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Making mushroom production process a zero waste enterprise

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ABSTRACT

Mushroom production is an eco- friendly technology which utilizes and recycles agro wastes for the production of nutritive and medicinal mushrooms. Another significant aspect of mushroom cultivation is using the biota in creating a pollution-free environment. Indirectly, mushroom cultivation also provides opportunities for improving the sustainability of small farming systems through the recycling of organic matter, which can be used as a growing substrate. The aim of the present investigation was to make mushroom technology a zero waste technology through the utilization of the wastes generated during mushroom spawn production process for biogas production and the spent mushroom substrate (SMS) generated during mushroom cultivation process for the on farm multiplication of the bioagent *Trichoderma harzianum* which is extensively used in Biological control of many plant diseases. Thus, this study helps to make mushroom technology as one of the most befitting green technologies for effective agrowaste management and a zero waste enterprise.

Key words: Biogas, SMS, Spawn, Trichoderma harzianum, Zero waste.

1. Introduction

Mushroom cultivation is a relatively new horticultural enterprise gaining importance in India. India produces about 40,600 tonnes of mushrooms per annum. An equal amount of spent mushroom substrate (SMS) is generated as waste by mushroom farms. Huge amounts of wastes in the form of starchy water, over boiled grains and contaminated spawn are produced by the mushroom spawn production labs and units. The present study was undertaken to utilize the wastes generated during mushroom spawn (seed) production for biogas production and the wastes generated during mushroom cultivation process for the multiplication of the bioagent *Trichoderma harzianum.*

Mushroom cultivation process essentially comprises of two aspects.

1. Mushroom spawn (seed) production
2. Mushroom cultivation

1.1 Spawn production

Mushroom spawn is produced by the inoculation of the desired fungus on sterile boiled sorghum (*Sorghum vulgare*) grains. The wastes produced during spawn production are

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1. Starchy water after grain boiling
2. The over boiled grains sticking to the machinery used in spawn production
3. Contaminated spawn

1.2 Mushroom cultivation

Mushroom cultivation involves the inoculation of sterilized agro wastes (paddy straw or other wastes) with the spawn of the desired mushroom fungus. The wastes produced during mushroom cultivation process are

1. Substrate (straw) left after harvest of mushrooms which is called as spent mushroom substrate (SMS)
2. Small stubs, stalks of mushrooms after harvest
3. Deformed mushrooms

2. Materials and methods

2.1 Recycling of wastes produced during spawn production

Experiment was conducted to study the utilization of wastes like starchy water, over boiled grains and contaminated spawn as feed material in Shakthi Surabhi biogas unit procured from Vivekananda Kendra, Kanyakumari. The biogas unit was initially activated by adding cow dung and water mixture in 1:1 ratio. Biogas production started after one month of the initiation process. The efficacy of various spawn production wastes for biogas production was studied by feeding various wastes and waste mixtures (5 kg per feed). The production of biogas was measured daily by recording the height of the drum in which the biogas was collected and the volume of biogas was expressed as volume of the cylinder depending on the increase in average height of the cylinder in one month period. The mixture of wastes used were

1. 5 litres of starchy water
2. Starchy water mixed with waste over boiled grains and chalk powder laden water (after machine wash), 5litres
3. Mixture of contaminated spawn (1 kg), starchy water (3 litres) and chalk powder (1 kg)
4. Mixture of contaminated spawn and chalk powder (5 kg).

