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Review

Potentiality of Mushrooms Cultivation as Resources for Food and Folk Medicine

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Abstract

The possibility to apply of higher fungi, mushroom, as a potential food and folk medicine sources is reviewed. The total world production of the cultivated edible mushroom has been increased dramatically since the end of the World War II. Some scientist has predicted that worldwide mushroom production will continue to increase at an average annual rate of 8% to the early of 21 century. Much interest has been aroused recently in the possibility of growing microorganism on a large scale to provide a cheap source of protein, food and folk medicine for antitumors and adult diseases. Large-scale culture for mushroom mycelium in submerged liquid culture has been realized. Despite the small contribution in terms of food and protein resources, mushrooms will remain a valuable food for many people. Not only does the mushroom have a unique flavor, its protein value lies somewhere between that of meat and vegetables. In addition mushrooms rank highly for their vitamin content and make an important contribution to the supply of some minerals in the diet. Lately it could not be negligible to have research the effect of the mushroom and its culture filtrates on cancer, antitumor and the other adult diseases and the possibility as very important folk medicines. In order to produce high value-added mushrooms, its fungal mycelia, and its use of food processing, certain technical difficulties in their productions could be overcome.

Key words: biomass, folk medicine, food, higher fungi, protein, submerged cultures, waste utilization.

Introduction

In the age of fast population growth and consequently greater demand for food, especially protein containing products, man attempts to solve the problem of food shortage in a number of ways. Apart from the conventional manners of protein production, new approaches are being examined. Among others, the microbiological methods seem to be hopeful for two basic reasons:

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fast growth of biomass and the possibility of utilization of industrial wastes (*e.g.* from food, pulp and paper industries). The idea of production of feed or food biomass by the submerged cultures of higher fungi in the agitated and aerated baffled tanks results from the experience carried out on penicillin and other antibiotic fermentation processes. As the substrates for fungal mycelium growth low-cost materials might be used. At the same time reduction of the biological oxygen requirement by waste byproducts to an acceptable level could be achieved. It results from the fact that higher fungi are equipped with efficient enzymatic apparatus, which can attack the substrates not acceptable for yeast and bacteria. Solid-stationary or submerged and agitated cultures have been carried in order to obtain either biomass or some nutritive substances such as vitamins, amino acids or mono saccharides.

The interest in higher fungi as a source of food protein comes from the fact that they have been used for many thousand years: their taste and aroma are pleasant for man and unlike yeast and bacteria they contain fewer nucleic acids which allow for their consumption without side effects. The overall contents of nucleic acids in bacteria amount to 17 g as calculated in its 100 g dry mass; in yeast over 5 g and in fungi only 500 mg (*e.g. Agaricus campestris*) (Grzybowski, 1978). However, even this considerably low amount of nucleic acids substantially reduces the nutrition value of fungal biomass. Therefore, modern biotechnology aims at their complete elimination (Newmark, 1980).

Out of about 2,000 species of edible fungi, only three are cultivated on a large up to 1970s, commercial scale under artificial conditions *e.g. Agaricus campestris* (Europe and North America) (Toth and Toth, 1972; Wuest *et al.*, 1987; Martin, 1982), *Lentinula edodes* (Japan, China and Korea) and *Volvaria volvacea* (China and South-East Asia) (Grzybowski, 1977). However, lately mainly in China, Korea and Japan, many edible mushrooms, *Pleurotus ostreatus, Flammulina velutipes, Agaricus bisporus, Pholiota nameko*, and *Grifola frondosa* are successfully cultivated, and commercialized. In addition, *Ganoderma lucidum, Coriolus versicolor, Phellinus pini* and the other fungi are largely cultivated and used as folk medicine drinks for adults diseases, such as tumors and cancer. It is believed that certain endogenic substances of a hormone character play a crucial role in the process. Their nature, however, has not been fully determined so far. In addition, a vast majority of other edible fungi defies classical cultivation for the fructification mechanism still remains largely unknown.

An alternative seems to be offered not by culture of fruiting bodies but rather by cultivation of vegetative mycelium. It exhibits identical biochemical properties and composition to fruiting bodies (Hadar and Cohen-Arazi, 1986). The vegetative mycelium contains a fairly good amount of protein (up to 50%) particularly rich in lysine and dicarboxylic amino acids. The quality and amount of protein and other important components can be regulated by means of various culture conditions. The amount of vitamins (especially of group B) equals that of yeast. The mycelium is always multi cellular and largely ramified which allows for the aggregation (Solomons, 1975; Platt *et al.*, 1982; Martin and Bailey, 1985; Tautorus, 1985).

Kinds of the fungal culture

The efficiency and quality of biomass depend on the fungal species and methods of culture growth. There are two known kinds of cultures: shallow stationary and submerged, mixed and aerated in the liquid phase. The latter is more advantageous because of the following factors:

- Fast vegetation (about 3 times or even more as fast as that of the shallow stationary).
- Easy separation of mycelium from the culture medium.
- Grow as a continuous culture occupying a relatively small area.
- Medium sterilization in the growing fermentor and keeping the culture aseptic.
- Control cultivation conditions during the fermentation process.

However, this kind of culture demands more work and energy than the shallow stationary culture. Research on the possibilities of vegetative mycelium culture was initiated on a worldwide scale more than 55 years ago (Treschow, 1944). The first successful attempt pertained to the culture of *Agaricus campestris* incubated under the submerged, agitated conditions in a synthetic nutrient medium containing glucose, urea, and mineral salts (Humfeld and Sugihara, 1952). Until 1959 there appeared several dozens of similar pilot works (Robinson and Davidson, 1959). The authors estimated the value of the research carried out so far and found it encouraging for the initial commercial production of mycelium as food. Another more comprehensive monographic analysis (Litchfield, 1967; Solomons, 1975; Grzybowski, 1977; Grzybowski, 1978) is a compendium of multidirectional research done up so far and a survey of the used fungal strains and the kinds of nutrient media (synthetic and waste) as well as physical and chemical parameters of the cultures. The fungal species referred to, in the monographs, as optimal producers of biomass for nutrition are the following: *Agaricus blazei, Agaricus campestris, Boletus indecius, Collybia velutipes, Cantharellus cibarius, Morchella hybrida, Morchella rimosipes, Tricholoma nudum* and *Xylaria polymorpha*. They may grow on the media containing such carbohydrates as glucose, maltose, lactose, and the waste materials or byproducts such as whey, spent molasses, sulfite waste liquor, wastes from the production of soybean oil and from corn and pumpkin preservation (Yoshikumi *et al.*, 1979; Yoshikumi *et al.*, 1980).

Commercial production

The cultures on a micro-, semi- and full technological scales are carried in the fermentors of some to several hundred and even thousand liters in volume. Humfeld *et al.* (1952) used a 20 L fermentor of 400 revs/min at the aeration rate 1-3 L of air per L of medium per min. After reaching the optimal yield, the *Agaricus campestris* culture was incubated 1-3 days longer in order to obtain strong, pleasent aroma. Finally, after 93 hrs of incubation, 15 kg of mycelium (13.6%of dry mass) were obtained from 150 L of medium and the aroma was satisfactory. In other micro- and semi-technological cases *Tricholoma nudum* stirred and aerated culture carried out by Reusser *et al.* (1958b), reached its optimum after 95 hrs. The aeration rate was of 0.3 of air per liter of the culture per minute at stirring rate of 400 revs/min. In this case as an antifoam agent, pork lard was used. Rehacek *et al.* (1962) incubated *Boletus edulis* in a 10 L

fermentor. The medium was inoculated with 500 ml of 7-day-old homogenized mycelium and aerated with 0.7 L of air per liter of the culture per minute at the agitation speed of 430 revs/min. Maximum yield of mycelium was reached after 120 hrs of incubation. However, after 72 hrs, the culture reached the maximum yield of protein (57.5%).

