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Effects of cultivation conditions on the growth of the basidiomycete *Coriolus hirsutus* in a medium with pentose wood hydrolyzate

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Abstract

The effects of physical–chemical parameters of cultivation (temperature, pH, intensity of medium aeration and degree of shear under agitation) and concentration of reducing sugars (RS) on the growth of the wood-rotting basidiomycete *Coriolus hirsutus* were studied in a medium with pentose wood hydrolyzate. The values of these parameters, which were optimal for mushroom biomass formation, were found. Optimum concentration of hydrolyzate RS for mushroom growth was shown to be 20–25 g/l. The culture under study was able to grow (increasing pH) at high acidity of the medium, although the optimum level of pH was 5.6–5.8. Maximum specific growth rate of mushroom was recorded at high temperatures of 30–36 °C. Due to these particular features, the basidium fungus *Coriolus hirsutus* can be suitable object for biotechnology. During shake-flask fermentation with the aeration level of 0.9–1.2 g O_2/l h^{-1} and corresponding to aeration the intensity of shearing forces, the mushroom grew in the form of small loose and fluffy pellets, which had more points of growth on a surface. This probably resulted in an increase of biomass concentration under these conditions and the very high efficiency of substrate utilization ($\eta_{x/s} = 0.68$).

Keywords: Basidiomycete; *Coriolus hirsutus*; Pentose wood hydrolyzate; Physical–chemical parameters; Batch cultivation

1. Introduction

Wood-rotting basidiomycetes are widely used in biotechnology to make a decision in solving economics and ecological problems. Production of edible mushrooms is realized in a number of European and Asian countries [1]. Fruit bodies of the wood-rotting basidiomycete *Lentinus edodes* (shii-take) are produced long since and are used as food in Japan. Worldwide large-scale production of 16 species of edible fungi fruit bodies, nine species of which belong to wood-rotting fungi, is realized [2]. Moreover wood-rotting basidium fungi are used for production of biologically active substances and enzymes [3–5].

Wood-rotting basidiomycetes possess powerful enzymes complex. They play a significant role in decomposition of highly molecular compounds of wood, which inaccessible for other microorganisms, and, consequently, in utilization of wood residues in the ecosystem. In this line, wood-rotting basidiomycetes are used for utilization of lignocellulosic wastes and their enrichment by protein [6–8]. Stepanova and Muchin [9] suggested that, at present, wood-rotting fungi of order the *Aphyllophorales* are the only known group of microorganisms which can decompose wood quickly with total mineralization.

Earlier, wood-rotting basidium fungi of the order *Aphyllophorales* were tested and the culture *Coriolus hirsutus* was chosen, due to the highest growth rate on products of wood processing and the highest efficiency of substrate utilization [10]. The purpose of this investigation was to study the effects of physical–chemical parameters of cultivation on the growth *Coriolus hirsutus* in the medium with pentose wood hydrolyzate.

2. Materials and methods

2.1. Organism

The study was performed using wood-rotting basidiomycete *Coriolus hirsutus* belonging to the genus *Coriolus*, the family *Poriaceae*, the order *Aphyllophorales*. The culture was obtained from the mushroom culture collection, Botanical Institute, Russian Academy of Sciences. The

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culture was maintained on malt agar slants at +4 °C and transferred every 6 months.

2.2. Inoculum preparation

The inoculum for submerged fermentation of basidiomycete *Coriolus hirsutus* was grown within two stages: first, surface inoculum; second, submerged inoculum.

To obtain a surface inoculum small pieces (20–40 mm^2) of mycelium without agar from 10- to 15-day-old (32 °C) malt agar slants were transferred to conical flasks with glass beads (Ø, 9–11 mm). Flasks contained 100 ml of liquid medium. Glass beads provided no possibility for the mycelium to submerge into the medium. Flasks were maintained static for 10 days at 32 °C until mycelium film covered all the surface of the medium. Mycelium films were then disintegrated by means of beads when the flasks were shaking. The mycelium suspension obtained was the surface inoculum.

