J. Mycopathol. Res. 38(2) : 65–69 (2000) Printed in India

Effect of pH on submerged mycelial production by *Pleurotus* spp. in a medium with lignocellulosic biomass

R. MUKHERJEE AND B. NANDI

Department of Botany, University of Burdwan, Burdwan 713104, West Bengal

The optimal pH required for mycelial growth of two *Pleurotus* spp. in submerged culture was investigated. Lignocellulosic wastes such as water hyacinth biomass and haulms of brassica used as carbon sources, produced significant mycelial growth of both species. An initial pH 6.4 and 6.2 proved to be optimum for cultivation of *P. florida* and *P. citrinopileatus* respectively, in submerged culture. Mycelial biomass and mycelial protein production both increased gradually upto 32 days of incubation and slightly decreased thereafter in all tested pH levels. *P. florida* showed higher production than *P. citrinopileatus* on brassica biomass. Extracellular protein showed maximum peaks after 32 days by both the test fungi, more so by *P. citrinopileatus* in growth medium with water hyacinth biomass. Reducing sugar content increased gradually after 16 days as incubation proggressed more so on water hyacinth biomass by *P. citrinopileatus*. Results clearly demonstrated that an incubation of 32 days was optimum for both species and also that brassica biomass was a better carbon source among the two lignocellulosic substrates for mycelial protein production.

Key words : *Pleurotus* spp., water hyacinth, brassica haulms, pH, submerged culture

INTRODUCTION

Attempts to solve the problem of protein rich food shortage, in the age of fast population growth, through non-conventional manner have met with limited success. In this context, microbiological methods seem to have great promise through fast biomass growth and utilization of lignocellulosic (LC) waste utilization (Ek and Erikkson, 1973). For production of mushroom mycelium, low cost LC wastes can be used as potential carbon source because these fungi are equipped with efficient enzymatic apparatus capable of converting the substrates into acceptable form (Guha and Banerjee, 1971; Hatakka, 1983; Tanaka and Matsuno, 1985; Leonowicz *et al.*, 1988). So, stationary submerged mycelial cultures of particularly edible white rot fungi (WRF) has been carried in order to obtain either mycelial biomass or some nutritive substances viz. soluble sugars, vitamins or amino acids (Gorzybowski, 1977; Saxena and Rai, 1990; Burla *et al.*, 1992)

An aquatic weed, water hyacinth [WHB (B = Biomass)] and the haulms of brassica [BHB] are two cheapest organic substances or LC wastes which can be transformed into protein by WRF. *Pleurotus* spp. (WRF) were reported as optimal producers of mycelial protein for food and ruminant feed (Urbanek *et al.*, 1977 ; Mitra and Nandi, 1989; Lena and Quaglia, 1992; Bushwell and Chang, 1994). Environmental factors such as pH and temperature of the medium play an important role that can significantly influence growth and metabolic activities of mushroom mycelia. On that point, different research workers studied the effect of pH on submerged mycelia production by *Pleurotus* (Bollag and Leonowicz, 1984; Burla *et al.*, 1992; Lena and Quaglia, 1992).

In the present study, mycelial growth, mycoprotein production, secretion of extracellular protein and changes in reducing sugar content by two *Pleurotus* species were investigated under different pH values of culture medium where the principal carbon sources were WHB and BHB.

MATERIALS AND METHODS

Organism

Cultures of *Pleurotus florida* and *P. citrinopileatus* were obtained from Mushroom Research Centre, Government of West Bengal, Chinsurah, India and maintained on 2% malt agar slants at 4°C.

Medium and inoculum

Composition (g/L) of the basal medium used for

Table 1 : Effect of different pH on change of MB (mg), MP (% of D.W.), ESP (μg/ml) and RS (μg/ml) content in growth medium with two ligno-cellulosic biomass during submerged culture of *Pleurotus citrinopileatus* [± means standard errors]

