Scientific Bulletin, Series F, Biotechnologies, Vol. XVI, 2012
ISSN Online 2285-5521, ISSN-L 2285-1364

THE SEMI-SOLID STATE CULTIVATION OF EDIBLE MUSHROOMS ON AGRICULTURAL ORGANIC WASTES

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Abstract

The main aim of this work was focused on testing new practical procedures in order to optimize the efficiency of edible mushroom cultivating by enhancing their fruit body formation during the semi-solid state cultivation on winery and fruit processing wastes. In this respect, the culture media for fungal growing were mainly prepared from winery and apple wastes mixed with relative small amounts of wheat and rye bran (15-30% w/w) as well as germinated barley seeds (3-5% w/w). After their steam sterilization at 121°C, 1.1 atm., for 15 min. they were transferred aseptically inside of 250 ml work volume flasks. These culture media were aseptically inoculated by using 10-20 ml suspension of fungal pellets collected from submerged cultures of the pure strains of Lentinula edodes (Berkeley) Pegler and Pleurotus ostreatus (Jacquin ex Fries) Kummer. After inoculation with mushroom pellets, the semi-solid cultivation was set up for each one of the tested mushroom species following the main parameters of culture media: constant temperature 23°C, pH level 5.7–6.0 units, relative humidity level of culture media 30-50%. During a period of semi-solid cultivation lasting between 30-50 days, the fungal mycelia were developed inside the culture media in the first 10-20 days and after that during the following 20-30 days the first mushroom primordia were emerged and developed the mushroom fruit bodies. In this final stage, the final mushroom fruit bodies were harvested and weighted, all results showing the percentage of 40-50% relative to the whole weight of culture media. Poplar, beech and birch sawdust were used as control samples for the tested culture media.

Keywords: *edible mushrooms, semi-solid state cultivation, apple and winery wastes*

INTRODUCTION

The agricultural works as well as the industrial activities related to grape and apple processing have generally been matched by a huge formation of wide range of cellulosic wastes that cause serious environmental pollution effects if they are allowed to accumulate in the environment or much worse they are burned on the soil [1, 2].
The solid substrate fermentation of plant wastes from agro-food industry is one of the challenging and technically demanding biotechnology that is known so far [3-5].
The major group of fungi which are able to degrade lignocellulose is represented by the edible mushrooms of Basidiomycetes Class [6-9].
The main aim of this work was to find out the best biotechnology of recycling the winery and apple wastes by using them as a growing source for edible mushrooms and, last but not least, to protect the environment [9-12].
Taking into consideration that most of the edible mushrooms species requires a specific micro-environment including complex nutrients, the influence of physical and chemical factors upon fungal biomass production and mushroom fruit bodies formation were studied by testing new biotechnological procedures [7-9].

MATERIAL AND METHOD

According to the main purposes of this work, two fungal species of Basidiomycetes group, namely *Lentinula edodes* (Berkeley) Pegler (folk name: Shiitake) as well as *Pleurotus ostreatus* (Jacquin ex Fries) Kummer (folk name: Oyster Mushroom) were used as pure

mushroom cultures isolated from the natural environment and now being preserved in the local collection of the University of Pitesti.

The stock cultures were maintained on malt-extract agar (MEA) slants (20% malt extract, 2% yeast extract, 20% agar-agar). Slants were incubated at 25°C for 120-168 h and stored at 4°C. The pure mushroom cultures were expanded by growing in 250-ml flasks containing 100 ml of liquid malt-extract medium at 23°C on rotary shaker incubators at 110 rev. min $^{-1}$ for 72-120 h. To prepare the inoculum for the spawn cultures of *L. edodes* and *P. ostreatus* the pure mushroom cultures were inoculated into 100 ml of liquid malt-yeast extract culture medium with 3-5% (v/v) and then maintained at 23-25°C in 250 ml rotary shake flasks.

After 10–12 d of incubation the fungal cultures were inoculated aseptically into glass vessels containing sterilized liquid culture media in order to produce the spawn necessary for the inoculation of 10 kg plastic bags filled with compost made of winery and apple wastes.