An American patent of Szuecs (1956) authority was the basis for the commercial cultivation of *Morchella esculenta* by Special Products, Inc. (Springfield, Missouri, USA) in the liquid medium containing glucose, ammonium phosphate, corn extract, calcium carbonate and silicone as an anti-foam. The mycelium from the agar slants was transferred sequent to 500 ml conical flasks, 10 L glass vessels, 500 L tanks and finally to 10,000 L tanks. The mycelium after 3-4 day cultivation at 21-24℃ reached the form of beads 2 cm in diameter. In the process of translocation the material undergoes sterile homogenization. The yield of a 10,000 L tank ranged on the average from 1.2 to 2 tons of wet biomass, which after centrifugation (1,000 revs/min) was dried in a way to improve its fragrance and flavor (optimal at 43.5℃). It has been assessed that culture aeration intensifies the yield, especially when the air is admitted into the fermentation tank while the culture is being stirred.

The efficient growth of *Morchella* mycelium was achieved by the aeration rate of 0.1 mM O_2/L/min. It is worth noting that the nutritious value of the product surpasses that of the *Morchella* fruiting bodies, found in their natural environment. According to Robinson and Davidson (1959), *Morchella* culture may be developed from the colony on a slant. The medium in a 250 ml flask was inoculated with the sterile homogenate and incubated at shaking (100 revs/min). Lower speeds caused the mycelium large agglomerations and their collecting in the flask neck; but higher speeds did not yield granules. After 3 days incubation, the content of the flask was used to inoculate the medium of a 7 L bottle. Then the material was further used to inoculate the medium of a 20 L fermentor. Consequently 400 and 75,000 L fermentors were inoculated. The incubation in big fermentors lasted 3 days at 25℃. This temperature gave the best results and substantially reduced the danger of infection. During incubation the pH of the culture lowered together with sugar consumption. When there was no more sugar left, pH began to rise, and that proved to be the best moment to collect the mycelium. Mycelial beads of 0.5-2.5 cm in diameter were received. From the 7,500 L fermentor containing 5,700 L of the medium 1.5-2.0 tons of wet mycelium were obtained. The product was rinsed with water in order to remove the smell coming from the fermented support. The obtained mycelium was dried powdered or pressed, and finally sold.

Another example of commercial production of mycelium is the *Fusarium graminearum* culture carried out in England (Solomons and Scammel, 1975; Newmark, 1980). The biomass produced in the continuous culture contained 45%of protein of a very advisable amino acid composition and a very low level of fat and cholesterol. An appropriate method, in which the mycelia are immediately heated up 64℃ for 20 min, allows for nucleic acids elimination. Under these conditions proteolytic enzymes are inactivated so that there is no breaking down of protein, while thermo-stable nucleases are stimulated to be active. The degradation products of nucleic acids can be washed off the cells. The yearly production of biomass amounted up to 100 tons. The

fungus was grown in the continuous culture at 30℃ in 1,300 L fermentation tanks in the medium containing glucose syrup and ammonia as the source of carbon and nitrogen respectively. The incoming myco-protein was the first product, which could be used for human consumption.

Polish contribution

Pilot attempts to cultivate fungi on a semi-technical scale have also been successful in our laboratory (Paszczynski *et al.*, 1975; Leonowicz *et al.*, 1978a). Fermentation tanks of 700 and 1,250 L were used. *Innonotus obliqous* mycelium was cultivated for 48 hours in the medium containing distillery brew (3.71% dry mass), 0.2% beet molasses and 0.2% calcium nitrate at the aeration rate of 10 L per hour for one liter of the culture. The stirring rate was 100 revs per minute. The yield was 250 kg of wet mycelium per 1,000 L of the medium i.e. 13.4 kg of dry mass including 6.2 kg of pure protein. During cultivation, the mycelium grew in the form of beads, which in the process of growth underwent spontaneous comminuting, so there was no need for homogenization when transferring the inoculum. After drying at 65℃, composition of the amino acids was determined and nutrition tests on animals were made. The hydrolizate included amino acids important for nutrition such as methionine, lysine and phenylalanine. Nutrition tests made on mice, rats, hamsters, and chickens in our laboratory have shown full applicability of biomass as protein supplementing animal feed. No side effects on health or growth rate were observed in pigs, which readily consumed the product (Paszczynski *et al.*, 1975; Leonowicz *et al.*, 1978a).

The results of laboratory research carried out by some other authors in Poland are also very encouraging. It was reported that the submerged culture of *Pleurotus ostreatus* in liquid mineral-saccharose medium, enriched with whey and sulfite waste liquor, produced 14 g of dry mass of mycelium per liter of the medium during 3 days (Urubanek *et al.*, 1977). It was also reported that *Trametes versicolor* and *Tyromyces albellus* in 120 hr submerged, aerated cultures on whey amounted 217 and 162 g of dry mass per liter of the medium respectively. It is important to note that an appropriate dilution of whey played a crucial role for the mycelium yield (Lobarzewski, 1976). Patent literature provides numerous examples of biomass production using the vegetative mycelium culture. It results in many reports describing as well the way of mycelium cultivation for biomass production using various byproducts *e.g.* sulfite waste liquors (Trojanowski and Leonowicz, 1974), distillery brew (Leonowicz *et al.*, 1974; 1977a; 1977b), molasses (Illnicka-Olejniczak and Malanowska, 1979), whey (Leonowicz and Trojanowski; 1975; 1977a; 1977b), sawdust (Trojanowski *et al.*, 1976) and straw (Majchrzak, 1977; Trojanowski and Leonowicz, 1980), as the optimization of fungal growth.

Mycelial biomass quality

Protein and amino acids

The protein content varies within extremely wide limits under different cultural conditions for any organism. It ranges some percent of mycelium dry mass. No precise data on the protein

content are available due to difficulties in complete extraction of the protein from the mycelium and the content of chitin. Thus an arbitrary figure of N×6.25 is fallaciously employed to determine the protein content in mycelium. Therefore some works suggest that nitrogency coefficient should range from 4.05 to 4.45 or even lower. For example in the case of *Agaricus bisporus*, the suggested value is 3.0; for *Cantharellus uberius* 2.8; for *Tricholoma equestra* 2.8 and for *Armillariella mellea* 3.4 (Grzybowski, 1978). However, Fitzpatrick *et al.* (1946) proposed in some examples of protein calculation to replace the commonly used coefficient 6.3 by 8.5. In such calculations also the level of digestive factor (NPUm *i.e.* nitrogen protein utilization coefficient for fungal mycelium) for particular fungal proteins should be taken into consideration. This value for *Boletus edulis* equals 64.9 and is 100% as high as that of *Agaricus campestris*. Despite the fact that the protein of *Boletus edulis* and *Agaricus campestris* are less valuable than the other vegetable proteins, it was established that when added to corn products, they substantially improve the biological value of gluten (Rafalski *et al.*, 1966). According to our experiments it was possible to replace the conventional protein source of animal feed, by the fungal mycelium (Leonowicz and W-Wasilewska, 1975).

