

MEDICINAL MUSHROOMS CULTIVATION THROUGH THE SOLID-STATE AND SUBMERGED FERMENTATIONS OF AGRICULTURAL WASTES

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ABSTRACT

The main aim of this work consists in screening the optimal biotechnology of medicinal mushroom cultivation from the solid-state fermentation and the submerged one by using different kinds of wastes coming from cereal crop processing as well as the agro-food industry. The both fermentation technologies were tested through the controlled cultivation of the medicinal mushrooms *Ganoderma lucidum* and *Lentinula edodes* on different growing substrates made of cereal, fruit and vegetable wastes. All the experiments were performed by using a modular system that provided the automatic operations of compost sterilization, aseptic inoculation in a completely clean chamber, incubation as well as mushroom fruit body formation in special growing chambers with controlled atmosphere and the picking up of mushroom fruit bodies. By applying these automatic procedures, all physical and chemical factors that could influence fungal biomass production as well as mushroom fruit body formation could be strictly driven and controlled in order to get high quality products through safe biotechnological models. Comparative results of the chemical investigations regarding some biological active compounds of both fungal pellets from submerged fermentation and mushroom fruit bodies from solid state cultivation are presented.

Keywords: Agricultural wastes; Biomass; Medicinal mushrooms; Solid state cultivation; Submerged fermentation.

INTRODUCTION

Solid-state fermentation of plant wastes from the agro-food industry is one of the challenging and technically demanding of all biotechnologies known to humankind so far [1, 2]. The industrial activities related to wine processing have generally been matched by a huge formation of wide range of wastes [3-5]. Many of these organic wastes cause serious environmental pollution effects if they are allowed to accumulate nearby wine making factories or much worse if they are incorporated in the soil matrix [6-9]. In this respect, the major group of fungi which is capable to degrade cellulose and lignocellulose wastes belongs to the Basidiomycetes Class [10].

MATERIALS AND METHODS

According to the main purposes of this work, two fungal species of the Basidiomycetes group, namely *Ganoderma lucidum* (folk name: Reishi) as well as *Lentinula edodes* (folk name:

Shiitake), were used as pure mushroom cultures isolated from the natural environment and preserved in the local collection of the University of Pitesti. Stock cultures were maintained on malt-extract agar (MEA) slants (20% malt extract, 2% yeast extract, 20% 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. After expanding, 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 [10-12].

Experiments of inoculum preparation were set up under the following conditions: constant temperature, 25 °C; agitation speed, 90-120 rev min⁻¹; initial pH, 5.5–6.5. All seed mushroom cultures were incubated for 120–168 h and were then inoculated into liquid culture media (20% malt extract, 10% wheat bran, 3% yeast extract, 1% peptone) at pH 6.5 previously distributed into rotary shake flasks of 1,000 ml.

During the incubation time period, all the spawn cultures were maintained in special culture rooms designed for optimal incubation at 25 °C. There were prepared three variants of culture compost made of marc grapes in the following ratios: 1:1, 1:2, 1:4 (w/w).

The winery wastes were mechanically pre-treated by using an electric grinding device to breakdown the lignin and cellulose structures to make them more susceptible to the enzyme actions [10-12].

All the culture compost variants made of winery wastes were transferred into 1,000 ml glass jars and disinfected by steam sterilization at 120 °C for 60 min. When the jars filled with composts were chilled, they were inoculated with the liquid spawn already prepared.

Each culture compost variant for mushroom growing was inoculated using liquid spawn having the age of 72–220 h and the volume size ranging between 3–9% (v/w).

RESULTS AND DISCUSSION

During a period of time of 18–20 d after inoculation, all the mushroom cultures had developed a significant mycelia biomass on the culture substrates made of marc of grapes [12]. The effects induced by some additional ingredients such as nitrogen and mineral sources upon the mycelial growth during the incubation were investigated. Of the tested nitrogen sources, wheat bran was the most efficient upon the mycelia growth and fungal biomass production at 35-40 g% fresh fungal biomass weight, being closely followed by malt extract at 25–30 g% [12]. Peptone, tryptone and yeast extract are well known nitrogen sources for fungal biomass synthesis but their efficiency in experiments was relatively lower than the mycelia growing and fungal biomass production induced by the wheat bran added as natural organic nitrogen source (Table 1).

