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Chapter 1

Environmental Biotechnology for Bioconversion of Agricultural and Forestry Wastes into Nutritive Biomass

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Additional information is available at the end of the chapter

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1. Introduction

The cellulose is the most widely distributed skeletal polysaccharide and represents about 50% of the cell wall material of plants. Beside hemicellulose and lignin, cellulose is a major component of agricultural wastes and municipal residues. The cellulose and hemicellulose comprise the major part of all green plants and this is the main reason of using such terms as "cellulosic wastes" or simply "cellulosics" for those materials which are produced especially as agricultural crop residues, fruit and vegetable wastes from industrial processing, and other solid wastes from canned food and drinks industries.

The cellulose biodegradation using fungal cells is essentially based on the complex interaction between biotic factors, such as the morphogenesis and physiology of fungi, as the cellulose composition and its complexness with hemicellulose and lignin (Andrews & Fonta, 1988; Carlile & Watkinson, 1996).

An efficient method to convert cellulose materials, in order to produce unconventional high-calorie foods or feeds, is the direct conversion by cellulolytic microorganisms. Theoretically, any microorganism that can grow as pure culture on cellulose substrata, used as carbon and energy sources, should be considered a potential organism for "single-cell protein" (SCP) or "protein rich feed" (PRF) producing.

2. Biotechnology of mycelia biomass producing through submerged bioconversion of agricultural crop wastes

The submerged cultivation of mushroom mycelia is a promising method which can be used in novel biotechnological processes for obtaining pharmaceutical substances of anticancer,

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antiviral, immuno-modulating, and anti-sclerotic action from fungal biomass and cultural liquids and also for the production of liquid spawn (Breene, 1990).

The researches that were carried out to get nutritive supplements from the biomass of *Ganoderma lucidum* species (Reishi) have shown that the nutritive value of its mycelia is owned to the huge protein content, carbohydrates and mineral salts. *Lentinula edodes* species (Shiitake) is a good source of proteins, carbohydrates (especially polysaccharides) and mineral elements with beneficial effects on human nutrition (Wasser & Weis, 1994; Mizuno et al., 1995).

It is well known the anti-tumor activity of polysaccharide fractions extracted from mycelia of *Pleurotus ostreatus*, known on its popular name as Oyster Mushroom (Mizuno et al., 1995; Hobbs, 1996).

The main purpose of this research work consists in the application of biotechnology for continuous cultivation of edible and medicinal mushrooms by submerged fermentation in agro-food industry which has a couple of effects by solving the ecological problems generated by the accumulation of plant wastes in agro-food industry through biological means to valorise them without pollutant effects as well as getting fungal biomass with high nutritive value which can be used to prepare functional food (Carlile & Watkinson, 1996; Moser, 1994).

The continuous cultivation of medicinal mushrooms was applied using the submerged fermentation of natural wastes of agro-food industry, such as different sorts of grain by-products as well as winery wastes that provided a fast growth as well as high biomass productivity of the investigated strains (Petre & Teodorescu, 2012; Petre & Teodorescu, 2011).

2.1. Materials and methods

Ganoderma lucidum (Curt. Fr.) P. Karst, *Lentinula edodes* (Berkeley) Pegler and *Pleurotus ostreatus* (Jacquin ex Fries) Kummer were used as pure strains. The stock cultures were maintained on malt-extract agar (MEA) slants, incubated at 25°C for 5-7 d and then stored at 4°C. The seed 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 incubator at 100 rev.min^{-1} for 7 d (Petre & Petre, 2008; Petre et al., 2007).

The fungal cultures were grown by inoculating 100 ml of culture medium using 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 conditions: temperature, 25°C; agitation speed, 120 rev. min^{-1}; initial pH, 4.5–5.5.

After 10–12 d of incubation the fungal cultures were ready to be inoculated aseptically into the glass vessel of a laboratory-scale bioreactor (Fig. 1).

For fungal growing inside the culture vessel of this bioreactor, certain special culture media were prepared by using liquid nutritive broth, having the following composition: 15% cellulose powder, 5% wheat bran, 3% malt extract, 0.5% yeast extract, 0.5% peptone, 0.3% powder of natural argillaceous materials. After the steam sterilization at 121°C, 1.1 atm., for 15 min. this nutritive broth was transferred aseptically inside the culture vessel of the laboratory scale bioreactor shown in figure 1.

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

The culture medium was aseptically inoculated with activated spores belonging to *G. lucidum*, *L. edodes* and *P. ostreatus* species. After inoculation into the bioreactor vessel, a slow constant flow of nutritive liquid broth was maintained inside the nutritive culture medium by recycling it and adding from time to time a fresh new one.

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 (Petre & Teodorescu, 2010; Petre & Teodorescu, 2009).

The experimental model of biotechnological installation, represented by the laboratory scale bioreactor shown in figure 1, was designed to be used in submerged cultivation of the mentioned mushroom species that were grown on substrata made of wastes resulted from the industrial processing of cereals and grapes (Table 1).

Variants of culture substrata	Composition
S1	Mixture of winery wastes and wheat bran 2.5%
S2	Mixture of winery wastes and barley bran 2.5%
S3	Mixture of winery wastes and rye bran 2.5%
Control	Pure cellulose

Table 1. The composition of compost variants used in mushroom cultures

2.2. Results and discussion

The whole process of mushroom mycelia growing lasts for a single cycle between 5-7 days in case of *L. edodes* and between 3 to 5 days for *G. lucidum* and *P. ostreatus*. All experiments regarding the fermentation process were carried out by inoculating the growing medium volume (15 L) with secondary mycelium inside the culture vessel of the laboratory-scale bioreactor (see Fig. 1).

