# Effects of Pulse Voltage Stimulation on Fruit Body Formation in *Lentinula Edodes* Cultivation

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**Abstract—Pulsed high voltage was applied to natural logs for mushroom cultivation to verify an effect of the pulse voltage stimulation on fruit body formation. Inductive energy storage (IES) system was employed to construct a pulsed power generator to generate narrow pulse over 100 kV with compact size. Copper thin fuse was used as an opening switch to interrupt large circuit current in short time. Four stages Marx generator was used to supply a large current to a secondary energy storage inductor. The output voltage of the IES pulsed power generator was 120 kV with 50 ns pulse width at 5 kV charging voltage to the primary energy storage capacitor. The output voltage of 50 ns pulse width was applied to the natural logs of *Lentinula edodes* (Shiitake mushroom) cultivation as the stimulation for the fruit body formation. The total weight harvested from fifteen logs was 2.29 kg at fifty times 50 kV pulse voltage stimulations and was larger by 1.09 kg at one 50 kV pulse stimulation case. The deviation of the mushroom yield among the logs decreased with the pulse voltage stimulation. The hydrophobic protein, which was predicted to contribute to the fruit body formations, was confirmed to be released from the vegetative hyphae when applying the pulse voltage.**

**Keywords—Pulsed power, electrical stimulation, inductive energy storage, mushroom, *Lentinula edodes***

## I. INTRODUCTION

Outbreak of mushrooms around a lightning strike point has been reported by some farmers, forestry workers, hikers, etc. The mechanism of the mushroom outbreak is not clear, but some researchers suggest two possibilities. One is that the mushroom hyphae are ruptured by the lightning because the rupture of hypha works as stimulation for fruit body formation of mushroom [1]. The other is the activation of enzymes. Some enzymes are activated by applying high voltage and consequently, mushroom fruit bodies develop abundantly [2].

An effectiveness of electrical sources such as high voltages in sinusoidal waves or pulses in cultivating mushrooms has been reported by several researchers since the 1950s. In 1987 Jitsufuchi *et al*. reported that the yields of shiitake mushroom (*L. edodes*) increased by electrical stimulation [3]. They obtained more than twice as high mushroom yield using an impulse generator which generated several hundreds kV high voltage with 40 μs pulse width. Some other researchers also achieved the improvement of mushroom yield using other electrical sources such as high voltages of sinusoidal waves or pulses [1-3].

Inductive energy storage (IES) pulsed power generators have favorable features for the mushroom cultivating applications e.g. they are compact, cost effective, light, have high voltage amplification compared with capacitive energy storage generators such as the impulse generator. Tsukamoto carried out high voltage pulse stimulation in *Lentinula edodes* (*L. edodes*) culturing using IES pulsed power generator [4]. They applied 140 kV pulse voltage to 120 cm length logs implanted with mushroom hypha and confirmed the increase of mushroom yield. The pulse voltage stimulation was also employed as the electrical stimulation on mushroom cultivation in a sawdust bottle [5].

The effect of the short pulse voltage stimulation on some other kinds mushroom, such as *Pholiota nameko* (*P. nameko*), *Lyophyllum decastes*, was also confirmed using IES generator developed for the improvement of mushroom yield as electrical stimulation [6, 7]. As a result of these studies, total harvested weight from logs and/or sawdust-based block for mushroom cultivation increased by applying pulse voltage as an electrical stimulation. However, the details of mushroom yield improvement, such as average weight of harvested mushroom, etc. are not known. Moreover, the mechanism from the stimulation to the improvement yield is also not clear.

This paper describes details of yield improvement achieved by pulse voltage stimulation produced with IES generator for natural log cultivations of *L. edodes*. The mechanism of the mushroom yield improvement is also investigated using polymerase chain reaction (PCR) technique for released protein analysis.

