0273–2289/87/1403–0221$01.80

Study of Operational Variables in the Submerged Growth of *Pleurotus ostreatus* Mushroom Mycelium

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Received May 12, 1986; Accepted August 4, 1986

ABSTRACT

Pleurotus ostreatus mushroom mycelium was cultivated in submerged culture in shake-flask experiments with acid extract from peat and yeast extract as nutrient sources. Different concentrations of water-diluted peat extract were tested in an attempt to overcome the effect of growth inhibitors apparently present in nondiluted peat extracts. The best results were obtained with a ratio of one part of peat extract diluted with one part of water. Several operating variables were studied to optimize the growth of mycelial biomass of *P. ostreatus*. The best results produced approximately 5 g/L dry biomass with a yield of 60% and an efficiency of 33%. These results were obtained in 8 d at 5% (v/v) inoculum ratio, 28°C, pH of 5.0, and 150 rpm.

Index Entries: *Pleurotus ostreatus*; mushroom mycelium; peat extract; submerged fermentation; fermentation operational variables.

INTRODUCTION

Although edible mushrooms are still collected wild in most parts of the world, their cultivation has been widely developed. The world production of mushrooms was estimated in 1981 as approximately 51.6 million kilograms (*1*). Among the edible mushrooms, *Pleurotus* species are considered a delicacy because of their flavor and taste (*2*).

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Several studies have been conducted in the past for the propagation of mushroom mycelium biomass in submerged culture. In 1950, Humfeld (3) observed that *Agaricus bisporus (campestris)* could be cultivated in the mycelial form in submerged culture. Szuecs (4) patented in 1958 methods for producing mycelium of several mushrooms. Other studies reported the use of a wide variety of substrates for the submerged production of mushroom mycelium (5–12). In addition to the objective of producing fungal mycelium protein, the main interest has been in the development of a flavoring agent, considering that mushroom flavor has been already accepted by the average consumer.

When subjected to wet-heat treatment using acid as a catalyst, peat produces a liquid extract. Peat extracts contain carbohydrates and other organic substances that could be employed as substrates for the growth of microbial populations (11–15). Recently, mycelia of *A. campestris* and *Morchella esculenta* mushrooms have been cultivated in peat extracts (11,12).

Although several studies have been conducted on the submerged culture of *P. ostreatus* mycelium (16–22), peat extracts have not been previously used as substrate for this fungus. This work reports the growth of *Pleurotus ostreatus* in acid extracts from peat and the optimization of several operating variables for this process.

MATERIALS AND METHODS

Culture

The culture of *P. ostreatus* was obtained from the Department of Plant Science, University of Western Ontario, London, Canada. It was maintained on slopes of peak extract–YM agar at 4°C and transferred every 4 mo.

Peat Extract

Ground sphagnum peat moss from Sundew Peat Bog, Newfoundland, Canada was mixed with 1.5% H_2SO_4 in a ratio of 20 g dry peat to 100 g solution and autoclaved at 15 psig (121°C) for 2 h. The extract was separated by pressing followed by filtration through Whatman filter paper.

Culture Conditions

The inocula for shake-flask culture were always prepared from fresh peat extract–agar slopes. The growth of one slope was blended with 50 mL sterile water for 30 s in a previously sterilized Waring blender.

The growth media consisted of peat extract supplemented with 5 g/L yeast extract and the pH adjusted by addition of 15N NaOH. Blended inoculum medium was aseptically introduced into 50 mL sterile growth

media in 250-mL shake flasks and were incubated in a Gyrotory water-bath shaker (model G76, New Brunswick Scientific Co., Inc., Edison, NJ). Inoculum ratio (2.5, 5, 7.5, and 10% v/v), temperature (19, 22, 25, 28, and 31°C), initial pH (4.0, 5.0, 6.0, and 7.0), fermentation time (6, 8, 10, and 12 d), agitation rates (100, 150, and 200 rpm), and peat extract:water dilution ratios (1:3, 1:2, 1:1, 1:0.5, and 1:0) were tested with the aim of obtaining the set of parameters that optimized the growth of *P. ostreatus* in peat extracts.