A) On water hyacinth biomass

pH		Days of Incubation						
		4	8	16	24	32	40	48
6	Change of pH	5.3	5.5	5.4	5.3	5.6	5.6	5.6
	MB	2.9 ± 0.1	16.2 ± 1.5	38.5 ± 0.9	72.2 ± 2.5	121.7 ± 4.6	115.9 ± 5.6	109.0 ± 0.4
	MP	0.4 ± 0.2	4.4 ± 0.6	6.3 ± 0.3	9.9 ± 0.9	10.8 ± 0.8	9.3 ± 0.4	7.2 ± 0.6
	ESP	6.0 ± 0.4	22.8 ± 0.3	56.1 ± 0.1	54.4 ± 0.2	87.2 ± 0.5	72.6 ± 0.6	59.1 ± 0.2
	RS	34.1 ± 1.1	57.4 ± 0.8	27.2 ± 1.0	74.5 ± 1.8	184.1 ± 1.5	222.3 ± 1.2	247.6 ± 0.7
6.2	Change of pH	5.5	5.7	5.4	5.3	5.5	5.6	5.6
	MB	4.1 ± 0.4	17.3 ± 1.8	41.8 ± 1.2	80.7 ± 2.9	131.2 ± 3.5	130.6 ± 2.3	118.5 ± 2.6
	MP	1.4 ± 0.1	4.9 ± 0.9	6.8 ± 1.1	10.8 ± 0.8	12.1 ± 0.5	10.5 ± 0.2	9.4 ± 0.4
	ESP	8.2 ± 0.2	28.7 ± 0.1	67.3 ± 0.5	81.1 ± 0.3	119.8 ± 0.9	94.5 ± 0.6	72.2 ± 0.1
	RS	42.2 ± 0.7	62.6 ± 0.5	34.8 ± 0.9	83.1 ± 1.2	197.1 ± 1.9	257.3 ± 0.8	296.2 ± 0.4
6.4	Change of pH	5.6	5.9	5.6	5.4	5.6	5.7	5.8
	MB	1.8 ± 0.2	12.1 ± 1.1	32.6 ± 1.6	61.1 ± 2.1	108.3 ± 3.1	103.5 ± 2.9	93.2 ± 4.3
	MP	0.2 ± 0.1	3.8 ± 0.1	6.0 ± 0.9	9.1 ± 1.0	9.7 ± 0.7	8.8 ± 0.2	7.5 ± 0.1
	ESP	5.0 ± 0.4	20.2 ± 0.1	45.6 ± 0.3	49.1 ± 0.7	82.4 ± 1.1	70.5 ± 0.2	60.2 ± 0.5
	RS	31.0 ± 0.5	52.4 ± 0.2	22.1 ± 0.9	66.5 ± 0.5	166.2 ± 0.5	209.1 ± 0.3	204.8 ± 1.1
6.6	Change of pH	5.8	6.2	5.7	5.5	5.6	5.9	5.9
	MB	1.8 ± 0.5	9.3 ± 0.2	22.4 ± 1.4	44.6 ± 2.6	98.1 ± 2.1	100.1 ± 0.2	89.5 ± 0.5
	MP	—	3.1 ± 0.1	5.5 ± .03	8.7 ± 0.1	9.1 ± 0.2	8.2 ± 0.4	7.1 ± 0.1
	ESP	2.1 ± 0.3	17.3 ± 0.1	40.5 ± 0.5	35.9 ± 0.2	76.1 ± 0.4	61.9 ± 0.5	50.0 ± 0.4
	RS	24.2 ± 1.2	48.1 ± 0.3	19.0 ± 0.2	62.4 ± 0.9	153.0 ± 0.4	196.4 ± 1.1	216.2 ± 0.5

