Application of Atmospheric Cold Plasma for Inactivation of Spoilage and Pathogen Microorganisms

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Abstract. The effect of cold atmospheric plasma (ACP) technique on inactivation of selected microorganisms was investigated. To study the effect of cold plasma on microorganism inactivation Dielectric Barrier Discharge (DBD) plasma was applied. The inactivation of yeast (Saccharomyces cerevisiae) and 4 pathogen microorganisms (Salmonella typhimurium, Listeria monocytogenes, Escherichia coli, and Staphylococcus aureus) using DBD cold plasma were investigated. The results have shown that yeast can be effectively inactivated on agar plate within 5 min cold plasma treatment. Adding H2O2 in concentration of 2 or 5% on agar plate improved the inactivation of microorganisms using cold plasma. Furthermore, it was observed that it is possible to inactive pathogen microorganisms on agar plate using DBD cold plasma within 3 to 5 min treatment time. Up to 57%, 96%, 91% and 94% pathogen microorganisms inactivation was achieved after 1 min DBD plasma treatments of S. aureus, L. monocytogenes, E. coli, and S. typhimurium respectively.

1. Introduction

Plasma is often referred to as the fourth state of matter, which exhibits unique properties. Any source of energy which can ionize a gas can be employed for generation of plasma. Plasma is composed of several excited atomic, molecular, ionic, and radical species, co-existing with numerous reactive species, UV and visible light [1]. The generation of uniform cold plasma at atmospheric pressures gives the opportunity to apply it to foods and biological surfaces [1]. Since many years Dielectric Barrier Discharge (DBD), as non-equilibrium discharge, has been applied for ozone generation at atmospheric pressure. The most important characteristic of dielectric-barrier discharges is its simplicity and flexibility with respect to geometrical configuration, operating medium and operating parameters. It can be easily scaled up for industrial scale application. Cold plasma generate reactive oxygen species (ROS), in air or oxygen-containing mixtures and produce of ozone, which have high antimicrobial effect, hydrogen peroxide, and singlet and atomic oxygen. The strong oxidative effect of species in cold plasma causes strong oxidative stress on cell membrane and lipid oxidation, enzyme inactivation, and DNA degradation [2]. Recently it is possible to produce cold plasma at atmospheric pressure and room temperature with spatially uniform, well-controlled cold plasma that can be applied for disinfection of foods at ambient pressure and temperature. Researchers [3] have studied the effect of cold plasma on sterilization of microorganisms using different gas types. They postulated that the sterilizing effect of cold plasma is not due to UV light, but is due to the action of reactive oxygen
radicals. Inactivation of *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* deposited on a nitrocellulose filter membrane or *Bacillus subtilis* spores deposited on polypropylene plates exposed to helium/oxygen plasmas generated with AC input power at 10 kHz, 6 kV results confirmed the sterilization effect of cold plasma on selected microorganisms with D-value of 18 to 60 sec. Dependent on microorganism types. Stareck et al. [4] have successfully applied the atmospheric cold plasma for inactivation of microorganisms in tomato juice while maintaining physicochemical properties in tomato juice. The effect of cold atmospheric plasma (CAP) on bacteria *E. coli*, *P. aeruginosa* and the yeast *C. albicans* using dielectric barrier discharge plasma with different temporal pulse characteristics were studied by Mertens et al. [5]. The results of their investigation have indicated that suitable both tested devices were suitable for microorganism inactivation but nanosecond pulsed plasma source has a higher efficacy based on a higher chemical discharge productivity. The aims of this study are to investigate the effect of treatment time of Dielectric Barrier discharge (DBD) atmospheric pressure cold plasma on yeast and pathogen bacteria microorganisms.

2. Material and methodology

2.1. Dielectric barrier discharge plasma (DBD) generator
The Dielectric Barrier Discharge (DBD) cold plasma generator was designed in our Department. The DBD consisting of 2 parallel plate of cupper disc (Ø=8 cm) covered by 5 mm thick acrylic plate. The gap between two plates was adjustable for 1 to 4 cm. The plasma was created by applying alternating current high voltage of 17 kV in magnitude (peak to peak), and 50 Hz frequency. The input power was 55 W. The gas flow rate during cold plasma treatment was fixed at 10 l/m. Argon gas was applied in this study.

