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A New Method to Sterilise Mushroom Substrate for Oyster Mushroom Cultivation

M.A.B. Sidik[1] --- Z. Buntat[2] --- M. Che Razali[3] --- Y. Buntat[4] --- Z. Nawawi[5] --- M.I. Jambak[6] --- I.R. Smith[7]

[1]*Institute of High Voltage and High Current, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, UTM Johor Bahru, Johor, Malaysia; Department of Electrical Engineering, Faculty of Engineering, Universitas Sriwijaya Indralaya, Organ Ilir, South Sumatra, Indonesia*
[2,3]*Institute of High Voltage and High Current, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, UTM Johor Bahru, Johor, Malaysia*
[4]*Faculty of Education, Universiti Teknologi Malaysia, UTM Johor Bahru, Johor, Malaysia*
[5,6]*Department of Electrical Engineering, Faculty of Engineering, Universitas Sriwijaya Indralaya, Organ Ilir, South Sumatra, Indonesia*
[7]*School of Electronic and Electrical Engineering, Loughborough University, Loughborough, Leicestershire, UK*

Abstract

One of the most important aspects in mushroom cultivation is substrate disinfection. The yield can be compromised if competitive microorganisms in the cellulosic materials are not killed. Steam sanitisation is usually used to disinfect the mushroom substrate. Large quantities of energy are required to generate the steam. The energy sources came from natural gas, diesel, electricity or firewood. The use of steam incurs large operational costs, and the process is time consuming. A more efficient ozone-based sterilisation technique needs to be developed to improve mushroom substrate sterilisation. Such a technique should be able to sterilise a large amount of substrate per day to enable growers to produce and sell more mushrooms, thereby increasing their revenue. This paper reports a new ozone-based sterilisation technique for mushroom substrate that is more effective in terms of production and time. This involves the injection of ozone into mushroom substrate with different concentration, and analysis to validate the use of ozone in the mushroom industry. The level of ozone and the treatment time needed to sterilise the entire volume of the substrate were analysed. The results revealed the optimum ozone concentration and the optimum time needed to sterilise the entire mushroom substrate. Ozone treatment is less time consuming compared to the conventional steam sterilisation technique. Thus, in the long term, it can increase the production of mushroom substrate and reduce costs.

Keywords: Ozone-based sterilisation, Mushroom substrate sterilisation, Mushroom industry.

1. Introduction

The market for mushrooms continues to grow due to the interest in their culinary, nutritional and health benefits (Vostrovsky and Jablonska, 2007). The production of mushrooms has increased in response to high customer demand and awareness on these health benefits (Aida, 2009). However, the government of Malaysia still has to import large quantities of mushrooms due to the inability of local mushroom growers to produce a sufficient amount to accommodate demand. Generally, mushrooms contain more protein, calcium, potassium, sodium, phosphorus, vitamins and a niacin than other vegetables (Jonathan, 2012) . They contain protein (30.4%), fat (2.2%), carbohydrate (57.6%), fibre (8.7%) and ash (9.8%) (Bhatti, 2007).

In Asian countries, mushrooms such as lingzhi (*Ganoderma lucidum)*, shiitake (Lentinusedodes), and yiner (*Tremella fuciformis*) are considered edible and medicinal resources (Wasser, 2002; Zhang, 2007). Oyster mushrooms (*Pleurotus* sp.) are one of the most highly valued mushrooms due to their significant nutritional value, very good taste and medicinal properties. Other varieties of mushroom found in the market are button, shiitake, abalone and

lingzhi. Mushrooms are considered a source of extraordinary power and virility and are used in the preparation of many continental dishes. They can be used treat a multitude of illnesses, such as migraine, headache, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, hypercholesterolemia, nephritis, dysmenorrhea, constipation, lupus erythematosis, hepatitis, leukopenia, cardiovascular problems and cancer, including leukaemia (Wasser, 2002; Cheung, 2012).

Oyster mushrooms are commonly cultivated by most local growers in Malaysia. Many countries, such as Africa, Asia and Latin America, are involved in the cultivation of oyster mushrooms (Kurtzman and Ralph, 2010). The production technology is relatively simple, and the materials used as substrate in oyster mushroom production are relatively cheap (Ficior, 2006). Oyster mushrooms show good adaptability to a variety of temperature ranges, making it possible to grow them almost all year round without the need for controlled climatic conditions (Ahmad, 2011; Chitamba, 2012). There are 40 species of oyster mushroom in the genus Pleurotus (Jayakumar, 2011). They can be cultivated on a wide range of cellulosic materials (Ficior, 2006).