The strains of these fungal species were characterized by morphological stability, manifested by its ability to maintain the phenotypic and taxonomic identity. Observations on morphological and physiological characters of these two tested species of fungi were made after each culture cycle, highlighting the following aspects:

- sphere-shaped structure of fungal pellets, sometimes elongated, irregular, with various sizes (from 7 to 12 mm in diameter), reddish-brown colour of *G. lucidum* specific culture (Fig. 2a);
- globular structures of fungal pellets, irregular with diameters of 5 up to 10 mm or mycelia congestion, which have developed specific hyphae of *L. edodes* (Fig. 2b);
- round-shaped pellets with diameter measuring between 5 and 15 mm, having a white-cream colour and showing compact structures of *P. ostreatus* mycelia (Fig. 2c).

The experiments were carried out in three repetitions. Samples for analysis were collected at the end of the fermentation process, when pellets formed specific shapes and characteristic sizes. For this purpose, fungal 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 (Petre at al., 2005a).

Biochemical analyses of fungal biomass samples obtained by submerged cultivation of edible and medicinal mushrooms were carried out separately for the solid fraction and extract fluid remaining after the separation of fungal biomass by pressing and filtering. Also, the most obvious sensory characteristics (color, odor, consistency) were evaluated and presented at this stage of biosynthesis taking into consideration that they are very important in the prospective view of fungal biomass using as raw matarials for nutraceuticals producing. In each experimental variant the amount of fresh biomass mycelia was analyzed.

Percentage amount of dry biomass was determined by dehydration at 70° C, until constant weight. The total protein content was investigated by using the biuret method, whose principle is similar to the Lowry method, being recommended for the protein content ranging from 0.5 to 20 mg/100 mg sample (Bae et al., 2000; Lamar et al., 1992).

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

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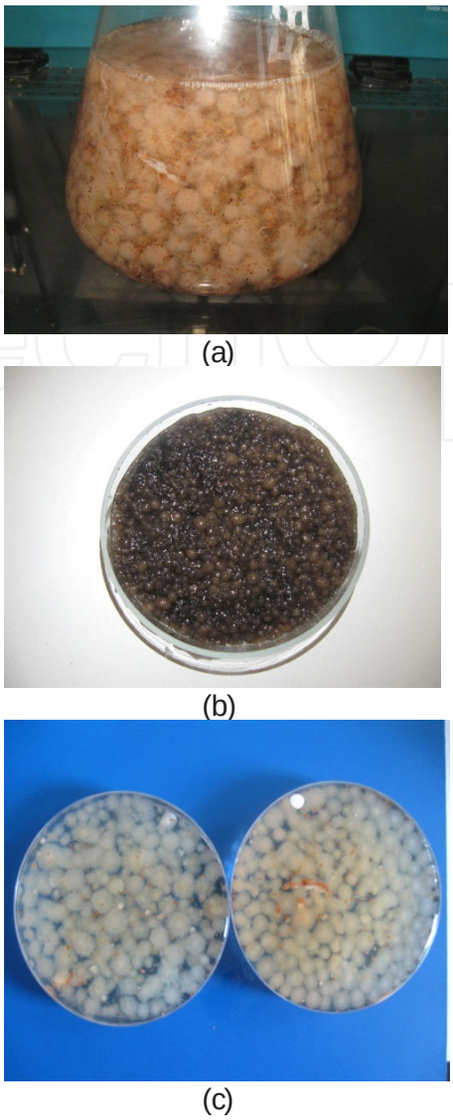


Figure 2. Fungal pellets of *G. lucidum,* b. Fungal pellets of *L. Edodes,*. c. Fungal pellets of *P. ostreatus*

In table 2 are presented the amounts of fresh and dry biomass as well as the protein contents for each fungal species and variants of culture media.

According to registered data, using a mixture of wheat bran 2.5% and winery wastes 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, the dry matter content was significantly higher when using barley bran 2.5% mixed with winery wastes for both species used. Protein accumulation was more intense when using barley bran compared with those of wheat bran and rye bran, at both mushroom species.

The sugar content of dried mushroom pellets collected after the biotechnological experiments was determined by using Dubois method. The mushroom 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 the precise temperature of 25 °C, in full darkness, with continuous homogenization to avoid the oxidation reactions.

Mushroom species	Culture variants	Fresh biomass (g)	Dry biomass (%)	Total proteins (g % d.w.)
G. lucidum	I	25.94	9.03	0.67
G. lucidum	II	22.45	10.70	0.55
G. lucidum	III	23.47	9.95	0.73
	Control	5.9	0.7	0.3
L. edodes	I	20.30	5.23	0.55
L. edodes	II	23.55	6.10	0.53
L. edodes	III	22.27	4.53	0.73
	Control	4.5	0.5	0.2
P. ostreatus	I	21.50	5.73	0.63
P. ostreatus	II	23.95	7.45	0.55
P. ostreatus	III	23.25	4.79	0.75
	Control	4.7	0.5	0.3

Table 2. Fresh and dry biomass and protein content of *G. lucidum, L. edodes* and *P. ostreatus* mycelia grown by submerged fermentation

After the removal of solid residues by filtration the samples were analyzed by the previous mention method (Wasser & Weis, 1994).

The nitrogen content of mushroom pellets was analyzed by 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).