B) On mustard haulm biomass

pH		4	8	16	24	32	40	48
6	Change of pH	5.4	5.7	5.6	5.3	5.4	5.5	5.6
	MB	3.2 ± 0.5	15.8 ± 1.2	39.4 ± 2.8	76.1 ± 3.2	128.9 ± 4.1	122.3 ± 3.7	112.5 ± 2.5
	MP	1.1 ± 0.1	5.8 ± 0.3	7.1 ± 0.2	10.4 ± 0.5	12.4 ± 0.8	10.2 ± 0.1	9.2 ± 0.2
	ESP	4.0 ± 0.5	19.5 ± 0.6	52.1 ± 0.2	51.3 ± 0.1	78.6 ± 0.6	71.2 ± 0.1	55.0 ± 0.5
	RS	41.1 ± 0.2	52.0 ± 0.5	36.0 ± 0.4	69.4 ± 0.8	171.0 ± 0.5	208.2 ± 1.1	232.5 ± 0.3
6.2	Change of pH	5.5	5.7	5.5	5.4	5.5	5.6	5.6
	MB	4.8 ± 2.1	17.9 ± 1.7	43.6 ± 0.9	86.0 ± 0.5	141.2 ± 3.2	136.5 ± 4.5	122.4 ± 2.8
	MP	1.8 ± 0.4	6.4 ± 0.1	8.2 ± 0.9	11.6 ± 0.3	13.2 ± 0.5	11.5 ± 0.2	9.8 ± 0.1
	ESP	6.2 ± 0.6	22.9 ± 0.9	59.2 ± 0.3	69.1 ± 0.2	108.1 ± 0.1	87.4 ± 0.5	68.0 ± 0.5
	RS	49.0 ± 0.5	58.2 ± 0.3	39.5 ± 0.6	76.3 ± 1.2	179.7 ± 0.9	231.0 ± 0.5	267.1 ± 1.2
6.4	Change of pH	5.7	6.0	5.6	5.4	5.7	5.7	5.8
	MB	2.1 ± 0.2	13.4 ± 1.5	35.2 ± 2.3	66.0 ± 0.5	114.5 ± 3.4	109.3 ± 4.1	99.1 ± 2.5
	MP	0.8 ± 0.1	5.6 ± 0.4	7.2 ± 0.7	10.1 ± 0.2	11.9 ± 0.1	9.8 ± 0.3	8.8 ± 0.5
	ESP	5.0 ± 0.5	16.2 ± 0.9	41.0 ± 0.5	43.1 ± 0.9	80.1 ± 0.1	69.2 ± 0.3	57.1 ± 0.1
	RS	37.2 ± 0.6	49.0 ± 0.5	34.2 ± 0.1	59.7 ± 0.9	152.4 ± 1.6	187.1 ± 1.1	211.2 ± 0.9
6.6	Change of pH	5.8	6.1	5.6	5.5	5.6	5.8	5.9
	MB	1.7 ± 2.1	9.1 ± 3.1	24.0 ± 0.5	52.4 ± 2.6	102.3 ± 1.6	101 ± 0.5	93.6 ± 2.1
	MP	0.2 ± 0.01	4.2 ± 0.3	6.1 ± .01	9.2 ± 0.7	11.1 ± 0.1	9.1 ± 0.5	7.4 ± 0.2
	ESP	1.8 ± 0.1	14.1 ± 0.5	36.4 ± 0.2	28.5 ± 0.7	71.2 ± 1.1	62.3 ± 0.1	52.3 ± 0.1
	RS	29.0 ± 0.5	42.4 ± 0.1	23.7 ± 0.9	45.2 ± 0.2	139.1 ± 0.9	162 ± 0.5	203.4 ± 0.8

MB = Mycelial biomass ; MP = Mycelial protein ; ESP = Extracellular protein ; RS = Reducing sugar submerged fungal culture was : KH_2PO_4, 1.5; $NaNO_3$, 2; KCL, 0.5; $FeSO_4$, 0.01; $MgSO_4$, $7H_2O$, 0.5; L- asparagine, 1; Tween-80, 2 ml/L. To the basal medium, 4% water hyacinth or 4% haulms of brassica were added separately to serve as the sole carbon source. Four parallel sets with pH adjusted to 6.0, 6.2, 6.4 and 6.6 using 2(M) KOH solution after addition of the carbon sources were used.

Table 2 : Effect of different pH on change of MB (mg), MP (% of D.W.), ESP (μg/ml) and RS (μg/ml) content in growth medium with two ligno-cellulosic biomass during submerged culture of *Pleurotus florida* [± means Standard Errors]

