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BIOTECHNOLOGY FOR CONTROLLED CULTIVATION OF EDIBLE MUSHROOMS THROUGH SUBMERGED FERMENTATION OF FRUIT WASTES

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Abstract

As a result of our recent scientific studies, the biotechnological controlled cultivation of edible mushrooms was tested through the submerged fermentation of different fruit wastes from organic horticulture that provided a fast growth as well as high biomass productivity of investigated strains in comparison with the sample. The research works were carried out by using the pure cultures of two edible mushroom species, namely Lentinulaedodes (Shiitake) and Pleurotusostreatus (Oyster Mushroom), which are well known as biological sources of proteins, carbohydrates and mineral elements with beneficial effects on human nutrition and health. All culture media used in experiments were prepared from different sorts of organic fruit wastes such as juice and pulps, resulted from the industrial processing of apples, pears and plums. The submerged fermentation was carried out inside the culture vessel of an automatic laboratory-scale bioreactor, all main cultivation parameters being set up at the following values: temperature, 23-23.5°C; agitation speed, 90-100 rev. min^{-1}; pH level, 5.5-6.7 units; dissolved oxygen tension within the range of 50-70%. During the cultivating cycles through submerged fermentation, lasting between 120-140 h, the mushroom biomass developed inside the culture media as fresh mycelia pellets. All registered results of these experiments were used to set up a laboratory-scale biotechnology for producing mycelia biomass of edible mushrooms in order to be used as raw biomass of natural fertilizer producing.

Key words: *biotechnology, edible mushrooms, fruit wastes, mycelia biomass, submerged fermentation.*

INTRODUCTION

The agricultural works as well as the industrial activities related to fruit processing have generally been matched by a huge formation of wide range of waste products. Submerged cultivation in liquid media of mushroom mycelium is a promising method which can be used in novel biotechnological processes for obtaining natural fertilizers from the mushroom biomass that could be grown using fruit wastes as culture substrates (Beguin and Aubert, 1994; Moser, 1994; Carlile and Watkinson, 1996).

Many researches for getting nutritive fertilizers from the biomass of *Lentinulaedodes* (Shiitake) and*Pleurotusostreatus* (Oyster Mushroom) specieshave shown that the nutritive value of edible mushrooms is own to the huge protein content, carbohydrates and mineral salts (Raaska, 1990; Chahal and Hachey, 1990; Verstraete and Top, 1992).

The main purpose of this work consists in testing the biotechnological controlled cultivation of edible mushrooms through submerged fermentation of different fruit wastes from organic horticulture that provided a fast growth as well as high biomass productivity of investigated strains in comparison with the pure cellulose used as sample.

MATERIALS AND METHODS

According to the main purpose of this work, two fungal species from Basidiomycetes, namely *Lentinulaedodes* (Berkeley) Pegler and *Pleurotusostreatus* (Jacquin ex Fries) Kummer were used as pure cultures in all carried out experiments.

The stock cultures were maintained on malt-extract agar (MEA) slants. Slants were incubated at 25° C for 5-7 d and then stored at 4° C. The mushroom cultures were grown in

250-ml flasks containing 100 ml of MEA medium (20% malt extract, 2% yeast extract, 20% agar-agar) at 23°C on rotary shaker incubators at 110 rev min^{-1} for 5-7 d (Ropars et al., 1992; Wainwright, 1992; Smith, 1998).
The mushroom culture media were prepared from different sorts of organic fruit wastes such as juice and pulps, resulted from the industrial processing of apples, pears and plums.
These compost variants were made by mixing apple, pear and plum wastes resulted from alcohol distillation with other needed natural ingredients, such as, barley and wheat bran, in small amounts (1.5-3% w/w), in order to improve the enzymatic activity of mushroom mycelia and convert the cellulose content of these fruit wastes into protein biomass.
The best composition of the five compost variants is presented in Table 1.

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

Variants of culture substrata	Substrate composition ratio
S1	Apple wastes and barley1.5 %
S2	Mixture of apple wastes and wheat bran 2%
S3	Mixture of pear wastes and barley bran 1.5%
S4	Mixture of plum wastes and barley bran 2.5%
S5	Mixture of apple wastes and wheat bran 3 %
Control	Pure cellulose

The mushroom cultures used in experiments were prepared by inoculating 100 ml of culture medium with 3-5% (v/v) of the seed culture and then cultivated at 23-25°C in rotary shake flasks of 250 ml.
The experiments were conducted under the following constant conditions of incubation: temperature, 25°C; agitation speed, 120 rev min^{-1}; initial pH 5.5 (Petre and Petre, 2013).
After 7-10 d of incubation, the mushroom cultures were ready to be inoculated aseptically into the glass vessel of laboratory-scale bioreactor (Figure 1).For the fungal growing in this bioreactor special culture medium was prepared by using liquid nutritive broth, having the following composition: 35% fruit wastes, 1-3% barley or wheat bran, 0.3% powder of natural argillaceous materials (Figure 1).

