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Fong Mun Oon
School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia

Agun, Linda
School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia

Mohd Fadthul Ikmal Misnal
School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia

Muhamad Nor Firdaus Zainal
School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia

他

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Effect of Cold Plasma Voltage and Treatment Duration on the Microstructure and Hydrophilicity of Mushroom Grain Spawn

Fong Mun Oon[1], Linda Agun[1], Mohd Fadthul Ikmal Misnal[1], Muhamad Nor Firdaus Zainal[1], Norizah Redzuan[1], Norhayati Binti Ahmad[1,*]

[1]School of Mechanical Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, Malaysia

*Corresponding author: nhayati@mail.fkm.utm.my

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Abstract: Nowadays, cold plasma treatment systems are widely used in many fields such as agriculture. It was proven that this technique can improve growth rate of mushroom grain spawn, but no research had been done on spawn treatment using cold plasma. Cold plasma surface treatment system was developed by using voltages of 2kV to 4 kV and treatment durations of 5s to 60 s were used. The results show an improvement of grain spawn hydrophilic properties and growth rate. The most suitable parameters to treat mushroom grain spawn were 2.5kV of voltage with treatment time of 15s resulting in increasing growth of mushroom until 5.7cm.

Keywords: cold plasma treatment; mushroom mycelium; applied voltages; treatment durations

1. Introduction

Mushrooms are defined as a type of macro fungus with a distinctive fruit body, and they can be grouped into two different types: epigeous which means growing above the earth or hypogenous which means growing underground. Mushrooms have two growth phases. The first phase involves the vegetative phase, which only happens in the soil. The second phase is the reproductive phase where the fruiting bodies is seen above the ground. Table 1 shows the nutritional value of a few common edible mushrooms.

Table 1. Nutritional value of mushrooms[1]

Nutritional parameters (dry wt. basis)	Mushroom Variety			
	Agaricus bisporus	*Pleurotus eous*	*Volvariella volvacea*	*Lentinula edodes*
Protein (%)	29.14	19.59	38.10	18.85
Carbohydrates (%)	51.05	64.34	42.30	63.60
Fat (%)	1.56	1.05	0.97	1.22
Vitamin D (IU/g)	984	487	462.04	205
Sodium (mg/kg)	500.8	208.87	345.34	82.49
Potassium (%)	4.21	2.70	4.16	2.10
Potassium : Sodium	84 : 1	129 : 1	120 : 1	255 : 1
Iron (mg/kg)	85.86	183.07	72.51	37.55
Manganese (mg/kg)	7.97	6.47	-	17.48
Zinc (mg/kg)	79.64	162.18	94.28	89.63
Selenium (mg/kg)	1.34	ND	ND	ND

Along with the vast advancements in modern technology findings and developments, many seek different strategies to improve agricultural yields in order to support today's ever-increasing population [2-3]. Many chemical or physical methods were developed, such as fungicides, ozone treatment, pulsed electric field processing, ultraviolet light, and so on. These efforts have shown positive effects in seed dormancy breaking, enhancing seed germination rates, and building resistance against diseases. However, these methods have their respective disadvantages, such as significant environmental risks [4], high machinery costs [5], radiation exposures, expensive processing methods or being ineffective at all [6]. For example, concerns of contamination possibilities from corroded electrodes of pulsed electric field equipment [7] and health implications from ozone treatment [8] were raised.

Cold plasma (CP) technology has proven to be a potential competitive approach to solve the problems mentioned earlier and provide better results. Cold plasma is generated when an electric current passes through a carrier gas or air between two electrodes at atmospheric or vacuum conditions [9]. During operation, various plasma species, electrons, ions, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are being generated [10]. For example, atomic oxygen (O), hydrogen peroxide (H2O2), hydroxyl (OH), and so on. The principle found behind the formation of these reactive species lies in the huge mass difference between electrons and neutral particles in the surrounding air, which led to improved levels of ionization and dissociation from the collision of activated electrons and heavy particles [11-12].

There are many positive effects brought upon by the

reactive plasma species generated in a cold plasma. Firstly, these species demonstrated bacterial inactivation behaviour. Yusupov et al. [13] found out from a molecular dynamic's simulation, that atomic oxygen can dissociate bonds which maintains the cell wall integrity of bacteria, leading to a breakdown of the cell wall and leakage of intercellular components. Other effects found are the causing of depressions on the surface of fungi, the accumulation of intracellular ROS, and the increase of antioxidant enzyme activities [14-15]. Furthermore, the reactive species are also capable of increasing seeds' wettability and water uptake, which led to the increased germination efficiency of crops [16-17].

