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Mushroom Biotechnology for Bioconversion of Fruit Tree Wastes into Nutritive Biomass

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Abstract

Annually, huge amounts of horticultural wastes are produced especially during the industrial food processing. One of the ecological means to solve this problem of environmental pollution is the biological recycling of such wastes through submerged cultivation of edible mushrooms on substrates made of different fruit tree wastes resulting from organic horticulture. The research works were carried out by using the pure cultures of three edible mushroom species, namely Ganoderma lucidum, Grifola frondosa and Lentinula edodes, and the culture substrates were prepared from different sorts of organic fruit wastes, such as juice and pulps, resulted from the industrial processing of apples, cherries and plums through natural fermentation made by yeasts and alcohol distillation. The submerged fermentation was carried out inside the culture vessel of an automatic laboratory-scale bioreactor that kept the main cultivation parameters at the following constant values: temperature, 23° C, agitation speed, 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, it was developed the mushroom biomass inside the culture media as fresh mycelia pellets. The biochemical investigations revealed the high contents of mushroom biomass in carbohydrates and proteins.

Keywords: bioreactor, mycelia pellets, submerged cultivation

1. Introduction

The agricultural works and industrial activities related to plant crops and their processing have generally been matched by a huge formation of wide range of vegetal wastes. All these wastes cause serious environmental troubles if they accumulate in the agro-ecosystems or they are burned on the soil (A. MOSER [1], P. BEGUIN & J.P. AUBERT [2], M.J. CARLILE & S.C. WATKINSON [3], M. MOO-YOUNG [4], J.T. BAE & al. [5]).

The use of other mushroom species than the edible ones (like those which were used in experiments) is a source of serious phytopathogenic effects upon the species used for cultivation in horticulture and much more than that for the potential toxigenic effects on the animal or human bodies.

As a result of our recent studies, the continuous cultivation of edible mushroom species was applied to convert different fruit tree wastes, such as apples, cherries and plums by using them as optimal substrates during controlled submerged fermentation in order to get nutritive biomass to be used as natural fertilizers in organic horticulture (R. COHEN & al. [6], F.G. CONFORTIN & al. [7], J.H. LIN & S.S. YANG [8], H.O. KIM [9]).

The main aim of this research work was to establish the optimal biotechnology for ecological recycling of fruit tree wastes to be valorised as natural-made fertilizers in organic

horticulture because these plant wastes are improper to be used as organic substrates for food or feed producing, due to their natural contamination with pathogenic mycotoxins that could produce carcinogenic effects on animal and human bodies even after their biotechnological processing.

2. Materials and methods

The following mushroom species, namely *Ganoderma lucidum* (Wm. Curtis: Fries) Karsten, *Grifola frondosa* (Dick: Fr.) S.F. Gray and *Lentinula edodes* (Berkeley) Pegler, were used as pure mushroom strains. These strains *G. lucidum* 14, *G. frondosa 3* and *L. edodes* 07 are now preserved in the collection of University of Pitesti, Faculty of Sciences in subculture and lyophilized. The stock cultures were maintained on Difco malt-extract agar (MEA) slants (Difco), incubated at 25 °C for 5-7 days and then stored at 4 °C. To prepare the inoculum for submerged fermentation of fruit tree wastes, the seed cultures were grown in 250-ml Erlenmayer flasks containing 100 mL of Difco malt-extract broth (MEB) at 23 °C on rotary shaker incubators at 150 rev. min^{-1} for 7 days (L. RAASKA [10], L.F. JIANG [11], J.P. PARK & al. [12], R. WAGNER & al. [13]).

The mushroom cultures were prepared by inoculating 100 mL of MEB culture medium using 3-5% (v/v) of the seed culture and then were grown at 23-25 °C inside the Erlenmayer flasks of 250 mL mounted on a rotary shaking incubator. The experiments were conducted under the following conditions: temperature, 25 °C; agitation speed, 120-180 rev. $mihen^{-1}$; initial pH, 4.5–5.5. After 10–12 days of incubation, the mushroom cultures were ready to be inoculated aseptically into the glass vessel of a 15 L laboratory-scale bioreactor, as it is shown in Fig. 1.