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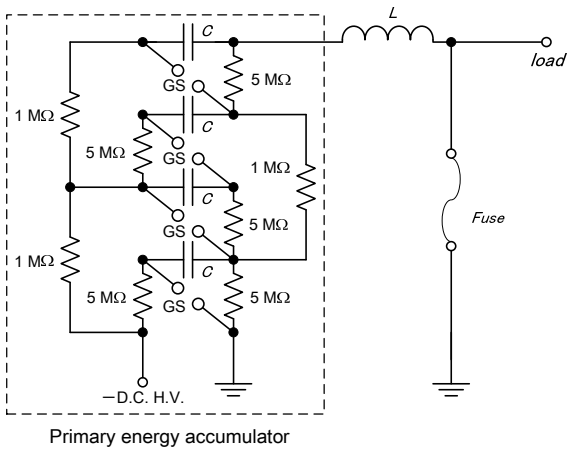



Fig. 1. Marx-IES pulsed power generator using fuse as an opening switch. (*C*: Primary energy storage capacitor, *L*: Secondary energy storage inductor).

## II. Experimental Setup

Fig. 1 shows the Marx-IES pulsed power generator circuit used for the high-voltage electric stimulation of mushrooms. The IES pulsed power generator basically consists of primary energy storage capacitors *C*, closing switches GS, a secondary energy storage inductor *L*, and an opening switch [8]. Copper fuse of 0.05 mm diameter was used as the opening switch to interrupt large current in short time. The four primary energy storage capacitors of 0.22 μF were connected in parallel and were charged up using high voltage dc power supply (50 kV maximum voltage). A charging voltage $V_C$ of the each primary energy storage capacitor was controlled in range from 5 to 7 kV. After charging up the capacitor, the gap switch GS was triggered externally. The closing switch GS changed the connection of the capacitors from parallel to series. As a result, the voltage was stepped up from $V_C$ to 4 $V_C$ in same manner to the Marx generator [7]. The fuse length $l$ and the total inductance $L$ of the secondary energy storage inductor and the generator circuit were changed in range from 5 to 20 cm and from 1.3 to 38 μH, respectively. The circuit current and the output voltage were measured with Pearson 110A current transformer and Pulse Electronics EP-100K high-voltage probe, respectively. The output signals from the current transformer and the voltage probe were stored with a Tektronix TDS3054B digitizing oscilloscope. The stored data were transferred to the computer for analysis of the electrical properties.

Fig. 2 shows typical circuit (fuse) current and output voltage waveforms without connection to the logs at 5 kV charging voltage. The fuse length and the inductance of the secondary energy storage inductor are chosen to be $l$ = 10 cm and $L$ = 10 μH, respectively. The time 0 means closing the switch GS. The circuit current starts to flow after closing the switch GS with *LC* oscillation. The peak value of the circuit current is about 920 A at 0.8 μs after closing the switch. After the current peak, the current decreases gradually from 920 to 600 A during 0.3 μs.

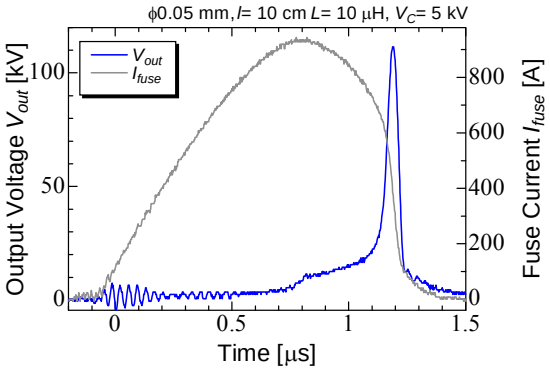



Fig. 2. Typical waveforms of fuse current and output voltage at $l$ =10 cm, $V_C$ = 5 kV and $L$ = 10 μH.