Total Carbohydrate Concentrations

The total carbohydrate (TCH) concentrations in the growth media before and after fermentations were determined by the anthrone reagent method (*23*).

Mycelium Dry Weight

The culture medium was filtered at the end of each fermentation process through oven-dried (105°C to constant weight), Whatman no. 1 filter paper. The filter paper with the mycelium was washed with distilled water and oven dried at 60–65°C to a constant weight, and the mycelium dry weight was found. The biomass yield was calculated as gram of mycelium dry weight produced per gram of TCH consumed, and the efficiency was calculated as gram of mycelium dry weight produced per gram of TCH supplied. Both yield and efficiency were expressed as percentages.

All reported results are the average of three determinations.

RESULTS AND DISCUSSION

Preliminary experiments were conducted to determine the appropriate substrate concentration (peat extract:water ratio) for the media composition and the best inoculum ratio (v/v). Then, the effect of temperature (5 levels), initial pH (4 levels), and agitation speed (3 levels) were investigated in 60 different sets of operating conditions. With the best set of temperature, pH, and agitation speed, new experiments were conducted to confirm the previously found best values of substrate concentration and inoculum ratio. Finally, different fermentation times were tested.

The effect on the growth of *P. ostreatus* of the different variables studied is presented in Table 1 and in Figs. 1–5 and discussed as follows:

Inoculum Ratio

The highest values of mycelium dry weight, yield, and efficiency were obtained with 5% (v/v) inoculum ratio (Table 1). The lower values for the growth parameters obtained at higher inoculum concentrations

TABLE 1
Effect of Inoculum Ratio on the Growth of *P. ostreatus* Mycelium[a,b]

Inoculum ratio, % v/v	Biomass dry weight, g/L	Yield,[c] %	Efficiency,[c] %
2.5	3.11 ± 0.16	43.02 ± 0.02	20.00 ± 0.02
5.0	4.98 ± 0.05	60.04 ± 0.01	32.02 ± 0.01
7.5	4.03 ± 0.14[a]	53.08 ± 0.05	25.99 ± 0.01
10.0	3.95 ± 0.03[a]	49.01 ± 0.03	25.05 ± 0.00

[a]In diluted peat extract, at 28°C, 150 rpm, 8 d of fermentation time, and initial pH of 5.

[b]Mean values ± standard deviations. Values in the same column with the same superscript are not significantly different at the 5% level.

[c]Percent yield and efficiency were subjected to angular transformation to find mean values ± standard deviations (*26*).

could be related to increased concentrations of inhibitory metabolites produced during the propagation stages and transported with the inoculum (*15*). An inoculum ratio of 5% (v/v) was used for the rest of the experiments.

Substrate Concentration

When peat extracts obtained from the hydrolysis process were inoculated with *P. ostreatus*, practically no growth was obtained. Because growth inhibitory effects have been observed with the use of peat extracts in the growth of mushroom mycelium, dilution of peat extracts has been attempted to decrease the inhibitors' concentrations (*24*). Although this dilution will produce a decrease of the nutrient concentrations present in the peat extract, this method was successful in allowing the growth of mushroom mycelium in the diluted media (Fig. 1). The initial TCH concentration in the nondiluted peat extract was approximately 30 g/L, which decreased proportionally in the diluted media, limiting the production of high biomass concentration in the process. Because the production of mushroom mycelium could be a more profitable operation than other fermented biomass productions, the resulting low biomass concentrations could be acceptable under specific, favorable conditions. Nevertheless, it appears that the removal of the growth inhibitors from peat extracts should be attempted to enhance the mycelial growth process.

Figure 1 shows that a dilution of one part of peat extract to one part of water produced the best results, with further dilutions hampering the growth, probably because of the lack of nutrients. The ratio of 1:1 peat extract:water was adopted as the dilution level for the experiments.

