The Efficiency of DBD Cold Plasma Pen Treatment on the Oyster Mushroom Bacterial Decontamination

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Abstract. Cold plasma provided bacterial inactivation role in food industry. In this study, the cold plasma play a crucial inactivation role when effectively reduces the bacteria colonies on oyster mushroom surface. By development of the dielectric barrier discharge-cold plasma pen (DBD-CPP) system, the mushroom surface was exposed to the cold plasma discharge with variable of exposure treatment time (0 min, 0.5 min, 1 min, 3 min and 5 min) with ~6 kV of power voltage and 5 SLM of atmospheric gas pressure flow rate. In order to identify the reduction of the microbial growth, isolation technique will be carry out by excising the mushroom sample into a suspension and serial dilution follows by identification of its colony morphologies and characteristics. Results screening shows increments of exposure treatment times up to 3 min shows none growth of bacteria colonies. This because the bacteria cell wall was disrupt and destruction by the plasma bombardment. Thus, this study able to extend the lifetime of the mushroom and produce a free microbial fresh mushroom by decontaminate the bacteria on the mushroom surface

1. Introduction

Mushroom was known as “Food of Gods” by Roman community when its supplied strength to their armies during battles on ancient times. This shown the mushroom good enough to maintain general health and might be chosen as staple food for certain places [1]. The unique taste of oyster mushroom and its nutritional values required oyster mushroom broadly consume and aggressively cultivate in Malaysia [2]. Nevertheless, the oyster mushrooms could not last longer before and after harvesting as it’s really sensitive to slightly change of cultivate environment [3;4] as shown in Figure 1. The mushrooms will rotten result from bacterial growth, cause infections for the whole fruiting body. Thus, it is responsible to ensure the mushrooms served freshly, could last longer and bacterial free for human consumption.

Cold plasma (CP) as bacteria inactivation is incontestable and has been demonstrated by numerous studied in food and agricultural industry [5-9]. The principal advantage of CP operate very close to the ambient temperature (below 50 ºC) and maintain the food quality, often found with thermal processing causes this technology get attention for apply in food industry [10]. Therefore the goal of this study is

to demonstrate the potential of developed dielectric barrier discharge cold plasma pen (DBD-CPP) system in inactivation the bacteria on the oyster mushroom. This study proved cold plasma can interact with bacterial cell wall, damage the cell wall and loss its dense causes the bacterial have less chance of getting infection. Thus the shelf lifetime of mushroom can be longer and consume safe mushrooms in human diet.

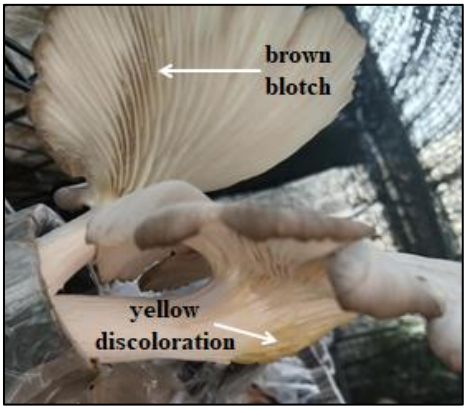

Figure 1. The mushroom problems during cultivation process

2. Materials and Methodology

2.1. Dielectric Barrier Discharge cold plasma pen (DBD-CPP) setup

The setup of DBD-CPP reactor (Figure 2) for mushroom treatment was developed and explained by [11]. The voltage applied for the pileus of oyster mushroom treatment was ~6kV with variable of exposure times of 0 min, 0.5 min, 1 min, 3 min and 5 min. The atmospheric pressure gas was flow by using 5 standard liter per minute (SLM) of flow rate.

2.2. Sample Preparations

A pack of commercial oyster mushroom was purchased from Aeon Taman Universiti, Skudai, Johor and cut into 1 cm X 1 cm prior the plasma treatment. Each of the treated samples was then immersed into separate universal bottles filled with 10 mL sterile distilled water. The samples were then shaken for 45 minutes to ensure the bacteria dispersed equally in the water.

2.3. Spread Plate Technique for Bacterial Growth Observation

Initially, 100 µL of each sample solutions were pipette out into the NA plate and spread out following the spread plate technique. All these steps were carried out in the laminar flow to maintain the sterility and to avoid contamination. The plates were then incubated at 37˚C overnight up to 3 days in order to monitor the bacteria growth.

