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SUBMERGED GROWTH OF MORCHELLA ESCULENTA IN PEAT HYDROLYSATES

Antonio M. Martin
Department of Biochemistry
Memorial University of Newfoundland
St. John's, N.F. Canada A1B 3X9

SUMMARY

Sphagnum peat acid hydrolysates have been successfully tested as a culture medium for the submerged growth of the fungus Morchella esculenta in batch fermentations. When glucose and $(NH_4)_2HPO_4$ are added to the hydrolysate, the concentration of biomass produced is lower than that obtained from nonsupplemented hydrolysates having an equivalent total carbohydrate (TCH) content. Therefore, it appears that the concentration of some essential nutrients(s), as well as the TCH content, is affected by the conditions of hydrolysis.

INTRODUCTION

Submerged culture production of edible fungi mycelium has potential as a food or a feed supplement. Because of the pleasant flavor of the morels, the Morchella species are organisms suitable for testing in the production of mycelium. Several substrates have been utilized in the submerged growth of Morchella spp. (Litchfield 1967a, Kosaric et al., 1973) and studies have been conducted in assessing their nutritional and flavor properties (Litchfield, 1967b, Le Duy et al., 1974).

Peat consists primarily of organic residues rich in carbohydrates, minerals and other substances. Some countries with extensive peatlands are interested in the efficient utilization of this resource. Besides the principal use of peat as fuel, several works have been done concerning the possible utilization of peat extracts as culture medium for the growth of valuable microorganisms, emphasizing the production of microbial protein (Le Duy, 1979). Although peat is an important component in the traditional production of commercial mushrooms, no previous attempt has been made to utilize peat extracts as nutrient source for the submerged culture of

edible fungi.

This letter deals with the submerged growth of the fungi Morchella esculenta utilizing peat hydrolysates as substrate. Initial findings are reported; work in progress includes analyzing more characteristics of peat as a nutrient source for the submerged growth of mushrooms.

MATERIALS AND METHODS

Peat hydrolysates: Sphagnum peat moss from Sundew Peat Bog, Newfoundland, Canada, 62% moisture content (determined by oven drying at 105°C for 24 hours), was ground in a Waring blender for one minute. Peat hydrolysates were prepared by mixing ground peat with 1.5% H_2SO_4 (v/v) and autoclaving the mixture at 121°C for 2 hours. The final total carbohydrate (TCH) concentration was determined by the peat:H_2SO_4 solution ratio. The hydrolysates were obtained at room temperature by pressing the autoclaved product in a Carver Laboratory Press Model C (F.S. Carver Inc., Wis.) followed by vacuum filtration of the liquid fraction obtained through Whatman No. 1 filter paper in a Büchner filter.

Organism: Morchella esculenta NRLL 2603 from the American Type Culture Collection was maintained in stock cultures of potato dextrose agar medium (Difco) at pH 5.6. Slants were inoculated with a portion of the mycelium, incubated at room temperature (approximately 25°C) for 7 days and stored at 4°C. To adapt the organism to the peat hydrolysate medium, the entire growth of one slant was transferred aseptically to a sterile Waring blender containing 50 mL of sterile water and blended for 30 s. The resulting suspension was used to inoculate sterile peat hydrolysate in shaker flasks (5 mL of inoculum per 100 mL medium).

Culture conditions: The pH of the peat hydrolysate was adjusted to 7.0 by addition of 2.5N NaOH. Erlenmeyer flasks (250 mL) containing 100 mL of medium were autoclaved at 15 psig (121°C) for 20 min. After inoculation, they were incubated for 6 days at 24°C and 100 RPM in a Gyrotory water bath shaker (Model G76, New Brunswick Sci. Co., Inc.). The content of each flask was blended in a sterile Waring blender before being used as an inoculum. Batch cultivations were conducted in a 2L Bioflo bench fermentor (New Brunswick Sci. Co., Inc.). The fermentor was filled with 950 mL medium, sterilized at 15 psig (121°C) for 45 min and cooled prior to inoculation of the content with 50 mL M. esculenta containing suspension from a shaker flask. The fermentations were carried out at 24°C, 1 vvm and 200 RPM. The pH was adjusted before inoculation and maintained at 7.0 by addition of 2.5 N NaOH. Samples of 5 mL were taken each 4 h and

filtered through Whatman No. 1 filter paper. The filtrate was utilized in measurements of pH and analysis of TCH content. Each process was ended when no further consumption of carbohydrates was observed.