3. Recycling of wastes produced during cultivation (SMS)

SMS produced after the cultivation of oyster mushroom, {*Pleurotus florida* (OSMS)} and milky mushroom, {*Calocybe indica* (CSMS)} were utilized for multiplication of the bioagent *Trichoderma harzianum*. The pure culture of *Trichoderma harzianum* was maintained on malt extract agar medium. The initial inoculum of the bioagent was multiplied on boiled sorghum grains. The spent mushroom substrate (SMS) of oyster (OSMS) and milky mushrooms (CSMS) after complete cropping was used as the carrier material for the multiplication of the bioagent *Trichoderma harzianum*. Neem cake as carrier material was used as control. The various treatment combinations used were pasteurized (steam pasteurization at 80°C for 120 minutes) and non-pasteurized Neem cake (NC), paddy straw

(PS), oyster mushroom spent substrate (OSMS), OSMS+25, 50 and 75% NC, milky mushroom substrate (CSMS), CSMS+ 25, 50 and 75% NC. The experiment was performed in small polypropylene bags (100 x 50 mm). 2g of *Trichoderma harzianum* inoculum grown on boiled sorghum grains (15 days old inoculum) was inoculated in each treatment of SMS combination. The bags were plugged with non absorbent cotton using PVC rings (25 mm internal diameter, 2 mm wall thick, 10 mm height) as neck. The bags were incubated at 26±2°C. Spore count and colony forming units (cfu) were recorded at an interval of 10 days for 40 days. Spore count was done by the standard haemocytometer technique. Colony forming units were counted by plating 1 ml of known spore load suspension on Malt extract agar medium in Petri plates (90mm diameter). The number of viable spores forming colonies was counted. Standard statistical analysis was done by natural log conversion and CD.

4. Results and discussion

Table-1 shows that all types of wastes generated during mushroom spawn production could be used as feed for the production of biogas. A pH of 6.5-7.0 was optimal for maximum biogas production. Among the various treatments, the cow dung water mixture produced the least biogas (1028.22 m^3) and the maximum biogas (figure 1) was produced by using mixture of contaminated spawn (5 kg) + chalk powder (8518.44 m^3). The biogas so produced was utilized in the mushroom Research lab for preparation of media and other related experimental work, thus saving Electrical energy. Shakti Surabhi is a Biomethanation plant developed by Vivekananda Kendra – Natural resources Development Project (nardep) for producing biogas from kitchen and vegetable waste. It is an improvement over the general floating drum type biogas plant. Due to its user friendly design, it is suitable for both Rural and Urban areas. It is easy to handle, very good for individual households and can be kept on terrace too. Easily available feed materials like leftover cooked food (Rice, chapatti, Dal, Subji etc.,), non-Veg. food wastes (Fish and Meat etc.,) vegetable wastes (waste from vegetable markets) waste material from flour mill (Wheat, Rice, Tapioca, Maize, etc.,) Non edible oil seed cakes (Neem, Jatropa, Rubber etc.,) can be used (www.vknardep.org). Utilization of mushroom waste was successfully experimented for the first time.

Table 1: Utilization of mushroom spawn waste for biogas production

Type of feed	pH	Biogas generated (m^3)
Standard feed - Cowdung + water (1:1)	8.5-9.0	1028.22
starchy water after grain boiling (5 lit)	5.9	5848.24
starchy water + waste machine wash =(5 lit)	6.2	6013.37
Contaminated spawn (1kg) + starchy water (4 lit)+chalk powder	7.8	5134.74
Contaminated spawn (5kg) with chalk powder	6.5 - 7	8518.44

The pasteurized SMS could support the growth and sporulation of *Trichoderma harzianum* (figure 2). Non – pasteurized SMS led to poor growth and contaminations. Some of the non pasteurized bags (oyster mushroom) showed sporophores too. Table-2 shows the spore count

of *T. harzianum* on various SMS combinations. The initial spore count on paddy straw (PS), Oyster mushroom spent substrate (OSMS) and OSMS + 25% NC, Milky mushroom spent substrate (CSMS) and CSMS + 25 and 50% NC was lower as compared to NC alone. The initial spore count on OSMS + 50% NC ($4.16x10^{7}$) and OSMS + 75% NC ($4.41x10^{7)}$ was similar to the spore count on NC alone ($4.38\ x10^{7)}$.