The chemical composition of the culture medium has no bearing on the protein quality though it has an effect on its content in the mycelium. For example *Agaricus campestris* accumulates 50.4% of protein, when nitrogen comes from casein hydrolisate and only 45.0% when ammonium nitrate is the source of nitrogen (Guha and Banerjee, 1970). *Trametes versicolor* accumulates 29-37% of protein in the mycelium with the same amino acid composition regardless of the kind of medium. The protein content in the cultures grown on molasse wastes ranged 30-58% (Rehacek *et al.*, 1962). The mycelium of the submerged culture usually contains more protein than in fruit bodies. Its digestibility is also much higher. For example the fruiting bodies of *Pleurotus ostreatus* contain 25% of protein, and its digestibility is 54%, whereas for mycelium these values are 76.2% and 84.7%, respectively (Grzybowski, 1978).

Mushroom protein is believed to be less nutritionally complete than meat protein due to its relatively lower contents of certain amino acids. Although mushroom protein contains threonine, valine and phenylalanine in similar amounts to meat protein, it may be slightly inferior in isoleucine, leucine, lysine and histidine. Mushroom protein has relatively more lysine and tryptophane than most vegetable proteins (Diem and Lenter, 1970). The protein hydrolizates of vegetative mycelium contain almost all amino acids, which can be found in other plant materials (Bano *et al.*, 1963; 1971; Lasota *et al.*, 1968; Mladecki *et al.*, 1968a). Indispensable amino acids usually occur in an acceptable amount (Mladecki *et al.*, 1968b). The amino acid composition is comparable to that of eggs or yeast and sometimes particularly in relation to lysine and tryptophane even better (*Tricholema nudum* and *Trametes versicolor*) (Block *et al.*, 1956; Norkrans, 1963; Bano *et al.*, 1971). The contents of amino acids undergo changes during fungal culture growth. For example the methionine was not discovered at an early stage of *Boletus edulus* cultivation though it appeared later on (Rehacek *et al.*, 1962). After 24 hrs of incubation the substantial increase of valine concentration was noticed though it did not appear at the beginning (Rehacek *et al.*, 1962). Some experimental data for mycelial amino acid composition

in comparison with that found in eggs or yeast and those recommended by FAO are given in Table 1.

Nucleic acids

The important, however, negative factor which limits the application of microorganism proteins as a source of feed or food is the content of nucleic acids. Their consumption in larger quantities affects the kidney diseases, *e.g.* urinary gout. As mentioned earlier, the amount of nucleic acids in the cells of higher fungi is substantially smaller than in bacteria and yeast. According to the data shown in Table 2 it is about 10 and 50 times smaller than in yeast and bacteria, respectively (Grzybowski, 1978). On the other hand, the positive effect of some nucleic acid metabolites is observed. Various purines and nucleotides cause specific "meat" aroma of mycelium, which makes fungi more attractive as food compared with other microorganisms (Reusser *et al.*, 1958b).

Table 1. Amino acid composition of some fungal mycelia in comparison with other protein sources and FAO recommendation (Rehacek *et al.*, 1962; Norkrans, 1963; Bano *et al.*, 1963; 1971; Lasota *et al.*, 1968; Mladecki *et al.*, 1968a; Mladecki *et al.*, 1968b; Leonowicz and W-Wasilewska, 1980).

Amino acid	*Tricholoma nudum*	*Inonotus obliqus*	*Trametes versicolor*	*Chaetomium cellulolyticum*	*Trichoderma viride*	Egg	Yeast	FAO recomendation
	g/100g of protein							
Arginine	4.2	3.0	4.9	-	-	-	8.6	-
Cystine	-	2.1	3.2	0.3	1.5	-	-	2.0
Histidine	2.6	1.5	2.2	-	-	-	2.8	-
Isoleucine	2.8	3.4	5.2	4.7	3.5	6.8	5.5	4.2
Leucine	6.0	6.3	9.3	7.5	5.8	9.0	8.3	4.8
Lysine	6.0	2.9	5.3	6.8	4.4	6.3	6.8	4.2
Methionine	1.6	1.7	1.7	2.3	1.4	3.1	2.6	2.2
Phenylalanine	3.4	3.2	4.6	3.8	3.7	6.0	4.5	2.8
Threonine	3.4	3.6	5.6	6.1	4.9	5.0	5.0	2.8
Trptophane	3.2	-	-	-	-	1.7	0.8	1.4
Valine	-	4.2	6.1	5.8	4.4	7.4	5.9	4.2

Table 2. Comparison of nucleic acid contents in dry mass of some fungal mycelia, yeast and bacteria cells (Grzybowski, 1978).

Organism	Nucleic acid content (mg/100 g)	
	RNA	DNA
Agaricus bisporus	404.5	116.1
Cantharellus cibarius	416.4	123.7
Helvella crispa	395.8	119.6
Yeast *Torula*	3,950	310.0
Escherichia coli	13,500	3,500

Carbohydrates

Carbohydrates appear in fungi as amino polysaccharide cell wall constituents in the form of matrix glycoproteins, free polysaccharides, some oligo saccharides, mono saccharides and sugar alcohols as well. The main cell wall component, chitin is a linear molecule constituted entirely of β-1, 4 linked N-acetyl glucosamine residues. The long chains of such units may achieve a molecular weight of cellulose (Foster and Webber, 1960). In the fungal cell wall there appear also other amino polysaccharides different from chitin *e.g.* polymer of galactosamine (Horold, 1962). Almost all free fungal proteins (including enzymes) appear in form of glycoptotein. They occur as a complex of amino sugar, mannan, glucan and protein. In the hydrolyzate of polysaccharide moiety, of *Neurospora crassa* laccase protein, mannose and glucosamine were found in large and small amounts respectively (Froehner and Eriksson, 1974). The structure of glycoprotein complexes is unknown, but it has been suggested that glucosamine serves as a link between protein and polysaccharides (Korn and Northcote, 1960). Among free polysaccharides, cellulose (Alexopoulos, 1962), glucans (*e.g.* glycogen (Kohlmunzer and Grzybek, 1972), pachyman (Warsi and Whelan, 1957) and lentinon (Chihara *et al.*, 1970)), mannans (B-Garcia and Nickerson, 1962) and polyuronides (Graham, 1960) are the main.

Among oligo saccharides the most popular is trehalose (Hegnauer, 1962), which appears in fruit bodies and mycelium as well in 90%of higher fungi (Paris *et al.*, 1957). Monosaccharides like galactose, glucose, fructose, mannose (Wakita, 1962; Kohlmunzer and Grzybek, 1972) and sedoheptulose (Benedict *et al.*, 1961) were identified in various fungal mycelia. Polyhydroxylalcohols (sugar alcohols) are represented by mannitol (first time discovered in Agaricus integer (Kohlmunzer and Grzybek, 1972), very popular in higher fungi (Hegnauer, 1962)), volemitol (isolated from *Lactarius volemus* (Kohlmunzer and Grzybek, 1972)), sorbitol (found in *Boletus bovinus* (Hegnauer, 1962)), erythriol (*Almilariella mellea* (Birkinshaw *et al.*, 1948)), arabitol (*Frustulina hepatica* and *Boletus bovinus* (Frejacque, 1943), inositol (*Ramariopsis croccea* (Kohlmunzer and Grzybek, 1972)) and xylitol (*Agaricus campestris* (Kratzl *et al.*, 1963).