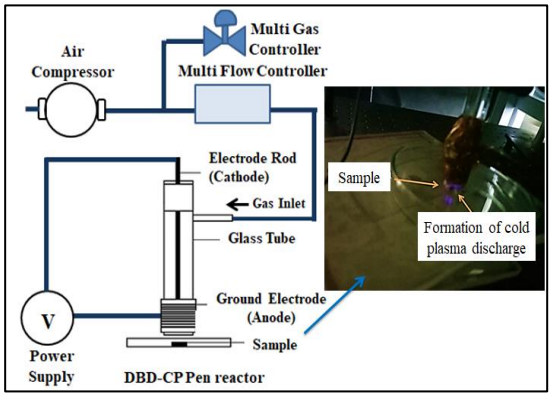

Figure 2. DBD-CPP system for Oyster Mushroom Treatment

3. Results and Discussions

The overall results for the oyster mushroom bacterial decontamination are summarized in Table 1. The 3 days experiments observation shows a reduction of bacterial growth in line with the increment of DBD-CPP treatment times. The 3 minutes and above compressed air plasma treatment gave the best results in bacterial inactivation. Although there was no bacterial growth for 0.5 minutes treated mushroom within first 24 hours, the bacterial however just started to grow on day 2. Similar to 1 minute of compressed air plasma treatment, there is almost no bacteria left on the plate in first 48 hours. However, there were a few bacterial colonies appeared on the plate on day 3. The bacterial could be considered as slow grower due to require more than 24 hours for displayed. Increasing the treatment time to 3 minutes leads to complete inactivation.

This phenomenon occurs because CP bombardment can caused shrinkage on the bacterial cell wall that lead to bacteriostatic effect when exposed up to 5 min treatment [12;13]. A similar trend by Lu et al., (2016) studied shows extending treatment time up to 30 s caused significant bacterial reduction with mode of action dependent on bacterial type compared to 5 s of treatment time [14].

Apart from that, the effectiveness of DBD-CPP for inactivates or reducing the bacteria on oyster mushroom for microbial safety is shows in Figure 4. The formation of bacteria colonies amount and size was reduced from too numerous to count for untreated sample and become undetectable for 3 min treatment and 5 minutes. Data gathered also displays untreated mushroom sample consist of two bacterial strains which were white and pink, smooth, convex colonies characteristics and less opaque morphology.

In general, the colonies were keeping growth as the bacteria were dividing. These bacteria colonies also keep maintain their colonies and its size for the next following days (images not include in the Table). According to Hong et al., (2009) observed severe cytoplasmic deformation and leakage of bacterial chromosome when received plasma treatment [15]. Furthermore, there was also bacteria cell wall aggregation found due to the stress received from the plasma treatment where the researchers observed the morphological alterations on the cells [16;17].

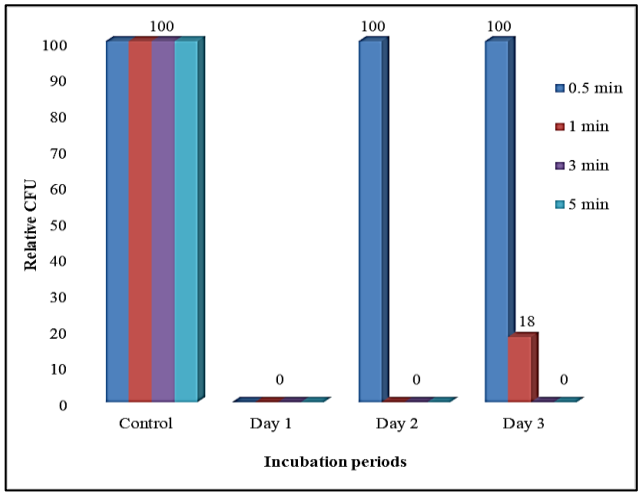

Figure 3. Existence of bacteria colonies for 10^{-1} dilution on oyster mushroom by DBD-CPP treatment

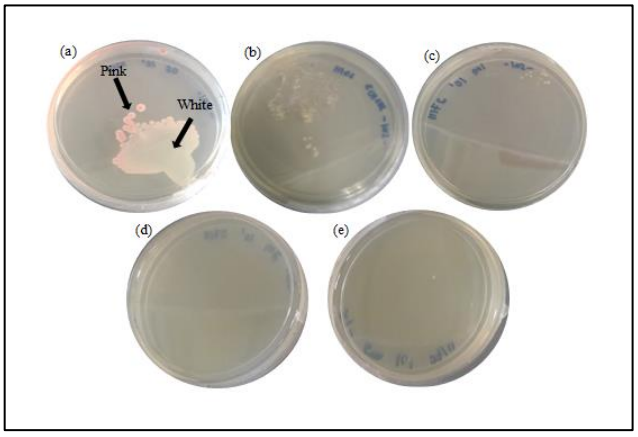

Figure 4. The Morphology and characteristics of bacteria colonies before and after DBD-CPP treatment: (a) 0 minute (b) 0.5 minutes (c) 1 minute (d) 3 minutes (e) 5 minutes