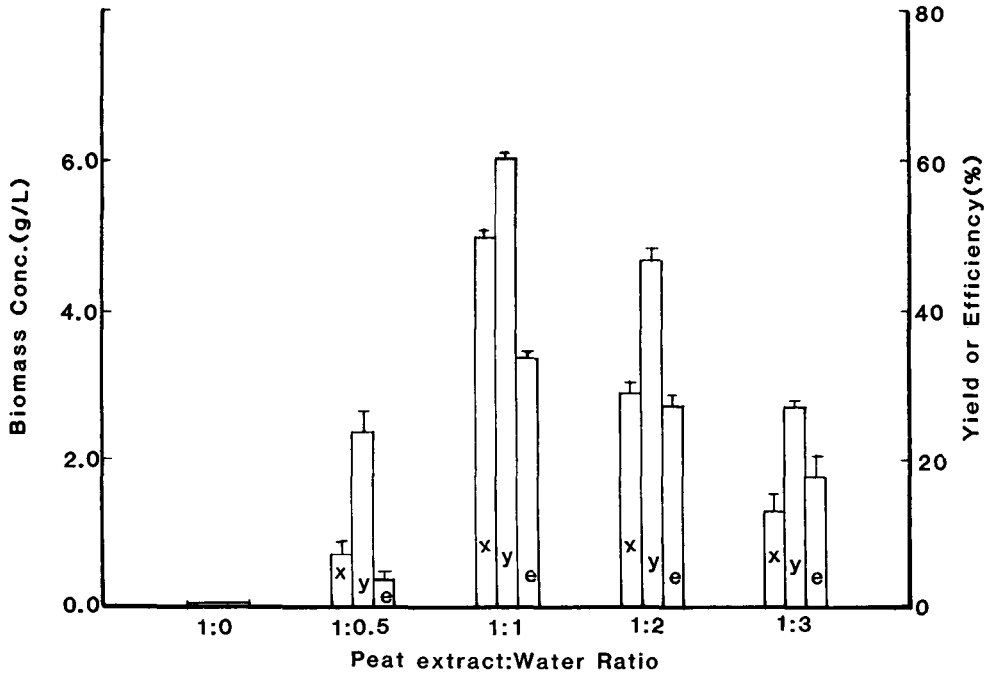

Fig. 1. Effect of substrate (peat extract) concentration on the growth of *P. ostreatus* mycelium at 28°C, 150 rpm, 8 d of fermentation time, and an initial pH of 5. x: dry biomass concentration; y: yield; e: efficiency.

Temperature

The range for optimum temperatures for the mycelial growth of *P. ostreatus* has been reported between 20–30°C (*1*). Figure 2 shows a smooth increase in the growth parameters when the temperature is increased from 19 ± 1 to 28 ± 1°C. At this temperature, the maximum values for the mycelial dry weight, yield, and efficiency were found. A further temperature increase to 31 ± 1°C produced a sharp decline in the growth.

Initial pH

The pH range reported for *Pleurotus* species varies from 5 to 6.5, with the optimum depending on the species (*25*). Figure 3 shows that an initial pH of 5 produced the best growth of *P. ostreatus* in a peat extract medium among the pH values tested. Further experiments in a pH controlled system are required to find the optimum pH value during the actual growth process.

Agitation Speed

Because of the filamentous form that characterizes the growth of fungal biomass, the possibility of enhanced mixing and dissolved oxygen