The total amount of carbohydrates in higher fungi ranges from 28%dry mass of *Lactarius deliciosus* to 76% in *Armillariella mellea*. The dry mass of *Agaricus campestris* contained 24.9%of carbohydrates, where glycogen, cellulose, some reducing sugar and mannitol were dominant (Szuecs, 1958). Alcohols with their respective ketones, especially octene-1-on-3 and octene-2-on-3 are partly responsible for fungal aroma. These substances are still perceptible at very low concentrations reaching 10^{-3} and 10^{-2} mg, respectively (Reusser *et al.*, 1958a). Mushrooms also contain fiber fraction, the main component is probably chitin, N-acetyl-D-glucosamine residues, which is an important structural polysaccharide in the mushroom cell wall (Kreger, 1954; Michalenko *et al.*, 1976) and accounts for 0.5-0.6%of the fresh weight of sporophore. On the other hand, mushroom carbohydrates are in lower proportion than in other vegetables, and would provide only a very small of energy. This low energy value of the mushroom enables it to be used in low-calorie diets.

Fats

Relatively small amounts of lipids (0.1-0.3%fresh weight) are present in mushroom. The composition of mycelium lipids changes during the culture growth. Unsafonifiable fats accounted for a high proportion of the total lipids and were more abundant in the white variety of *Agaricus campestris*. A large amount of free sterol was present with smaller amount of triglycerides, 1,2- and 1,3- diglycerides, mono glyceride, phospholipid and a sterol esterol. In three *Agaricus campestris* varieties, 14 fatty acids were found as in Table 3 (Hughes, 1962). The mycelium of edible fungi contains various fat compounds where palmitic, oleic and linoleic acids are the main constituents (Leegwater and Craig, 1962). It is suggested that the fungal aroma depends partly on the autooxidation of these unsaturated fatty acids (Reusser *et al.*, 1958a). The amount of triglycerides in young cultures is 20%, whereas in the older ones decreases to 1.7%(Reusser *et al.*, 1958a).

Table 3. The fatty acid composition of three common varieties of *Agaricus campestris*.

Fatty acid	No. Carbon chain	White	Golden white	Cream
		Percent of the total fatty acids		
Capric acid	C10	0.34	-	0.31
Lauric acid	C12	2.55	-	1.37
Myristic acid	C14	0.98	0.26	0.80
Palmitic acid	C16	11.9	14.76	11.68
Margaric acid	C17	1.15	-	0.63
Stearic acid	C18	5.48	4.88	4.26
Oleic acid	C18	2.64	3.33	1.17
Linoleic acid	C18	63.42	73.88	74.14
Linolenic acid	C18	-	-	0.50
Arachidic acid	C20	9.04	2.28	3.45
Unknown				
Total		99.63	99.97	99.92

Table 4. Vitamin content in some fungal mycelia and yeast (Litchfield, 1964).

Vitamins	*Agaricus campestris*	*Morchella esculenta*	*Tricholoma nudum*	*Yeast (torula)*
	μ/g of dry mass			
Biotin	-	0.8	-	1.8
Folic acid	-	3.5	-	2.8
Niacin	146	82	150	500
Pantothenic acid	690	8.7	145	130
Pyridoxin	-	5.8	-	-
Riboflavin	34	24.6	53	49
Thiamin	2.0	3.9	11	6.2

Vitamins

The higher fungi are a relatively good source of some important vitamins. It has been established for example that 100 g of fresh fungi may provide 20%of human day demand of riboflavin (B_2) and 25%of niacin (Szuecs, 1958). The amount of riboflavin and niacin in fungi places them between higher plant and yeast, and in some cases (*e.g. Boletus edulis*) even higher (Grzybowski, 1978). A vegetative mycelium is especially rich in vitamins of B group. According to Bell *et al.* (1958) 5 % of *Morchella* esculenta mycelium in a synthetic diet fully satisfies the demand for the group B vitamins. It contains 9 vitamins this group, and niacin, panthotenic acid, choline and inositol as well. Vegetables are poor sources of the vitamin (Diem and Lentner, 1970) and B_{12} deficiency is commonly associated with strict vegetarian diets. Hayes and Hand (1981) were found that mushroom contains 0.32 to 0.65μg B_{12} per g of it, depending on the development stage and fruiting cycle. Table 4 shows a vitamin content in mycelia of some fungi in comparison with that of yeast. From the data by Diem and Lentner (1970), the vitamin content of mushroom comprises favorable with that of most vegetables which lack folic acid.

Minerals

There is little data for minerals on the composition of mushrooms. One of the most comprehensive mineral analyses of muton mushroom published by Varo *et al.* (1980) as in Table 5. Since more than 1% of the fresh weight of mushrooms is ash, the mineral content is generally higher than in many fruits and vegetables. Mushroom contains considerable amounts of K, P, Cu and Fe but do not contain appreciable quantity of Ca. A significant proportion of the recommended daily dietary need for P (Diem and Lentner, 1970), which is predominant in the gills of the sporophore, could be supplied by the mushroom. The same is also true for Fe, which is found mainly in the surface layer. Potassium, a mineral that is evenly distributed throughout the sporophore is present in quantity that 200 g mushroom could provide the full daily requirement of this element. Copper is most abundant in the outer layers and in the cap and gills. In general daily need for Cu in the adult diet is 1.5-2.0 mg and more than half of this could be supplied by a

Table 5. Mineral composition of *Agaricus bisporus* (Varo et al., 1980).

Element	Quantity/kg fresh weight	Element	Quantity/kg fresh weight
N	6.9g	Ni	0.02mg
K	6.2g	Cr	10μg
Ca	0.04g	Se	30μg
Mg	0.16g	Rb	4.2mg
P	0.75g	Al	14mg
S	0.48g	B	0.29mg
Fe	7.8mg	Hg	220μg
Cu	9.4mg	Cd	10μg
Mn	0.83mg	Pb	10μg
Zn	8.6mg	Ash	13g
Co	<5μg		

single meal. The mushroom supplies significant quantities of other elements associated with enzyme function, including manganese, molybdenum and particularly Zn.

Mushroom flavor

Flavor is one of the most characteristic features of edible fungi (Moustafa, 1960), and most important single factor to account for the historical and present day widespread consumption of wild and commercially available cultivated mushroom. It is not clear which chemical fractions are responsible for the flavor property of the mushroom. Some researchers reported that certain non-volatile substances may contribute to mushroom flavor and among these glutamate (Guha and Banerjee, 1970), short-chain fatty acids (Stauble and Rast, 1971) and carbohydrates (Litchfield, 1967) have been suggested. Proteins would be contribute to the overall flavor, as well as non-protein nitrogenenous substances such as nucleotides which are well-known flavor components in other foods. Altamura *et al.* (1970) identified some amino acids contribute to mushroom flavor, especially upon heating. By Maga (1981) mushroom flavor are closely related to the volatile fraction, and about 150 different volatile compounds were identified in varous mushroom species. Some of the important flavour volatiles are believed to be a series of eight-carbon (C8), and less volatile ten-carbon (C10) compounds as in Table 6. Tressl *et al.* (1982) reported that some of C 8 and C10 compounds could be formed enzymatically from linoleic acid and linolenic acids, both of which are normally present in mushrooms. The most important volatile component, 1-octen-3-ol, has a typical intensive flavor of many mushrooms, and is known as mushroom alcohol. It occurs as two typically active isomers, the natural (-) form having a strong flavor than the (+) form. Cronin and Ward (1971) reported mushroom like flavors detected from GLC, which could not be ascribed to a particular peak on a gas chromatogram. This suggests that there may still by unidentified compounds with mushroom flavor.

