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Mycelial protein production from agroindustrial wastes

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Wastes like saw dust from timber industries and rice straw from agriculture showed promise for more purposeful use as carbon sources for production of mycoprotein by *Volvariella volvacea*. Mycelial biomass and mycoprotein production showed further promotion by different nitrogen and thiamine enrichment of the media. Varying C : N ratios and concentration of the nitrogen sources also promoted growth and protein production of the organism.

Key words : Mycelial protein, Production, Agroindustrial waste, *Volvariella volvacea*

Protein deficiency in food and feed of many developing countries has led to searches for some cheap sources of protein. One outcome of these efforts is the prospect of production of fungal protein from various agro-industrial wastes.

For quite sometimes paddy straw has been shown to be the best substrate for the cultivation of fructification of *V. volvacea* in tropics and sub-tropics. It is quite likely that the vast amout of paddy straw or similar agricultural plant remains as well as saw dust from timber industries can be effectively transformed into mycelial biomass (Ammerman and Block, 1964 ; Heltay and Petofi, 1965 ; Zadrazil, 1974) and protein by using suitable fungi. Both rice straw and saw dust contain small amount of soluble carbohydrates but mostly long chain polysaccharides molecule. The ratio of lignin to polysaccharides in these lignocellulose materials ranges from 1 : 2 to 1 : 4 and in combination of these polymers make upto 70-90% of the biomass of vascular plants. With the exception of a recent report of the bacterial conversion of lignin to a water soluble polyphenol (Crawford *et al*., 1984) most other bioconversion involves the polysaccharides directly or the carbohydrates resulting from solubilization of the polysaccharides.

The present investigation was to study mycelial biomass and mycoprotein production of *Volvariella volvacea* (Bnll ex Fr.) Sing. under different *p*H, nitrogen sources and thiamine concentrations using saw dust and rice sraw dust as carbon sources.

MATERIALS AND METHODS

The test fungus was procured from the culture collection of Indian Agricultural Research Institute, New Delhi. The stock culture was maintained on 2% Malt-agar at 27±1°C. The rice straw used as substrate was procured from Crop Research Farm of Burdwan University. The saw dust collected from local saw mill and consists mainly of powered wood from *Magnifera indica, Shorea robusta* and *Atrocarpus integrifolia*.

Substrates and media

For production of mycelial biomass of *V. volvacea* the liquid salt solution I of Hofsten and Ryden (1975), containing 0.2% phosphoric acid, 0.2% ammonium sulphate and 0.02% magnesium sulphate ($MgSO_4$, $7H_2O$), was selected as basal medium. Both the saw dust and rice straw were further ground to about 40 mesh in a electric grinder and added separately in different concentrations (2%, 4% and 8%) to salt solution I as the carbon source. The *p*H was adjusted to 6, 6.4, 6.8 and 7.2 with 2(M)KOH after addition of the carbon sources.

Eeffect of nitrogen sources

Nitrogen source of salt soln. I (ammonium sulphate) was replaced by using equivalent amount of nitrogen present in different sources. A set without any nitrogen source was kept as control. The media containing the carbon sources (2%) and the different compounds (50 ml medium in each 250 ml Erlenmeyer's flask) were plugged, sterilized, inoculated with mycelial discs (5 mm in diameter) and finally incubated at 27±1°C (optimum recorded in an earlier experiment) for 14 days in darkness. The mycelia were then harvested, washed and dried to constant weights at 50°C. Final *p*H of the media were noted.

Production of Mycoprotein

The total protein in the mycelial biomass was estimated following Lowry *et. al.* (1951).

Dried mycelial powder (100 mg) was estimated with 5 ml of 1.0(M) NaOH and incubated at 80°C for one hour with periodic agitation of the mixture by a glass rod. It was then diluted with 5 ml of distilled water, The mixture was then centrifuged (5000 rpm for 10 mins) and the supernatant used for protein estimation.

To 1 ml of the supernatant, 1 ml of a mixture of copper tartrate and sodium carbonate-sodium hydroxide [Na_2CO_3-NaOH 1:10] was added. It was shaken thoroughly and allowed to stand for 10 mins. Three ml of the Folin-Ciocalteu reagent (diluted 1:10 with distilled water) was then added to the mixture, shaken well and after 5 mins the colour intensity of the reaction mixture was measured in a SICOSPEC-100 at 610 nm. The results have been presented in Tables 1 and 2.

Effect of C : N ratio

The C : N ratio of the carbon sources used as substrates (rice straw dust and saw dust) were calculated by estimating total carbon and total nitrogen following Jackson (1973) and Vogel (1961) respectively. Different C : N ratios were reconstituted by the enrichment of the substrates with different amounts of the nitrogen sources keeping the carbon source constant in the medium. The media were then plugged, sterilized and incubated as before. Mycelial growth was noted and myco protein production was estimated. The results have been recorded in the Table 3.