Comparing all the registered data, it could be noticed 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.

From these mushroom species that were tested in biotechnological experiments *G. lucidum* (variant III) showed the best values concerning the sugar and total nitrogen content. On the very next places, *L. edodes* (variant I) and *G. lucidum* (variant II) could be mentioned from these points of view.

The registered results concerning the sugar and total nitrogen contents have higher values than those obtained by other researchers (Bae et al., 2000; Jones, 1995; Moo-Young, 1993). The nitrogen content in fungal biomass is a key factor for assessing its nutraceutical potential, but the assessing of differential protein nitrogen compounds requires additional investigations.

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Mushroom species	Culture variants	Mushroom pellets d. w. (%)	Sugar content of dried pellets (mg/ml)	Kjeldahl nitrogen of dried pellets (%)
G. lucidum	I	17.64	4.93	5.15
G. lucidum	II	14.51	3.70	5.35
G. lucidum	III	20.16	5.23	6.28
	Control	0.7	0.45	0.30
L. edodes	I	19.67	4.35	6.34
L. edodes	II	17,43	3.40	5.03
L. edodes	III	15.55	4.75	6.05
	Control	0.5	0.45	0.35
P. ostreatus	I	19.70	5.15	6.43
P. ostreatus	II	14.93	4.93	6.25
P. ostreatus	III	15.63	5.10	5.83
	Control	0.55	0.50	0.35

Table 3. The sugar and total nitrogen contents of dried mushroom pellets

3. Laboratory-scale biotechnology of edible mushroom producing on growing composts of apple and winery wastes

The agricultural works as well as the industrial activities related to apple and grape processing have generally been matched by a huge formation of wide range of cellulosic wastes that cause environmental pollution effects if they are allowed to accumulate in the environment or much worse they are burned on the soil (Petre, 2009; Verstrate & Top, 1992).

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 (Petre & Petre, 2008; Carlile & Watkinson, 1996).

The major group of fungi which are able to degrade lignocellulose is represented by the edible mushrooms of Basidiomycetes Class. 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 (Petre & Petre, 2008; Moser, 1994; Beguin & Aubert, 1994; Chahal & Hachey, 1990).

The main aim of research was to find out the best biotechnology of recycling the apple and winery wastes by using them as a growing source for edible mushrooms and, last but not least, to protect the environment (Petre et al., 2008; Smith, 1998; Raaska, 1990).

3.1. Materials and methods

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 4.

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)

Table 4. The composition of five compost variants used in mushroom culture cycles

In this way, the whole bags filled with compost were steam sterilized at 121°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 10-15 d, on the surface of sterilized plastic bags filled with compost, the first buttons of mushroom fruit bodies emerged. For 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 (Petre et al., 2012; Oei, 2003; Stamets, 1993; Wainwright, 1992; Ropars et al., 1992).

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3.2. Results and discussion

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 (Stamets, 1993; Wainwright, 1992).

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 3 and the data regarding *L. edodes* biomass could be seen in figure 4.

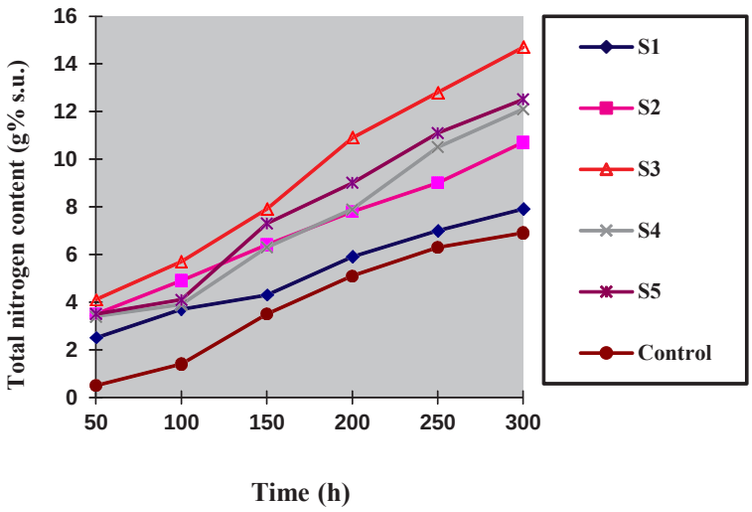


Figure 3. The evolution of total nitrogen content in *P. ostreatus* biomass

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, 15–17°C;
- the air flow volume, 5–6m^3/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.

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. 5).

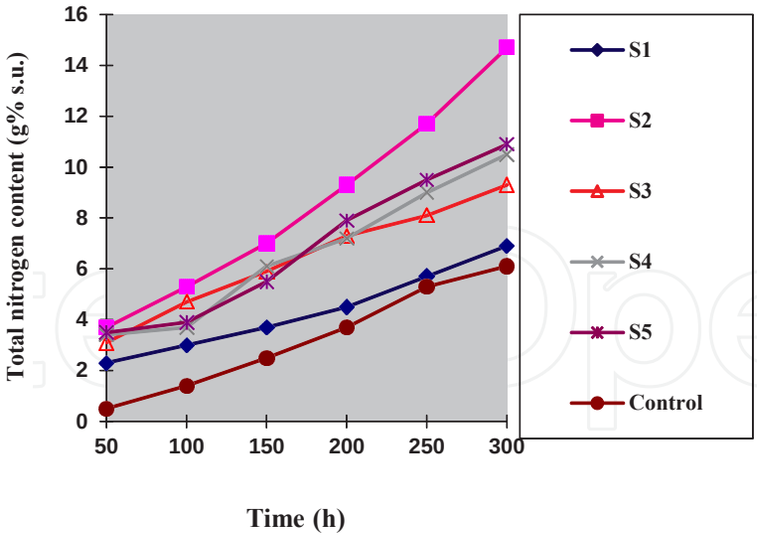


Figure 4. The evolution of total nitrogen content in *L. edodes* biomass

As it is shown in figure 5, 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.