In order to achieve the stronger flavor it was proved that the culture must be grown one or two days longer than it was needed to reach the maximum of fungal growth. It was also intensified

Table 6. Relative concentration of some volatile compounds in fresh *Agaricus bisporus* mushroom and their organoleptic properties (Pyssalo, 1978).

Compound	Relative concentration (%)	Threshold value (ppm)	Flavor
1-octen-3-ol	33	0.01	mushroom-like
1-octen-3-one	0.02	0.004	boiled mushroom
trans-2-octen-1-ol	6	0.04	a little medical, oily, sweet
trans-2-octenol	0.05	0.003	sweet, phenolic
3-octanol	1	0.018	cod liver oil
3-octanone	4	0.05	sweet, fruity, musty
octanol	0.3	0.48	soap or detergent
1-octen-3-yl acetate	0.05	0.09	mushroom-like, soapy
1-octen-3-yl	0.4	0.022	rich odor, sweet, fruity,
propionate	0.05	0.09	mushroom-like
nonanol			soap or detergent, sweet

by mixing mycelium with NaCl and incubating at 4℃. After one day of such treatment some improvement of flavor was observed but the best results were achieved after seven days (Szuecs, 1954). The positive effects were also observed when lecitin or vegetable oil or milk was added into the growth culture (Szuecs, 1958). In order to achieve good flavor, the concentration of N, P, K, S, Fe and Zn should be far greater than that needed to obtain the maximum yield of the mycelium (Humfeld and Sugihara, 1952).

Much interest has developed in using flavor components of mushroom mycelium as flavor materials of the other foods. Grove (1981) has examined the volatile compounds from the mycelium of two commercial strains of *Agaricus bisporus* grown axenically on a semi-defined medium. The pattern of compounds from the mycelium of both strains was the same and was found to be similar to that of sporophore. It can be said that more concentrated medium and slower growth of the culture ensure stronger and more pleasant flavor. Research should aim at finding a mutant which could, while growing fast, produce desired flavor.

In addition any form of processing of mushrooms can be expected to change the some composition of the volatile components, many of which are chemically reactive. New compounds are formed during mushroom cooking. Picardi and Issenberg (1973) found that the main difference in volatiles of *Agaricus bisporus* present after cooking was an increase in content of 1-octen-3-one, a compound with a very strong flavor. This compound is believed to be formed by the oxidation of 1-octen-3-ol. Card and Avisse (1977) also found that benzaldehyde and 3-octanone concentrations increased while furfural and methylfurfural were newly formed. Various methods of drying mushrooms have been found to result in major losses (as much as 90%) of 1-octen-3-ol (Dijkstra, 1976; Sulkowska and Kaminski, 1977).

Medicinal value

Mushroom have been used an important folk medicine as a theraphy for cancer and the other adult diseases. In China, Korea and Japan Basidiomycetes of the Polyporaceae family were believed to have antitumor properties. Modern scientific examination of antitumor fungal products has led to the discovery of the antitumor activity of zymosan (Brander *et al.*, 1958) and several glucans and mannans (Mankowski *et al.*, 1957; Diller *et al.*, 1963; 1964) from the yeast cell wall.

Most of antitumor studies on mushrooms and its products have been concerned to fungal polysaccarides. A review by Chihara (1978) of fungal polysaccharides reported the antitumor and immunological properties of extracts from sporophores of popular edible mushroom in Japan, such as *Lentinus edodes, Tricholoma matsutake* and *Pholiata nameko*. These mushrooms contain polysaccharides that strongly inhibit the development of transplanted tumors in mice (Ikekawa *et al.*, 1969). Lentinan, one of 6 polysaccharides isolated from fruit body of *Lentinus edodes* has especially potent antitumor activity (Chihara *et al.*, 1970). Though fungal polysaccarides active against cancer cells may vary in their strength of activity, they all appear to act indirectly against cancer cells by restoring and stimulating the immune response of the host against cancer. Another compound having antitumor activity, retine, has been reported

(Chedd, 1967). Retine, the simplest member of a group of compounds known as α-keto aldehydes, is also present in animal tissues. The basis of the antitumor activity was thought to be related to its ability to react with-SH (thiol) groups and thus to inhibit protein synthesis. Anyway since fungal polysaccharides are virtually non-toxic and do not produce harmful side effects, it is expected that they will play an increasingly important role in cancer research.

Toxic elements

Although numerous fungal species have been used as food for thousands of years without any side effects, it is hard to assume that the mycelium at edible fungi grown in the submerged culture is equally safe. So far, its toxicity has not been observed (Bell *et al.*, 1958; Jennison *et al.*, 1957). On absorption experiments of toxic elements from polluted substrates, Enke *et al.* (1979) found that Cd and Hg were accumulated significantly but that Pb uptake was very low (Tyler, 1980). Other studies (Seeger *et al.*, 1976; Laub *et al.*, 1977) also reported that Cd accumulated in mushrooms. Ag, another toxic metal, was found relatively large amounts (5μg/g fr wt) in the sporophore (Ramage, 1930). Byrne *et al.* (1979) also showed that all species of Agaricus had high Ag content (10-133μg/g fr wt) with an average value of 30.5μg/g. But the effect of trace amounts of those elements, Ag, Ti, Va, Rb, and Li, on the diet have almost been ignored.

Factors influencing fungal growth and biomass quantity

Environmental conditions

Temperature

The higher fungi usually require lower temperature for vegetation than yeast and mould (ranging 25-28℃). It was established that even slight temperature changes substantially influenced the mycelium growth (Moustafa, 1960). The temperature higher than 30℃ usually inhibits the fungal growth, lower than 15℃ slows it down. Below 15℃ fungi still grow. The temperature dose to 0 ℃ usually does not kill higher fungi, but their growth in this case is extremely poor. The optimum temperature for *Agaricus campestris* ranges 25-30℃ (Moustafa, 1960; Fraser and Fujikawa, 1960), for *Boletus edulis* 25℃ (Grzybowski, 1977), for *Morchella* hybrida 20-25℃, for *Phlebia radiata* 28℃ (Hatakka *et al.*, 1986), for *Trametes versicolor*, *Pholiota mutabilis* and *Pleurotus ostreatus* 27℃ (Leonowicz *et al.*, 1978b).

pH

The higher fungi usually require lower pH of the culture than bacteria, but not so low as moulds. It ranges usually between pH 4.0 and 7.5. For example pH optimum for *Agaricus campestris* submerged culture is between pH 5.1 and 7.5 (Moustafa, 1960; Guha and Banerjee, 1970; Guha and Banerjee, 1971), for *Morchella* hybrida pH 4.0 - 6.2 (Litchfield,

1967), for *Boletus edulis* pH 4.5 - 5.5 (Grzybowski, 1977) and for *Polyporus anceps* pH 4.0 - 7.0 (Perlman, 1949). Wood rotting fungi like *Trametes versicolor*, *Pholiota mutabilis*, *Pleurotus ostreatus*, *Phlebia radiata* or *Phanerochaete chrysosporium* prefer slightly lower pH range 3.5-5.5 (Bollag and Leonowicz, 1984; Hatakka *et al.*, 1986; Asther *et al.*, 1988). The optimal pH of *Lentinula edodes* ranges 5.0-6.5 (Ohga, 1999b).