Effect of Thiamine

Stock solutions of thiamine was placed in double distilled water and was mixed with the basal medium (made vitamin free by boiling with activated charcoal) in desired quantities (100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm) separately in flasks. 25 ml of the medium was then poured in each 250 ml Erlenmeyer flask and plugged. One set of flasks with the vitamin free medium was kept as control. The *p*H was maintained at 6.4 (recorded optimum in an earlier experiment). The flasks were sterilized for 15 minutes at 10 lbs pressure and then inoculated aseptically wirh uniform discs of mycelia (5 mm in diameter) grown on vitamin-free malt-agar medium in petri dishes and incubated as before. After the incubation period, the mycelia were harvested by filtering, washed thoroughly over previously dried and weighed Whatman's filter paper. Final *p*H of the filtrates were recorded. The mycelia on filter papers were then dried to constant weights at 50°C and weighed. Protein content of the mycelia was estimated as before.

RESULTS AND DISCUSSION

In *V. volvacea* some mycelial growth was recorded even in control (containing rice straw or saw dust but without any nitrogen enrichment). On addition of different inorganic nitrogen compounds to rice straw dust, mycelial biomass was considerably promoted except with ammonium nitrate where an inhibition was recorded. Ammonium sulphate induced maximum promotion ; di-ammonium hydrogen phosphate, potassium nitrate and urea exhibited moderate promotion while others only low promotion. Workers like Lilly and Barnett (1951), Nicholas (1965) while working on the nutrition physiology of fungi have shown that fungi can utilise a wide source of simple inorganic nitrate, nitrite and ammonium compounds.

In case of saw dust also different degrees of promotion of mycelial biomass were recorded on addition of nitrogen amendments except potassium nitrate which showed prominent inhibition. Maximum growth occured in ammonium sulphate and urea whereas ammonium chloride, ammonium nitrate, sodium nitrate gave fair promotion. The rest showed only marginal promotion.

In both the carbon sources the amount of protein varied significantly in different salts. Among the nitrogen sources, ammonium sulphate exhibited maximum protein production in both the carbon sources. Ammonium nitrate in rice straw dust and sodium nitrate in wood dust showed minimum protein production.

Table 1. Mycelial growth* and protein production* of *Volvariella volvacea* in rice straw dust (2%) and saw dust (2%) substrates enriched with some nitrogen sources after 14 days incubation at 27±1°C

Nitrogen sources	Rice straw dust				Saw dust			
	Initial *p*H	Final *p*H	Mycelial Biomass (mg)	Protein (%)	Initial *p*H	Final *p*H	Mycelial Biomass (mg)	Protein (%)
Control	6.0	5.94	30.10	9.40	6.0	5.90	82.6	6.30
$(NH_4)_2SO_4$	6.0	4.83	42.40	18.45	6.0	5.67	184.0	16.38
NH_4NO_3	6.0	5.17	36.00	13.59	6.0	5.78	114.6	10.29
NH_4Cl	6.0	4.88	37.10	14.20	6.0	5.90	100.0	11.80
$NH_4H_2PO_4$	6.0	4.59	36.51	16.50	6.0	5.87	112.0	10.68
$(NH_4)_2HPO_4$	6.0	4.97	41.00	16.82	6.0	5.06	132.0	7.90
KNO_3	6.0	5.44	40.20	17.00	6.0	5.89	57.0	9.10
$NaNO_3$	6.0	5.96	38.34	14.32	6.0	5.16	130.0	6.20
$CO(NH_2)_2$	6.0	5.82	41.40	17.79	6.0	5.92	180.5	7.59
LSD			1.28	0.25			1.01	0.06

*Average of three replicates

Effect of pH and carbon sources

V. volvacea showed very low mycelial biomass in carbon source-free control. However, on addition of rice straw dust and saw dust there was significant increase in mycelial biomass. The amount of mycelia increased with increase in carbon source to 8% in case of rice straw whereas maximum mycelial growth was evident at 4% saw dust followed by a decrease with further increase in concentration. Mycelial growth was maximum at *p*H 6.4 in all series.

Small amount of protein obtained even in the carbon source free control particularly at *p*H 6.4, was evidently from growth on the inoculum.

Protein content also increased with increase in the amount of both rice straw dust being more pronounced at *p*H 6.4.

Table 2. Mycelial growth* and protein production* of *Volvariella volvacea* on rice straw dust and saw dust substrate in salt solution I after 14 days incubation at 27 ± 1°C

Treatment	*p*H 6		*p*H 6.4		*p*H 6.8		*p*H 7.2	
	Mycelial Biomass (mg)	Protein (%)	Mycelial Biomass (mg)	Protein (%)	Mycelial Biomass (mg)	Protein (%)	Mycelial Biomass (mg)	Protein (%)
Control	2.16	6.40	2.76	7.10	2.00	4.80	2.16	4.23
Straw 2%	42.33	18.40	83.33	25.90	61.66	24.40	47.66	20.30
Straw 4%	97.33	21.70	108.33	24.80	121 66	20.90	103.33	18.40
Straw 8%	215.33	29.80	207.30	32.90	204.00	29.40	238.00	23.30
LSD	1.06	0.38	1.68	0.26	2.89	0.34	2.93	0.44
Control	2.16	6.40	2.76	7.10	2.00	4.80	2.16	4.23
Saw 2%	186.00	16.40	180.66	23.30	204.30	20.10	199.66	14.90
Saw 4%	270.00	24 00	256.00	31.80	246.66	25.60	256.00	18 80
Saw 8%	202.66	20 20	215.66	29 70	204.00	20.60	231.66	17.60
LSD	3.31	1.11	1.00	0 19	2.08	0.33	2.10	0.39

*Mean of three replicates

Effect of C : N ratio

Total carbon in rice straw was found to be much lower than that in saw dust. On the otherhand, total nitrogen was much higher in the former than in the later, thus resulting into a C : N ratio of about 66.7 : 1 in rice straw dust and 117 : 1 in wood dust.