The registered data revealed that by applying such biotechnology, the winery and apple wastes can 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 these two mushroom species was registered as being between 20–28 kg relative to 100 kg of composts made of apple and winery wastes.

4. Biotechnology of forestry wastes recycling as growing composts for edible and medicinal mushroom cultures

The most part of wastes produced all over the world arise from industrial, agricultural and domestic activities. These wastes represent the final stage of the technical and economical life of products (Verstraete & Top 1992).

As a matter of fact, the forestry works as well as the industrial activities related to forest management and wood processing have generally been matched by a huge formation of wide range of waste products (Beguin & Aubert 1994, Wainwright 1992).

Many of these lignocellulosic wastes cause serious environmental pollution effects, if they are allowed to accumulate in the forests or much worse to be burned for uncontrolled domestic purposes. So far, the basis of most studies on lignocellulose-degrading fungi has been eco-

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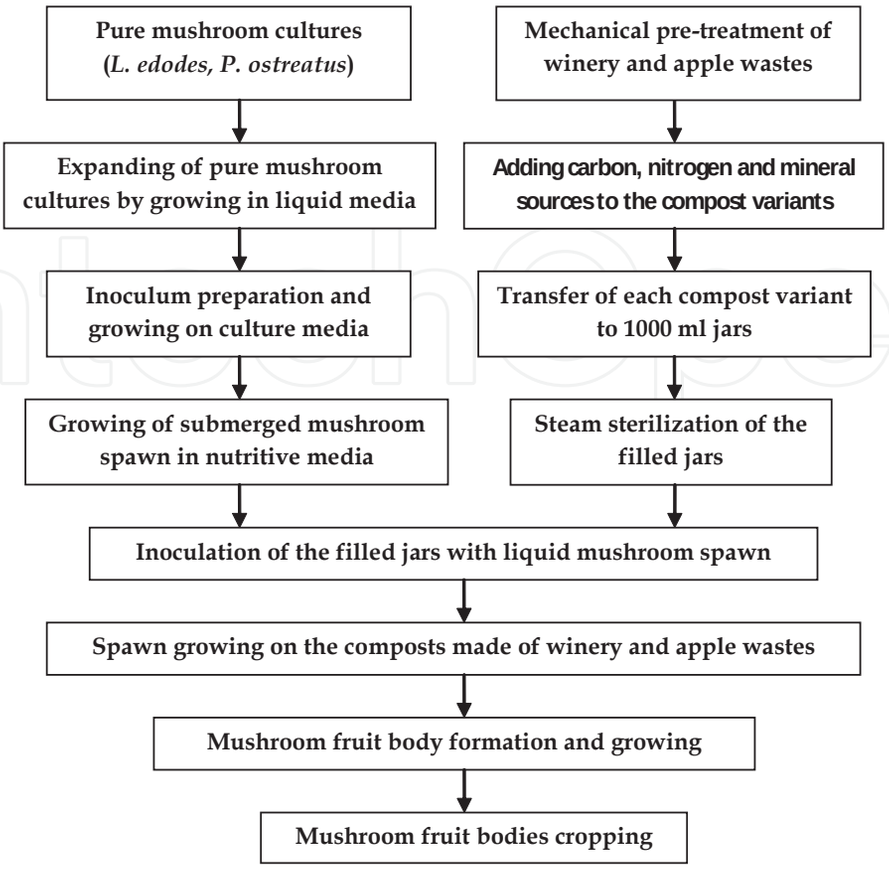


Figure 5. Scheme of laboratory-scale biotechnology for edible mushroom producing on winery and apple wastes

nomic rather than ecological, with emphasize on the applied aspects of lignin and cellulose decomposition, including biodegradation and bioconversion (Carlile & Watkinson 1996).

In this respect, the main aim of this work was focused on finding out the best way to convert the wood wastes into useful food supplements, such as mushroom fruit bodies, by using them as growing sources for the edible and medicinal mushrooms (Smith, 1998).

4.1. Materials and methods

4.1.1. Fungal species and culture media

According to the main purpose of this work, three fungal species from Basidiomycetes, namely *Ganoderma lucidum* (Curt.:Fr.) P. Karst, *Lentinus edodes* (Berkeley) Pegler and *Pleurotus ostreatus* (Jacquin ex Fries) Kummer were used as pure mushroom cultures during all experiments. The stock mushroom cultures were maintained by cultivating on malt-extract agar (MEA) slants. After that, they were incubated at 25° C for 5-7 d and then stored at 4° C. These pure mushroom cultures were grown in 250-ml flasks containing 100 ml of MEA medium (20% malt extract, 2% yeast extract) at 23°C on rotary shaker incubators at 110 rev min^{-1} for 5-7 d.

4.1.2. Methods used in experiments

4.1.2.1. Preparation of submerged mycelia inoculum

The pure mushroom cultures for 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 conditions:

- temperature, 25°C;
- agitation speed, 90-120 rev min^{-1};
- initial pH, 4.5–5.5.