Among the various mineral sources examined, CaCO₃ yielded the best mycelia growth as well as fungal biomass production at 28-32 g% and for this reason it was registered as the most appropriate mineral source.

The experiments were carried out for 288 h at 25 °C with the initial pH 6.5 and all data are the means of triplicate determinations carried out on the compost variants containing marc of grapes in the ratio 1:4, as it is shown in Table 2.

Table 1: The effect of nitrogen source on mycelial growth of *L. edodes* and *G. lucidum*

Nitrogen source (1%, w/v)	Fungal biomass dry weight (g/l)		Fungal biomass fresh weight (g/l)		Final pH	
	<i>L. edodes</i>	<i>G. lucidum</i>	<i>L. edodes</i>	<i>G. lucidum</i>	<i>L. edodes</i>	<i>G. lucidum</i>
Rice bran	6.47 ± 0.14	7.05 ± 0.10	57 ± 0.05	63 ± 0.23	5.5	5.1
Malt extract	6.41 ± 0.23	6.83 ± 0.12	55 ± 0.03	69 ± 0.20	5.3	5.7
Peptone	4.45 ± 0.15	5.43 ± 0.03	41 ± 0.12	57 ± 0.15	4.6	5.3
Tryptone	5.23 ± 0.09	6.95 ± 0.15	28 ± 0.70	55 ± 0.17	5.1	5.9
Yeast extract	5.83 ± 0.35	7.15 ± 0.21	30 ± 0.01	61 ± 0.14	4.3	5.1

All data are the means ± S.D of triplicate determinations

Table 2: The effect of mineral source on mycelial growth of *L. edodes* and *G. lucidum*

Mineral source (5 mg)	Fungal biomass dry weight (g/l)		Fungal biomass fresh weight (g/l)		Final pH	
	<i>L. edodes</i>	<i>G. lucidum</i>	<i>L. edodes</i>	<i>G. lucidum</i>	<i>L. edodes</i>	<i>G. lucidum</i>
KH ₂ PO ₄	5.71 ± 0.09	6.05 ± 0.15	45 ± 0.07	53 ± 0.12	5.5	5.9
K ₂ HPO ₄	6.98 ± 0.13	5.93 ± 0.07	57 ± 0.05	59 ± 0.07	5.1	5.7
MgSO ₄ ·5H ₂ O	6.18 ± 0.20	7.01 ± 0.25	55 ± 0.09	63 ± 0.28	5.6	6.1

* All data are the means ± S.D. of triplicate determinations

During the stage of mushroom fruit body formation, the culture parameters were set up and maintained at the following levels: air temperature, 15–17 °C; air flow volume, 5–6m³/h; air flow speed, 0.2–0.3 m/s; relative moisture content, 80–85%, light intensity, 500–1,000 lux for 8–10 h/d.

The final fruit body production of the mushroom species used was registered between 1.5-2.8 kg per 10 kg of compost made from winery wastes. The whole mycelia growing during the incubation period from the moment of inoculation up to the mushroom fruit body formation lasted between 30–60 d, depending on the fungal species used in experiments.

In order to characterize the chemical composition of the collected mushroom pellets, the sugar as well the nitrogen content were investigated. Firstly, the dry weight of the fungal biomass was established for each mushroom species. Secondly, the sugar content of dried mushroom pellets collected after the biotechnological experiments was determined by using Dubois method. Mushroom extracts were prepared by suspension of dried pellets in a solution of NaOH (pH 9) in a ratio of 1:5. All these dispersed solutions containing the dried pellets were maintained 24 h at the precise temperature of 25 °C, in full darkness, with continuous homogenization to avoid oxidation reactions. After removal of solid residues by filtration, the samples were analyzed as above.

