RESULTS AND DISCUSSION

4.1 Chemical composition of cellulosic waste materials:

The major chemical constituents of the cellulosic wastes under study are presented in Table(1)and included cellulose, hemicellulose, crude protein, total sugars, lignin, and ash contents. Determinations were made on the finely ground beet pulp, mango wastes, pear wastes, and tomato residues,

It is obvious from the data that the holocellulose represented more than 48% of all waste materials except in case of mango wastes which contained about 35% holocellulose. The beet pulp showed the highest cellulose and ash contents. In the same time, it had a low lignin content as compared to other by-products under study. These results support the possibility of using beet pulp as a substrate for SCP production by microorganisms. Also it could be noticed that tomato wastes (mixture of seeds and peels), contained about 20% protein, This reltaively high protein content can be attributed to the high protein content of the seeds. Accordingly, one can deduce that it would be perferable to use tomato wastes directly, if possible, as animal fodder instead

Asp. paraziticus, Asp. tamarii, Asp. terreus Asp. versicolor, Asp. foetidius DSM 734, Asp. oryzaeDSM1964 Asp. sp, Alternaria sp, Cephalosporium sp, Chaetomiuna sp Fusarium oxysporum, Fusarium sp, Neurospora sp, Penicillum lilacinum DSM62851, pen. sp, Phoma sp, Rhizoctonia solani Trichoderma viride, Trichoderma sp, Tricothecium sp, Mucor hiemalis DSM6397, Mucor meihi, Myrothecium verrucaria. and Trichoderma reesei. These cultures were tested for their ability to utilize cellulose as a sole carbon source, since holocellulose constitutes the major component of food industery wastes under study.

It is clear from the data in table (2) that the tested cultures of Myrothecium verrucaria, Trichoderma viride, Trichoderma reesei, Aspergillus humicola, and Aspergillus terreus gave the best growth and showed a potent ability for cellulose degradation appeared through clear zones formation on a solid Czapek medium containing cellulose as a sole carbon source. Although other cultures showed similar potencies they were not selected with the other mentioned cultures since it was thought preferable to use a more safer strains known as SCP producers.

The selected cultures were subject to another confirmational test where they were grown on a modified

Table (2): Screening for the ability of some fungal culture to utilize α cellulose as a main carbon source in Czapeck solid medium.

FUNGAL CULTURE	AFTER 3 DAYS		AFTER 6 DAYS		AFTER 8 DAYS	
	clear zones	growth	clear zones	growth	clear zones	growh
Aspergillus foetidius DSM 734	-	-	-	-	-	-
Asp. oryzae DSM 1864	+	+	+	+	+	+
Asp. humicola	+	++	+	++	+	++
Asp. niger	-	+	-	+	-	+
Asp. ochrecus	-	-	-	+	-	+
Asp. tamarii	+	+	+	+	+	+
Asp. paraziticus	-	+	-	+	-	+
Asp. terreus	-	++	+	++	+	++
Asp. versicolor	-	+	+	+	+	+
Asp. sp	-	+	++	++	++	+
Alternaria. sp.	-	+	-	+	-	+
Cephalosporium. sp.	-	+	-	+	-	+
Chaetomiuna. sp.	-	-	-	+	-	+
Fusarium oxysporium	-	+	+	+	+	+
Fusarium sp.	-	+	-	+	-	+
Neurospora sp.	-	+	-	+	-	+
Penicillium. lilacinum DSM62851	+	+	+	+	+	+
Penicillium. sp.	+	++	+	+	+	+
Phoma sp.	-	+	+	+	+	+
Rhizoctonia solani	-	-	+	+	+	+
Trichoderma viride	+	++	+	+	+	+
Trichoderma reesei	+	+	+	+	+	+
Trichoderma sp.	+	++	+	++	+	+
Trichothecium sp.	+	++	+	+	+	+
Mucor himalis DSM63297	+	+	+	+	+	+
Mucor meihi	-	-	-	-	-	-
Myrothecium verrucaria	++	+++	++	++	++	+
Control	-	-	-	-	-	-

\+ Small growth.
++ Medium growth
+++ Ewcessive growth.
Control =Solid Czapek medium without inoculation.

liquid Czapek medium. One-quarter litre flarks were used after distrbuting 50 ml of the liquid medium containing 2% of cellulose as a main carbon source. The extent of cellulose degradation and the amount of growth were visually estimated after four days incubation at 28± 1°C on rotary shaker at 120 r.p.m,. Data given in table (3) show that M. Verrucaria was superior cellulytic fungal culture that gave excellent growth and high extent of cellulose utilization. Also good growth and cellulose decomposition were obtained with Trichoderma viride, Trichoderma reesei and Penecillium lilacinum cultures.