The seed culture was transferred to the fungal culture medium and cultivated for 7–12 d (Petre et al., 2005a; Glazebrook et al., 1992).

4.1.2.2. Incubation of mushroom cultures

The experiments were performed by growing all the previous mentioned fungal species in special culture rooms, where all the culture parameters were kept at optimal levels in order to get the highest production of fruit bodies. The effects of culture compost composition (carbon, nitrogen and mineral sources) as well as other physical and chemical factors (such as: temperature, inoculum size and volume and incubation time) on mycelial net formation and especially, on fruit body induction were investigated (Petre & Petre, 2008).

All the culture composts for mushroom growing were inoculated using liquid inoculum with the age of 5–7 days and the volume size ranging between 3-7% (v/w). During the period of time of 18–20 d after this inoculation, all the fungal cultures had developed a significant biomass on the culture substrata made of wood wastes, such as: white poplar and beech wood sawdusts. These woody wastes were used as main ingredients to prepare natural composts for mushroom growing. The optimal temperatures for incubation and mycelia growth were maintained between 23–25°C. The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30–60 days, depending on each fungal species used in experiments (Petre & Teodorescu, 2010).

4.1.2.3. Preparation of mushroom culture composts

The lignocellulosic materials were mechanical pre-treated to breakdown the lignin and cellulose structures in order to induce their susceptibility to the enzyme actions during the mushroom growing. All these pre-treated lignocellulosic wastes were disinfected by steam sterilization at 120° C for 60 min (Petre et al., 2005b; Leahy & Colwell 1990).

The final composition of culture composts was improved by adding the following ingredients: 15-20% grain seeds (wheat, rye, rice) in the ratio 2:1:1, 0.7–0.9% $CaCO_3$, 0.3–0.5% $NH_4H_2PO_4$, each kind of culture medium composition depending on the fungal species used to be grown. As control samples for each variant of culture composts used for the experimental growing of

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all these fungal species were used wood logs of white poplar and beech that were kept in water three days before the experiments and after that they were steam sterilized to be disinfected.

4.1.2.4. Preparation of mushroom spawn

3000 g of white poplar sawdust and 1500 g of beech sawdust were mixed with cleaned and ground rye grain, 640 g of $CaCO_3$, 50 g of $NH_4H_2PO_4$ and 3550 ml of water, in order to obtain the growth substratum for mushroom spawn. The ingredients of such smal compost were mixed and then they were sterilized at 121° C, for 20 min. and allowed to cool until the mixture temperature decreased below 35° C. The spawn mixture was inoculated with 100-200 ml of liquid fungal inoculums and mixed for 10 min. to ensure complete homogeneity. Sterile polyethylene bags, containing microporus filtration strips, were filled with the smal composts and incubated at 25° C, until the spawn fully colonized the whole composts. At this point the spawn may be used to inoculate the mushroom growing substrate or alternatively it may be stored for up to 6 months at 4° C before use (Chahal & Hachey, 1990).

All the culture composts were inoculated using inoculum with the age of 5–7 d and the volume size ranging between 3-7% (v/w). The optimal temperatures for incubation and mycelia growth were maintained between 23–25°C. The whole period of mushroom growing from the inoculation to the fruit body formation lasted between 30–50 days.

4.1.2.5. Mushroom cultivation

The experiments were carried out inside such *in vitro* growing rooms, where the main culture parameters (temperature, humidity, aeration) were kept at optimal levels to get the highest production of mushroom fruit bodies (Moser, 1994).

In order to find a suitable carbon source for the mycelia growth and consequently for fungal biomass synthesis, the pure cultures of *P. ostreatus* (Oyster Mushroom), as well as *L. edodes* (Shiitake) and *G. lucidum* (Reishi) were cultivated in different nutritive culture media containing various carbon sources, and each carbon source was added to the basal medium at a concentration level of 1.5% (w/v) for 7-12 d (Raaska, 1990).

To investigate the effect of nitrogen sources on mycelia growth and fungal biomass production, the pure cultures of these two fungal species were cultivated in media containing various nitrogen sources, where each nitrogen source was added to the basal medium at a concentration level of 10 g/l. At the same time, malt extract was one of the better nitrogen sources for a high mycelia growth. Peptone, tryptone and yeast extract are also known as efficient nitrogen sources for fungal biomass production by using the pure cultures of such fungal species (Chang & Hayes, 1978). In comparison with organic nitrogen sources, inorganic nitrogen sources gave rise to relatively lower mycelia growth and fungal biomass production (Bae et al., 2000).

The influence of mineral sources on fungal biomass production was examined at a standard concentration level of 5 mg. In order to study the effects of initial pH correlated with the incubation temperature upon fruit body formation, *G. lucidum*, *P. ostreatus* and *L. edodes* were

cultivated on substrates made of wood wastes of white poplar and beech at different initial pH values (4.5–6.0). The experiments were carried out for 6 days at 25°C with the initial pH 5.5. Similar observations were made by Stamets (1993), during the experiments. K_2HPO_4 could improve the productivity through its buffering action, being favourable for mycelia growth. The experiments were carried out between 30-60 days at 25°C.

4.2. Results and discussion

The effects of carbon, nitrogen and mineral sources as well as other physical and chemical factors on mycelial net formation and especially, on fruit body induction were investigated by adding them to the main composts made of white poplar and beech sawdusts in the ratio 2:1. For the experimental growing of all these fungal species white poplar and beech logs were used as control samples.