The spore count on Milky mushroom spent substrate (CSMS) was lower on CSMS alone or in combination with 25 and 50% NC combination. However on CSMS + 75% NC combination the spore count was similar ($4.26\ x10^{7)}$ as on NC. The spore count on all substrate combinations decreased with time. The spore count on paddy straw alone (PS) decreased by 28.18% by 40th day after inoculation as compared to 13.24% on Neem cake (NC) alone, 23.04% on oyster mushroom spent substrate (OSMS) and 22.88% on Milky mushroom spent substrate (CSMS). The decrease in spore count by 40th day on OSMS + 25% NC was 16.21%, 13.70% on OSMS + 50% NC and 18.59% on OSMS + 75% NC. Similarly, there was a decrease of spore count by 40th day on CSMS + 25% NC (24.33%), CSMS + 50% NC (28.08%) and CSMS + 75% NC (15.25%) respectively.

Table 2: Spore count of *T. harzianum* on spent mushroom substrate (SMS)

Substrate	Spore count/g of spent substrate			
	10 DAI	**20 DAI**	**30 DAI**	**40 DAI**
NC	4.38 x 107 (17.572)*	4.19 x 107 (17.551)	4.08 x 107 (17.525)	3.8 x 107 (17.453)
PS	2.2 x 107 (16.902)	2.06 x 107 (16.838)	1.95 x 107 (16.787)	1.58 x 107 (16.581)
OSMS	2.82 x 107 (17.156)	2.63 x 107 (17.088)	2.35 x 107 (16.972)	2.17 x 107 (16.895)
CSMS	2.84 x 107 (17.162)	2.57 x 107 (17.064)	2.38 x 107 (16.987)	2.19 x 107 (16.902)
OSMS+25% NC	2.59 x 107 (17.065)	2.52 x 107 (17.045)	2.27 x 107 (16.940)	2.17 x 107 (16.894)
OSMS+ 50% NC	4.16 x 107 (17.544)	4.07 x 107 (17.523)	3.77 x 107 (17.447)	3.59 x 107 (17.398)
OSMS+ 75% NC	4.41 x 107 (17.603)	4.14 x 107 (17.540)	3.87 x 107 (17.472)	3.59 x 107 (17.396)
CSMS+ 25% NC	2.63 x 107 (17.085)	2.43 x 107 (17.006)	2.19 x 107 (16.903)	1.99 x 107 (16.809)
CSMS+ 50% NC	2.92 x 107 (17.188)	2.52 x 107 (17.043)	2.62 x 107 (17.054)	2.1 x 107 (16.859)
CSMS+ 75% NC	4.26 x 107 (17.563)	4.22 x 107 (17.558)	4.01 x 107 (17.508)	3.61 x 107 (17.403)
Interaction CD AT 1% = 0 .0122 Interaction CD at 5% = 9.245 *Figures in parenthesis are natural log transformed				

The number of colony forming units (cfu) is a measure of the viability of the *Trichoderma* spores, hence a very important deciding factor for the effectiveness of the bioagent in controlling the disease. Cfu decides the colonization ability of the bioagent in the soil and its effectiveness to control the targeted pathogens. As seen from ~~figure 2~~ table 3, there was a general decrease (10 times) of colony forming units (cfu) on all combinations as compared to spore count. The cfu value decreased with increase in time. At the end of 40th day, the cfu value on OSMS and its 25, 50 and 75% combination with Neem cake was $1.94\ x10^{6}$**,** 1.83

$x10^6$, 3.47 $x10^6$ and 3.47 $x10^6$ per gram substrate as compared to 3.61 $x10^6$ in Neem cake alone. In OSMS the cfu value became constant after 30th day. In CSMS the cfu varied as 1.94 $x10^6$, 1.92 $x10^6$, 1.96 $x10^6$ and 3.54 $x10^6$ respectively. The cfu value on paddy straw was very poor. However the cfu on NC alone and other substrate combinations was similar. The cfu count on various combinations of OSMS and CSMS was almost equivalent to the recommended dose of $2x10^7$ found in the commercial formulations.