4.3. Growth, biomass and protein yields of selected fungi :

In a series of experiments the four selected fungal cultures, namely, Myrothecium verrucaria, Trichoderma viride Trichoderma reesei and penicillium lilacinum were tested for their growth, biomass and protein yields when grown on each of the food industry wastes under study. Thus, they were grown in liquid modified Czapek medium containing 2% waste substrate which served the sole carbon source.

Data presented in tables4,5,6 and 7 illustrate that Myrothecium verrucaria gave the highest protein content (23.65, 15.82, 22.68, 25.55%) when tomato wastes, pear wastes, mango wastes, and beet pulp were used as substrates, respectively. However, the biomass yield

Table (3): Screening for the ability of some eslected fungal strains to utilize ∝ cellulose grown on a liquid medium containing 2% ∝ cellulose as the main carbon source.

Fungal strain	Growth amount	Cellulose utilization
Aspergillus terreus	+	+
Aspergillus humicola	+	+
Trichoderma reesei	++	++
Trichoderma viride	++	++
Penicillium lilacinum	++	++
Myrothecium verrucaria	+++	+++

(g/l) and subsequently the protein yield, were rahter low. Garg and Nàelakantan (1981), working with Aspergillus terreus, stated that the biomass dry matter yield of this organism, in general, was low with higher crude protein content of biomass, and conversely, with higher dry matter yield the crude protein content was low. On the other hand, Trichoderma viride gave slightly less protein percent (22.40, 12.95, 21.05, 23.80).on tomato wastes, mango wastes, pear wastes, and beet pulp, respectively. However, the protein content of this organism was higher than that produced by Penicillium lilacinum or Trichoderma reesei which gave the highest biomass yield as shown in the tabulated results. In fact, this higher biomass yield is largly attributed to indigestable substrate part associated with biomass harvested.

By summing up the available data Fig(1) it appears that beet pulp in general gave good results with respect to protein production from tested Fungi. The highest protein precentage and protein yield were produced by Myrothecium verrucaria and Trichoderma viride on this substrate. These results in addition to those obtained from the chemical analysis support the selection of beet pulp as substrate and the choice of the cultures Myrothecium verrucaria and Trichoderma viride for further detailed studies.

Table (4): Protein content, biomass and protein yield of selected fungi grown on tomato wastes (seeds and peels).

Micro-organism	Biomass Yields		Protein Yield		Crude protein
	g/L	g/g substrate	g/L	g/g substrate	%
Penicillium lilacinum	16.500	0.8250	3.028	0.1514	18.34
Trichoderma viride	14.776	0.7388	3.310	0.1655	22.40
Trichoderma reesei	15.160	0.7580	3.364	0.1682	22.19
Myrothecium verrucaria	11.456	0.7528	2.710	0.1355	23.78

Table (5): Protein content, biomass and protein yield of selected fungi grown on pear wastes.

Micro-organism	Biomass Yield		Protein Yield		Crude Protein
	g/L	g/g substrate	g/L	g/g substrate	%
Penicillium lilacium	14.570	0.0785	1.570	0.0785	10.780
Trichoderma viride	16.780	0.8390	2.174	0.1087	12.950
Trichoderma reesei	17.636	0.8818	2.222	0.1111	12.600
Myrothecium verrucaria	15.156	0.7528	2.382	0.1191	15.820

Table (6): Protein content, biomass and protein yield of selected fungi grown on mango wastes.

Micro-organism	Biomass Yields		Protein Yield		Crude protein
	g/L	g/g sub-strate	g/L	g/g sub-strate	%
Penicillium lilacinum	14.540	0.7270	1.964	0.0982	13.51
Trichoderma viride	13.728	0.6860	2.890	0.1445	21.05
Trichoderma reesei	15.540	0.7770	2.798	0.1399	17.99
Myrothecium verrucaria	11.940	0.5970	2.708	0.1354	22.68

Table (7): Protein content, biomass and protein yield of selected fungi grown on beet pulp.