2.2. Microorganism preparation
For preparation of yeast microorganism suspension was yeast (*Saccharomyces cerevisiae*), added into a sterile nutrient solution containing 0.5% peptone, and 0.3% yeast extract) and incubate for 24 h at 37 ± 1 °C. Microorganism suspension of *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* (*TISTR Culture Collection, Thailand Institute of Scientific and Technology Research, Thailand*) were prepared by adding lyophilized microorganism in sterile condition in to sterile Muller Hilton Broth (MHB) and incubated for 24 h at 37 ± 1 °C. After that 20 µl of each microorganism suspension was separately added on nutrient agar plate and spread it on nutrient agar surface. All the nutrients were purchased from HIMEDIA (India). For DBD cold plasma treatment was the agar plate containing microorganism (yeast *Saccharomyces cerevisiae* and pathogen bacteria were placed in DBD plasma generator and treated at different treatment time. To study the effect of aerosolized hydrogen peroxide (H₂O₂) combined with cold plasma treatment was agar plate containing yeast sprayed with about 0.2 ml of 2 % and 5% H₂O₂ solution. One part of agar plate containing yeast and H₂O₂ was treated with DBD cold plasma at different treatment time, the other part of agar plate containing yeast and H₂O₂ was kept as reference to investigated the effect of combined H₂O₂ and DBD cold plasma compare with sample containing H₂O₂ without cold plasma treatment.

3. Results and discussions
In figure 1 is the produced DBD cold plasma at 17 kV between two cupper electrodes covered with acrylic plate demonstrated. The intensive blue color between two electrodes is due to ionizing of argon gas and producing UV and visible light among other active species produced during plasma generation.
Cold plasma treatment of yeast induced inactivation of microorganism on agar plate. With increasing of treatment time from 1 min to 5 min increased extend of yeast inactivation. After 5 min DBD cold plasma treatment at 17 kV and DBD gap of 2 cm and argon as ionizing gas was more than 2 log inactivation of yeast observed (table 1). After 5 min DBD cold plasma treatment was no yeast on agar plate. This indicated that is possible to remove yeast microorganism from surface of sample using DBD cold plasma. Cold plasma treatment of yeast induced inactivation of microorganism on agar plate. With increasing of treatment time from 1 min to 5 min increased extend of yeast inactivation. After 5 min DBD cold plasma treatment at 17 kV and DBD gap of 2 cm and argon as ionizing gas was more than 2 log inactivation of yeast observed (table 1). After 5 min DBD cold plasma treatment was no yeast on agar plate. This indicated that is possible to remove yeast microorganism from surface of sample using DBD cold plasma. Colona et al [6] have reported that yeast cells were killed by exposing high-voltage atmospheric cold plasma in dry air and oxygen-rich modified air. In addition, they reported higher yeast sensivity to cold plasma at lower cell densities and suspension volume. In contrast, yeast was less sensitive to cold plasma treatment in grape juice compared to water.

**Table 1.** Effect of dielectric barrier discharge plasma to yeast inactivation

| Condition | Treatment Time (min) | Yeast count (CFU/ml) | Microorganism inactivation (%) |
|-----------|----------------------|----------------------|-------------------------------|
| Untreated | -                    | $1.32 \times 10^4$   | -                             |
| DBD       | 0.5                  | $1.15 \times 10^4$   | 12.7737                       |
| DBD       | 1                    | $0.85 \times 10^4$   | 34.7628                       |
| DBD       | 2                    | $0.3 \times 10^4$    | 77.1137                       |
| DBD       | 3                    | $0.23 \times 10^4$   | 81.8621                       |
| DBD       | 5                    | 0.00                 | More than 99%                 |

Adding aerosolized hydrogen peroxide (2% solution) showed synergistic effect of DBD cold plasma for inactivation of yeast. Whereas aerosolizing of hydrogen peroxide in 2% concentration alone can inactivate up to 43% of initial yeast count, was strong inactivation effect of combined hydrogen peroxide and DBD cold plasma on yeast inactivation observed (table 2). After 1 min. DBD cold
plasma treatment was up to 52.28% yeast inactivation observed. Further increasing the treatment time to 3 min and longer resulted total inactivation of yeast microorganisms on agar plate (figure 2). Jiang et al., [7] have also reported the positive effect of aerosolized hydrogen peroxide (7.8%) during cold plasma treatment of *Escherichia coli O157:H7*, *Salmonella Typhimurium*, and *Listeria innocua*.