Aeration and stirring

Higher fungi usually require lower intensity of aeration and stirring than yeast and moulds. Sometimes they even prefer the stationary shallow culture to the submerged and agitated one giving in this case better aroma and producing more enzymes (Norkrans, 1963). The results obtained during the fermentor cultivation of *Morchella hybrida* show that it requires rather low speed of stirring (below 100 revs/min) and very weak aeration (0.08 -0.15 μM O_2 per liter of the medium per minute) in comparison with the other investigated fungi. For example *Agaricus campestris* grows the best in a fermentor when the speed of culture stirring is 180-350 revs/min and aeration about hundred per cent more intensive than in the case of *Morchella* (Litchfield *et al.*, 1963). In some experiments stirring of the fermentor culture lowered the mycelium yield, therefore aeration was only applied (Litchfield *et al.*, 1963).

Water potential

Water condition is one of the most important factors to the mycelial growth and fructification (Ohga, 1990; 1999a). A slight reduction of water potential (ψ), -0.5 MPa stimulated mycelial and colony growth on liquid, agar and sawdust-based substrates. *Lentinula edodes* has been found to grow well at a ψ around -0.5 MPa, which corresponds to a moisture content around 55%. The ψ of well-colonized mature substrate was -0.7 MPa before and -4.0 MPa after the fruiting. It is suggested that the water-holding capacity of a substrate is related to culture maturity. Excellent water-providing capacity (higher ψ) is expected in the substrate of well-matured cultures with a high density of mycelial colonization.

Substrate conditions

Carbon source

The carbon utilization by higher fungi depends not only on its source but on the other components of the medium as well. Presence of vitamins and relation of carbon to nitrogen in the medium seems to be of special importance. According to Perlman (1949) glucose utilization by *Polyporus anceps* and consequently mycelium growth is much greater (about four times) in the presence of thiamine. The Perlman's experiment also proved that this fungus utilizes starch twice as much as fructose. From Table 7 it can be seen that among low molecular sugars, mannitol is the best carbon source for both biomass and protein production by *Agaricus campestris*. It is also interesting to note that lactose and saccharose produced worse results. On the other hand, *Pleurotus ostreatus* as the best carbon source preferred glucose, especially, when fer-

Table 7. The effect of various carbon sources on biomass and protein yield in the submerged culture of *Agaricus campestris* (Guha and Banerjee, 1971).

Carbon source	Mycelium dry weight (g/l)	Protein content (%)
Mannitol	3.8	31.0
Glucose	4.4	28.2
D (+)-xylose	3.2	27.1
D (+)-fructose	2.6	28.0
Maltose	2.4	27.5
Lactose	1.9	27.0
Saccharose	1.4	24.0

mentor culture was additively supported with mollases (Hadar and Cohen-Arazi, 1986). In this case, besides glucose, sucrose, fructose, galactose and lactose were tested.

Though the laboratory experiments provided some information how to fit the best conditions for the cultivation of fungi, they cannot be used on a wider scale because of the high price. To avoid the high cost of low molecular carbon sources the conceptions are evaluated to utilize natural industrial byproducts like ligno-celluloses and wastes of organic origin. These experiments will be described in detail in the further part of the paper.

Nitrogen source

Utilization of various sources of nitrogen depends on individual features of the fungus. It was established that only few strains of higher fungi could utilize inorganic nitrogen sources (*e.g. Lentinus lepidus*, *Ptychogaster rubescens*). Other fungi prefer to utilize organic nitrogen sources, though with some differences: more of them grow better on amino acids but some prefer organic ammonium salts (*e.g. Fomes annosus* which grows the best in the presence of ammonium tartrate). Among inorganic nitrogen sources, ammonium phosphate seems to be the best utilizable, but with much lower extent than organic one (Guha and Banerjee, 1971).

Some natural products like yeast extract influence fungal demand for carbon and nitrogen sources. It depends on carbon and nitrogen ratio which proves to be the most favourable at 20-30 : 1 (Moustafa, 1960). The yeast extract serves probably as a vitamin source. The yield of *Agaricus campestris* biomass, grown on asparagine, phenylalanine or proline as a source of nitrogen, in the presence of thiamine or yeast extract was about 4 -10 times greater than that devoided of vitamins at all (Fraser and Fujikawa, 1960). The mixtures of various nitrogen sources are sometimes efficient *e.g. Tricholoma nudum* grows well on ammonium sulphate, ammonium chloride, urea, ammonium tartrate and nitrates. The best results for protein production are obtained with the mixture of ammonium sulphate, tartrate and chloride (Reusser *et al.*, 1958). However, the best source of nitrogen is aminoacids, especially valine, glutamine, asparagine, glutamic acid, aspartic acid or arginine. Lysine and β-alanine prove to be weaker nitrogen sources (Guha and Banerjee, 1971). As in the case of carbon, attempts are made to use

cheaper but equally effective nitrogen sources. The whey proteins and distillery brew may serve as examples (Leonowicz *et al.*, 1974; 1977a; 1977b; Leonowicz and Trojanowski; 1975; 1977a; 1977b). Some other byproducts of the food industry were also effectively used (Trojanowski and Leonowicz, 1974; Trojanowski *et al.*, 1976; Trojanowski and Leonowicz, 1980).

Mineral components

The mineral components of the medium like phosphorus, sulphur, magnesium and potassium influence not only yield of the fungal biomass but also its quality *e.g.* fragrance. It should be noted that optimum for biomass production usually does not coincide with that for aroma. For example *Agaricus campestris* culture grows best in the medium with 50 mg/L of phosphorus (Grzybowski, 1977). The optimum aroma, however, requires much higher phosphorus concentration (300-400 mg/L). In the case of potassium, the optimum concentration in the medium for biomass yield was 50 mg/L. In the absence of this component, the mycelium yield falls down by 10%. The best fragrance was obtained at the potassium concentration between 100 and 300 mg/L depending on the cultivation time. Sulphur gave the best aroma at the concentration of 200 mg/L of medium after 8 days cultivation. Magnesium at the concentration of 20 mg/L in the medium yielded the highest amount of mycelium (Grzybowski, 1977).

Plant oils and their constituents

The respective research shows that some natural oils, *e.g.* sunflower, cottonseed, soybean or olive added to the fungal medium at the concentration of about 0.2%, substantially improved the stationary culture growth. The similar effect was obtained with esters of fatty acids. Free acids, however, inhibited the growth of the submerged culture (Grzybowski, 1977). It is interesting that the positive effect of plant oils was observed also during ligninase production. This enzyme was highly stimulated by sunflower and olive oils (Asther *et al.*, 1988). Welsh onion also stimulates the growth and fructification (Ohga, 1986). Nucleic acids components contributed to the fungal growth of L. edodes (Ohga, 1988).