Prior to spawning, the mushroom substrate has to undergo a disinfection process to prevent different pathogens (bacteria, moulds or pests) affecting the mushroom development and yield. Killing competitive fungi will permit faster, better and more uniform spawning and will assure better resistance to future infections (Ficior, 2006). Therefore, the mushroom yield will be higher and qualitatively superior. Several disinfection methods have been implemented, including pasteurisation (Royse, 2003). In pasteurisation, the substrate is pasteurised by passing an air-steam mixture through the substrate at 650° C for 1 hour and using electrical stimulation to reduce unwanted fungi (Takaki, 2007). The conventional method uses a steam oven (autoclave).

The steam oven inactivates all fungi, bacteria, viruses and bacteria spores in the mushroom substrate as shown in Figure 1. High-temperature steam must be constantly produced at around 121° C (250° F) or 0.5 bar at average cycle times of 6–8 hours to effectively sanitise the mushroom substrate and to ensure complete biological inactivation. Subsequently, the steamed substrate is allowed to cool down for approximately 6 hours before spawning. The total time consumed is about 12–14 hours. To complete the process, a large amount of heat must be generated, and large quantities of energy are needed. Energy produced from natural gas, diesel and electricity is essential for the mushroom substrate disinfection process. Rapid increases in energy costs and the time-consuming nature of the sanitisation process system have major implications for operating margins and costs of mushroom farms. This, in turn, has a dramatic impact on the ability of mushroom farms to fulfil the needs of their customers.

Fig-1. Conventional (oven)/autoclave treatment system

Ozone treatment have been widely used in industry to control odours, water and wastewater treatment (Omer and Walker, 2011). Interest in ozone applications for agriculture and food processing has increased in recent years. In 2001, ozone was declared as a GRAS (generally recognised as safe) substance by the FDA (Smilanick, 2003) .

Ozone is a highly reactive form of oxygen where three molecules are bonded together. One of the oxygen atoms has a weak bond and is readily available to transfer electrons. This allows for the transfer of electrons between the contaminant and the ozone molecule. Thus, when one atom of ozone detects a contaminant molecule, it breaks away from the other two atoms and attaches itself to the contaminant. When the single oxygen atom combines with the other organic substances, it results in a biocidal action and creates a microscopic explosion. This particular biocidal action is triggered by the reaction of ozone with the double bonds of fatty acids in bacterial cell walls, membranes and the protein capsid of viruses. In bacteria, the oxidation results in a change in cell permeability and leakage of cell contents into solution.

Ozone attacks these cell walls, breaking down membranes and the ultrastructural components of the organism (Bocci, 2009; Arvanitoyannis, 2012).

Fig-2. Ozone treatment system

As ozone treatment is less time consuming then the convention disinfection technique, it will be more cost effective in the long term. By using an ozone sterilising process, a greater amount of substrate (estimated as six times higher than with the conventional technique) can be sterilised per day. The time taken to kill the bacteria and fungi in the substrate is about half an hour or less, depending on the concentration of ozone, with less than 10 minutes of ozone settle-down time.

In the present study, the ozone treatment system shown in Figure 2 was introduced as a new method of sterilising mushroom substrate. The aim was to reduce the time taken to disinfect the mushroom substrate and to identify the optimum ozone concentrations that effectively kill the pathogens inside the substrate. Consequently, more mushrooms can be grown and cultivated.

2. Material and Methods

The study was carried out at the Institute of High Voltage and High Current, Faculty of Electrical Engineering, Universiti Teknologi Malaysia, Malaysia. A laboratory experiment was conducted using 10 bags of mushroom substrate to study the viability of ozone as a sterilising agent for mushroom substrate. The process flow of mushroom cultivation and the ozone sterilisation system are shown in Figure 3 and Figure 4, respectively.

The substrate was prepared using sawdust (93%), rice bran (5%) and gypsum (2%) on a dry condition, and the substrate was mixed thoroughly with water. The substrate was placed in polypropylene bags. About 800 g of medium was packed into each bag, and 10 bags of mushroom substrate were used as the samples for the study.