Micro-organism	Biomass	Yield	Protein	Yield	Crude Protein
	g/L	g/g sub-strate	g/L	g/g sub-strate	%
Penicillium lilacium	15.390	0.769	1.874	0.094	12.18
Trichoderma viride	13.808	0.690	3.286	0.164	23.80
Trichoderma reesei	13.854	0.693	2.532	0.126	18.27
Myrothecium verrucaria	11.716	0.586	2.994	0.149	25.55

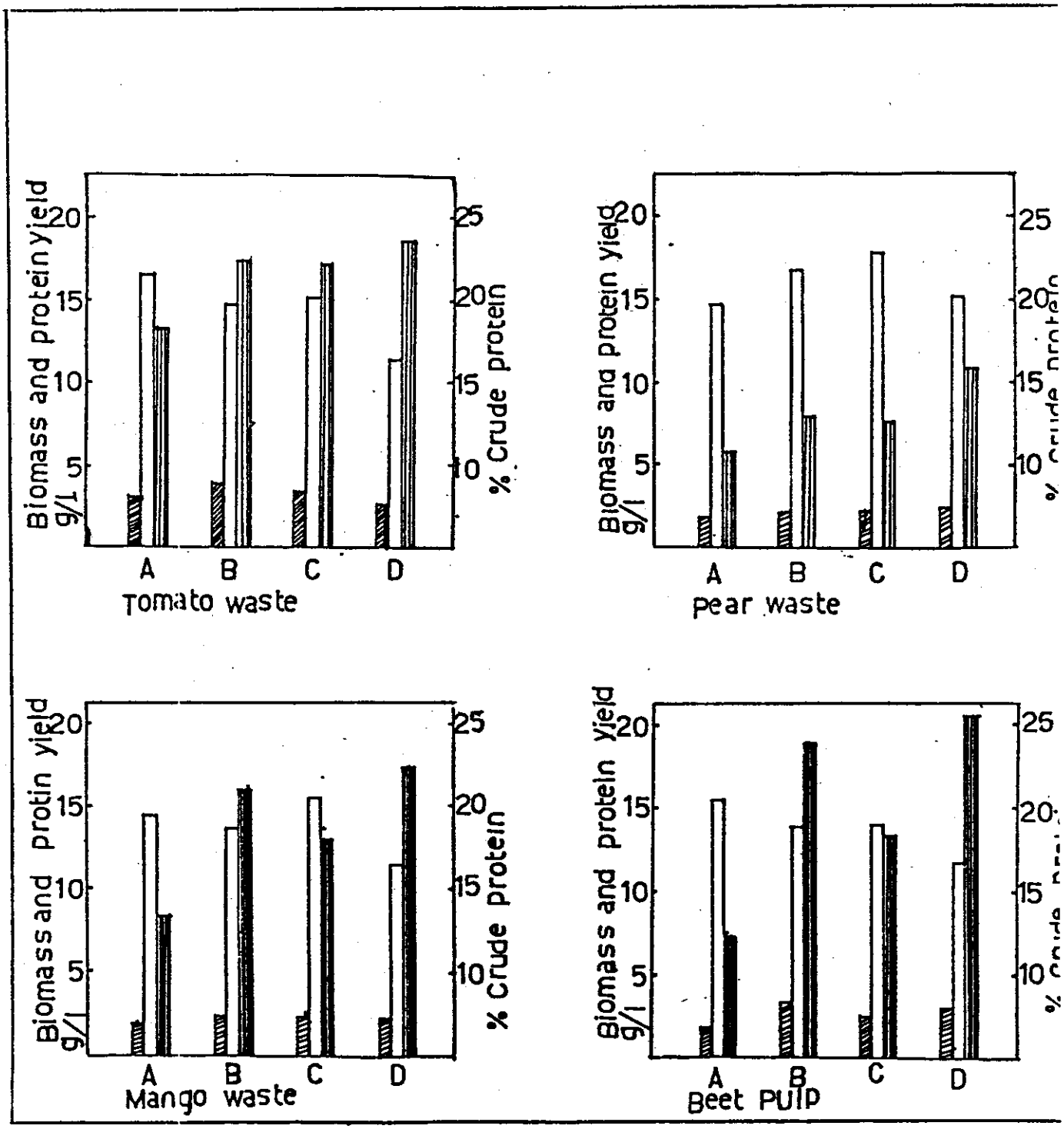


Fig. (1): Comparative growth parameters of some fungal strains grown on different food processing wastes.
(A, P. lilacinum; B, T. reesei; D, M. verrucaria.
▨ Protein Yield, □ Biomass yield, ▥ % Crude protein.)

4.4 Pretreatment of sugar beet pulp :

The degradation of natural celluloses is often limited because of their association with lignin and hemicellulose. In the literature many pretreatment methods have been demonstrated to be effective in disrupting the lignin-carbohydrate complex, whereas others are used to disrupt the highly ordered cellulose strsture itself (Fan et al,. 1982).