A) On water hyacinth biomass

pH		Days of Incubation						
		4	8	16	24	32	40	48
6	Change of pH	5.2	5.6	5.3	5.3	5.5	5.6	5.6
	MB	1.0 ± 0.5	13.4 ± 0.9	29.5 ± 1.7	61.2 ± 1.1	104.7 ± 4.2	101.1 ± 0.7	96.5 ± 2.3
	MP	0.3 ± 0.1	4.1 ± 0.5	6.8 ± 0.2	9.3 ± 0.4	10.1 ± 0.8	9.2 ± 0.1	7.9 ± 0.1
	ESP	3.0 ± 0.5	18.2 ± 0.3	54.0 ± 0.5	51.1 ± 0.1	76.2 ± 0.7	62.5 ± 0.2	55.1 ± 0.4
	RS	22.0 ± 0.4	39.5 ± 0.9	15.2 ± 1.5	52.0 ± 0.4	129.2 ± 1.1	178.4 ± 0.9	205.7 ± 0.2
6.2	Change of pH	5.4	5.8	5.4	5.3	5.5	5.5	5.7
	MB	2.7 ± 1.2	14.3 ± 1.9	34.7 ± 2.5	74.5 ± 1.4	117.1 ± 3.6	111.3 ± 2.1	104.8 ± 0.9
	MP	1.6 ± 0.2	5.3 ± 0.6	7.5 ± 0.1	10.7 ± 0.2	11.8 ± 0.4	10.3 ± 0.1	9.1 ± 0.2
	ESP	5.0 ± 0.4	19.4 ± 0.1	55.6 ± 0.5	52.1 ± 0.9	89.4 ± 0.4	81.0 ± 0.5	69.3 ± 0.3
	RS	31.4 ± 0.4	45.7 ± 0.9	34.1 ± 0.5	65.3 ± 1.1	142.5 ± 1.9	196.1 ± 0.9	237.2 ± 1.3
6.4	Change of pH	5.6	5.8	5.5	5.4	5.5	5.6	5.7
	MB	5.0 ± 0.5	21.2 ± 1.2	58.7 ± 1.7	104.5 ± 2.5	149.3 ± 1.1	138.9 ± 4.1	123.2 ± 3.3
	MP	1.9 ± 0.7	5.8 ± 0.2	7.8 ± 0.4	11.2 ± 0.2	13.6 ± 0.6	107.0 ± 0.1	9.8 ± 0.2
	ESP	5.0 ± 0.5	22.2 ± 0.1	61.5 ± 0.3	59.9 ± 1.1	101.3 ± 0.5	82.1 ± 0.9	73.8 ± 0.2
	RS	36.2 ± 0.2	49.5 ± 0.6	37.1 ± 0.5	72.8 ± 0.9	169.5 ± 0.2	206.0 ± 0.4	251.4 ± 1.1
6.6	Change of pH	5.7	5.9	5.6	5.4	5.5	5.8	5.9
	MB	3.2 ± 0.4	15.2 ± 1.1	36.5 ± 2.1	71.1 ± 3.2	124.0 ± 0.9	118.7 ± 1.7	112.5 ± 3.5
	MP	1.1 ± 0.1	4.4 ± 0.5	6.2 ± 0.0	9.9 ± 0.1	12.1 ± 0.3	10.3 ± 0.4	9.4 ± 0.1
	ESP	3.2 ± 0.4	15.2 ± 0.1	51.1 ± 0.2	46.4 ± 0.5	71.1 ± 0.1	60.7 ± 0.9	51.3 ± 0.5
	RS	24.1 ± 0.1	33.4 ± 0.9	17.0 ± 0.5	42.3 ± 0.5	127.4 ± 1.1	170.6 ± 0.7	193.2 ± 1.1