The experiment involved the sterilising process of mushroom substrate by injecting certain amount of ozone concentration. These processes were carried out to determine the optimum concentration of ozone applied to effectively kill all fungi, microbes, bacteria, viruses and bacteria spores in the substrate. This had been verified by monitoring the process of mycelium colonisation inside the substrate after inoculation of oyster mushroom spawn. Complete mycelium colonisation throughout the substrate indicated the successful of sterilizing process and the optimum ozone concentration could be determined.

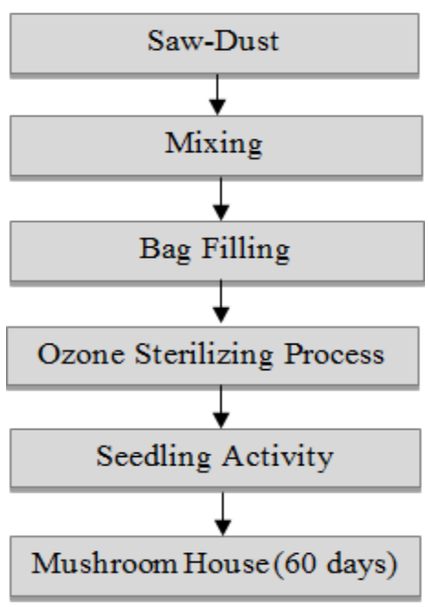

Fig-3. Flow chart of mushroom cultivation

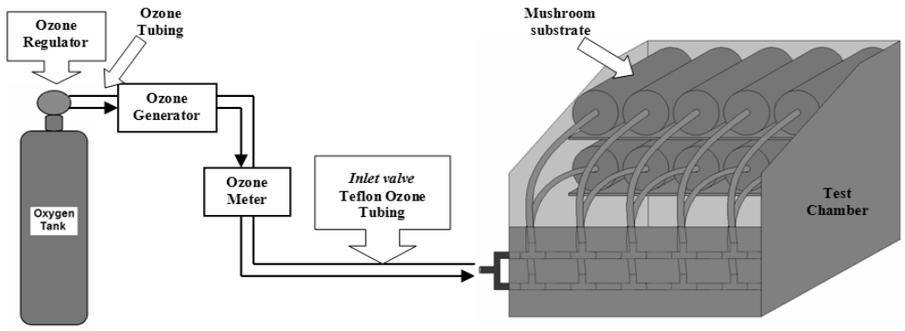

Fig-4. Ozone sterilising system

Figure 4 shows the ozone sterilising system used in the experiment. Ten samples of substrates filled in black PVC bags were injected with different concentrations of ozone at a concentration ranging from 50 ppm to 500 ppm for a period of 30 minutes. The treated sawdust substrate in the bags was then inoculated with approximately 2 g of oyster mushroom spawn and incubated in a dark chamber for 60 days at a temperature of 28–30° C until full colonisation by mycelium was attained. The growth of the mycelium (linear length) in each bag was measured with a measuring tape at 6-day intervals. Using these data, the spawn run rate (cm/day) was determined for every spawn type. When the mycelium fully covered the substrate bag (spawn run completed) and the completion of spawn running in the substrate bag was recorded. The bags were later transferred to the mushroom cropping room where they were opened to trigger fructification.

The optimum concentration of ozone was defined as the minimum concentration able to kill all the bacteria and fungi in the substrate within a specified time. The success of the ozone treatment at different concentrations was monitored and expressed in terms of the growth rate of the mycelium in each sawdust substrate. The treatment time and the ozone concentration were used to evaluate the effectiveness of the ozone treatment, and the optimum concentration and optimum treatment time were determined.

3. Results and Discussion

Figure 5(a) shows 10 samples of mushroom substrate treated with different ozone concentrations after inoculated with oyster mushroom spawn. Figure 5(b) shows the growth of mycelium in the 10 samples of mushroom substrate after 10 days. The results showed that the mushroom samples treated with 150 ppm ozone concentration and higher led to normal growth of mycelium in the substrate. This result indicates that an ozone concentration of 150 ppm or higher is sufficient to kill bacteria and microorganisms in mushroom substrate within a specified time (half an hour). However, at an ozone concentration of 100 ppm and lower, the samples showed no development of mycelium.