Table 3: Colony forming units (CFU) of *T. Harzianum* on spent mushroom substrate

Substrate	Colony forming units (cfu)/g of substrate			
	10 DAI	**20 DAI**	**30 DAI**	**40 DAI**
NC	4.0 x 10^6	3.9 x 10^6	3.8 x 10^6	3.6 x 10^6
PS	2.0 x 10^6	1.8 x 10^6	1.8 x 10^6	1.5 x 10^6
OSMS	2.8 x 10^6	2.5 x 10^6	2.3 x 10^6	1.9 x 10^6
CSMS	2.6 x 10^6	2.5 x 10^6	2.4 x 10^6	1.9 x 10^6
OSMS+25% NC	2.5 x 10^6	2.3 x 10^6	2.1 x 10^6	1.8 x 10^6
OSMS+ 50% NC	4.2 x 10^6	4.0 x 10^6	3.7 x 10^6	3.5 x 10^6
OSMS+ 75% NC	4.5 x 10^6	4.0 x 10^6	3.9 x 10^6	3.5 x 10^6
CSMS+ 25% NC	2.5 x 10^6	2.2 x 10^6	1.9 x 10^6	1.9 x 10^6
CSMS+ 50% NC	2.9 x 10^6	2.5 x 10^6	2.3 x 10^6	1.9 x 10^6
CSMS+ 75% NC	4.6 x 10^6	4.2 x 10^6	3.9 x 10^6	3.5 x 10^6
CD at 1% = 3.8642 5% = 2.9236				

Figure 1: Biogas production using mushroom spawn waste

Figure 2: *Trichoderma harzianum* multiplication on SMS

The SMS left over after mushroom harvest contains enough digestible nutrition which can be used in various profitable ways like animal feed (Kakkar and Dhanda 1998, Zadrazil 1977 and 1984), organic manure for soil conditioning (Abdallah et al 2000, Batista et al 2000, Nguyen et al 1987, FAO bulletin), bioremediation (Chiu et al 1998, Eggen 1999, Martiriani et 1996), Pest management (Hibbett and Thorn 1994), enzyme production (Tan and Wahab 1997), reuse for mushroom cultivation (Kim et al 1998) etc which help the farmer in earning extra profit. Demand for organic cultivation of fruits and vegetables are on the rise. One aspect of organic farming is the use of biological control agents like *Trichoderma harzianum* for the control of diseases as against chemicals. The utilization of Mushroom SMS for the multiplication of the *Trichoderma harzianum* is an additional advantage resulting through the second recycling of the same waste which was earlier used for mushroom production.

5. Conclusion

Mushroom production is one of the most eco-friendly methods of recycling agro wastes. The present studies show that the wastes which are generated during mushroom spawn production and mushroom cultivation can be further utilized for the production of biogas and as base material for the mass multiplication of *Trichoderma harzianum*. The utilization of these wastes is an additional advantage resulting in biogas production which can save electrical energy especially in rural areas and the second recycling of the agro waste in the form of SMS can help in the production of the bioagent *Trichoderma harzianum* thus leading to non chemical method of plant disease control. Thus the process of mushroom production starts with the utilization of agro wastes for mushroom cultivation, utilizes the wastes produced during the process for bioagent multiplication and energy production and no waste is produced in the process. Hence mushroom production has the potential to become a technology of total recycling with zero waste production.

List of symbols and abbreviations

NC = Neem cake

PS = Paddy straw

OSMS = *Pleurotus florida* (oyster mushroom) spent mushroom substrate

CSMS = *Calocybe indica* (milky mushroom) spent mushroom substrate

DAI = Days after inoculation

SMS = Spent mushroom substrate

cfu = Colony forming units

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