4.2.1. The effect of carbon sources upon mushroom mycelia growth

When the cells were grown in the maltose medium, the fungal biomass production was the highest among the tested variants. Data presented in the following table are the means ± S.D. of triple determinations (Table 5).

Carbon source (g/l)	**Fresh Fungal Biomass Weight (g/l)**			**Final pH**		
	G. lucidum	*L. edodes*	*P. ostreatus*	*G. l*	*L. e*	*P. o*
Glucose	27±0.10	41±0.05	43±0.03	5.5	5.3	5.1
Maltose	27±0.14	45±0.12	49±0.05	5.8	5.4	5.3
Sucrose	25±0.23	35±0.03	37±0.09	5.1	5.1	5.7
Xylose	26±0.07	38±0.07	35±0.07	5.3	5.5	5.9

Table 5. The effect of carbon sources upon the mycelia growth of pure mushroom cultures on white poplar and beech composts

What is very important to be noticed is that the maltose has a significant effect upon the increasing of mycelia growth and fungal biomass synthesis. The experiments were carried out for 12 days at 25 °C with the initial pH 5.5 (Petre, 2002).

4.2.2. The effect of nitrogen sources upon mushroom mycelia growth

Among five nitrogen sources examined, rice bran was the most efficient for mycelia growth and fungal biomass production. The experiments were carried out for 12 days at 25 °C with the initial pH 5.5 (Table 6).

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Nitrogen sources (1%, w/v)	Fresh Fungal Biomass Weight (g/l)			Final pH		
	G. lucidum	*L. edodes*	*P. ostreatus*	*G. l*	*L. e*	*P. o*
Rice bran	37±0.21	57±0.05	73±0.23	5.5	5.5	5.1
Malt extract	36±0.12	55±0.03	69±0.20	5.3	5.2	5.7
Peptone	35±0.03	41±0.12	57±0.15	4.6	4.9	5.3
Tryptone	36±0.15	38±0.07	55±0.17	5.1	5.3	5.9
Yeast extract	37±0.20	30±0.01	61±0.14	4.3.	5.1	5.1

Data presented in table 6 are the means ± S.D. of triple determinations.

Table 6. The effect of nitrogen sources upon the mycelia growth of pure mushroom cultures on white poplar and beech composts

4.2.3. The effect of mineral sources upon mushroom mycelia growth

Among the various mineral sources examined, K_2HPO_4 yielded good mycelia growth as well as fungal biomass production and for this reason it was recognized as a favourable mineral source (Table 7). Data presented in table 7 are the means ± S.D. of triple determinations

Mineral Sources (5 mg)	Fresh Fungal Biomass Weight (g/l)			Final pH		
	G. lucidum	*L. edodes*	*P. ostreatus*	*G. l*	*L. e*	*P. o*
KH_2PO_4	37±0.15	45±0.07	53±0.12	5.5	5.3	5.9
K_2HPO_4	45±0.07	57±0.05	59±0.07	5.1	5.1	5.7
$MgSO_4 \cdot 5H_2O$	35±0.25	55±0.09	63±0.28	5.6	5.4	6.1

Table 7. The effect of mineral source upon mycelia growth of pure mushroom cultures on white poplar and beech composts

4.2.4 The influence of initial pH and temperature upon mushroom fruit body formation

The optimal pH and temperature levels for fungal fruit body production were 5.0–5.5 and 21–23°C (Table 8).

To find the optimal incubation temperature for mycelia growth, these fungal species were cultivated at different temperatures ranging from 20-25°C, and, finally, the optimum level of temperature was found at 23°C, being correlated with the appropriate pH level 5.5, at it is shown in Table 8. All data presented in the previous table are the means ± S.D. of triple determinations

Initial pH (pH units)	Initial temperature (t°)	Final Weight of Fresh Mushroom Fruit Bodies (g / kg substratum)		
		G. lucidum	*L. edode*	*P. ostreatus*
4.5	18	175±0.23	191±0.10	180±0.02
5.0	21	193±0.15	203±0.05	297±0.14
5.5	23	198±0.10	195±0.15	351±0.23
6.0	26	181±0.12	179±0.12	280±0.03
6.5	29	173±0.09	105±0.23	257±0.15

Table 8. The effects of initial pH and temperature upon mushroom fruit body formation on white poplar and beech composts

4.2.5. The influence of inoculum age and inoculum volume upon mushroom fruit body formation

Amongst several fungal physiological properties, the age and volume of mycelia inoculum may play an important role in fungal hyphae development as well as in fruit body formation (Petre & Teodorescu, 2012).

To examine the effect of inoculum age and inoculum volume, mushroom species *G. lucidum*, *P. ostreatus* and *L. edodes* were grown on substrates made of vineyard wastes during different time periods between 30 and 60 days, varying the inoculum volume (5 - 7 v/w).

All the experiments were carried out at 25°C and initial pH 5.5. As it is shown in Tables 9 and 10, the inoculum age of 120 h as well as an inoculum volume of 6.0 (v/w) have beneficial effects on the fungal biomass production.