B) On mustard haulm biomass

pH		4	8	16	24	32	40	48
6	Change of pH	5.2	5.5	5.3	5.3	5.3	5.4	5.5
	MB	1.4 ± 0.5	14.2 ± 1.2	33.6 ± 2.6	71.3 ± 2.5	112.5 ± 3.7	106.7 ± 3.1	98.1 ± 2.6
	MP	0.8 ± 0.1	5.0 ± 0.4	8.9 ± 0.3	10.2 ± 0.8	12.1 ± 0.5	10.6 ± 0.2	8.7 ± 0.3
	ESP	1.5 ± 0.4	15.1 ± 0.7	43.4 ± 0.1	37.5 ± 0.3	71.1 ± 0.9	59.5 ± 0.5	51.3 ± 0.7
	RS	17.2 ± 0.2	37.5 ± 0.1	19.7 ± 0.5	49.1 ± 0.9	121.0 ± 0.5	167.5 ± 0.7	192.3 ± 0.4
6.2	Change of pH	5.4	5.7	5.5	5.3	5.5	5.6	5.7
	MB	3.5 ± 0.1	15.8 ± 0.9	39.4 ± 1.2	82.0 ± 0.4	124.9 ± 1.6	120.3 ± 4.7	109.5 ± 2.3
	MP	2.1 ± 0.2	6.1 ± 0.5	9.0 ± 0.4	12.1 ± 0.6	13.5 ± 0.9	11.4 ± 0.1	10.2 ± 0.5
	ESP	3.2 ± 0.1	17.6 ± 0.3	49.5 ± 0.1	43.1 ± 0.2	76.5 ± 0.8	70.9 ± 0.1	64.0 ± 0.4
	RS	24.2 ± 0.7	39.7 ± 0.2	28.1 ± 0.9	57.3 ± 0.1	125.5 ± 0.3	181.1 ± 1.1	219.2 ± 0.9
6.4	Change of pH	5.5	5.8	5.5	5.4	5.4	5.6	5.6
	MB	7.1 ± 0.2	28.4 ± 1.5	69.2 ± 2.8	113.0 ± 0.4	161.9 ± 0.9	150.5 ± 1.1	137.1 ± 0.5
	MP	2.6 ± 0.1	6.6 ± 0.1	9.6 ± 0.2	12.9 ± 0.3	14.2 ± 0.5	11.9 ± 0.1	10.8 ± 0.3
	ESP	6.0 ± 0.4	21.1 ± 0.9	57.3 ± 0.3	48.3 ± 0.2	97.2 ± 0.6	76.9 ± 0.9	72.0 ± 0.4
	RS	37.4 ± 0.4	44.9 ± 0.9	35.2 ± 0.3	67.5 ± 0.1	153.7 ± 0.2	197.2 ± 0.5	239.1 ± 0.5
6.6	Change of pH	5.7	6.0	5.6	5.4	5.7	5.8	5.8
	MB	3.2 ± 0.4	15.9 ± 1.3	36.8 ± 2.1	89.5 ± 0.6	129.9 ± 3.6	122.3 ± 4.1	113.5 ± 0.7
	MP	1.4 ± 0.35.3 ± 0.98.6 ± .06		12.5 ± 0.3	13.7 ± 0.9	11.5 ± 0.5	10.5 ± 0.3	
	ESP	2.1 ± 0.1	15.5 ± 0.1	43.1 ± 0.9	37.9 ± 0.2	62.5 ± 1.1	51.4 ± 0.5	49.7 ± 0.1
	RS	19.7 ± 0.5	34.2 ± 0.3	23.5 ± 0.9	45.9 ± 0.3	107.1 ± 0.7	159.2 ± 0.7	173.5 ± 0.8

MB = Mycelial biomass ; MP = Mycelial protein ; ESP = Extracellular protein ; RS = Reducing sugar

Erlenmeyer flasks (250 ml) containing basal medium (50 ml) with the substrate were sterilized at 121°C for 20 min., cooled, inoculated with four agar discs which were cut out from the margin of 6 days old growing colony of individual fungus and incubated at 26 ± 1°C. The flasks were taken after 4 days and 8 days, and thereafter at 8 days intervals.

For production of mycelial biomass (MB), the flasks were kept in static condition, and for extracellular protein (ESP) and reducing sugar (RS) in gyratory shaker at 150 rev./ min.

Estimation

MB was harvested by filtering through copper net after thorough washing with cold water and dried at 60°C to constant weight (mg).

Fresh MB was used to extract mycelial protein (MP) following the method of Lowery *et al.* (1951). ESP and RS contents were estimated from the culture filtrate after cold centrifugation at 15000 g for 20 min.

MP (% of dry weight) and ESP (μg/ml) were estimated following dye-binding method of Bradford (1976) and RS (μg/ml) following Miller (1959).

The changes of pH during incubation was also monitored in all sets.

RESULTS

Production of MB, MP, ESP and RS as well as changes of the pH values of the media under submerged conditions have been shown in Tables 1 and 2.