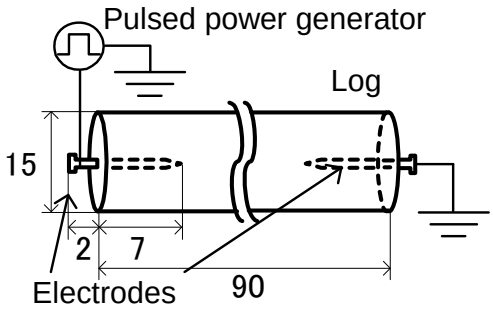



Fig. 3. Experimental setup for pulsed power stimulation to the *L. edodes* cultivating logs.

This time duration corresponds to a fuse melting phase. The circuit current is interrupted after fuse melting phase within 100 ns. The output voltage increases rapidly and has a maximum voltage of 110 kV, which corresponds to 22 of an amplification factor defined as ratio of the maximum output voltage to the charging voltage. The pulse width of the output voltage is 50 ns in FWHM (full-width at half-maximum). The high voltage pulse is produced by the total circuit inductance and a rapid current interruption produces a high voltage pulse expressed as

$$v = V_C - \frac{1}{C}\int i\,dt - L\frac{di}{dt} \approx -L\frac{di}{dt} \qquad (1)$$

where $i$ means the circuit current.

Fig. 3 shows an experimental setup of the pulse voltage stimulation for the natural log cultivation. The *L. edodes* mushroom was used as a specimen. Fungi were inoculated in the natural logs around two years before the experiment. The logs dimension was 90 cm in length and 10 cm in diameter. The needle electrode of 3 mm diameter and 9 cm length was driven in the logs 7 cm and subsequently the pulse voltage was applied by the Marx-IES pulsed power generator as an electrical stimulation. The resistance of the log was roughly obtained as 10 kΩ using a multi-mater. As the result, a pulse current of 11A in peak amplitude and 50 ns in width flows through the logs when the pulse voltage of 110 kV is applied to the logs. Under the experimental condition, the current flows

through the log uniformly because the skin depth is roughly calculated to be 2.1 m.

The fruiting season of the mushroom species is mainly early autumn, partially spring. After the inoculation, the fungi matured in the logs under environmentally controlled conditions. The experiment was carried out an attempt to increase the yields from 2007 to 2008. For the purposes, the experiment fifteen logs were treated as one crop, because normally each log gives different harvest size. The stimulation effect was subsequently roughly estimated taking into consideration aggregate harvest from fifteen logs. Next, the effect was thoroughly evaluated by comparison of the yields from every particular log, first before and then after the stimulation.

## III. Results and Discussion

### A. Mushroom yield

Fig. 4 shows the crop of the *L. edodes* harvested under five different pulse voltage stimulation conditions. One group was cultured without pulse voltage stimulation and was used as a reference i.e. control group. Three groups were stimulated by single high-voltage pulse with three different amplitudes; 50, 90 and 125 kV. The last group was stimulated with 50 times of 50 kV pulse voltage. The yield of the fruit body is evaluated as total weight harvested during four seasons. It includes the crops from all 15 logs, appropriately averaged. The yield of the control group is only 2 g on the first harvesting season, 2007 in autumn, because the *L. edodes* species used in present experiment mainly fruits in spring. In this case, the 30 g weight of fruit bodies was harvested from only one log. Therefore, standard deviation is obtained to be 7.5 g which is larger value than 2 g average weight. This result indicates that the mushroom species employed in the experiment usually does not develop to the fruit bodies based on statistical analysis. However, the yield of the first season increases from 2 to 73 g when 50 kV pulse voltage was applied. The yield increases from 73 to 153g when voltage number increases from 1 to 50 times. In this case, the standard deviation was obtained to be 73.0 g which was lower value than 153 g average weight. This result indicates that the employed mushroom develops to the fruit bodies by applying the pulse voltages.