It is clear that, cold plasma technology is a better alternative in the food and agricultural field. However, no in-depth research has been done and the mechanisms behind the plasma-induced effects on mushrooms.

Better understanding of cold plasma towards the mushroom industry is important, as the demand for mushrooms in Malaysia is estimated to increase along with the population due to its health benefits [18]. Besides that, there exist a contradictory part, where cold plasma was found to speed up the growth rate of mushroom mycelium and increased the production rates [19-20]. But we knew from previous research that cold plasma has fungi deactivation behaviours, which was not the case in this study. Hence, there exist a certain knowledge gap which became the basis for this research.

The objectives of this research were to determine the effects of cold plasma on the changes of hydrophilicity, surface morphology, and chemical properties of the mushroom grain spawn. In addition to that, this research aimed to identify the changes in the growth of the mushroom mycelium under different applied voltages and treatment durations when the cold plasma generator was being operated. The scope for this research includes design of a cold plasma treatment system, fabricating, and assembling the prototype, treating the mushroom grain spawns under the parameters tested, characterization, and finally post-inoculation observation.

2. Methodology

2.1 Prototype design improvement and assembly

Figure 1 shows the design of a cold plasma treatment system. Then, it was fabricated and some of the parts were printed out using 3D printer.

Pleurotus sajor-caju (P. sajor-caju) mushroom grain spawns, or commonly known as grey oyster mushrooms were used as the target specimen to be treated by using cold plasma. The mushroom grain spawns came in different shapes, such as circular, ellipsoid, dextrinoid, peanut-shaped, and many more irregular shapes. The prototype was connected to a PVM500 Plasma Driver as a power source for cold plasma generation. The desired voltage and treatment duration was set accordingly as shown in Table 2.

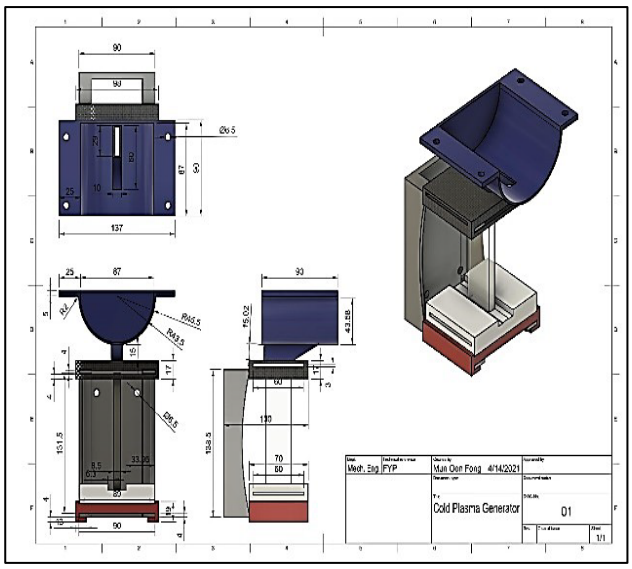

Fig. 1: Technical drawing of the final design for the cold plasma generator.

Table 2. Cold plasma treatment parameters

No	Applied voltage (kV)	Treatment duration (s)
1	2.0 kV	5
2		15
3		30
4		45
5		60
6	2.5 kV	5
7		15
8		30
9		45
10		60
11	3.0 kV	5
12		15
13		30
14		45
15		60
16	3.5 kV	5
17		15
18		30
19		45
20		60
21	4.0 kV	5
22		15
23		30
24		45
25		60

2.2 Characterisation of the samples

The surface morphology and microstructure of the treated and untreated mushroom grain spawns were analysed by using scanning electron microscopy (SEM) The samples were prepared accordingly as described by Mehdizadeh-Kashi A et al. [21] for biological samples. A Fourier-transform infrared spectrometry (FTIR) analysis was carried out to determine the main chemical groups present in the mycelium structure and its changes after cold plasma treatment. To generate the FTIR spectra, the samples were placed directly on the cleaned diamond/ZnSe crystal, and the pressure arm was rotated clockwise to allow good contact of the sample with the crystal. The scanning was performed by attenuated total reflection (ATR) from wavelength of 650 to 4000 cm-1 with a Perkin-Elmer FT-IR Spectrometer Frontier equipped with a universal ATR sampling accessory. This wavelength range was chosen to ensure full detection coverage of the OH and fingerprint regions of the samples [22]. To identify the changes in the hydrophilicity of the sample's surface, an apparent contact angle (ACA) sessile drop technique was applied with an OCA 15EC contact angle measuring device. The samples were first placed on the sample table, and a small syringe would dispense 0.3 µL of water at dosing rate of 1.00 µL/s in contact with the sample's surface. The water droplet would form a hemispherical shape on the surface, where the angle is defined as the angle between the solid surface and the tangent of the droplet outline. This angle was then captured automatically by the device camera for both left and right parts of the droplet, and the average angle was calculated using the formula as below:

$$Average\ angle = \frac{Left° + right°}{2} \qquad (1)$$

3. Results and Discussion

Based on the characterisation results obtained on mushroom spawn, it is obvious that cold plasma treatment had on effect on the surface morphology, chemical functional group changes, and surface hydrophilicity. From Fig. 2, it shows that the treated grain spawns demonstrated a few obvious changes, such as increasing depressions and cracks on the mycelium surface (red arrows), appearance of sclerotia structures (S), and cell wall degradation (Cwd). The presence of oxidative stress caused by ROS will promote the formation of sclerotia for better survival rate [23]. When the treatment duration was increased gradually, the mycelium structures appeared more folded and collapsed due to the oxidation and interactions of the reactive plasma species with the mycelium.

The FTIR analysis was being performed to validate the effect of cold plasma towards the mycelium surface as seen previously in the SEM results. Figure 3 illustrates the changes of the FTIR spectrum for the untreated and treated (5, 15, 30, 45, 60 s) grain spawns, whereas Fig. 4 shows the spectrum when different voltages were being applied (2.0, 2.5, 3.0, 3.5, 4.0 kV). From the data, it can be observed on the peak of the O-H signals increase when the treatment duration was increased as well. This could be due to the incorporation of O-H groups on the mycelium surface during the treatment [24]. When the voltage applied was raise, the peak of the OH signals showed a decreasing trend as shown in Fig. 4. This could be an indication that dehydration was taking place with potential cell wall changes and the intensified absorption of OH groups onto the surface [25]. The increase in intensities for other peaks like C-H and C=C inferred the presence of chemical etching and oxidation of the biomolecules at the surface.

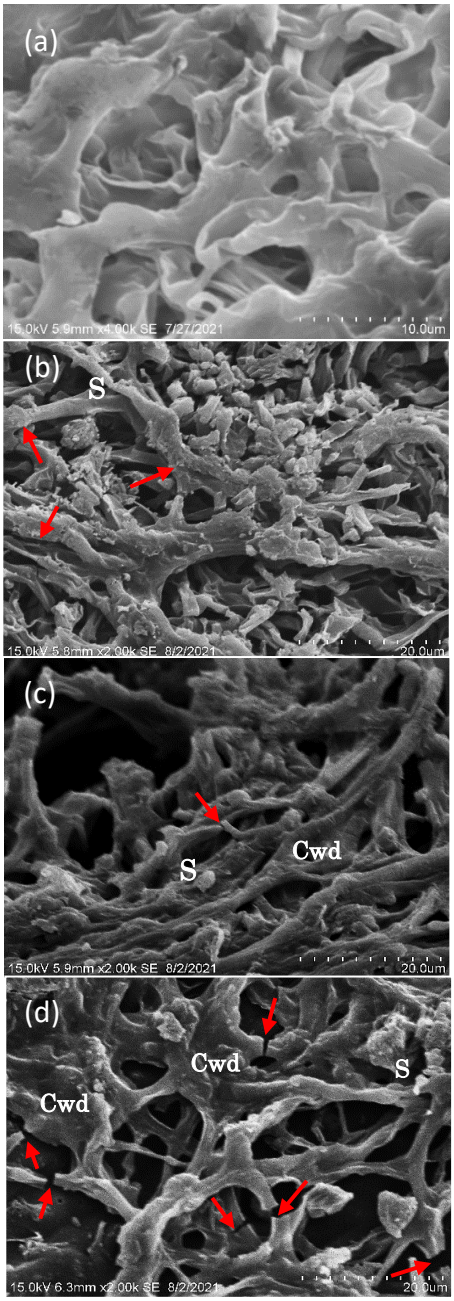

Fig. 2: SEM results of grain spawns (a) untreated, (b) 5s, (c) 30s, and (d) 60s treatment time.