In the present work several pretreatments have been attempted to increase the biodegradability of the raw materials(beet pulp) by the selected f... cultures. Some of these procedures were reported in the literature and are based on soaking the cellulosic by-product in acid or alkali for disruption of lignin structure and decrease of the cellulose crystallinity(Gupta et al., 1972; Garg and Neelakantan 1981). Other methods are based on the removal of lignin and formation of holocellulose by treatment with sodium chlorite (Sidhu and Sandhu, 1980). In addition, we have also attempted to test new approaches of chemical pretreatments in order to increase digestibility of the by-products under study. Table (8) summarizes different pretreatments used for beet pulp and their effects on the residual part as well as the nitrogen content in the post-treated by-product.

Table (8 : Effect of different pretreatments on nitrogen content and amount of the residual part of beet pulp.

Pretreatment	Brief description of pretreatment	Residual Part %	Nitrogen %
1	Preparation of holo-cellulose by removal of lignin using sodium chlorite. (Sidhu and Sandhu, 1980).	61	1.664
2	Preparation of cellulose from holocellulose (Sidhu and Sandhu, 1980).	50	0.155
3	Acid treatment (Gupta et al., 1972) beet pulp was soaked in N/10 Hydrochloric acid for 24 hr.	51	2.302
4	Pretreatment with sodium hydroxide. (Garg and Neelakantan, 1981).	40	2.053
5	Alkali treatment (Gupta et al., 1976) the beet pulp was soaked in N/10 sodium hydroxide for 24 hr.	55	2.224
6	A suggested modification for procedure of Garg and Neelakantan, 1981.	68	1.897
7	A suggested acid-heat pretreatment using 0.5 Hydrochloric acid.	96	1.757
8	A suggested acid-heat pretreatment followed by neutralization with ammonium hydroxide.	95	2.484
9	Control (untreated beet pulp)		1.568

present in the beet pulp. This may be considered as a negative factor. Accordingly, it was necessary to examine such pretreatments with respect to their effect on fungal biomass and protein yield upon using the treated beet pulp as substrate in growth media of the selected fungal cultures.

4.5. Production of fungal protein from beet pulp :

No doubt that the production of fungal protein depends on the fungal growth, and the bioconversion of the substrate to cell material. Such bioconversion processes are in turn resultant of growth and enviromental factors and conditions prevalent during the fungal multiplication. In the next series of experiments detailed studies were made to optimize such conditions in order to obtaine maximal protein yields using M. verrucaria and T.viride grown on pretreated beet pulp.

4.5.1. Effect of beet pulp pretreatments on biomass and protein yields of M.verrucaria and T.viride :

Two selected fungal cultures, i.e. M.verrucaria

Table (9): Biomass and protein yield of Myrothecium verrucaria and Trichoderma viride grown on beet pulp treated with eight different pretreatments.

Pretreatment No.	Myrothecium verrucaria					Tricoderma viride				
	Biomass Yields		Protein Yield		Crude protein	Biomass Yields		Protein Yield		Crude protein
	g/L	g/g sub-strate	g/L	g/g sub-strate	%	g/L	g/g sub-strate	g/L	g/g sub-strate	%
1	9.094	0.4547	1.773	0.086	19.50	9.362	0.468	1.938	0.096	20.70
2	8.678	0.4340	1.539	0.085	17.74	18.648	0.932	0.817	0.040	4.38
3	13.330	0.6660	4.182	0.166	31.27	12.400	0.622	2.852	0.145	23.46
4	9.624	0.4810	2.877	0.145	29.90	9.620	0.481	2.213	0.109	23.13
5	9.984	0.4990	2.895	0.128	29.06	12.576	0.628	2.264	0.114	18.81
6	3.178	0.1590	0.763	0.037	24.08	7.200	0.362	0.720	0.036	10.06
7	9.984	0.5000	1.354	0.070	13.56	12.000	0.600	1.836	0.092	15.32
8	6.942	0.3740	2.741	0.127	39.49	7.400	0.371	2.760	0.139	37.38