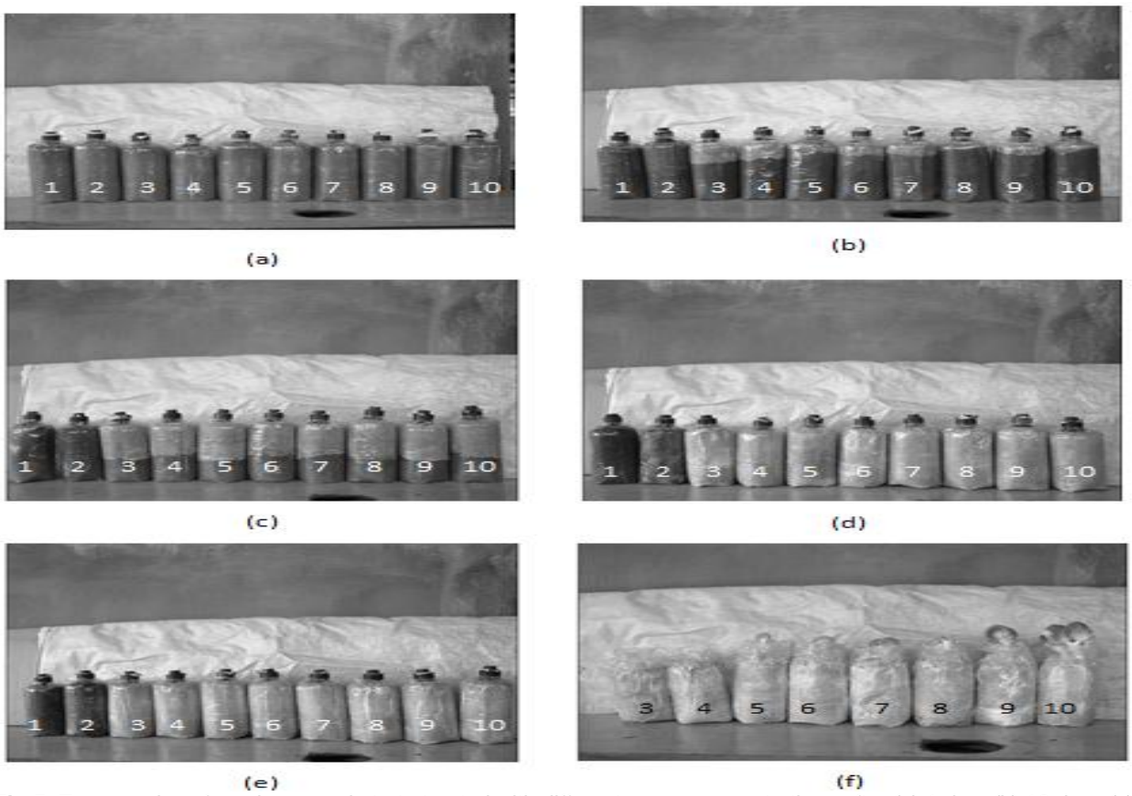

Fig-5. Ten samples of mushroom substrate treated with different ozone concentrations after (a) 1 day, (b) 10 days (c) 30 days, (d) 40 days, (e) 50 days and (f) 60 days, except Figure 5(f), which had only 8 samples. 1=50 ppm, 2=100 ppm, 3=150 ppm, 4=200 ppm, 5=250 ppm, 6=300 ppm, 7=350 ppm, 8=400 ppm, 9=450 ppm, 10=500 ppm.

Figure 5(c) shows that the substrate treated with the 150 ppm ozone concentration and higher after 30 days showed increased growth of mycelium. In contrast, the samples treated with an ozone concentration of 100 ppm and lower started to show bacterial and fungal growth. Figure 5(d) shows the mushroom samples treated with an ozone concentration of 150 ppm and higher after 40 days. As can be seen, the sample was characterised by increased growth of mycelium, which filled the entire volume of the substrate, indicating the success of the treatment. However, the sample treated with the ozone concentration of 100 ppm and lower continued to show increased fungal growth.

Figure 5(e) shows samples of mushroom substrate after 50 days. The results show that at this stage, the mushroom samples treated with the 150 ppm ozone concentration and higher started to produce mushroom roots and were therefore ready to produce mushrooms. However, the samples treated with the ozone concentration of 100 ppm and lower exhibited increased growth of fungi, and the substrate turned black and rotten. Lastly, Figure 5(f) shows the successful treatment of eight samples of mushroom substrate after 60 days. At this point, they started to produce mushrooms and were ready for harvesting after a few days. It can also be seen that the treatment with the higher concentration of ozone produced faster growth of mushrooms. The results are summarised in Table 1.