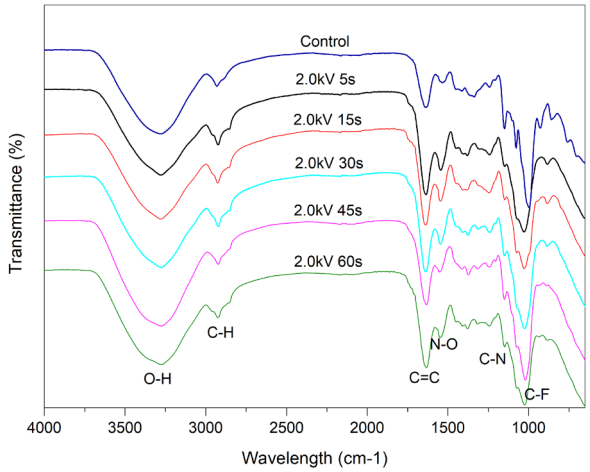

Fig. 3: FTIR spectrum under different treatment durations.

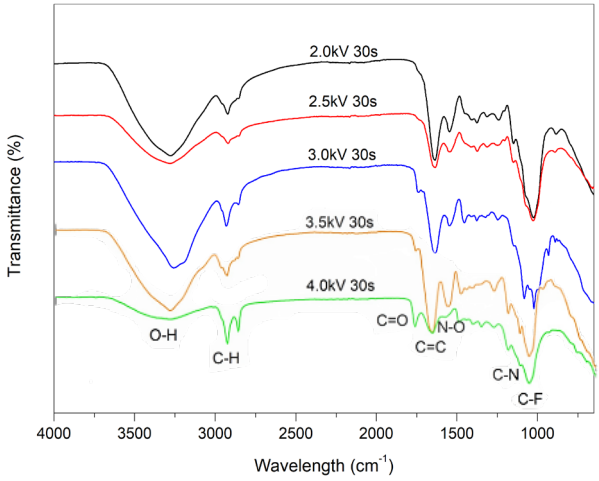

Fig. 4: FTIR spectrum under different applied voltages

The changes in hydrophilicity of the treated grain spawns were investigated by using the ACA sessile drop technique. Figure 5 shows a section of the apparent contact angles for both untreated and treated samples. The detailed data is tabulated in both Table 2 and Table 3 for samples of various treatment durations and different applied voltages respectively. A higher value of apparent contact angle indicates that the surface is hydrophobic, whereas a small value shows that the surface is keen to have hydrophilic properties.

When the treatment duration was increased, it is found that the water droplet appeared to be easily absorbed into the surface and the apparent contact angles were decreasing as well. The improvement of surface hydrophilicity was primarily caused by the reactive species generated during the cold plasma treatment, OH and O species in particular. OH plays a major role in the oxidation-reduction biochemical processes on the surface, which causes physical and chemical modifications [26]. Another reason could be the that the presence of OH and O radicals led to the formation of other polar functional groups such as: carbonyl (C=O) and carboxyl (COOH), which resulted in a much more hydrophilic surface [27].

Table 2. ACA of samples for different treatment durations

Parameters	Contact Angle (left)	Contact Angle (right)	Average
Untreated	118.3°	119.1°	118.7°
2.0 kV, 5 s	92.2°	94.6°	93.4°
2.0 kV, 15 s	67.4°	71.8°	69.6°
2.0 kV, 30 s	67.1°	55.7°	61.4°
2.0 kV, 45 s	42.6°	47.0°	44.8°
2.0 kV, 60 s	30.6°	29.0°	29.8°

Table 3. ACA of samples for different applied voltages

Parameters	Contact Angle (left)	Contact Angle (right)	Average
2.5 kV, 30 s	59.7°	59.6°	59.65°
3.0 kV, 30 s	61.6°	61.6°	61.6°
3.5 kV, 30 s	56.5°	52.1°	54.3°
4.0 kV, 30 s	32.3°	32.3°	32.3°