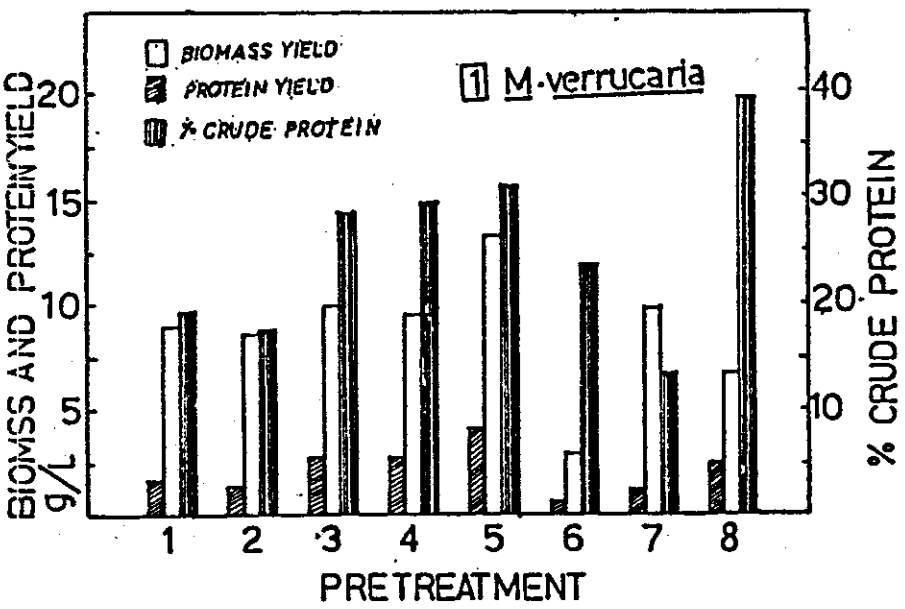


Fig (2): Growth parameters of M. verrucaria grown on beet pulp subjected to eight different pretreatments. (□ Biomass yield, ▨ Protein yield, ▩ Protein content %)

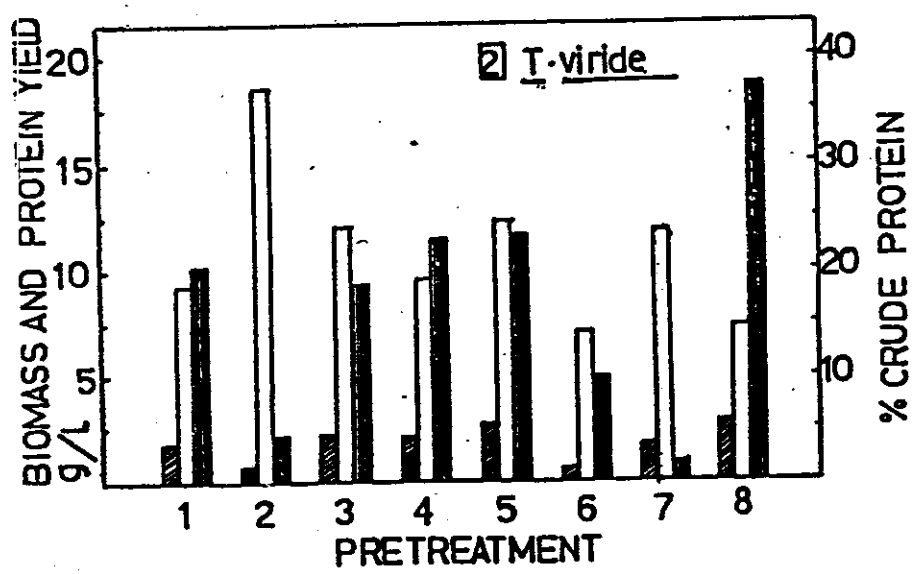


Fig (3): Growth parameters of T. viride grown on beet pulp subjected to eight different pretreatments. (□ Biomass yield, ▨ Protein yield, ▩ Protein content %).

pretreatments as mentioned before to examine their effect on biomass and protein yield. These two parameters in addition to the protein content were collectively taken as criteria for selecting the best pretreatment. Data are presented in Table (9) and graphically illustrated in fig.(2and3). Results indicate that the maximum protein contents were 37.38% and 39.49% for T.viride and M. verrucria, respectively, when grown on beet pulp treated with acid followed by neutralizations with ammonium hydroxide.

On the other hand, the lowest protein content (4.3%) was produced by T.viride grown on cellulose obtained from holocellulose of beet pulp (pretreatment NO.2), while the lowest protein content produced by M.verrucria was given by the alkali treatment followed by neutralization with nitric acid (Pretreatment NO.7). The highest biomass yield was 18.65g/l given by T.viride on product of pretreatment NO.2, in which cellulose was obtained from holocellulose, and 13.33 g/l given by M.verrucaira on acid pretreatment NO.3 prepared according to Gupta et al., (1972). This latter pretreatment gave also the highest protein yield (4.18 g/l), and (2.85 g/l) for both M. verrucria and T.viride, respectively.

With regard to protein content the pretreatment with sodium hydroxid (NO.4) prepared according to Garg and Neelakantan, 1981 procedure gave also good results.