Figure 1. Laboratoy-scale bioreactor for submerged cultivation of edible mushrooms

After the steam sterilization at 121°C, 1.1 atm., for 15 min. the nutritive broth was transferred aseptically inside the culture vessel of a laboratory scale bioreactor. This culture medium was aseptically inoculated with 30 h activated spores of *P. ostreatus* and *L.edodes* species in a glucose liquid solution 1%.
After inoculation into the bioreactor vessel, the submerged fermentation was set up at the following parameters: constant temperature, 23°C; agitation speed, 80-100 rev. min^{-1}; pH level, 5.7-6.0 units; dissolved oxygen tension within the range of 30-70%. After a period of submerged fermentation lasting up to 120 h, small fungal pellets were developed inside the broth.
The experimental model of biotechnological installation, represented by the laboratory scale bioreactor (Figure 1), was designed to be used in submerged cultivation of the mentioned edible mushrooms on substrata made of wastes resulted from the industrial processing of fruits for nutritive fungal biomass production (Petre et al., 2012; Stamets, 1993).

RESULTS AND DISCUSSIONS

In order to increase the specific processes of cellulose biodegradation of fruit wastes and finally induce its bioconversion into protein mycelia biomass, there were performed experiments to cultivate 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 (Petre and Petre, 2008; Verstraete and Top, 1992; Stamets, 1998).
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 the fact that by applying this biotechnology, the fruit wastes could be recycled as useful raw materials for mushroom compost preparation in order to get significant mushroom biomass production.
In this respect, the final nitrogen content of mycelia biomass production achieved through the cultivation of these two mushroom species on composts made of fruit wastes was registered as being between 7.1-14.7 g% d.w.
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 analysed by using Kjeldahl method.

The registered results concerning the evolution of total nitrogen content in *P. ostreatus* biomass are presented in Figure 2 and the data regarding *L. edodes* biomass could be seen in Figure 3.

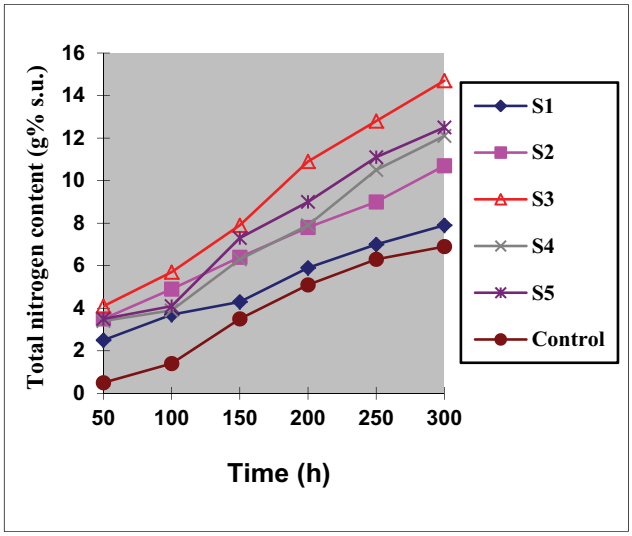

Figure 2. The evolution of total nitrogen content in *P. ostreatus* biomass

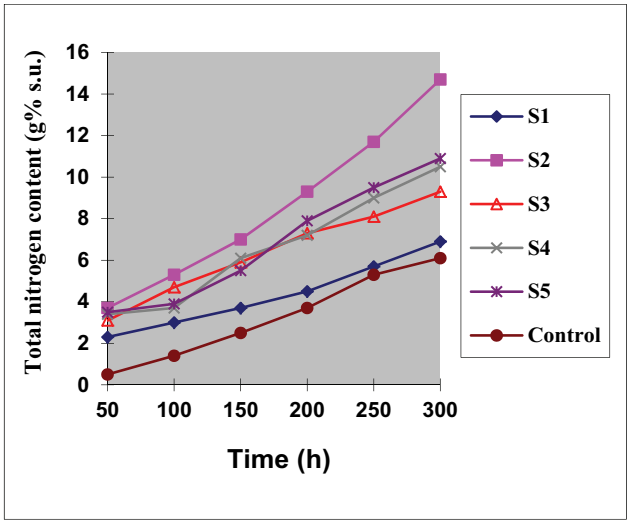

Figure 3. The evolution of total nitrogen content in *L. edodes* biomass

The significant increasing of fruit wastes biodegradation and conversion into protein mycelia biomass by using the biotechnological model of continuous controlled cultivation of edible mushrooms could be achieved by:
a) using pure strains of the mushroom species *P. ostreatus* and *L. edodes* of which biomass has got nutritive and functional properties proved by the research results of some achieved projects or others that are running now;

b) excluding any potential contamination sources for the edible mushrooms by using total sterilization or filtration equipment in each production module, by controlling all row and auxiliary materials, water and air.

CONCLUSIONS

The final nitrogen content of mycelia biomass production achieved through the cultivation of *L. edodes* and *P. ostreatus* mushroom species on different composts made of fruit wastes was registered as being between 7.1-14.7 g% d.w.

To increase the bioconversion of fruit wastes by using the biotechnological model of continuous controlled cultivation of edible mushrooms it is compulsory to exclude any potential contamination sources for the edible mushrooms by using the total sterilization or filtration equipment in each production module, by controlling all row and auxiliary materials, water and air.

The registered data revealed that by applying this biotechnology, the fruit wastes could be recycled as useful raw materials for mushroom compost preparation in order to get significant production of natural fertilizers and protect the natural environment surrounding any fruit processing farm.

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