Table 2. Combined effect of DBD plasma with 2% H$_2$O$_2$ on yeast

| Condition                        | Treatment Time (min) | Yeast count (log CFU/mL) | Yeast inactivation (%) |
|----------------------------------|----------------------|--------------------------|------------------------|
| Untreated                        | -                    | 1.31*10$^4$              | -                      |
| 2% H$_2$O$_2$ sprayed            | -                    | 0.74*10$^4$              | 43.30                  |
| 2% H$_2$O$_2$ sprayed + DBD      | 0.5                  | 0.6*10$^4$               | 47.56                  |
| 2% H$_2$O$_2$ sprayed + DBD      | 1                    | 0.63*10$^4$              | 52.28                  |
| 2% H$_2$O$_2$ sprayed + DBD      | 2                    | 0                        | 100                    |
| 2% H$_2$O$_2$ sprayed + DBD      | 3                    | 0                        | 100                    |
| 2% H$_2$O$_2$ sprayed + DBD      | 5                    | 0                        | 100                    |

Figure 2. Growth inhibition of yeast: A) untreated, B) cold plasma treated for 30s, C) cold plasma treated for 1 min, D) cold plasma treated for 2 min, E) cold plasma treated for 3 min, F) cold plasma treated for 5 min.

Higher concentration of hydrogen peroxide up to 5% aerosolized on yeast containing agar plate confirmed the synergistic effect of physical (cold plasma) and chemical (hydrogen peroxide) treatment on yeast inactivation. Spraying of 5% H$_2$O$_2$ on nutrient agar plate with yeast led to only 46.59% inactivation of yeast. In contrast, combined spraying of 5% H$_2$O$_2$ and subsequent DBD cold plasma
treatment led total yeast inactivation on agar plate after 1 min and longer cold plasma treatment (table 3).

| Condition        | Treatment Time (min) | Yeast count (log CFU/mL) | Yeast inactivation (%) |
|------------------|----------------------|--------------------------|------------------------|
| Untreated        | -                    | 1.31*10^4                | -                      |
| 5% H2O2 sprayed  | -                    | 1.2*10^4                 | 46.59                  |
| 5% H2O2 sprayed  | 0.5                  | 0.42*10^4                | 67.78                  |
| + DBD            |                      |                          |                        |
| 5% H2O2 sprayed  | 1                    | 0                        | 100                    |
| + DBD            |                      |                          |                        |
| 5% H2O2 sprayed  | 2                    | 0                        | 100                    |
| + DBD            |                      |                          |                        |
| 5% H2O2 sprayed  | 3                    | 0                        | 100                    |
| + DBD            |                      |                          |                        |
| 5% H2O2 sprayed  | 5                    | 0                        | 100                    |
| + DBD            |                      |                          |                        |

The effect of aerosolized hydrogen peroxide during cold plasma treatment on microorganism inactivation was reported by Jiang et al. [7]. They reported effective inactivation of *Escherichia coli* O157:H7, *Salmonella Typhimurium*, *Listeria innocua* on vegetable foods by using combined ACP and aerosolized H2O2 (7.8%) for 45 s treatment. To study the effect of cold plasma for inactivation of pathogen microorganisms such as *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* were these microorganisms inoculated on nutrient agar plate and treated by DBD cold plasma. The treatment gas was argon gas at 5 L/min gas flow rate. The results of this study have indicated that the DBD cold plasma is effective to inactive pathogen microorganisms in very short time. After 1 min DBD treatment the number of colony forming *S. aureus* microorganism decreased drastic. Treatment time of 2 min and longer (3min) was sufficient to inactive all the microorganism (*S. aureus*) on agar plate as it shown in figure 3 and in table 4.

![Figure 3](image-url)

**Figure 3.** *S. aureus* on agar plate with condition untreated (0 minute), treat with DBD 1 minute, 3 minutes and 5 minutes.
Table 4. Inactivation of *S. aureus* during DBD with 5L/min argon flow rate.

| Treatment time (minute) | Microorganism count (CFU/ml) | Reduction of microorganism (%) |
|-------------------------|------------------------------|--------------------------------|
| 0                       | $1.22 \times 10^4$          | 0 %                            |
| 1                       | $5.2 \times 10^3$           | 57.55 %                        |
| 3                       | $1.5 \times 10^2$           | 98.78 %                        |
| 5                       | Less than $10^3$            | 99.59 %                        |