Fig. 1. General view of the laboratory-scale bioreactor

For the optimal growing of the mushroom biomass inside the culture vessel of this bioreactor five culture substrates were prepared from the fruit wastes (juices, pulps and seeds resulted from the industrial processing of apples, cherries and plums after their natural fermentation made by yeasts and alcohol distillation) mixed with natural supplements like barley and wheat bran, having the following composition that is presented in table 1.

As control, pure cellulose (Merck) was used.

Table 1. The composition of substrates used for mushroom submerged fermentation of apple, cherry and plum wastes

Variants of substrates	Composition
I	Apple wastes 50%, barley bran 15%, limestone powder 10%, water 25%
II	Apple wastes 50%, wheat bran 15%, limestone powder 10%, water 25%
III	Cherry wastes 60%, barley bran 15%, limestone powder 5%, water 20%
IV	Cherry wastes 60%, wheat bran 15%, limestone powder 5%, water 20%
V	Plum wastes 50%, barley bran 15%, limestone powder 10%, water 25%
VI	Plum wastes 50%, wheat bran 15%, limestone powder 10%, water 25%
Control	Pure cellulose

These substrates were steam sterilized at 121 ºC, 1.1 atm., for 15 min. and transferred aseptically inside of the culture vessel of a laboratory scale bioreactor where the pure cultures of *G. lucidum*, *G. frondosa* and *L. edodes* species were inoculated. After the 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%. A lot of mycelia pellets were developed inside the nutritive culture substrates after a period of submerged fermentation lasting up to 120 h (M. PETRE, V. PETRE [14], M. PETRE & al. [15]).

Samples for analysis were collected at the end of the fermentation process, when mycelia pellets took specific shapes and characteristic sizes. For this purpose, mycelia biomass was washed repeatedly with double distilled water in a sieve with 2 mm diameter eye, to remove the remained bran in each culture medium. All experiments were carried out in three replicates.

Biochemical analyses of mycelia biomass samples obtained through submerged cultivation of mushrooms were carried out for the solid fractions after their separation from the remaining fluid by pressing and filtering. In each experimental variant the amount of fresh biomass mycelia was determined. Percentage amount of dry biomass was investigated by dehydration obtained at a temperature of 70° C, until constant weight.

The total protein content was determined by biuret method, whose principle is similar to the Lowry method, this method being recommended for the protein content ranging from 0.5 to 20 mg/100 mg sample (L. RAASKA [10], J.P. PARK & al. [12], C. SANCHEZ [16], I.L. SHIH [17]).

This method requires only one sample incubation period for 20 min. In this way the interference with various chemical agents (ammonium salts, for example) is eliminated. The principle method is based on the reaction that takes place between copper salts and compounds with two or more peptides in which results a red-purple complex, whose absorbance is read in the visible domain (λ = 550 nm) of a spectrophotometer.

The sugar content of dried mycelia pellets collected after the biotechnological experiments was determined by using Dubois method (M. PETRE, V. PETRE [18], P. STAMETS [19], [20] L.M. PAPASPYRIDI & al. [20]).

The mycelia extracts were prepared by immersion of dried pellets inside a solution of NaOH pH 9, in the ratio 1:5. All dispersed solutions containing the dried pellets were

maintained 24 h at a precise temperature of 25 ºC, in full darkness, with continuous homogenization to avoid the oxidation reactions. After the removal of solid residues by filtration the samples were analysed by the previous mentioned method.

The nitrogen content of mycelia pellets was analysed by using Kjeldahl method (M. PETRE, V. PETRE [18], P. STAMETS [19], W. VERSTRAETE & E. TOP [21].

3. Results and discussion

The amounts of fresh and dry mycelia biomass and the protein contents of each mushroom species as well as the substrate variants that were used in experiments are presented in table 2 by comparison with the control samples.

All experiments were carried out three times for each mushroom species and substrate variant. The reported results are the means of these repeated experiments.