Inoculum age (h)	Final Weight of Fresh Mushroom Fruit Bodies (g /kg substratum)		
	G. lucidum	*L. edodes*	*P. ostreatus*
264	123±0.14	128±0.05	135±0.23
240	141±0.10	150±0.28	157±0.17
216	154±0.12	195±0.90	193±0.15
192	155±0.23	221±0.25	215±0.05
168	169±0.37	235±0.78	241±0.07
144	210±0.20	248±0.03	259±0.12
120	230±0.15	253±0.05	264±0.21
96	215±0.09	230±0.15	253±0.10
72	183±0.05	205±0.23	210±0.05

Table 9. The effect of inoculum age upon mushroom fruit body formation on white poplar and beech composts

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Inoculum Volume (v/w)	Final Weight of Fresh Mushroom Fruit Bodies (g /kg substratum)		
	G. lucidum	*L. edodes*	*P. ostreatus*
7.0	234±0.12	215±0.20	220±0.05
6.5	245±0.15	248±0.23	251±0.20
6.0	253±0.1	257±0.07	280±0.15
5.5	243±0.12	235±0.03	247±0.07
5.0	255±0.23	215±0.15	235±0.03

Table 10. The effect of inoculum volume upon mushroom fruit body formation on white poplar and beech composts

From all these fungal species tested, *P. ostreatus* was registered as the fastest mushroom (25–30 days), then *L. edodes* (35–45 days) and eventually, *G. lucidum* as the longest mushroom culture (40–50 days).

The registered data revealed that the white poplar and beech wood wastes have to be used as substrates for mushroom growing only after some mechanical pre-treatments (such as grinding) that could breakdown the whole lignocellulose structure in order to be more susceptible to the fungal enzyme action (Chahal, 1994).

Due to their high content of carbohydrates and nitrogen, the variants of culture composts supplemented with wheat grains at the ratio 1:10 and rice grains at the ratio 1:5 as well as a water content of 60% were optimal for the fruit body production of *P. ostreatus* and, respectively, *L. edodes*. The mushroom culture of *G. lucidum* does not need such supplements (Ropars et al., 1992; Lamar et al., 1992).

So far, lignocellulose biodegradation made by mushroom species of *Ganoderma* genus had been little studied, mostly because of their slow growth, difficulty in culturing as well as little apparent biotechnological potential. Only, Stamets (1993) reported a few experimental data concerning the cultivation of such fungal species in natural sites and he noticed its slowly growing.

In spite of these facts, some strains of *G. lucidum* were grown in our experiments on culture substrates made of wood wastes of white poplar and beech mixed with rye grains at the ratio 1:7 and a water content of 50%.

Higher ratio of rye grains might lead to an increase of total dry weight of fruit body, but also could induce the formation of antler branches and smaller fruit bodies than those of the control samples.

The final fruit body mushroom production ranged between 15 and 20 kg relative to 100 kg of compost made of wood, depending on the specific strains of those tested mushroom species.

5. Conclusions

1. The cereal by-products and winery wastes used as substrata for growing the fungal species *G. lucidum, L. edodes* and *P. ostreatus* by controlled submerged fermentation showed optimal effects on the mycelia development in order to get high nutritive biomass.
2. The dry matter content of fungal biomass produced by submerged fermentation of barley bran was higher for both tested species.
3. The protein accumulation is more intense when using barley bran compared with those of wheat and rye, at both fungal species.
4. *G. lucidum* (variant III) registered the best values of sugar and total nitrogen contents, being followed by *L. edodes* (variant I)
5. The winery and apple wastes can 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.
6. By applying the biotechnology of recycling the grape and apple wastes can be produced between 20–28 kg of mushroom fruit bodies relative to 100 kg of composts made of winery and apple wastes.
7. From all these fungal species tested in experiments, *P. ostreatus* was registered as the fastest mushroom culture (25–30 days), then *L. edodes* (35–45 days) and finally, *G. lucidum* as the longest mushroom culture (40–50 days).
8. The registered data revealed that when the cells were grown in the maltose medium, the fungal biomass production was the highest among the tested variants.
9. From five nitrogen sources examined, rice bran was the most efficient for mycelia growth and fungal biomass production
10. Among the various mineral sources examined, K_2HPO_4 yielded good mycelia growth as well as fungal biomass production and for this reason it was as a favourable mineral source.
11. The inoculum age of 120 h as well as an inoculum volume of 6.0 (v/w) have beneficial effects on the fungal biomass production and the optimal pH and temperature levels for fungal fruit body production were 5.0–5.5 and 21–23° C.
12. The final fruit body mushroom production ranged between 15 and 20 kg relative to 100 kg compost made of wood, depending on the specific strains of those tested mushroom species.

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References

[1] Bae, J. T, Sinha, J, Park, J. P, Song, C. H, & Yun, J. W. (2000). Optimization of submerged culture conditions for exo-biopolymer production by *Paecilomyces japonica*. *Journal of Microbiology and Biotechnology*, 1017-7825, 10, 482-487.

[2] Beguin, P, & Aubert, J. P. (1994). The biological degradation of cellulose. *FEMS Microbiol. Rev.*, , 13, 25-58.

[3] Breene, W. M. (1990). Nutritional and medicinal values of mushrooms. *J. Food Prot.*, , 53, 833-894.

[4] Carlile, M. J, & Watkinson, S. C. (1996). Fungi and biotechnology. In: *The Fungi*, M.J. Carlile, S.C. Watkinson (Eds.), Academic Press, 0-12159-960-4England, 253-264.

[5] Chahal, D. S. (1994). Biological disposal of lignocellulosic wastes and alleviation of their toxic effluents. In: *Biological Degradation and Bioremediation of Toxic Chemicals*, G.R. Chaudry (Ed.), Chapman & Hall, 978-0-41262-290-8London, England, 347-356.

[6] Chahal, D. S, & Hachey, J. M. (1990). Use of hemicellulose and cellulose system and degradation of lignin by *Pleurotus sajor-caju* grown on corn stalks. *Am. Chem. Soc. Symp.*, , 433, 304-310.