These compost variants were mixed with other needed natural ingredients in order to improve the enzymatic activity of mushroom mycelia and convert the cellulose content of winery and apple wastes into protein biomass. The best compositions of five compost variants are presented in Table 1.

Table 1. The composition of five compost variants used in mushroom culture cycles

Compost variants	Compost composition
S1	Winery and apple wastes (1:1)
S2	Winery wastes + wheat bran (9:1)
S3	Winery wastes and rye bran (9:1)
S4	Apple wastes and wheat bran (9:1)
S5	Apple wastes + rye bran (9:1)
Control	Poplar, beech and birch sawdust (1:1:1)

In this way, the whole bags filled with compost were steam sterilized at 121^{o}C, 1.1 atm., for 30 min. In the next stage, all the sterilized bags were inoculated with liquid mycelia, and then, all inoculated bags were transferred into the growing chambers for incubation. After a time period of 10-15 d, on the surface of sterilized plastic bags filled with compost, the first buttons of mushroom fruit bodies emerged. During a period of 20-30 d there were harvested between 1.5–3.5 kg of mushroom fruit bodies per 10 kg compost of one bag [10-14].

RESULTS AND DISCUSSIONS

To increase the specific processes of winery and apple wastes bioconversion into protein of fungal biomass, there were performed experiments to grow the mushroom species of *P. ostreatus* and *L. edodes* on the previous mentioned variants of culture substrata (see Table 1). During the mushroom growing cycles the specific rates of cellulose biodegradation were determined using the direct method of biomass weighing the results being expressed as percentage of dry weight (d.w.) before and after their cultivation [14, 15]. The registered data are presented in Table 2 and Table 3.

Table 2. The rate of cellulose degradation during the growing cycle of *P. ostreatus*

Variants of culture substrata	Before cultivation (g% d.w.)	After cultivation (g% d.w.)
S1	2,7-2,9	0,9
S2	2,5-2,8	0,7
S3	2,3-2,5	0,4
S4	2,5 -2,7	0,8
S5	2,5-2,7	0,7
(Control	3,0	1,5

Table 3. The rate of cellulose degradation during the growing cycle of *L. edodes*

Variants of culture substrata	Before cultivation (g% d.w.)	After cultivation (g% d.w.)
S1	2,6-2,7	0,5
S2	2,3-2,5	0,4
S3	2,3-2,5	0,5
S4	2,5 -2,7	0,7
S5	2,7-2,9	0,5
Control	3,0	1,4

The registered data revealed that by applying this biotechnology, the winery and apple wastes could be recycled as useful raw materials for mushroom compost preparation in order to get significant mushroom production.

In this respect, the final fruit body production of the cultivation of these two mushroom species was registered as being between 20–28 kg relative to 100 kg of composts made of apple and winery wastes. In order to determine the evolution of the total nitrogen content in the

fungal biomass there were collected samples at precise time intervals of 50 h and they were analyzed by using Kjeldahl method. The registered results concerning the evolution of total nitrogen content in *P. ostreatus* biomass are presented in figure 1 and the data regarding *L. edodes* biomass could be seen in figure 2.

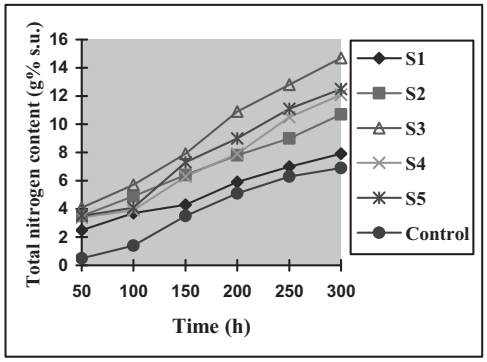

Fig. 1. The evolution of total nitrogen content in *P. ostreatus* biomass

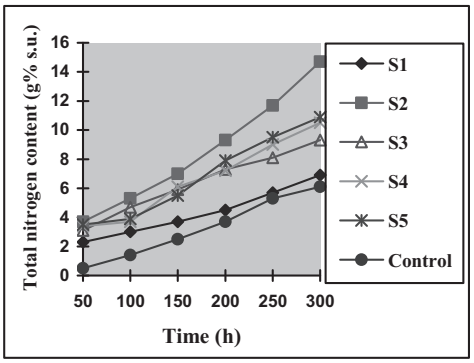

Fig. 2. The evolution of total nitrogen content in *L. edodes* biomass

According to the registered results of the performed experiments the optimal laboratory-scale biotechnology for edible mushroom cultivation on composts made of marc of grapes and apples was established.