4. Conclusions

The experiments of DBD-CPP effects on bacterial decontamination were investigated and the application showed an inactivation effect on the bacterial CFUs on oyster mushroom samples. Promising results were obtained from the treatments of more than 3 minutes. However, shorter treatment duration was not very efficient in decontaminating the bacterial, where a lot of CFU's were still present on the plate within 3 days observation. In general, the bacterial colonies growth strongly correlated with DBD-CPP treatment time. Thus, this study demonstrated DBD-CPP can be applied in food industry as potential approach for improving microbial safety.

Acknowledgment

The authors sincerely acknowledge UTM and MOHE for the financial support (UTMPR grant 00L65).

References

[1] Zhang, Y. et al., (2014). Edible Mushroom Cultivation for Food Security and Rural Development in China: Bio-Inn., Technol. Dissemination and Marketing. Sustainability, 6, 2961-2973.
[2] Mat Amin, M. Z. et al., (2017). A study of consumer behaviour towards mushroom-based products in Malaysia. Eco. and Technol. Management Review, 12, 55–63.
[3] Bellettini, M. B. et al., (2018). Diseases and pests noxious to *Pleurotus spp* . mushroom crops. Revista Argentina de Microbiología, 50, 216–226.
[4] Navarro, M. J. et al., (2018). Identification, incidence and control of bacterial blotch disease in mushroom crops by management of environmental conditions. Sci. Horticulturae, 229, 10–18.
[5] Agun, L. et al. (a) (in press). Sterilization of oyster mushroom crop residue substrate by using cold plasma technology. Materials Today: Proceedings.
[6] Huang, M. et al. (2020). Differences in cellular damages induced by dielectric barrier discharge plasma between *Salmonella typhimurium* and *Staphylococcus aureus*. Bioelectrochemistry, 132, 107445.
[7] Zhang, Y. et al., (2019). Bactericidal effect of cold plasma on microbiota of commercial fish balls. Inn. Food Sci. and Emerging. Technol., 52, 394–405.
[8] Liao, X. et al. (2017). Inactivation mechanisms of non-thermal plasma on microbes: A review. Food Control. 75, 83–91
[9] Surowsky, B. et al., (2014). Impact of cold plasma on Citrobacter freundii in apple juice: Inactivation kinetics and mechanisms. Int. J. of Food Microbiology, 174, 63–71.
[10] Pérez-Andrés, J. M. et al. (2020). Effects of cold atmospheric plasma on mackerel lipid and protein oxidation during storage. Lwt- Food Sci. and Technol., 118, 108697.
[11] Agun, L., et al., (2020b). Spawn Treatment by Cold Plasma for Increase Mushroom Germination and Production. *IOP Conference Series: Materials Science and Engineering*, *884*, 012004.
[12] Han, L. et al. (2016) Mechanisms of inactivation by high-voltage atmospheric cold plasma differ

for *Escherichia coli* and *Staphylococcus aureus*. Appl. and Environmental Microbiology. 82, 450–458.

[13] Kannan, S. et al., (2019). Microbial Pathogenesis Decrease of growth, bio fi lm and secreted virulence in opportunistic nosocomial Pseudomonas aeruginosa ATCC 25619 by glycyrrhetinic acid. Microbial Pathogenesis, 126, 332–342.

[14] Lu, H., et al., (2014). Bacterial inactivation by high-voltage atmospheric cold plasma: Influence of process parameters and effects on cell leakage and DNA. J. of Applied Microbiology, 116(4), 784–794.

[15] Hong, Y. et al., (2009). Sterilization effect of atmospheric plasma on *Escherichia coli* and *Bacillus subtilis* endospores. Letters in Applied Microbiology. 48, 33-37.

[16] Min, S. C. et al. (2018) In-package atmospheric cold plasma treatment of bulk grape tomatoes for microbiological safety and preservation. Food Research Int. 108, 378–386.

[17] Ulbin-Figlewicz, N. et al., (2015). Antimicrobial activity of low-pressure plasma treatment against selected foodborne bacteria and meat microbiota. Ann. Microbiol. 65**,** 1537-1546.