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The Influence Of Hydrological Processing Of Vegetable Material As The Basic Component Of Substrate, On The Conversion Of Substances By Mycelium And The Yield Of Fungus Ganoderma Lucidum (Curt.:Fr.) P. Karst.

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ABSTRACT

`The article contains information on the perspectives for using the methods of preliminary acid hydrolysis of a lignocellulosic substrate for the cultivation of a lingzhi mushroom. The studies which were the basis of the work, concerned the study of carbohydrates structure of the lignocellulosic substrate, the possibilities of their modification and partial destruction of polymers. The increase in the concentration of sugars and reducing substances in the hydrolyzed substrate is reliably shown in comparison with the control. The stimulation of the yield of fungi on the hydrolyzed substrate is shown. The hydrolyzed substrate provides a more effective conversion of the solid substance, and also determines the increase in the biological efficiency of growing the fungus.
Keywords: acid hydrolysis, lignocellulosic substrate, biotechnology, mycelium, cultivation of fungi, bioconversion.

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INTRODUCTION

In recent decades, research on the biology and possibilities of using the biotechnological potential of xylotrophic basidiomycota fungi, which have therapeutic and prophylactic properties, has become more active throughout the world. Medicinal products based on fungal metabolites can successfully substitute preparations of plant and animal origin that are similar in biological effect. According to a number of researchers, the lingzhi mushroom (Ganoderma lucidum (Curtis) P. Karst) is a species that "... demonstrated a phenomenal healing potential" [1,2]. The results of the study of the regulation possibilities of the productive potential of G. lucidum in artificial conditions can be used as a theoretical basis for the development of methods for the industrial cultivation of fruit bodies and for the production of metabolites of different chemical nature and direction of biological action. The process of artificial cultivation of G. lucidum is associated with a number of technical difficulties. Special attention should be paid to the selection of the optimal formulation of the substrate, which should take into account the trophic needs of the species [3,4]. We have developed a promising substrate, as well as a method for growing fruit bodies of this species [5,6]. However, an optimization of the organic substrate, which will increase the coefficient of bioconversion of the substance, seems to be a promising direction of research [7].

RESEARCH METHODOLOGY

The subjects of the study were 12 strains of G. lucidum. Cultures of fungi cultivated in Petri dishes were incubated in a thermostat at +24 - + 26 ° C. Cultivation in deep conditions was carried out in Erlenmeyer flasks on a circular shaker at a rotation speed of 225 rpm with an eccentricity of 2.5 cm, and a temperature of +24 - + 26 ° C. The glucose concentration in the substrate was determined by the glucose oxidase method. Optical density measurements were performed on a KFK-3 spectrophotometer at a wavelength of 500 nm. The content of the mass fraction of reducing substances was determined by the method of McAnn and Shorel [8].

To carry out the hydrolysis of the substrate, the material was treated with a 0.4% hydrochloric acid solution with a 1: 2 hydro module and placed in an autoclave where it was exposed to a temperature of 120 ° C for 1 hour. Residual amounts of introduced acid were neutralized with calcium carbonate to pH 6.5-6.8. Similar amount of acid was added to the control samples, and it was neutralized in the same way. Distillation of fruit bodies was carried out according to generally accepted methods with intensive way of cultivation on solid organic substrates. Biological efficiency of growing fruit bodies was defined as the ratio of the raw weight of fungi to the dry mass of the substrate. According to this indicator, the yield of fungi is determined in various substrates with different composition and humidity [9,10]. The conversion ratio was calculated as the ratio of the dry weight of the fungi to the dry weight of the substrate. The values of this indicator allow analyzing the conversion of nutrients by the fruit bodies obtained on a hydrolyzed and regular substrate.

The statistical processing of the results was carried out with the help of a professional computer program for the processing and analysis of statistical data "Statistica 6.0". To assess the reliability of the influence of the structure and composition of substrates on the parameters to be determined, an analysis of the resulting data set (ANOVA) was carried out. In order to study the effect of the structure and the composition of substrates on some of the components being determined, a correlation analysis was performed. To assess the significance of the correlation coefficients obtained, Student's t-test was used at a significance level of 0.95 [11].

RESULTS

In the course of the experiments, the growth rate and the ability to utilize cellulose as the sole source of carbohydrates by selected fungal cultures were evaluated. In the experiment we used a model cellulose-containing material (filter paper) subjected to acid hydrolysis at a temperature of 120 ° C. The control was a medium containing a cellulosic material that had not undergone preliminary hydrolysis. The used substrates differed significantly in the content of the reducing substances (Table 1).