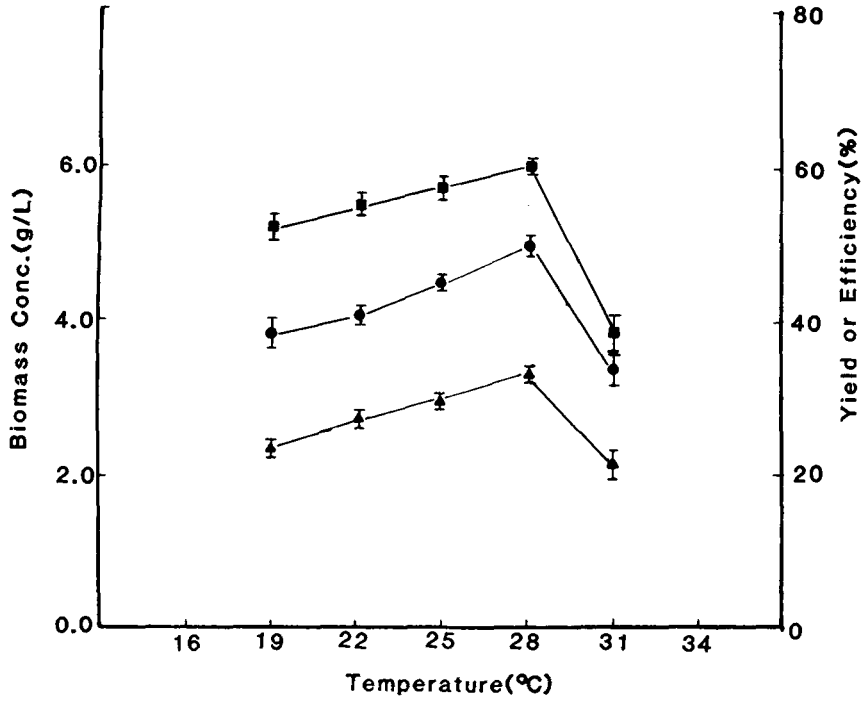

Fig. 2. Effect of temperature on the growth of *P. ostreatus* mycelium in diluted peat extract at 150 rpm, 8 d of fermentation time, and an initial pH of 5. Dry biomass concentration: ●; yield: ■; efficiency: ▲.

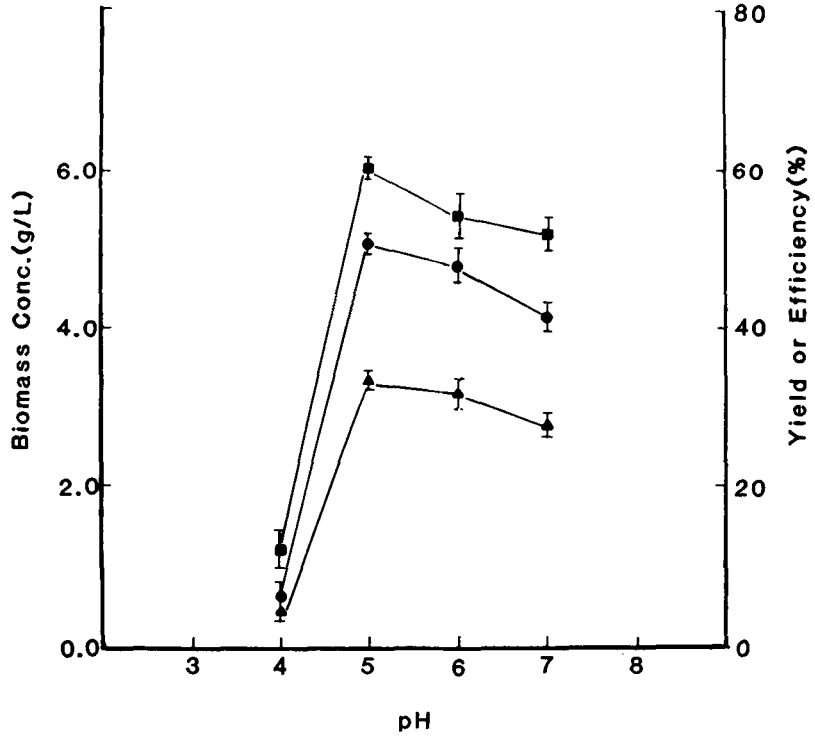

Fig. 3. Effect of pH on the growth of *P. ostreatus* mycelium in diluted peat extract at 28°C, 150 rpm, and 8 d of fermentation time. Dry biomass concentration: ●; yield: ■; efficiency: ▲.

concentration created by mechanical agitation is limited by the detrimental effect of increased shear stress on the mycelium. This phenomenon was already observed in the production of *A. campestris* mushroom mycelium in submerged fermentation (*11*). In this work, the maximum biomass concentration, yield, and efficiency were obtained at 150 rpm, with those values decreasing at 100 and 200 rpm (Fig. 4).