ANALYTICAL METHODS

Total carbohydrates: TCH in peat hydrolysates and in the fermented media were determined by the anthrone reagent method (Morris, 1948).

Mycelium dry weight: The culture medium after fermentation was filtered through oven dried (105°C to constant weight) Whatman No. 1 filter paper. The filter paper with mycelium was oven dried (105°C) to constant weight, and the mycelium dry weight determined.

RESULTS AND DISCUSSIONS

The yields of M. esculenta grown in batch fermentations with peat hydrolysates as the only substrate source are comparable to reported yields of the same organism on various supplemented substrates (Table 1).

Table 1 Yields obtained in the submerged growth of Morchella esculenta in different substrates

Substrate	Carbohydrate (%)		Yield (g dry biomass produced per g carbohydrate consumed.)	References
	Supplied	Residual		
Glucose	2.5	0.87	0.48	
Lactose	1.25	0.87	0.43	
Maltose	1.25	0.53	0.48	Litchfield 1967a
Cheese Whey	4.00	3.60	0.32	
Corn Canning waste	0.54	0.28	0.33	
Pumpkin canning waste	2.75	1.18	0.50	
Peat hydrolysate	3.30	1.50	0.37	This work, 1981
	2.10	1.10	0.35	

Peat is a complex material containing many potential nutrients. In addition to celluloses and hemicelluloses, N, P, K, Ca, Mg, Mn, Fe and Zn are present (Pollet, 1972). However, the value of a fermentation substrate depends on both the concentration of nutrients and their assimilation by the organism being studied. Quierzy et al. (Quierzy et al; 1979), working with Candida utilis in peat hydrolysates, observed that in batch cultivations only 55-

60% of initial TCH were consumed. They suggested that the residual carbohydrates are nonassimilable by C. utilis.

Litchfield and Overbeck (Litchfield and Overbeck, 1965) reported that the addition of phosphates to the substrate improves the yields of Morchella spp. Similarily, McLoughlin (McLoughlin, 1971) found that KH_2PO_4 significantly increased the yields of C. utilis grown in peat extracts, and Quierzy et al. (Quierzy et al., 1979) observed that the addition of nitrogen and phosphate resulted in better growth of C. utilis in peat hydrolysates.

To evaluate the effect of increased N and P concentrations on the growth of M. esculenta in peat hydrolysates, 5 g/L of $(NH_4)_2HPO_4$ were added to the two hydrolysates obtained (A: 33 g TCH/L, B: 21 g TCH/L). Fermentations were carried out as previously. In both cases no important increases in the final biomass concentrations were obtained. The yields of biomass produced to TCH consumed were practically the same as before. To determine if the growth limiting factor is the absence of assimilable carbohydrates, as suggested by the variation in biomass production with different initial TCH concentrations, new batch fermentations were conducted supplementing the hydrolysate B with 12 g/L glucose or with 12 g/L glucose plus 5 g/L $(NH_4)_2HPO_4$. In both instances, the initial TCH concentration of the medium was 33 g/L (i.e., the same TCH concentration present in the hydrolysate A). Table 2 summarizes the results of the batch fermentations conducted. Although the biomass concentration increased in the glucose plus $(NH_4)_2HPO_4$ supplemented medium relative to the same nonsupplemented medium, this concentration is also much lower that the biomass concentration produced by the peat hydrolysate with higher original TCH concentration (A).

Table 2 Results obtained in the batch fermentations of Morchella esculenta in peat hydrolysates.

Medium	TCH concentration (w/v)%		Total biomass produced (w/v)%	Yield (g dry biomass produced per g TCH consumed)
	Initial	Final		
Peat hydrolysate A	3.3	1.5	0.67	0.37
A + 5 g/L $(NH_4)_2PO_4$	3.3	1.5	0.68	0.38
Peat hydrolysate B	2.1	1.1	0.35	0.35
B + 5 g/L $(NH_4)_2PO_4$	2.1	1.0	0.40	0.36
B + 12 g/L glucose	3.3	2.2	0.35	0.32
B + 12 g/L glucose + 5 g/L $(NH_4)_2PO_4$	3.3	2.0	0.49	0.38

These results suggest that some other nutrient is acting as the growth limiting factor in the peat hydrolysate B medium. The apparent greater availability of this nutrient in peat hydrolysate A suggests that hydrolysis conditions expressed in different peat:H_2SO_4 solution ratios affect the release of other nutrient(s), as well as of carbohydrates.

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