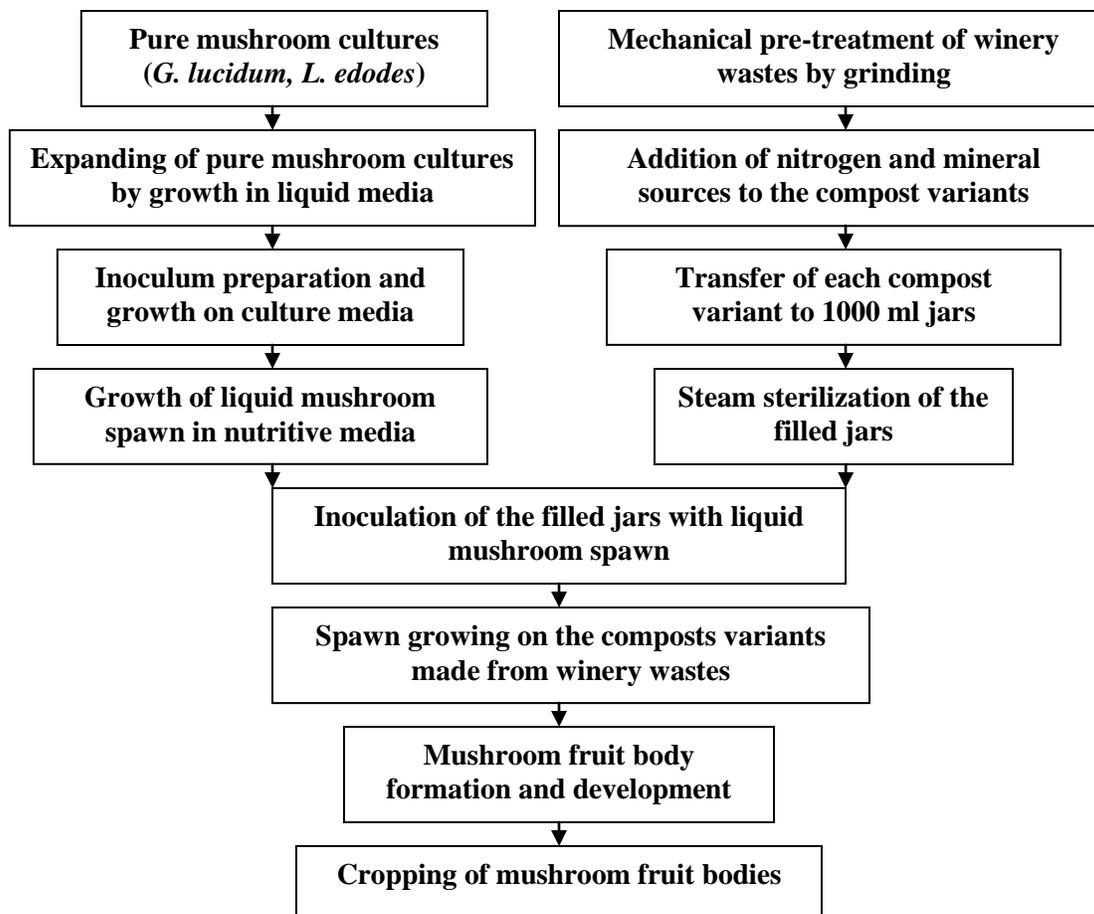


Figure 1: Scheme of enhanced cultivation of edible mushrooms on organic winery wastes

The nitrogen content of the same samples of mushroom pellets was analyzed by the Kjeldahl method. All the registered results are related to the dry weight of mushroom pellets that were collected at the end of each biotechnological culture cycle (Table 3).