It could be noted with reference to the data of previous experment that there is a positive relationship between residual nitrogen content of the beet pulp treated with different pretreatments and the protein content produced from using the products of such pretreatments. Thus, the highest protein contents produced by M.verrucaria, and T.viride, (39.49, 37.38%, respectively)., were obtained with pretreatment NO.(8) yielding the highest nitrogen content (2.48%). On the other hand, in pretreatment No. (2) which was used for obtaining cellulose from the beet pulp holocellulose, the nitrogen content was the lowest (0.16%), and consequentlly, the protein content produced by T.Viride (4.38%) was very low. The corresponding protein content of M. verrucaria grown on beet pulp of the same pretreatment gave a higher protein content (17.74%) but still lower than the majority of protein contents obtained with other pretreatments for the same fungal culture.

Data gave evidence that M.verrucaria is more capable of digest cellulosic materials than T. viride . This result was confirmed by growing the two cultures on cellulose as a sole carbon source where protein content was (5.32%), and (1.56%) for M.verrucaria and

T.viride, respectively. This also indicates that pure cellulose was a poor substrate to be converted to fungal protein. These results are in agreement with those of Sidhu and Sandhu (1979) who stated that, cellulose alone did not show high production of SCP as when was in combination with hemicellulose perhaps because of less utilisation as substrate by Trichoderma longibrachiatum.

4.5.2. Effect of exposure time of beet pulp to acid pretreatment :

Based on the data obtained with the beet pulp using different pretreatments (Table 10), it could be concluded that pretreatments with acids gave much better protein yields of the fungi tested as compared to alkali pretreatments. Furthermore, acid pretreatments followed by ammonium hydroxide gave the highest protein content in the fungal biomass reaching 39.5%, and 37.4% for M.verrucaria and T.viride, respectively. There fore, pretreatment NO.(8) was adopted and the effect of time of exposure to acid on biomass and protein yield of M.verrucaria and T.viride was evaluated. The same procedure was carried out but with varying time of exposure of beet pulp to acid treatment in the range of 1 to 20 hours followed by ammonium hydroxide neutralization. The resulting pretreated pulps were dried and used in proportion of 2% in a modified Czapek medium used in

Table (10): Effect of exposure time of pulp to 0.5N hydrochloric acid pretreatment on protein content, biomass and protein yield of Myrothecium verrucaria and Trichoderma viride.

Exposure time (hr)	Myrothecium verrucaria					Tricoderma viride				
	Biomass	Yield	Protein	Yield	Crude protein	Biomass	Yield	Protein	Yield	Crude protein
	g/L	g/g substrate	g/L	g/g substrate	%	g/L	g/g substrate	g/L	g/g substrate	%
1	11.628	0.5814	4.66	0.233	40.10	11.488	0.5744	3.280	0.164	28.677
2	11.966	0.5983	4.66	0.233	38.88	11.736	0.5868	3.840	0.192	32.809
4	12.012	0.6006	4.66	0.233	38.88	11.972	0.5986	3.300	0.165	27.706
8	12.000	0.6000	5.06	0.253	42.29	12.664	0.6032	3.740	0.187	31.108
24	11.866	0.5933	4.90	0.245	41.31	10.998	0.5499	3.100	0.155	28.192

the previous experments the results obtained in table (11).

Indicated that the protein content and protein yield given by M.verrucaria were nearly the same for the pretreatment of beet pulp with acid of one, two, and four hours followed by notable increase in the protein yield and protein content for the organism grown in beet pulp acid treated for eight and twenty hours. The maximum protein percentage and protein yield were 42.3% and 5.1 g/l, respectively, for M.verrucaria grown on beet pulp treated with acid for eight hours. On the other hand, T.viride did not exhibit regular pattern with respect to its protein content and protein yield in relation to the extent of acid treatment of the pulp The biomass yield showed no significant variation for the two fungal culture with respect to exposure time of beet pulp to acid treatment.

Several cellulosic by-products have been reported in the literature as possible substrates for production of SCP by fungi. Sugar-can bagasse was subjected to extensive studies for SCP production by Menezes et al, (1976), who concluded that M.verrucaria, Trichoderma viride and Geotrichum were the most effecient for conversion sugar cane bagasse into protein.

They reported that medium composition, substrate concentration, and pretreatment of the bagasse have a great effect on protein yield and productivity of the fungus Meanwhile, Chahal et al. (1976) cultivated Chatomium celluloticum for protein production using wheat straw they found that more protein was produced from pretreated straw than untreated one. Moo-Young et al. (1978) showed that Chatomium celluloticum was faster in growth than T.viride using sawdust as carbon source. Singh et al. (1978) recommended the use of M.verrucaria for SCP production from corn cobs. They found that the maximum protein production was 20.4% in the biomass recovery.