At the optimum *p*H and the C : N ratios present in rice straw as well as saw dust, the mycelial growth was low. On addition of different concentrations of the nitrogen source to the medium mycelial growth increased and reached maximum at the C : N ratio of 2.7 : 1 in case of rice straw and 3.3 : 1 in saw dust. With

Table 3. Mycelial growth* and protein production* of *Volvariella volvacea* on rice straw dust (2%) and saw dust (2%) substrate in optimum *p*H 6.4. in different C : N ratio at 27 ± 1°C after 14 days incubation

Rice straw dust					Saw dust				
C : N ratio	Initial *p*H	Final *p*H	Mycelial Biomass (mg)	Protein (%)	C : N ratio	Initial *p*H	Final *p*H	Mycelial Biomass (mg)	Protein (%)
66.7 : 1 (Control)	6.4	5.19	55.00	15.10	117.1 : 1 (Control)	6.4	6.23	156.00	14.50
3.8 : 1	6.4	6.04	65.40	21.00	4.7 : 1	6.4	6.25	165.00	17.90
3.1 : 1	6.4	6.00	66.62	23.30	3.9 : 1	6.4	6.21	172.00	18.35
2.7 : 1	6.4	5.90	83.30	25.90	3.3 : 1	6.4	6.20	180.60	23.30
2.4 : 1	6.4	5.92	81.00	24.20	2.9 : 1	6.4	5 94	176.00	21.30
2.1 : 1	6.4	6.10	72.60	22.40	2.6 : 1	6.4	5 86	179.00	18.00
1.9 : 1	6.4	6.01	64.30	21.35	2.4 : 1	6.4	5.72	135.00	16.80
1.7 : 1	6.4	5.90	63.00	20.20	2.1 : 1	6.4	5.61	118.00	13.80
1.6 : 1	6.4	5.89	61.20	18.30	1.95 : 1	6.4	5.34	118.20	13.50
LSD			0.63	0.21				0.87	0.18

*Mean of three replicates

further increase in nitrogen, mycelial growth gradually decreased to the minimum at 1.6 : 1 and 1.95 : 1 in rice straw and saw dust respectively.

During microbial utilization of plant substrate nitrogen is known to be mobilised into the cells of the colonizers while most of the carbon is released as carbon dioxide (Alexander, 1977). Proportion of carbon and nitrogen which are different in different plant tissues, are an important factor that governs the rate of such decomposition (Flaig, 1964). As a result of nitrogen being captive in organic combination, the percentage of nitrogen continuously rises in the residual substance, and hence the C : N ratio shows a decrease as has been shown during decomposition of straw and roots of cereals and grasses (Pal and Broadbent, 1975 and Herman *et al.*, 1977). Depending on the organism mineralisation of nitrogen takes place only when the ratio crosses a certain lower level.

Protein content of the mycelia also increased on nitrogen enrichment in the media. It was maximum when C : N ratio was 2.7 : 1 in rice straw and 3.3 : 1 in case of saw dust.

From the above discussion, it was evident that considerable enhancement of growth and protein production could be induced by suitably manipulating the C : N ratios using carbon sources from agroindustrial wastes and adding proper nitrogen sources.

Table 4. Mycelial growth* and protein production* of *Volvariella volvacea* on rice straw dust (2%) at optimum *p*H 6.4 and in optimum nitrogen source using Thiamine at different concentrations at 27±1°C after 14 days incubation

Concentration of Thiamine	Initial *p*H	Final *p*H	Mycelial Biomass (mg)	Protein (%)
Nil	6.4	5.90	83.00	25.50
100 ppm	6.4	5.77	98.00	27.30
10 ppm	6 4	5.81	86.00	25.34
1 ppm	6.4	5 31	87.00	25.96
0.1 ppm	6.4	5.55	84.00	25.92
0 01 ppm	6.4	5.40	83.25	25.90
LSD			0.31	0.14

*Mean of three replicates.

Effect of Thiamine

Mycelial biomass as well as protein production did not show any promotion at 0.01 ppm. However, at 10 ppm of thiamine, small promotion of mycelial production was recorded. At 100 ppm both mycelial biomass and protein production was considerably increased. Thus, *V. volvacea* appeared to be a deficient species for thiamine. Promotion of mycelial growth with added thiamine in the medium signified that either the optimum amount of thiamine was not present in the media or the fungus was unable to synthesize the required amount for its optimum growth. In the present case, the fungus was incapable of synthesizing the required level of thiamine and hence, was partially deficient as it showed some amount of growth even in media without the added vitamin.

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