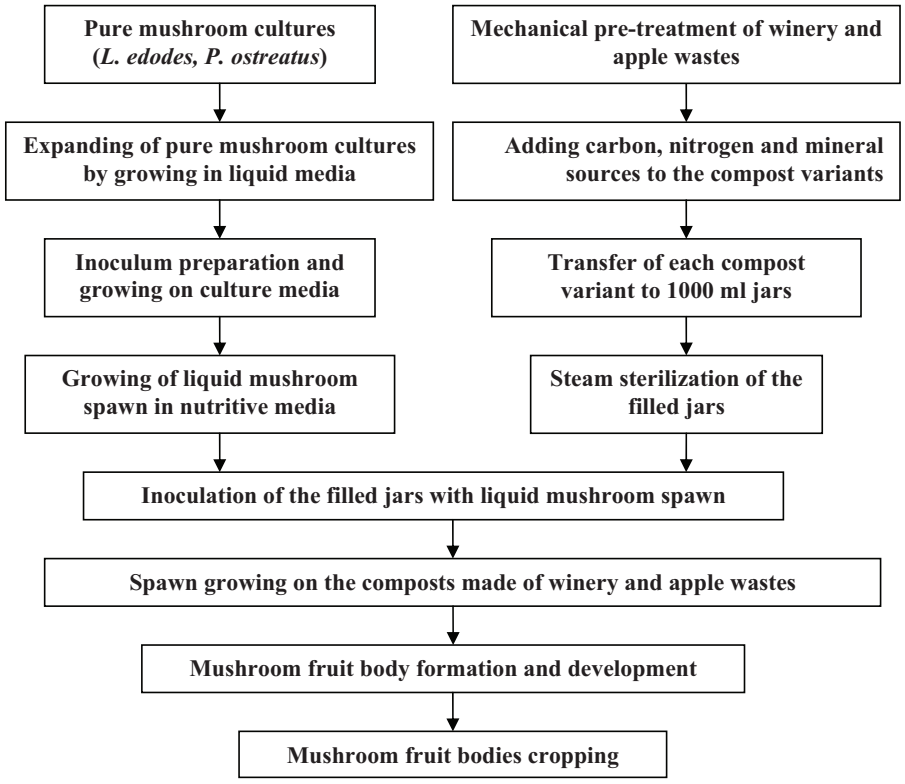

Fig. 3. Scheme of laboratory-scale biotechnology for edible mushroom production by recycling winery and apple wastes

As it is shown in figure 3, two technological flows were carried out simultaneously until the first common stages of the inoculation of composts with liquid mushroom spawn followed by the mushroom fruit body formation.
The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30–60 d, depending on each fungal species used in experiments.
During the whole period of fruit body formation, the culture parameters were set up and maintained at the following levels, depending on each mushroom species: air temperature, 15–170C; the air flow volume, 5–6m3/h; air flow speed, 0.2–0.3 m/s; the relative moisture content, 80–85%, light intensity, 500–1,000 luces for 8–10 h/d. The final fruit body production of these mushroom species used in experiments was registered between 1.5 – 2.8 kg relative to 10 kg of composts made of winery and apple wastes.

CONCLUSIONS

1. The registered data revealed that by applying this biotechnology, the winery and apple wastes could be recycled as useful raw materials for culture compost preparation to get edible mushrooms
2. By applying this biotechnology, the winery and apple wastes could be recycled as useful raw materials for mushroom compost preparation in order to get significant mushroom fruit body production and protect the natural environment surrounding apple juice factories as well as wine making industrial plants.
3. The fruit body productions of these two mushroom species were registered as being between 20–28 kg relative to 100 kg of composts made of vineyard and apple wastes.

ACKNOWLEDGEMENTS

This work was carried out in the framework of National Research Plan PN II, the 4th Program - “Partnership in priority domains”, through the contract no. 51-002/2007, granted by The Romanian Ministry of Education and Research.

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