Table 2. The fresh and dry biomass as well as total proteins of mycelia biomass after submerged fermentation of apple, cherry and plum wastes

Mushroom species	Substrate variants	Fresh biomass (g)	Dry biomass (%)	Total protein (g% d.w.)
G. lucidum	I	25.94	10.70	3.67
	II	22.45	9.35	3.35
	III	23.47	9.95	2.95
	IV	21.97	9.15	3.30
	V	25.64	10.05	3.55
	VI	25.03	9.95	3.10
	Control	5.9	0.7	0.3
G. frondosa	I	23.75	7.97	3.53
	II	21.23	7.05	3.55
	III	21.10	6.75	2.95
	IV	20.97	7.45	3.25
	V	23.56	6.50	2.75
	VI	21.95	6.95	2.80
	Control	5.3	0.5	0.2
L. edodes	I	20.30	5.95	2.55
	II	23.95	5.10	2.43
	III	22.27	6.10	3.60
	IV	20.10	5.21	2.35
	V	23.45	5.50	2.70
	VI	21.23	5.90	3.50
	Control	4.7	0.5	0.2

According to the registered data, using wheat bran the growth of *G. lucidum* biomass was stimulated, while the barley bran led to increased growth of *L. edodes* mycelium and *G. lucidum* as well.

In contrast, dry matter content is significantly higher when using barley bran for both species used. At the same time, the protein accumulation is more intense when using barley bran as supplementary ingredient comparing with wheat bran, at both mentioned species of mushrooms (Table 2).

All registered results about sugar and nitrogen contents are related to the weight of mycelia pellets collected at the end of each culture cycle and they are compared with the values of control samples, as it is shown in Table 3.

The experiments were carried out three times for each mushroom species and substrate variant. The reported results are the means of these repeated experiments.

Table 3. The contents in sugar (mg/g) and Kjeldahl nitrogen (%) of dried mycelia pellets after submerged fermentation of fruit tree wastes used as culture substrates

Mushroom species	Substrate variants	Sugar content (mg/g)	Kjeldahl nitrogen (%)
G. lucidum	I	5.15	6.55
	II	4.93	5.35
	III	5.55	6.70
	IV	4.35	5.75
	V	5.50	6.90
	VI	5.30	6.10
	Control	0.55	0.30
G. frondosa	I	4.95	5.95
	II	5.65	6.55
	III	5.55	6.53
	IV	4.70	5.05
	V	5.30	6.10
	VI	4.90	5.50
	Control	0.45	0.35
L. edodes	I	5.50	6.10
	II	4.75	5.95
	III	5.73	6.70
	IV	4.95	5.45
	V	5.30	5.75
	VI	5.60	5.90
	Control	0.40	0.35

Comparing all registered data, it could be noticed that the correlation between the dry weight of mycelia pellets and their protein, sugar and nitrogen contents of is kept at a balanced ratio like in case of the fruit bodies of each tested mushroom species, as it is mentioned in a few scientific works (L. RAASKA [10], J.P. PARK & al. [12], C. SANCHEZ [16], I.L. SHIH [17], P. STAMETS [19], [20] L.M. PAPASPYRIDI & al. [20]).

Among the mushroom species that were tested in biotechnological experiments *G. lucidum* – culture variant I showed the best values concerning the protein, sugar and total nitrogen contents. In order, on the very next places, *L. edodes* – culture variant III and *G. frondosa* - culture variant II could be mentioned from these points of view.

The registered results concerning the sugar and total nitrogen contents highlighted higher values than those obtained by other researchers (P. BEGUIN & J.P. AUBERT [2], M.J. CARLILE & S.C. WATKINSON [3], M. MOO-YOUNG [4], F.G. CONFORTIN & al. [7], J.H. LIN & S.S. YANG [8], H.O. KIM [9]).

As a matter of fact, the nitrogen and protein contents of mycelia biomass represents key factors for assessing its nutritive potential, but the assessing of differential protein nitrogen compounds requires additional investigations.