Lignocellulosic by-products as a support fungal biomass production

As mentioned, it is possible to cultivate higher fungi not only on expensive synthetic media but also on byproducts or even waste materials. It is important, therefore, to proceed in the search for cheap, abundant and easily accessible supports, which can simultaneously meet the physiological demands of fungi. One should mention the cheapest organic substance, which could be transformed into protein by fungi is ligno-cellulose - a component of a great number of industrial wastes, especially those of paper and wood industries. According to Bellamy (1974), the world production of cellulose by all plants on the earth amounts to 24 tons per capita a year. A meaning part of that mass used in paper industry is responsible for waste (mostly lignin waste) ranging to about 500 billion tons a year on a worldwide scale. Therefore it would be desirable to find such organisms, which could utilize the lingo-cellulose and especially their lignin component and at the same time eliminate any constraints in treating the material as a source of feed biomass. In fact,

these requirements are fulfilled by arboreal fungi for which ligno-cellulose is the natural growth environment.

Already in the 30s, the first attempts were made to determine the process of wood delignification caused by the activity of various white rot Basidiomycetes. It has been shown that *Trametes versicolor* (Campbell, 1930) and *Trametes pini* (Wiertelak, 1932) are the most active wood delignifiers. The second of above mentioned pioneer work in this field is of particular importance for us because it was prepared by the Polish scientist. Several years later, a more detailed analysis was offered on beech-tree sawdust delignification by such species as *Polyporus abietinus*, *Stereum rugosum* and *Marasimius scorodonius* (Robak, 1942; Fahraeus *et al.*, 1949). These works for the first time stressed the fact that delignification and cellulose decomposition were parallel processes. It took 9 months and the consumption of both components ranged from 45 to 65%depending on the species. This relatively low ligno-cellulose complex degradation results from lignin resistance to the biological attack. It has been stated that in the wood, cellulose fibers are surrounded with lignin forming a three-dimensional network, which protects cellulose from cellulolytic enzymes. As a result of such lignin barrier appearance, wood carbohydrates are not decomposed by the majority of carbohydrate degrading microbes (Fengel, 1971).

The problem of "lignin barrier" has been the focal point of a number of biotechnological studies. There are two possible ways of solving it. The first consists in using as a support, for the fungal culture, cellulose waste or its hydrolysates which can be chemically obtained or derived from the cellulase complex activity isolated from cellulose-degrading fungi. The second consists in using ligno-cellulose waste as a support for fungi possessing both ligno- and cellulolytic activity (in the case of only cellulolytic fungi, the extent of degradation was limited and the product will always contain indigested lignin).

More information about the first way can be found in many reviews and papers (Bellamy, 1974; Labendzinski, 1974; Leonowicz *et al.*, 1975; Ek and Eriksson, 1977; Seaman, 1977; Trojanowski *et al.*, 1977; Chahal and Wang, 1978; Moo-Young *et al.*, 1979; Chahal *et al*, 1979; 1983; Piotrowski, 1980; Matteau and Bone, 1980; Chahal and Moo-Young, 1981; Taniguchi *et al.*, 1982; Hatakka, 1983; Hatakka and Pirhonen, 1984; 1985; Eriksson, 1985; Tanaka and Matsuno, 1985). Therefore we shall limit our presentation to the most popular and typical examples of biotechnological methods already applied or intended to be applied soon. Murray Moo-Young of the Canadian Waterloo University developed a method by which paper industry waste is converted into glucose (Moo-Young *et al.*, 1977). This copyright process is carried out in special fermentation tanks for selected lower fungus species. The product is further used as a support for yeast cultivation and finally the valuable feed biomass is obtained (Can. For. Ind., 1975). Swedish researchers headed by Prof. Eriksson of the Forest Products Laboratory in Stockholm described a method of paper mill waste utilization by means of Sporotrichum pulverulentum. This culture gave biomass containing 14%protein. To improve the yield and quality of biomass a synergic culture of *Sporotrichum pulvelurentum* and *Candida utilis* was suggested (Ek and Eriksson, 1975). Another species *Trichoderma viride* could

vegetate efficiently on straw autoclaved previously with 5 %sodium hydroxide. After the 5 days growth, the protein content in product was 25%and the degradation of straw cellulose reached 75%(Peiterson, 1975). A method of *Paecilomyces variotti* culture on sulfite waste liquors from the cellulose production was patented in Finland and introduced on a commercial scale (the process "PEKILO"). In this method, carbohydrate fractions of the sulfite wastes are mostly consumed by the mycelium. The yearly yield of biomass (containing 55%protein amounts to 10,000 tons and is appropriated for animal feed (Romantschuk, 1974; 1976). An American patent which points to the possibility of the submerged culture on sulfite wastes liquor of such higher fungi as *Tricholoma nudum*, *Collybia velutipes*, *Lepiota neuryna* and *Agaricus blazei* is of a pioneering character. An agar mycelium is transferred into the medium containing sulfite waste liquor enriched with 0.1%ammonium phosphate and then moved again into the sulfite waste of a concentration in the medium corresponding to 15 g/L of reducing sugars, neutralized by calcium carbonate to pH 5 and enriched as in the previous case- with 0.1% ammonium phosphate. After 8 hrs of the aerated culture, the yield was fairly small and ranged to 10 g of dry mass per 1 L of the culture (Crestwood *et al.*, 1960).

Pretreatment of cellulose wastes with cellulase complex preparations is a very useful starting-point for further cultivation of specialized fungi. *Trichoderma viride* (especially QM 9123 and QM 9414 strains) is known as an efficient cellulase producer (Andren *et al.*, 1975), so its enzymatic system was used to obtain the glucose syrup from paper mill waste. This syrup (free of fungal metabolites) served as a support in biomass production for appropriate species of fungi or was used directly as a substrate in rafinery or other industry (Andren *et al.*, 1975; Wilke and Yong, 1975; Su and Paulavicus, 1975). Because paper mill waste contains a certain amount of lignin which renders cellulolytic enzyme access difficult, preliminary cultivation on those waste materials of *Trametes versicolor* widely known as a lignolytic species, was suggested. The partly digested waste substrate by *Trametes versicolor* no longer contains lignin and thus becomes more accessible for cellulolytic enzymes of *Trichoderma viride* (Yamanaka *et al.*, 1977). Some authors suggest application of cellulolytic fungus as a direct protein biomass producer (Moo-Young *et al.*, 1977). The thermo-resistant *Chaetomium celulolyticum* strain proves to be such a species (Chahal and Hawkworth, 1976). It surpasses *Trichoderma viride* by 100%as far as the vegetation rate is concerned and has the possibility to grow at a relatively high temperature (37℃). This temperature serves as a barrier against the invasion of many fungal species, which would infect the culture. However the attempts described so far do not authoritatively solve the problem of the lignin component of lingo-cellulosic supports. The remained not digested lignin substantially reduces the biomass nutrient value.