Fermentation Time

In general, the highest growth parameters were observed after 8 d of fermentation time (Fig. 5). Although the dry biomass concentration was higher at 10 d, the yield decreased, which indicates that the accelerated phase of growth has been surpassed. This optimum fermentation time is similar to those found for the submerged growth of other mushroom species (*11,12*).

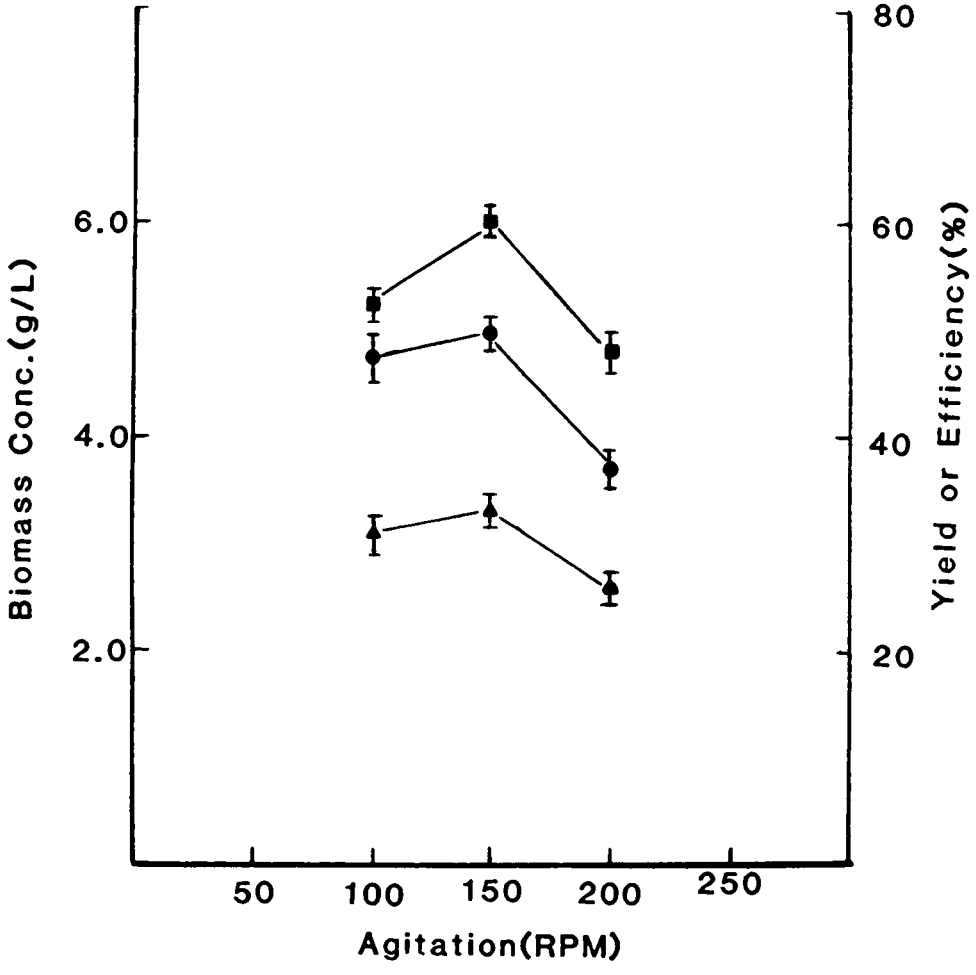

Fig. 4. Effect of agitation speed on the growth of *P. ostreatus* mycelium in diluted peat extract at 28°C, 8 d of fermentation time, and an initial pH of 5. Dry biomass concentration: ●; yield; ■; efficiency: ▲.