Taking into consideration all registered results it was established the biotechnology for bioconversion of fruit tree wastes into organic fertilizers through submerged fermentation made by the edible mushroom species *G. lucidum, G. frondosa* and *L. edodes*.

The main stages of this biotechnology are presented in Figure 2.

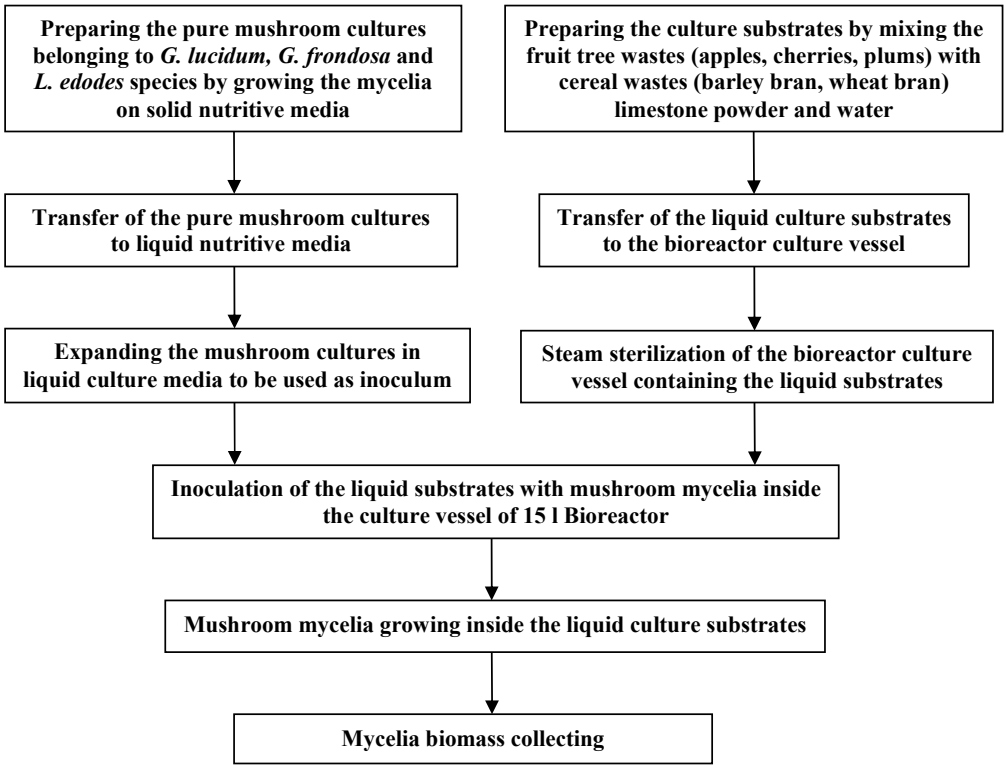

Figure 2. Schematic flow of biotechnology for bioconversion of fruit tree wastes into organic fertilizers through submerged fermentation made by the edible mushroom species *G. lucidum, G. frondosa* and *L. edodes*

4. Conclusions

The fruit tree wastes from apples, cherries and plums processing were used as main substrates for optimal growing of mushroom species *G. lucidum, G. frondosa* and *L. edodes* through controlled submerged fermentation inside the culture vessel of laboratoy-scale bioreactor.

The dry matter content of mycelia biomass produced by submerged fermentation supplemented with barley bran was higher for all tested species and the protein accumulation was more intense when using apple and plum wastes mixed with barley bran compared with the wheat bran.

The correlation between the dry weight and protein of mycelia biomass as well as between their sugar and nitrogen contents is kept at a balanced ratio for each one of the tested mushroom species. *G. lucidum* - culture variant I showed the best values of protein, sugar and total nitrogen contents, being followed by *L. edodes* – culture variant III and finally by *G. frondosa* culture variant II.

The applied biotechnology for bioconversion of fruit tree wastes into organic fertilizers through submerged fermentation made by the edible mushroom species *G. lucidum, G. frondosa* and *L. edodes* has shown the optimal results concerning the mycelia development in order to get high nutritive biomass to be used as natural fertilizers in organic horticulture.

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