The fluctuation of pH during the experiment were monitored by a rapid drop in the early period of incubation (4 days) which thereby increased upto 8 days., but again decreased between 9 days to 24 days and finally slightly increased but remained lower than the initial pH in all sets.

MB and MP content both increased gradually upto 32 days of incubation but slightly decreased thereafter in all tested pH levels. MP content did always correspond significantly to the MB. *P. citrinopileatus* showed highest mycelial growth as well as MP content at the initial pH 6.2 when BHB was used as sole carbon source [Table 1(B), 141.2 ± 3.2 mg MB, 13.2 ± 0.5% d.w. MP, after 32 days]. *P. florida*, on the other hand, showed maximum MB and MP content [Table 2(B), 161.9 ± 0.9 mg MB, 14.2 ± 0.5% d.w. , after 32days], which was even higher than *P. citrinopileatus*, at the initial pH 6.4 with BHB in the medium.

ESP content increased upto 16 days of incubation, then slightly declined between 17 days to 24 days, again rose between 25 days to 32 days and finally decreased markedly in all cases. ESP of both fungi showed maximum peak after 32 days at all tested pH levels. Maximum ESP was recorded at pH 6.2 after 32 days of mycelial growth of *P. citrinopileatus* on WHB [119.8 ± 0.9 μg/ml, [Table 1(A)]. *P. florida* showed highest ESP at pH 6.4 [Table 2(A), 101.3 ± 0.5 μg/ml] after 32 days on WHB in the growth medium which incidentally was lower than that by *P. citrinopileatus*.

RS content showed sharp decline between 9 days to 16 days at all pH levels but increased gradually thereafter till the end. Maximum RS in growth medium of both *P. florida* and *P. citrinopileatus* was recorded at the initial pH 6.4 [Table 2(A), 251.4 ± 1.1 μg/ml] and 6.2 [Table 1(A), 296.2 ± 0.4 μg/ml] respectively with WHB in the medium, while minimum at pH 6.0 and 6.6 respectively which was more lower in the BHB supplemented medium.

DISCUSSION

In the present study, patterns of pH change were characterized by a rapid fluctuations concominant with active growth phase of the mycelia and finally followed by a slight increase when active growth ceased. In all sets, MB gradually reached peak at higher values after 32 days due to increasingly longer log phase. During growth periods of both fungi at all pH levels, MP content increased gradually upto 32 days but decreased thereafter. The increment of MP content always corresponded to the production of MB.

The above mentioned type of pH change, particularly lowering pattern, were also observed by Saxena and Rai (1990), and Lena and Quaglia (1992) during submerged growth of *Pleurotus* spp. Semichavsky *et al.* (1985) reported that *P. ostreatus* had the ability to regulate the pH of the medium to the optimal range for growth. Burla *et al.* (1992) reported maximum biomass production by *P. ostreatus* with an initial pH value of 6 in the synthetic media.

During growth on the LC materials in the medium, mycelium of the tested organisms must have secreted various extracellular hydrolytic as well as oxidizing enzymes which accounted for the ESP production. ESP concentration initially increased, then slightly decreased for a short period, thereafter steadily increased showing the peak and finally decreased again possibly due to proteolysis or from lowering of enzyme secretion, by both fungi in all pH levels. In the present study, the optimum pH for ESP production was 6.4 and 6.2 for *P. florida* and *P. citrinopileatus* respectivelsy. Ghosh *et al.* (1998) reported lowering of ESP content in submerged cultures of *P. sajorcaju* and *P. ostraeatus* after 24 days and 32 days respectively. Bushwell and Chang (1994) studied protease activity in *Volvariella volvacea* and *Tricholoma lobayense* with an increasing days of incubation.

Patterns of RS change were characterised by an initial increment due to release of RS in the medium, then by a rapid drop corresponding with a phase of active primary mycelial growth arising from utilization of the simple sugars. This was followed by a gradual increase when products of cellulolysis and hemicellulolysis began to accumulate in the medium. Saxena and Rai (1990) recorded sharp decline of soluble sugars between 5 to 10 days but slower decline thereafter followed by and insignificant rise after 20 days during submerged growth of *P. sajorcaju* on rice straw. Lena and Quaglia (1992) reported lowering of RS content in submerged culture of *P. florida* with sugar beet pulp upto 14 days but did not make any further observation. They also concluded that both mycelial growth and sugar release could be positively correlated to the amount of LC biomass.