To take up the problem of lignin barrier (above mentioned second way) it is necessary to look for appropriate organisms or enzymatic systems directly degrading lignin. Another problem is to find an adequate model of the lignin polymer. Such a model preparation would serve either as a source of carbon for fungi or as a substrate for the lignolytic enzyme system. In the first case, the fungal biomass would be directly obtained but in the second it would be possible to achieve a better acceptable support for fungal growth. Moreover, the fungi growing directly on a lignin

model would produce the inducible enzymatic system useful in the second case. Lignin degradation products obtained in the enzymatic way, would serve as a valuable substrate for biotechnology. Recently, some authors suggest, that such adequate lignin model would to be the so-called lignosulphonic acids (Leonowicz *et al.*, 1985; Szklarz and Leonowicz, 1986). Such high-molecular compounds appear as the byproducts when cellulose is obtained by the sulfite method. In this case the whole amount of lignin in the form of calcium lignosulfonates soluble in water is transferred. Sulfite waste liquors are usually accompanied by simple saccharides in the form of free hexoses and high molecular pentosans, which can be eliminated by means of yeasting. Such a form of lignin preparation was used by several groups of authors as a source of carbon for lignin degrading fungi. Among these investigations, the worthy of remembering seems to be the following:

- The Polish patent (Trojanowski and Leonowicz, 1974), describing the way of cultivation of some known as lignin degrading basidiomycete fungi like *Trametes versicolor*, *Trametes pini*, and *Pleurotus ostreatus* in the lignosulphonates containing medium.
- The utilization of strain *Mycelium sterilium* (isolated by authors from Czechoslovakia) for very effective degradation of lignosulfonic acids (32-35%protein containing biomass was yielded (Jilek *et al.*, 1976)).
- The fungal utilization of sulfite waste liquor previously deprived of carbohydrates by yeasting was successfully performed in our country on a semi-technological scale. In this case the lignosulphonate containing medium was enriched with whey, yeast extract, sodium nitrate, superphosphate, some microelements and aneurine. *Trametes versicolor* culture was grown in a 4,000 L fermentation tank and the support was sterilized by overheated water steam. After 4 days incubation of the culture, the yield of mycelium reached 40 g of dry mass per 1 L (Trojanowski *et al.*, 1975).

The whole natural lignocellulosic material (byproducts containing both lignin and cellulose, *e.g.* various kinds of sawdust or various straws) was directly utilized in our laboratory by using of the fungal cultures. Preliminary screening tests proved *Trametes versicolor*, *Pleurotus ostreatus* and *Chaetomium piluliferum* grew the best on ligno-cellulose supports. The composition of the supports for these fungi was further optimized. Under the optimal conditions, 30 and 40% of lignin and cellulose degradation were achieved respectively (Trojanowski *et al.*, 1975). Possibility of bark utilization for fungal biomass production should also be mentioned. Acidic pine bark extracts were used to grow about 200 pure fungal cultures. Out of all tested species, 38 exhibited a fairly rapid vegetation process, among them the lignin degraders *Trametes versicolor* and *Polyporus brennis* (Uptergraff and Grant, 1975). In our laboratory, delignification of the pine bark was achieved in the stationary culture of some Basidiomycetes. The most active degrader was *Inonotus obliquus*, which removed from the support 23.3%of lignin and 29.5% of cellulose. Some industrial wastes which served as a nitrogen source stimulated the growth of the fungus and degradation of support significantly (Leonowicz *et al*, 1978).

Lignin, as pointed out, is extremely resistant to the attack of microorganisms. In the last two decades a substantial progress has been made towards the full examination of its biological degra-

dation (Buswell and Odier, 1987; Evans, 1987; Kirk and Farrell, 1987; Leonowicz *et al.*, 1987). First of all, a number of organisms responsible for that has substantially increased and it has been stated that not only white rot fungi are responsible for lignin degradation but soft-rot fungi (Haider and Trojanowski, 1975), brown-rot fungi (Ander and Eriksson, 1978) and bacteria (Haider *et al.*, 1978; Vicuna *et al.*, 1987) as well. Recently, a number of enzymes active in lignin degradation were markedly enlarged. Despite ligninase (considered as a main lignin degrading enzyme) (Litchfield, 1967; Kirk *et al.*, 1986; Hatakka *et al.*, 1987; Jonsson *et al.*, 1987; Miki *et al.*, 1987; Shimada *et al.*, 1987; Kantelinen *et al.*, 1988) cellobiose: quinone oxidoreductase (Westermark and Eriksson, 1974), manganase peroxidase (Glenn and Golg, 1985; Johansson and Nyman, 1987), methanol oxidase (Nishida and Eriksson, 1987), formaldehyde dismutase (Kato *et al.*, 1988), glucose- 2 -oxidase (Eriksson *et al.*, 1986), glyoxal oxidase (Kersten and Kirk, 1987), demethylase (Gold *et al.*, 1982; Frick and Crawford, 1983; Hatakka, 1985) and methylase (Frazer *et al.*, 1986) were discovered. Knowledge about a role of the other enzymes such as laccase (Leonowicz *et al.*, 1985; Szklarz and Leonowicz, 1986; Ohga *et al.*, ; Ohga and Royse, 2001), peroxidase (Lobarzewski *et al.*, 1982; Evans, 1987; Kersten *et al.*, 1987), glucose oxidase (Szklarz and Leonowicz, 1986; Leonowicz *et al.*, 1986), and dioxyganase (W-Wasilewska and Luterek, 1987; W-Wasilewska *et al.*, 1988) participating in lignin degradation has markedly increased. The cooperation among these enzymes is taken into consideration (Westermark and Eriksson, 1974; Lobarzewski and Paszczynski, 1985; Szklarz and Leonowicz, 1986; Leonowicz *et al.*, 1986; Evans, 1987). In this case, simultaneous transformation of cellulose and lignin by means of cellulase and oxygenase complexes may occur (Westermark and Eriksson, 1974; Leonowicz *et al.*, 1986; Leonowicz *et al.*, 1987; Ohga and Royse, 2001). The existence of the enzyme connecting the two metabolical sequences *i.e.* cellobiose:quinone oxidoreductase (Westermark and Eriksson, 1974) or glucose oxidase (Leonowicz *et al.*, 1986) was shown. All these achievements give a hopeful outlook for solution of the "lignin barrier" problem in no distant future.

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きのこの食材および漢方薬としての可能性

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抄　　録

高等菌類であるきのこについて，蛋白源や食材および漢方薬としての活用への可能性について総説した．きのこの生産量は第二次世界大戦以後，急激に増加してきた．世界的な生産量の伸びは21世紀まで毎年８％の規模で増えつづけると予想されてきた．最近きのこの菌糸体を大規模に培養して，蛋白源や食材，癌や成人病に対する漢方薬として活用することに興味が持たれはじめている．液体浸漬培養によるきのこ菌糸体の大量培養が成功している．きのこは貴重な食材をしての価値を持ち続けるであろう．きのこは独特の香りを持っているばかりでなく，蛋白質含有量の観点からみると肉と野菜の中間である．またきのこはビタミン含有量が高く，数種の無機物が健康維持に有効である．近年，きのこや菌糸体の癌や成人病に対する効果について研究され，重要な漢方薬としての可能性が注目されている．高付加価値を持ったきのことして，菌糸体の生産や食材としての活用のために，種々の技術的な問題が解決されてきた．

キーワード：菌体量・漢方薬・食料・高等菌類・きのこ・蛋白質・浸漬培養・廃棄物利用

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