In the literature several chemical pretreatments of cellulosic by-products were reported as means to enhance both digestibility of the by-product and protein yield resulting during the fermentation processes by the appropriate microorganism. Han and Callihan. (1974) showed that treatment of rice straw and sugarcane bagasse with ammonia increased digestibility of cellulose. Dhawan and Gupta., (1977) reported that alkali treatment increased the degradation of sugarcane bagasse from 7% to 48% by T.viride.

On the other hand, Kausar et al. (1976) found that soaking in 2N HCI and 2% NaOH for 1 hr. at room temperature released 2.8 and 1.85 mg/g sugar from bagasse

and rice husk respectively. Ferrer et al. (1977) investigated the effect of bagasse treatmentwith NaOH and Tween 80 on fermentation by T.viride. They reported that alkali treated bagasse was a good substrate for SCP production from this organism. Fan et al (1982)experessed the opinion that the acids are used mainly for hydrolysis of cellulose rather than a pretreatment reagent. However, in the present work the combination of acid treatment of beet pulp neutralized with ammonium hydroxide gave better protein yield and protein content in the biomass of M.verrucaria and T.viride. Thus, this pretreatment was adopted for further study. The acid exposure time was selected to be eight hours based on data obtained previously .

4.6 Optimization of growth conditions for SCP production:

Based upon the data obtained in previous section both M.verrucaria, and T.viride were selected for SCP production using acid pretreated beet pulp. In the present section a series of experiments was conducted in order to reveal the optimum growth conditions of fungal cultures leading to the production of maximum biomass yield with the highest protein content.

4.6.1. Effect of incubation period :

This experiment aimed to study the effect of incubation period of M.verrucaria, and T.viride grown

on beet pulp treated with three different pretreatments selected from the previous studies. The cultures were prepared under the same conditions employed in the previous experiments except cultures were incubated on rotary shaker for three, five, and seven days. Results tabulated in Table (11 and 12) and illustrated in Figs (4, 5).indicate. That biomass yield, protein yield, and protein percentage of M.verrucaria and T.viride were affected by two main factors i.e. the incubation period and the type of pretreatment. In case of M.verrucaria the maximal protein content (47.40% and 33.50%) and protein yield (3.7 g/l and 4.3 g/l) were attained after three days incubation period. On the other hand, T.viride gave its maximal protein content (47.10 and 29.20%) and protein yield (3.70 g/l and 3.04 g/l) after five days incubation. These maximum values of protein content and protein yield for the two fungal cultures were obtained with the acid pretreatment followed by neutralization with ammonium hydroxide and the untreated beet pulp, respectively,

Data also revealed that with increasing the incubation period to seven days the two fungal cultures have undergone notable decrease on their protein percentage protein yields, and mycelial biomass. These results were in agreement with those reported by Martin

Table (11): Effect of incubation period on protein content, biomass and protein yield of Myrothecium verruceria grown on beet pulp treated with three selected pretreatments.

No. of the treatment	Incubation Periods: 3 days Biomass g/L	Yield g/g*	Protein g/L	Yield g/g*	Crude protein %	5 days Biomass g/L	Yield g/g*	Protein g/L	Yield g/g*	Crude protein %	7 days Biomass g/L	Yield g/g*	Protein g/L	Yield g/g*	Crude protein %
3	14.94	0.747	3.80	0.190	25.52	13.60	0.680	4.52	0.226	33.29	12.10	0.605	3.58	0.179	29.65
4	15.23	0.761	3.76	0.188	24.79	13.92	0.696	3.86	0.193	27.70	13.15	0.657	3.32	0.166	25.27
8	7.86	0.393	3.72	0.186	47.39	6.70	0.325	3.02	0.151	45.20	7.54	0.377	2.44	0.122	32.32
Control	12.77	0.638	4.28	0.214	33.54	13.54	0.677	3.72	0.186	27.46	10.34	0.517	2.82	0.141	27.22

* g/g = g/g substrate.

Table (12) Effect of incubation period on protein content, biomass and protein yield of Tricoderma viride grown on beet pulp treated with three selected pretreatments.

No. of the treatment	Incubation Periods: 3 days Biomass g/L	Yield g/g*	Protein g/L	Yield g/g*	Crude protein %	5 days Biomass g/L	Yield g/g*	Protein g/L	Yield g/g*	Crude protein %	7 days Biomass g/L	Yield g/g*	Protein g/L	Yield g/g*	Crude protein %
3	14.77	0.738	3.66	0.186	24.79	10.44	0.689	3.04	0.200	29.16	10.71	0.555	3.06	0.148	28.68
4	14.25	0.713	2.52	0.126	17.74	11.77	0.588	2.80	0.140	23.82	12.05	0.603	2.22	0.111	18.47
8	8.43	0.421	3.72	0.177	44.23	7.79	0.389	3.72	0.186	47.15	7.66	0.383	3.08	0.154	40.10
Control	11.78	0.589	2.92	0.146	24.79	10.44	0.522	3.04	0.152	29.16	10.70	0.535	3.06	0.153	28.68

* g/g = g/g substrate.