Table 1: Effect of cellulose hydrolysis on the content of reducing substances (including glucose) in the model material (p <0.05)

Variant	Content of a material air-dry weight, mg / g		
	cellulose	free glucose	reducing substances
Hydrolysis at120°C	872,1± 0,18	12,1±0,1	118,4±2,1
Control	980,5± 0,12	not detected *	10,2±0,1

*below detection level

A significant stimulation of fungal growth, expressed in a greater rate of development of the substrate, as well as the degree of expression of both air and substrate mycelium was noted in the experiment. From practical positions, it is advisable to study the development of the mycelium of the lingzhi mushroom on secondary lignocellulose substrates, which are waste from plant growing and wood processing. For this purpose, a lignocellulosic substrate based on straw of cereals was used in the experiment. The substrate was subjected to preliminary hydrolysis treatment using the same technological scheme as for pure cellulosic material. Taking into account the specificity of the polysaccharide composition of natural plant materials, the study of the substrate was carried out for the content of holocellulose (cellulose complex with hemicelluloses). The evaluation of the final results made it possible to reveal the comparability of the quantitative changes in holocellulose (in terms of its residual content and hydrolysis products) in a natural substrate with similar changes in the model pure cellulose material after hydrolytic action (Table 2).

Table 2: Influence of different regimes of hydrolysis of lignocellulosic substrate on the content of reducing substances (including glucose) in the substrate (p <0.05)

Variant	Content of a material air-dry weight, mg / g		
	cellulose	free glucose	reducing substances
Hydrolysis at120°C	672,5± 7,8	6,1±0,4	107,6±8,1
Control	770,9± 6,2	not detected *	9,5±0,6

*below detection level

In our experiments we studied the fruit formation of G. lucidum on lignocellulose hydrolyzed substrates. The positive effect of preliminary acid hydrolysis of substrates on fruit formation of the studied cultures is shown. In the control, lignocellulosic substrates containing cereal straw and hardwood sawdust in a ratio of 10: 1, neutralized with chalk or gypsum to the optimum pH, were inoculated with deep mycelium. In the experiment, preliminary acid hydrolysis of the substrates was carried out at a temperature of 120 ° C. The substrates subjected to hydrolysis were assimilated by the mycelium most actively, an earlier formation of primordia was noted, and then the most active formation of differentiated fruit bodies. The yield of fungi was calculated as the ratio of the raw weight of fungi to the moist mass of the substrate (Table 3).

Table 3: Effect of acid hydrolysis of lignocellulosic substrate on the yield of G. lucidum strains (p <0.05)

Strains of G. lucidum	Yield, g / kg of substrate		% to control
	Control	Hydrolysis at120°C	
Gl-1	156,0±5,1 (15,6%)	198,4±7,3 (19,8%)	+26,9
Gl-6	131,0±3,3 (13,1%)	149,0±6,9 (14,9%)	+13,7
GD-I-2	119,2±2,1 (11,9%)	142,1±6,3 (14,2%)	+19,3
GD-ун2	130,6±10,6 (13,1%)	164,4±6,0 (17,4%)	+26,2

Biological efficiency of growing fruir bodies was defined as the ratio of the raw weight of fungi to the dry mass of the substrate. According to this indicator, the yield of fungi is determined in different substrates in composition and humidity. The conversion ratio was calculated as the ratio of the dry weight of the fungi to the dry weight of the substrate. The values of this indicator allow analyzing the conversion of nutrients by the fruiting bodies of fungi obtained on a hydrolyzed and regular substrate. The yield of fungi on the hydrolyzed substrate, as mentioned above, exceeded the benchmarks by 13.7 ... 26.9%. Hydrolyzed substrate biological efficiency exceeded the control indicators by 11.7 ... 25.0%. Hydrolyzed substrate conversion factor was from 9.8 to 11.9% and significantly exceeded the benchmarks (Table 4).

Table 4: Influence of acid hydrolysis of lignocellulosic substrate on biological efficiency and conversion of nutrient substrate substances by G. lucidum cultures (p <0.05)

Strains of G. lucidum	Control			Hydrolyzed substrate		
	Mass fraction of dry substances in fruit bodies,%	Biological efficiency,%	Conversion rate,%	Mass fraction of dry substances in fruit bodies,%	Biological efficiency,%	Conversion rate,%
Gl-1	18,4	52,4	11,9	29,8	64,2	13,3
Gl-6	15,7	36,7	11,3	29,6	49,8	13,8
GD-I-2	15,9	33,1	12,8	26,5	42,2	15,4
GD-ун2	17,8	49,2	11,2	29,9	60,3	14,0

The formation of large single fruit bodies with a relatively low moisture content, the absence of pronounced fruiting waves, and the comparison of the results obtained with the information available in the literature on the large-scale cultivated species [7,8] reflect the characteristic biological features of G. lucidum. The process of acid hydrolysis made it possible to significantly increase the biological efficiency and the conversion coefficient of the substances of the substrate. Such methods of modification of the substrate can be justified for several reasons. First, there is an increase in the share of low-molecular, and therefore more affordable, carbohydrates, and secondly, hydrolysis does not selectively harm the contaminant forms of microorganisms contained in the substrate, bringing their numbers almost to zero, which allows further sterilization of the substrate in a softer mode. This makes it possible to preserve the presence of various biofactors in the substrate, which is expedient in the processes of industrial cultivation of edible fungi.

CONCLUSION

It can be recommended to use the process of hydrolysis of lignocellulosic substrate to increase the concentration of available sugars and as a result to intensify the growth and development of fungi. The use of such material is promising with intensive technology of cultivation of G. lucidum.

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