[7] Chang, S. T, & Hayes, W. A. Eds.), ((1978). The Biology and Cultivation of Edible Mushrooms. Academic Press, New York, , 147-156.

[8] Jones, K. (1995). *Shiitake- The Healing Mushroom*. Healing Arts Press, Rochester, 0-89281-499-3USA

[9] Glazebrook, M. A, Vining, L. C, & White, R. L. (1992). Growth morphology of *Streptomyces akiyoshiensis* in submerged culture: influence of pH, inoculum, and nutrients. *Canadian Journal of Microbiology*, , 38, 98-103.

[10] Hobbs, C. (1996). Medicinal mushrooms. Santa Cruz, Botanika Press, , 251-270.

[11] Lamar, R. T, Glaser, J. A, & Kirk, T. K. (1992). White rot fungi in the treatment of hazardous chemicals and wastes. In: Leatham, G.F. (ed.), *Frontiers in industrial mycology*, Chapman & Hall, New York, , 223-245.

[12] Leahy, J. G, & Colwell, R. R. (1990). Microbial Degradation of Hydrocarbons in the Environment, *Microbial Rev.*, , 54, 305-315.

[13] Moo-young, M. (1993). Fermentation of cellulose materials to mycoprotein foods, *Biotechnology Advances*, 0734-9750, 11(3), 469-482.

[14] Moser, A. (1994). Sustainable biotechnology development: from high-tech to ecotech. *Acta Biotechnologica*, 0138-4988, 12(2), 10-15.

[15] Oei, P. (2003). Mushroom Cultivation. 3rd Edition, Backhuys Publishers, 9-05782-137-0The Netherlands

[16] Petre, M, & Teodorescu, A. (2012). Biotechnology of Agricultural Wastes Recycling Through Controlled Cultivation of Mushrooms. In: Advances in Applied Biotechnology (M. Petre Editor), InTech Open Access Publisher, 978-9-53307-820-5, 3-23.

[17] Petre, M, Teodorescu, A, & Giosanu, D. (2012). Advanced Biotechnological Procedures of Mushroom Cultivation. In: Mushroom Biotechnology and Bioengineering, M. Petre and M. Berovic Editors, CD Press, Bucharest, 978-6-06528-146-2, 1-21.

[18] Petre, M, & Teodorescu, A. (2011). Recycling of Vineyard and Winery Wastes as Nutritive Composts for Edible Mushroom Cultivation. Proc. of the International Conference on Advances in Materials and Processing Technologies AMPT, American Institute of Physics (978-0-73540-871-5

[19] Petre, M, & Teodorescu, A. (2010). Handbook of submerged cultivation of eatable and medicinal mushrooms. CD Press, 978-6-06528-087-8Bucharest, Romania

[20] Petre, M. & Petre, V. (2008). Environmental Biotechnology to Produce Edible Mushrooms by Recycling the Winery and Vineyard Wastes. *Journal of Environmental Protection and Ecology*, Vol. 9, No.1, pp. 88-95, ISSN: 1311-5065

[21] Petre, M, & Petre, V. (2008). Environmental Biotechnology to Produce Edible Mushrooms by Recycling the Winery and Vineyard Wastes. *Journal of Environmental Protection and Ecology*, 1311-5065, 9(1), 88-95.

[22] Petre, M, Peng, M-X, & Mao, L-X. (2005a). The influence of culture conditions on fungal pellets formation by submerged fermentation of *Cordyceps sinensis* (*Paecilomyces hepiali*)- *Cs 4*. *Acta Edulis Fungi*, Suppl.), , 12, 345-353.

[23] Petre, M, Teodorescu, A, & Dicu, G. (2005b). The Growing Effect of Vineyard and Winery Wastes on the Production of Mycelia and Fruit Bodies of Edible and Medicinal Fungi. *International Journal of Medicinal Mushrooms*, 1521-9437, 7(3), 444-445.

[24] Petre, M, Zarnea, G, Adrian, P, & Gheorghiu, E. (2001). Biocontrol of cellulose waste pollution by using immobilized filamentous fungi. In: Environmental Monitoring and Biodiagnostics of Hazardous Contaminants (Healy, M., Wise, D.L. Moo-Young, M, eds), Kluwer Academic Publishers, The Netherlands, 079236869, 227-241.

[25] Raaska, L. (1990). Production of *Lentinus edodes* mycelia in liquid media: Improvement of mycelial growth by medium modification. *Mushroom Journal of the Tropics*, , 8, 93-98.

[26] Ropars, M, Marchal, R, Pourquie, J, & Vandercasteele, J. P. (1992). Large scale enzymatic hydrolysis of agricultural lignocellulosic biomass. *Biores. Technol.*, , 42, 197-203.

[27] Smith, J. (1998). Biotechnology. 3rd Edition. Cambridge University Press, 0-52144-911-1England

[28] Stamets, P. (2000). Growing Gourmet and Medicinal Mushrooms. Ten Speed Press, 1-58008-175-4Toronto, Canada

[29] Verstraete, W, & Top, E. (1992). Holistic Environmental Biotechnology. Cambridge University Press, 0-52142-078-4England

[30] Wainwright, M. (1992). An Introduction to Fungal Biotechnology. Wiley, Chichester, , 103-115.

[31] Wasser, S. P, & Weis, A. L. (1994). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Critical Reviews in Immunology*, 1040-8401, 19, 65-96.

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