The total harvested weight during four seasons is 167 g in the control group. This value increases to 322 and 319 g when pulse voltage of 50 and 100 kV was applied. However, the yield decreased to 243 g when voltage was increased to 125 kV. This result indicates that the optimum of amplitude of the pulse voltage for stimulation *L. edodes* exists and it is estimated to range from 50 to 100 kV/m. The number of fruit bodies harvested cropped in 2008 were 46, 48, 36, 40 and 25 in the control, 50 kV x 1, 100 kV x 1, 125 kV x 1 and 50 kV x 50 times, respectively. The average weight of one mushroom was 34.2, 43.1, 70.0, 32.3 and 48.6 g. This was calculated using the total crop weight of 1575, 2070, 2520, 1290 and 1215 g. Therefore, the dominant effect of electrical stimulation for the improvement of total mushroom yield in spring is an increase of the size of each fruit body.

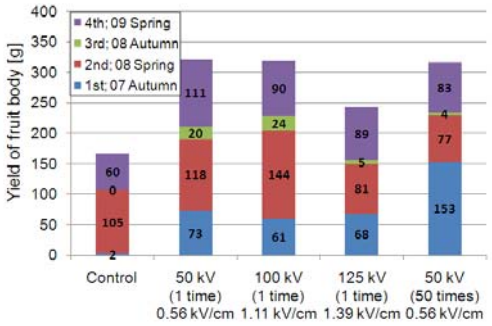
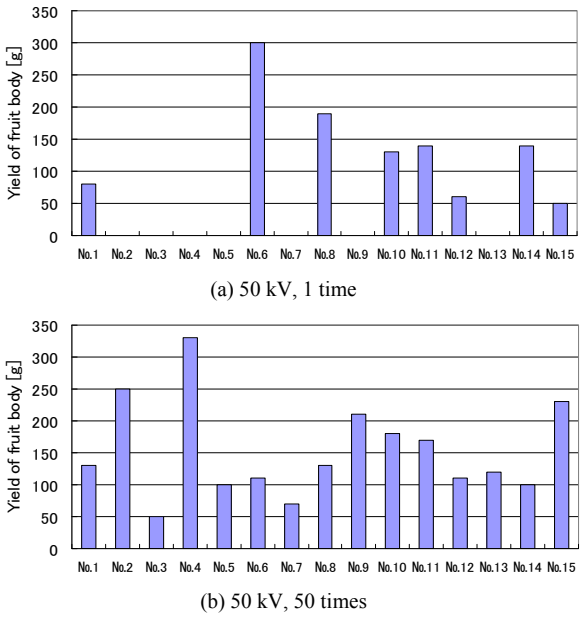



Fig. 4. Total weight of cultured *L. edodes* for various electrical stimulation conditions.




(a) 50 kV, 1 time

(b) 50 kV, 50 times

Fig. 5. Difference of yield of fruit body of *L.edodes* on the number of applied voltage.

Fig. 5 shows the weights of the *L. edodes* harvested from each log at two different pulse voltage stimulation values. The applied voltage was controlled to be 50 kV in all cases. The total weight from the logs after fifty times stimulations is 2.29 kg (= 153 g x 15) as shown in Fig. 4, which is larger than 1.09 kg (= 73 g x 15) harvested after single stimulation. The maximum value of the harvested fruit body from one log at single stimulation is 300 g, and is similar to 320 g obtained after fifty times stimulation. Although there were no logs observed without mushroom formation at fifty-time stimulation, after single stimulation no fruit bodies grew on seven logs. The average yield for one log is around 73 g (= 1090/15) after single stimulation. Only six logs show the larger yield than 73 g average value, whereas fourteen logs show yield larger than 73 g in case of 50 times stimulation. This result indicates the fact that on particular logs the pulse voltage decreases deviation in the mushroom formation.