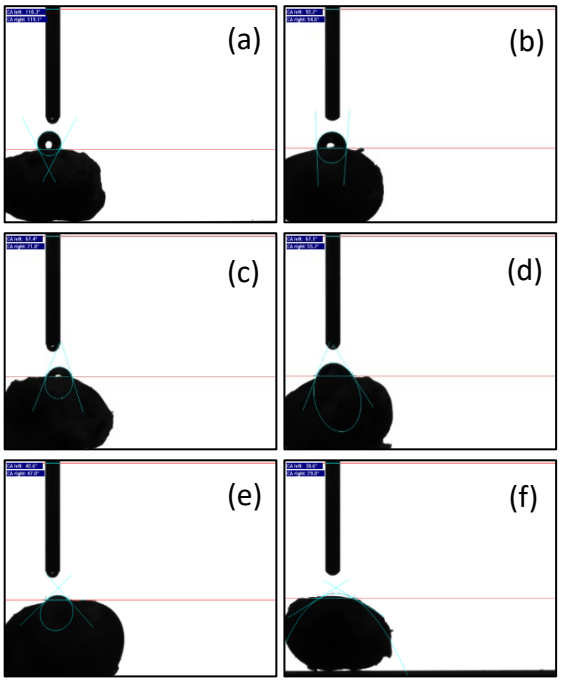

Fig. 5: ACA of samples treated for (a) 0 s, (b) 5 s, (c) 15 s, (d) 30 s, (e) 45 s, (f) 60s.

From Fig. 6, it is clear that after cold plasma treatments, most of the samples demonstrated an improvement of mushroom growth compared to the untreated samples. The untreated sample had an initial 4.95 cm growth within the first week. When 2 kV voltage was applied, most of the samples did not show significant growth improvements. The highest growth of 5.29 cm was achieved during the 60s treatment time. It could be a reasoned that 2 kV might not be sufficient to generate the reactive plasma species for the faster mycelium growth. 2.5 kV applied voltage gave the most prominent results

among the rest, with three different durations obtaining the greatest mycelium growth of 5.69, 5.7, and 5.7 cm during 5, 15, and 45 s of treatment time respectively.

From this data, it can be expecting that 2.5 kV is the most suitable applied voltage compared to the other voltages, whereas the most suitable treatment duration lies between 5 s to 45 s. When the voltage was being increased to 3 kV, only the first three durations (5, 15, 30 s) were able to improve the mycelium growth. Beyond 45 s treatment, an inhibitory effect on the growth could be observed since the mycelium growth was even lesser than the control sample. The same goes for 4 kV, where almost no samples demonstrated mycelium growth after treatment. This could infer that 3.5 kV and 4 kV might not be suitable for the mushroom grain spawns, as the high voltage would have killed off the mycelium structures on the grain spawn surface.

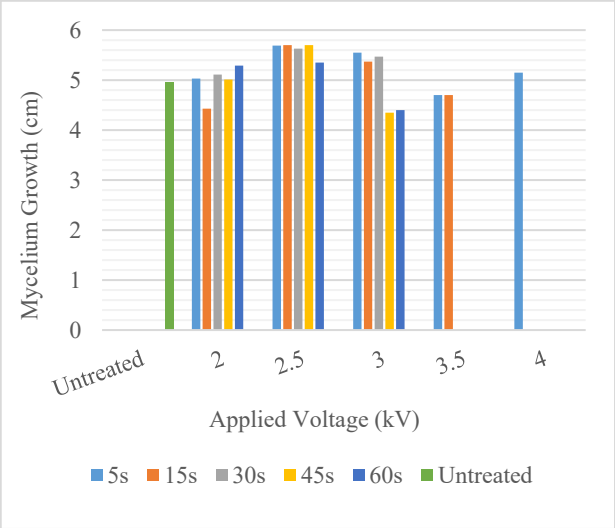

Fig. 6: Graphs of mycelium growth for untreated and treated samples.

4. Conclusion

It can be concluded that reactive plasma species generated during a cold plasma treatment able to modify the surface morphology of the mycelium structures found in P. sajor-caju mushroom grain spawns. After the treatment process, cracks, folding, depressions, cell wall degradations could be observed with various degrees of severity. These species could also affect the surface chemical properties of the mycelium, which include the accumulation of OH groups, formation of new functional groups (C=O), oxidation of biomolecules was found to be taking place in the treated samples. Another finding was that improvement of hydrophilic properties on mushroom spawn grain was got by reactive plasma species as well. This could be the presence of polar and hydrophilic groups on the surface after the treatment. By increasing both the applied voltage and treatment durations, it shows that there were more drastic effects compared to the untreated samples. It was proven from the data that voltage of 2.5 kV and duration of 15 s were most suitable to improve the growth of P. sajor-caju mushroom grain spawns.

Acknowledgements

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Nomenclature

CP	cold plasma
ROS	reactive oxygen species
RNS	reactive nitrogen species
O	atomic oxygen
H_2O_2	hydrogen peroxide
OH	hydroxyl
kV	kilovolts
s	seconds
SEM	scanning electron microscopy
FTIR	Fourier-transform infrared spectrometry
ATR	attenuated total reflection
ACA	apparent contact angle
µL	microliters
µL/s	microliters per second
S	sclerotia
Cwd	cell wall degradation

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