Similarly, it was observed that *L. monocytogenes* can be effectively inactivated on agar plate using DBD cold plasma at treatment time of 1 min to 3 min. After 1 min DBD treatment was more than 96% of microorganism *L. monocytogenes* inactivated (figure 4 and 5). In addition to microorganisms *S. aureus* and *L. monocytogenes* it was possible to inactive *E. coli* using DBD cold plasma. The shortest treatment time applied in this study was sufficient to inactive *E. coli* microorganism effectively (more than 90% inactivation). Longer DBD treatment time led to total *E. coli* microorganisms on agar plate as it shown in figure 5 and table 5. The last pathogen microorganism investigated in this study was *S. typhimurium*. Similar to inactivation of other pathogen microorganisms investigated in this research it was possible to reduce the number of this microorganism up to 94% on agar plate after 2 min DBD cold plasma treatment Longer DBD treatment time of 3 min led to nearly 100% microorganism reduction (figure 5).

![Image of L. monocytogenes on agar plate](image_url)

**Figure 4.** *L. monocytogenes* on agar plate. From left to right: untreated (0 minute), treat with DBD 1 minute, 3 minutes and 5 minutes.

Table 5. Inactivation of *listeria monocytogenes*, *escherichia coli* and *salmonella typhimurium* during dbd with 5l/min argon flow rate.

| Treating time (minute) | Percent reduction of microorganism |
|------------------------|-----------------------------------|
|                        | *Listeria monocytogenes* | *Escherichia coli* | *Salmonella typhimurium* |
| 1 minute               | 96.20 %                     | 91.30 %           | 94.38 %                   |
| 3 minute               | 95.11 %                     | 10.15 %           | - %                       |
| 5 minute               | 100 %                       | 98.55 %           | 99.58 %                   |
From the above results it is obvious the DBD cold plasma technique at 17 kV, 50 Hz, gap = 2 cm, and Argon gas flow rate of 5 L/min is a suitable method to inactive most common pathogen microorganisms in food. It is to note that these experiments were conducted on agar plate with very flat surface so that the active ions produced by cold plasma could directly contact the microorganism on agar media surface.

Min et al., [8] studied the effects of dielectric barrier discharge atmospheric cold plasma (DACP) treatment on the inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on Romaine lettuce. They found that cold plasma can reduce the *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* microorganism about 1 to 1.3 log CFU/g. In another study Rød et al. [9] investigate the application of cold atmospheric pressure plasma for decontamination of a sliced ready-to-eat (RTE) meat product (bresaola) inoculated with *Listeria innocua* (10^5 CFU/cm^2) and packed in polymer bags made of linear-low density- polyethylene, polyethylene phthalate and polyethylene terephthalate. Cold plasma treatment at 27.8 kHz and 27 kV resulted *L. innocua* reduction between 0.8 ± 0.4 and 1.6 ± 0.5 log CFU/g on bresaola slices.

Ying Wang et al. [10] reported the inactivation of yeast (*Zygosaccharomyces rouxii* LB and 1130) in apple juice using cold plasma at 21.3kVand 50 Hz and 30 min. treatment time. Under these process conditions they have observed more than 6 log microorganism reduction. Lee et al., [3] applied helium/oxygen mixture as ionizing gas during cold plasma treatment of sliced bacon to inactive *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella Typhimurium*. They have observed that with increasing of treatment time and power the inactivation of microorganism was increased. The microorganism count inactivation using cold plasma and mixture of helium/oxygen reduced the microorganism count on bacon from 7 log CFU/g to 4.65 log after 60 s. Lee et al., [11] have investigated the effect of atmospheric pressure plasma jet on inactivation of *Listeria monocytogenes* on the surface of agar plates and slices of cooked chicken breast and ham. They found that the antimicrobial effect of cold plasma is dependent on gas type used for generating plasma. They find that the relation of N_2 and O_2 in gas mixture is important inactive *L. monocytogenes*.

4. Conclusion

Cold Plasma was produced by using simple geometry of DBD plasma generator at voltage of 17 kV and 50 Hz. The DBD generator was able to decrease yeast *S. cerevisiae* and pathogen microorganism *S. aureus* microorganism count on nutrient agar plate effectively. Aerosolized hydrogen peroxide in concentration of 2 and 5 % H_2O_2 showed synergistic effect for yeast inactivation combined with DBD
cold plasma treatment. The treatment time is important factor for yeast and \textit{S. aureus} inactivation. In general cold plasma treatment for 3 min. and longer can decrease the microorganism count rapidly.

5. References

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