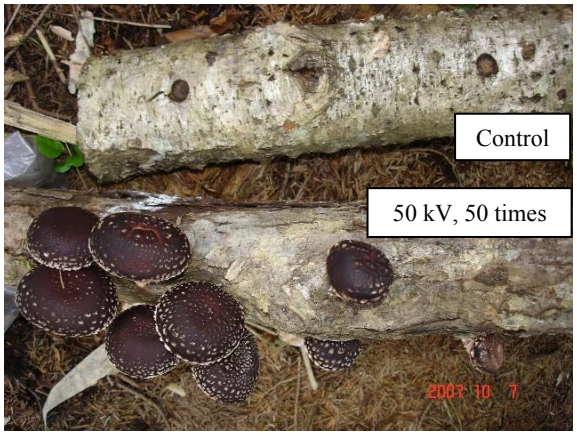



Fig. 6. Typical photographs of the cultured *L.edodes* with (lower side) and without (upper side) the electrical stimulation.

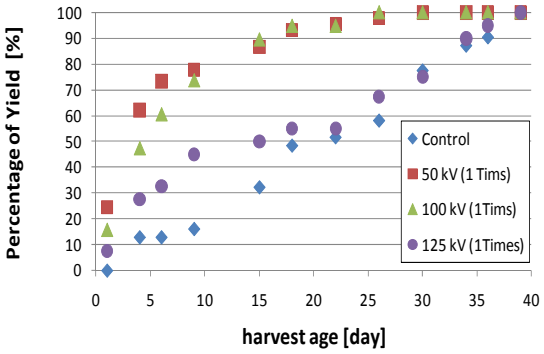



Fig. 7. History of total amount of harvested fruit body for various stimulation conditions.

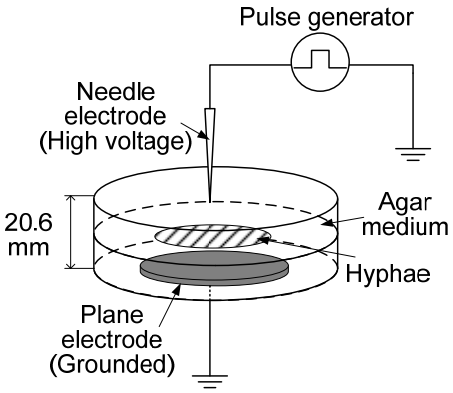



Fig. 8. Experimental setup about effect of pulse voltage stimulation on the hypha activity.

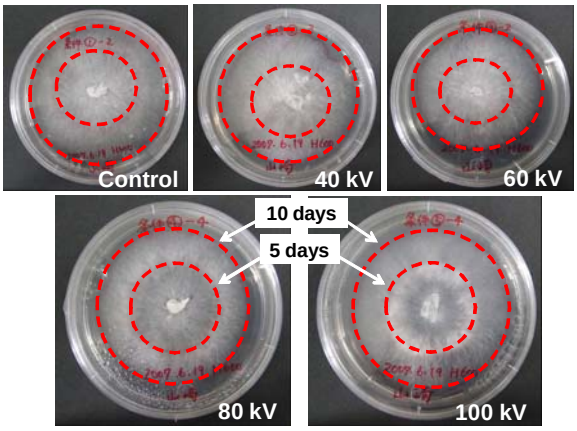



Fig. 9. Influence of pulse voltage stimulation on hypha growth in agar medium cultivation.

### *B. History of mushroom yield*

Fig. 6 shows the photographs of cultured *L. edodes* taken on the same day. The *L. edodes* in the stimulation group of 50 kV x 50 times grow faster than those in the control group. The high voltage electrode is located on the left side of the log. The fruit bodies mainly grow near the high voltage electrode. Fig. 7 shows the history of total amount of mushroom cultured under various stimulation conditions in spring, 2009. The all logs were checked every morning and the grown fruit bodies were harvested whole the harvesting season. The number and the weight of the fruit body harvested from the logs were measured during 40 days. The yield is normalized by total crop weight of one harvesting season and is evaluated as aggregate of all crops. The total crop weights are 60, 111, 90 and 89 g in the control group, 50 kV x 1, 100 kV x 1, 125 kV x 1 and 50 kV x 50 times groups, respectively, as shown in Fig. 4. The total output increases when applying voltage of 50 and 100 kV compared with that of the control group. The harvested weight during 15 days after the first crop is around 50% in the control group. However, the amount of the crop weight increases to 86% when applying voltage of 50 and 100 kV. This result indicates that the mushroom can be harvested in short days by applying pulse voltage stimulation.