The findings of this study can be expected to benefit the mushroom-related industry by providing a new treatment method and reducing the treatment time, thereby yielding cost savings. The results point to the optimum ozone concentration level needed for treatment and the optimum treatment time. The treatment method described herein can be used to enhance existing treatment systems. The findings establish the benefits of ozone treatment and highlight its potential in large-scale mushroom cultivation.

Table-1. Characteristics of mushroom substrate following ozone treatment for different periods

Sample	**Ozone concentration (ppm)**	**Characteristics of the mushroom substrate (days)**					
		1	**10**	**30**	**40**	**50**	**60**
1	50	No progress	No progress	Growth of fungi	Increased growth of fungi	Became black and rotten	Totally damaged
2	100	No progress	No progress	Growth of fungi	Increased growth of fungi	Became black and rotten	Totally damaged
3	150	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	No sign of mushroom pinheads
4	200	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	No sign of mushroom pinheads
5	250	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	Sixth sample to produce mushroom pinheads
6	300	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	Fifth sample to produce mushroom pinheads
7	350	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	Fourth sample to produce mushroom pinheads
8	400	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	Third sample to produce mushroom pinheads
9	450	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	Second sample to produce mushroom pinheads
10	500	No progress	Normal mycelium growth	Increased mycelium growth	Bags show mycelium growth	Mycelium growth in entire substrate	First sample to produce mushroom pinheads

This cultivation method may further strengthen aspirations of commercial scale mushroom growers in Malaysia to become a major player in biotechnology frontier. This comparatively new method will give a much-needed boost to the mushroom industry worldwide. In adding, the outcomes of this project should have an impact on small and medium industry. Expected sectors that will gain mutual benefits from this project are the food, manufacturing, pharmaceutical and chemical industries. High value of mushrooms (i.e. ganoderma and shiitake that have anticancer properties) can also be produced using this method.

A very effective procedure is needed to kill the bacteria and microorganisms that accumulate inside the mushroom substrate, and complete penetration of ozone gas into the

whole volume of the substrate is vital. Care must be taken to ensure that the experimental environment is be suitable for the mushroom spawning process. Furthermore, during the spawning process, other factors need to be considered, such as ensuring that the operators who are conducting the experiment are bacteria free. This can be achieved by having the operators wash their hands with methylated spirit. The quality of the mushroom seeds is also important, as is the time taken to kill the bacteria. To ensure the success of the whole process, certain procedures must be strictly followed, and each step must be prepared carefully, especially during the seedling process.

The growth of mycelium in the whole volume of the substrate indicates the success of the ozone injection treatment and the elimination of bacteria. From the observations during the experiment, it can be seen that the mushroom seeds did not grow well in the substrates treated with the lower ozone treatment (50 ppm and 100 ppm). After a few days of the ozone treatment, fungi appeared at the top of the substrate, indicating the failure of the treatment and the existence of bacteria in the substrate. This can be clearly observed from the results depicted in Figures 5(a) to 5(e).

There are several factors that may result in treatment failure. The first is perhaps the concentration of ozone injected into the mushroom substrate, with the amount insufficient to kill all the bacteria in the substrate. Another factor may be an insufficient treatment time for the ozone to penetrate the whole volume of the substrate and to kill all the bacteria in the substrate. Thus, at lower ozone concentrations, a longer treatment time appears to be necessary.

As clearly seen in Figures 5(a) to 5(f), the injection of higher ozone concentrations (150 to 500 ppm) yielded very good results with respect to the growth of mycelium in the substrate. The results indicate that all the bacteria in the entire volume of the mushroom substrate were killed within 30 minutes of the ozone treatment, proving the effectiveness of the ozone treatment system.

4. Conclusion

The results of this study have implications for large-scale production of mushroom cultivation in general. Based on the experimental results, the injection of ozone as a treatment system for sterilising mushroom substrate is effective, less time consuming and more cost-effective than current methods. The results also reveal the optimum ozone concentration, the minimum time required for the treatment and the potential use of the system in large-scale mushroom cultivation.

Hopefully, these findings will spur innovation in the mushroom industry. The mushroom industry will be able to generate far better economic value using this method compared to conventional techniques. The success of the ozone treatment system could also lead financial institutions to disburse more loans to mushroom growers.

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