Table 3: Sugar and total nitrogen contents of dried mushroom pellets

Sample number	Mushroom species	Mushroom pellets d. w. (%)	Sugar content of dried pellets (mg/ml)	Kjeldahl nitrogen of dried pellets (%)
G-1	<i>G. lucidum</i>	17.64	4.93	5.15
G-2	<i>G. lucidum</i>	14.51	3.70	5.35
G-3	<i>G. lucidum</i>	20.16	5.23	6.28
L-1	<i>L. edodes</i>	19.67	4.35	6.34
L-2	<i>L. edodes</i>	17.43	3.40	5.03
L-3	<i>L. edodes</i>	15.55	4.75	6.05

Comparing all the registered data, it can be seen that the correlation between the dry weight of mushroom pellets and their sugar and nitrogen contents is kept at a balanced ratio for

each tested mushroom species. Among all the mushroom samples that were analyzed, *G. lucidum* G-3 showed the best sugar composition values and total nitrogen content (Table 3).

In this stage, 70-80% of the former fungal pellets were separated by collecting them from the culture vessel of the bioreactor and separating from the broth by slow vacuum filtration. On the base of registered results the optimal *in vitro* culture conditions were evaluated and the best values of all physical and chemical factors which could influence the evolution of biotechnological processes for fungal biomass synthesis were established. Taking into consideration all these registered results, it was established the biotechnology of mushroom pellets producing by controlled submerged fermentation including the most important stages (Fig. 2).

