ozonation/UV-C fume using the standard hand-washing protocol [24] up to 60 sec produced various degrees of microbial disinfection depending on the levels of initial *E. coli* contaminations and the concentrations of H_2O_2 used. At 1-2 $\log_{10}$ CFU/cm^2, all *E. coli* cells can be removed from volunteer's hands within 15 sec using the H_2O_2 concentration as low as 0.5%. At the higher initial *E. coli* contaminations, only 3% H_2O_2/Ozonation/UV-C fume was able to reduce the *E. coli* count to zero in 30 sec. The lower H_2O_2 concentrations were unable to produce complete disinfection even after 60 sec of constant rubbing in the H_2O_2/Ozonation/UV-C fume. Higher concentration of H_2O_2 may be needed to achieve total sterility using brief rubbing treatment or else a different mechanism (e.g., spraying) may be required to collect more mass of disinfecting solution containing •OH onto the hands. The ultrasonic fume generate very fine airborne aerosols that only lightly wetted and hardly accumulated on the skin surfaces.



a) 1-2 $\log_{10}$ CFU/cm^2 **b)** 3-4 $\log_{10}$ CFU/cm^2 **c)** 5-6 $\log_{10}$ CFU/cm^2

Figure 4 Effect of a) *E. coli* population on hands treated by 0.5% were combined with ozone and UV b) *E. coli* on hands treated by 1% were combined with ozone and UV-C and c) 3% H_2O_2 respectively, used for hand-washing. The inoculate at the initial cell loading of 1, 3 and 5 $\log_{10}$ CFU/cm^2

Conclusion

The use of advanced oxidation technology by applying ozonation and UV-C photocatalysis was able to enhance the bactericidal effectiveness of H_2O_2 fume. The generation of •OH in the H_2O_2/Ozonation/UV-C fume was used to explain the significant improvement from the H_2O_2 fume alone. Owing to the non-selectiveness oxidation towards any bacteria, the H_2O_2/Ozonation/UV-C fume were demonstrated for hand and surface sanitization. Depending on the degree of contamination, the concentration of H_2O_2 can be selected to produce substaintial reduction of contamination or completely elimination on the intended surfaces. At approximately 3-4 log10 CFU/cm^2, it was compulsory to use at least 3% H_2O_2/Ozonation/UV-C fume to achieve complete surface sanitation in 30 sec. The glove juice confirmed the effectiveness of the 3% H_2O_2/Ozonation/UV-C fume for 30-sec hand sanitation.

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