### *C. Hypha activity*

Fig. 8 shows an experimental setup of the pulse voltage stimulation for an agar medium cultivation of *L. edodes* hypha. The *L. edodes* mushroom (Hokken No. 600) was used as a specimen. Fungi were inoculated in the center of the agar medium in Petri dishes 5 days before the electrical stimulation. Petri dishes dimension was 2 cm in depth and 10 cm in diameter. The needle electrode of around 1 mm diameter was located in the center of cover of the Petri dishes and subsequently the pulse voltages were applied by Blumlein-line type pulsed power generator [9] as an electrical stimulation. The applied voltage was 20-100 kV in amplitude and 100 ns in pulse width. The number of pulse stimulation was fixed to 100 times. Fig. 9 shows typical photographs after 10 days cultivation at various amplitudes of the applied voltage. From the microscopic observation, the growth direction of the hyphae changes to perpendicular to the surface of the agar medium from surface by applying high-voltage. The hypha activity was evaluated by amount of a hydrophobin release which was mainly observed before the fruit body formation [10, 11].

Fig. 10 shows a hydrophobin release from two different parts of the hypha for various days after 100 kV

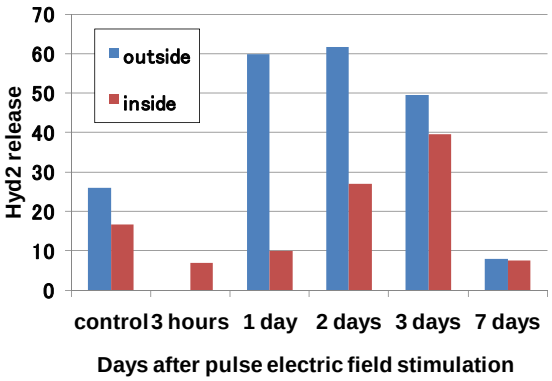



Fig. 10. Hydrophobin release for various days after 100 kV pulse high-voltage stimulation at two different parts of hypha.

pulse voltage stimulation. The pulse voltage is applied after five days cultivation of *L. edodes* hypha. The tip position of the hyphae after 5 days cultivation is marked by inner dotted circles shown in Fig. 9. The hydrophobin releases are analyzed at two parts; vegetative hyphae (outside of the inner dotted circles shown in Fig. 9) and inactive hyphae (inside of the inner dotted circles). The real time polymerase chain reaction technique is used to amplify the DNA of the hydrophobin Hyd2 and to measure Hyd2 release [10, 11] using a PCR Applied Biosystems 7500.

The hydrophobin release decreases during three hours after stimulation. However, the hydrophobin release from the vegetative hyphae increases to 2.3 times larger value on one day after the stimulation. The hydrophobin release decreases with time three days after the stimulation. The hydrophobin release from the inactive hypha has maximum point on the third day after the stimulation. This result indicates that the inactive hypha is also activated with the pulse voltage stimulation. The hyphae of the mushroom can be expressed as series connection of a capacitance and resistance components [12, 13]. The application of the pulse electric field generates the forces to the hyphae. Many parts of the hyphae are displaced by the force and some hyphae are sheared. This is candidate of triggering the increase of the hypha activity.

## IV. Conclusion

The pulsed high voltage was applied to logs for mushroom culturing to verify the effect of the pulse high-voltage stimulation on fruit body formation of *basidiomycete*. The high voltage short pulse was applied to natural logs of *L. edodes* as an electrical stimulation. The experimental results clearly show that the weight of the fruit body formed after the pulse voltage electrical stimulation is greater by 1.9 times in those without the stimulation. The deviation of the mushroom yield among the cultivation logs decreases when applying pulse voltage as stimulation for fruit body formation.

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