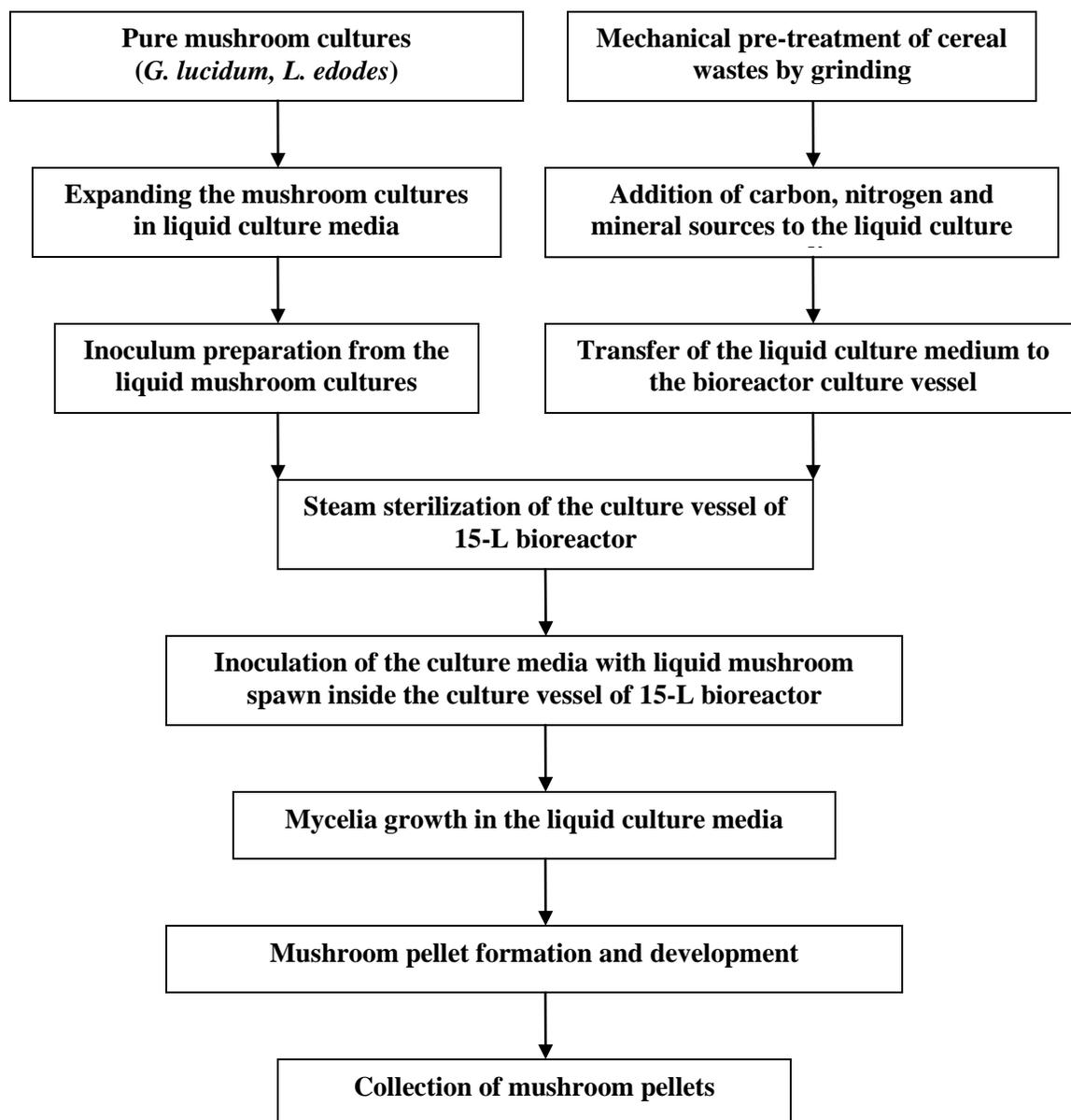


Figure 2: Flow chart showing the biotechnology of mushroom pellet production in submerged culture

CONCLUSIONS

1. Among the five nitrogen sources examined, wheat bran was the most efficient upon the mycelia growing and fungal biomass production of *L. edodes* and *P. ostreatus*, at 35-40 g% fresh fungal biomass weight, closely followed by malt extract at 25–30 g%.
2. The best mineral source was CaCO₃ that yielded the optimal mycelia growing as well as fungal biomass production at 28-32 g%.
3. The final fruit body production by the two mushroom species was registered between 1.5–2.8 kg per 10 kg of solid composts made from winery wastes.
4. Among all mushroom samples that were analyzed, only *G. lucidum* G-3 had shown the best values of their composition in sugars and total nitrogen content.
5. The collected mushroom pellets having high nutritional value were produced by applying the biotechnology of controlled submerged fermentation in order to be used in functional food producing.

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REFERENCES

- [1] Carlile, M.J. and Watkinson, S.C. (1996). Fungi and biotechnology. In: *The Fungi*. Carlile M.J. and Watkinson S.C. Eds. pp 253-264
- [2] Stamets, P. (1993). *Growing Gourmet and Medicinal Mushrooms*. Ten Speed Press, Berkeley, Toronto, 128-143.
- [3] Raaska, L. (1990). Production of *Lentinus edodes* mycelia in liquid media: Improvement of mycelial growth by medium modification. *Mushroom J. Tropics*. 8: 93-97.
- [4] Smith, J. (1998). *Biotechnology*. 3rd Edition. Cambridge University Press.
- [5] Moser A. (1994). Sustainable biotechnology development: from high-tech to eco-tech. *Acta Biotechnol.* 12: 10-15.
- [6] Verstraete W. and Top E. (1992). *Holistic Environmental Biotechnology*. Verstraete W. and Top E. Eds. pp 203-210.
- [7] Petre M. and Petre V. (2008). Environmental biotechnology to produce edible mushrooms by recycling the winery and vineyard wastes. *J. Environ. Protection Ecol.* 9: 88-95.
- [8] Wainwright M. (1992). *An Introduction to Fungal Biotechnology*, Wiley, Chichester.
- [9] Lamar R.T. et al. (1992). White rot fungi in the treatment of hazardous chemicals and wastes. In: *Frontiers in Industrial Mycology*. Leatham G.F. Ed. pp 123-128.
- [10] Petre M. et al. (2007). Mycotechnology for optimal recycling of winery and vine wastes. *Int. J. Med. Mushr.* 9: 241-243.
- [11] Chang S.T. and Hayes W.A. (1978). *The Biology and Cultivation of Edible Mushrooms*, Academic Press, New York, pp 64-70.
- [12] Petre M. (2008). Ecological biotechnology for agro-food wastes valorization. In: *Biotechnology of Environmental Protection (Vol. 2)*. Petre M. Ed. pp 143-150.