From the present study, it was evident that initial pH 6.4 was optimum for mycelial growth as well as production of ESP and RS by *P. florida* while pH 6.2 was optimum for *P. citrinopileatus*. Of the two LC biomass, BHB proved to be more effective substrate for maximum mycelial growth, but WHB for both ESP and RS production.

REFERENCES

Bollag, J.M. and Leonowicz, A. (1984). Comparative studies of extracellular fungal laccases. *Appl. Environ. Microbiol.*, **48**, 849-854.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protenin-dye binding. *Anal. Biochem.*, **72**, 218-254.

Burla, G. ; Grazillo, A.M., Luna, M., Cardelli, L.E. and Schiesser, A. (1992). Effects of different growth conditions on enzyme production by *Pleurotus ostreatus* in submerged culture *Bioresource Technol.*, **42**, 89-94.

Bushwell, J.A. and Chang, S.T. (1994). Biomass and extracellular hydrolytic enzyme production by six mushroom species grown on soyabean waste. *Biotechnol. Lett.*, **16**(12), 1317-1322.

Ek, M. and Eriksson, K.E. (1977). Conversion of wastes fibres into protein, in : T.K. Ghosh (Ed.), Proc. Int. Symposium on Bioconversion, Haus Khas, N. Delhi, India, Feb. 21-23, 1-6.

Ghosh, M., Mudherjee, R. and Nandi, B. (1998). Production of extracellular enzymes by two *Pleurotus* species using banana pseudostem biomass. *Acta Biotechnol.*, **18**(3), 243-254.

Grazybowski, R. (1977). The submerged culture of higher fungi as a potential protein sources *Przem. Spoz.*, **31**, 371-374.

Guha, A. K. and Banerjee, A.B. (1971). Effect of different carbon compounds on the submerged production of *Agaricus campestris* mycelium. *J. Food Sci. Techn.*, **8**, 82-83.

Hatakka, A.I. (1983). Pretreatment of wheat straw by white rot fungi for enzymic saccharification of cellulose. *Eur. J. Appl. Microbiol. Biotechnol.*, **18**, 350-357.

Lena, G. Di. and Quaglia, G.B., (1992). Saccharification and protein enrichment of sugar beet pulp by *Pleurotus florida, Biotch. tech.*, **6**(6), 571-574.

Leonowicz, A., Wojtas-Wasilewska, M., Rogalski, J. and Luterek, J. (1988). Biological decomposition of lignocellulose, in : A. Blazej and J. Zeinek (Eds.), Interbiotech '87 : Enzyme Technologie-Progress in Biotechnology, Elsevier, Amsterdam, Oxford, New York, Tokyo, **4**, 415-451.

Lowery, O.H., Rosenburg, U.J., Farr, A.I. and Randll, R.J. (1951). Protein Mesurement with the Folin-phenol reagent. *J. Biol. Chem.*, **193**, 265-275.

Miller, G.L. (1959). Use of Dinitro salicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, **31**, 426-428.

Mitra, S. and Nandi, B. (1989). Mycoprotein from agroindustrial wastes. *Mush. J. Tropics*, **9**, 121-126.

Saxena, S. and Rai, R.D. (1992). Effect of nitrogen on production of extracellular degradative enzymes by *Pleurotus sajor-caju* (Ff) Sing. on wheat straw. *Mushroom Res.*, **1**(1), 45-48.

Semichaevsky, V.D., Dudchenko, L.G. and Melnichuk, G.G. (1985). Effect of pH on the formation of extracellular enzymes by *P. ostreatus* : a wood rotting basidiomycete. *Mikrobiol. Zh.*, **47**(5), 72-76.

Tanaka, M. and Matsuno, R. (1985). Conversion of lignocellulosic materials to single cell Protein (SCP) : recent developments and problems. *Enz. Microb. Technol.*, **7**, 197-206.

Urbanek, H., Yirdaw, G. and Borwinska, A. (1977). Production of *Pleurotus ostreatus* biomass for feed by means of the submerged culture., *Przem. Ferm. Rolny*, **21**, 22-27.

(Accepted for publication September 12 2000)