To prepare a submerged inoculum a 10% (v/v) of surface inoculum was used to inoculate the medium for submerged inoculum. The inoculum was grown in 750-ml flasks containing 100 ml of liquid medium. These were incubated on a rotary shaker ($n = 240$ rpm) at 28 °C for 4 days. A 10% (v/v) of submerged inoculum was used to inoculate the submerged batch cultures.

2.3. Growth conditions

Coriolus hirsutus (inoculum and fermentation) was grown in medium containing [in g/l]: nitrogen [in the form of $(NH_4)_2SO_4$], 0.6; KH_2PO_4, 1.3; K_2HPO_4, 1.4; K_2SO_4, 0.22; $MgSO_4$, 0.22. The medium contained reducing substances (RS) of hydrolyzate as the carbon source. D-Xylose was the main component of hydrolyzate (Fig. 1). The initial pH value of the medium for fermentation (except of those when the effect of pH on the growth was studied) was adjusted with 40% NaOH to 6.0. The batch fermentations of mushroom were carried out in 750-ml flasks containing 100 ml of liquid medium and incubated at 28 °C on a rotary shaker ($n = 240$ rpm). pH of the medium was not maintained during cultivation.

2.4. Analytical procedures

A weight method was used to determine the mycelium content in the culture broth. Dry weight of biomass was determined by filtering a known amount of mycelium suspension, washing the biomass with distilled water and drying at 105 °C.

The reducing substances contents in the medium and the culture broth were assayed according to the method of Somogyi and Nelson [11,12]. Assays of reducing substances in the culture broth were performed in samples where the mycelium was removed by filtration.

The sulphite method was used to determine the oxygen mass transfer in the medium in the shake-flasks [13].

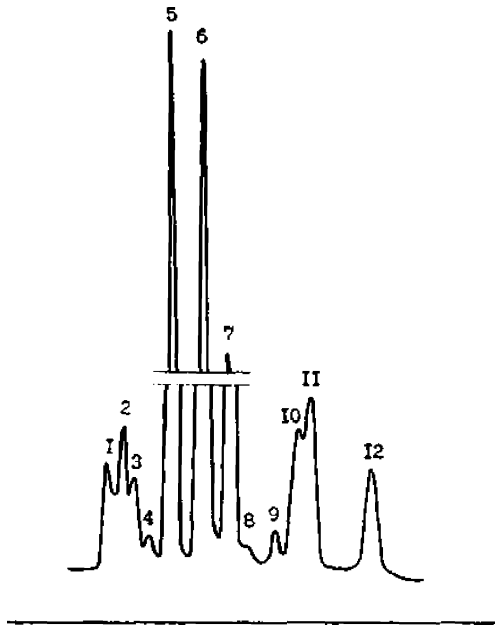

Fig. 1. Chromatogram of hydrolysate monosaccharides trimethulsilylderivatives: (1) β-L-arabinose; (2) γ-D-xylose; (3) α-L-arabinose; (4) γ-L-arabinose; (5) α-D-xylose; (6) β-D-xylose; (7) α-D-mannose; (8) γ-D-galactose; (9) α-D-galactose; (10) β-D-mannose; (11) α-D-glucose + β-D-galactose; (12) β-D-glucose.

Upper and lower pH limits and optimum pH for mushroom growth were assayed according to the method of Rypáčhek [14]: by placing the equal amounts of mushroom tissues in solutions with the various initial pH values, determining the final pH values after incubation and carrying out a graphic analysis of data obtained.

3. Result and discussion

3.1. Effect of concentration of hydrolyzate RS on mushroom growth

Accordingly to literature data [15], different concentrations of monosaccharides in medium are recommended for submerged cultivation of mushroom. To determine the influence of the hydrolyzate RS on the *Coriolus hirsutus* growth, basidiomycete was cultivated in the medium containing hydrolyzate ranging from 5 to 50.0 g RS/l. Varying RS concentration in the medium led to the alteration both of the maximum amount of biomass obtained and the duration of mushroom cultivation. In this case, specific growth rate was an inadequate criterion for estimation of culture growth efficiency. The specific growth rate is the rate of growth per unit amount of biomass, e.g. it is the reciprocal of the biomass amount. Therefore, the higher the RS concentration in the medium, the higher will be the biomass concentration in the medium (because of the presence of substances, which inhibit culture growth in the hydrolyzate, the concentration