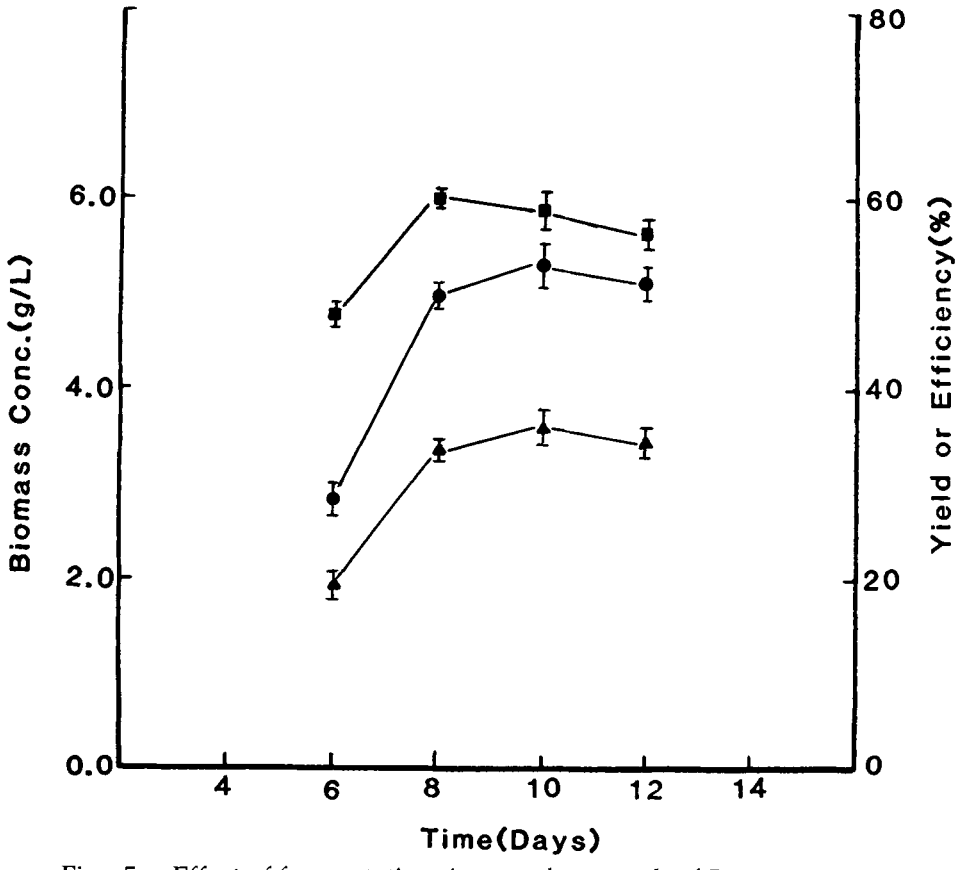

Fig. 5. Effect of fermentation time on the growth of *P. ostreatus* mycelium in diluted peat extract at 28°C, 150 rpm, and an initial pH of 5. Dry biomass concentration: ●; yield, ■; efficiency: ▲.

CONCLUSIONS

The present work has established the optimal range of values for the most important operational variables involved in the submerged growth of *P. ostreatus* mycelium in a peat-based culture medium. These results, together with the studies presently being conducted on the nutrient supplementation of peat extracts based on the specific requirements of *P. ostreatus*, will enable the scale-up of this process to aerated and agitated fermenters. The latter scale will facilitate further research on the *P. ostreatus* biomass production.

The numerical results obtained for the growth parameters suggested that part of the carbohydrates available in the media are not utilized by the *P. ostreatus* fungus. This is indicated by the low values of efficiency found, though the yield values at the best operating conditions were high. The complex chemical composition of peat might account for the presence of carbohydrate products, measured as TCH, not assimilable by the organisms used in this work. Therefore, in addition to the study of growth inhibitors present in peat and peat extracts, research on the

methods for more efficient hydrolysis and extraction of carbohydrates should also be conducted.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Natural Sciences and Engineering Research Council of Canada. The authors would like to thank Ms. Susan Legeza, Head Technician of the Department of Plant Sciences, University of Western Ontario, for supplying the culture of *P. ostreatus* used in this work.

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