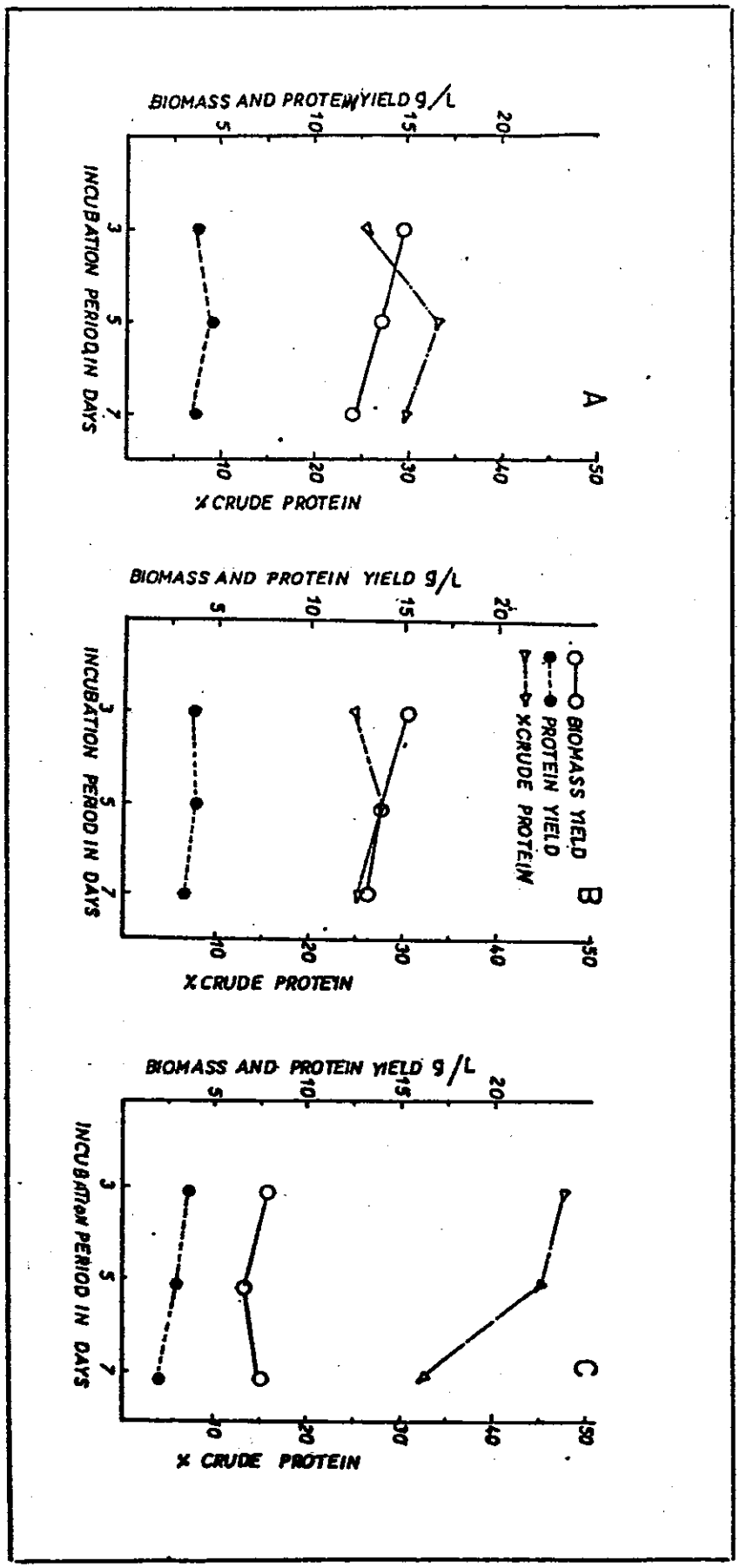


Fig ( ): Protein content, biomass, and protein yield of M. verrucaria grown on beet pulp treated with three selected pretreatments as influenced by incubation period. (A, acid pretreatment No.3; B, alkaline pretreatment; C, proposed acid-heat pretreatment).

(1983) who concluded that biomass yield of Agricus Compestris generally increased from 4-6 days and afterward it decreased due to the onset of mycelial autolysis. The present data also indicate that acid pretreatment followed by ammonium hydroxide neutralization is the best pretreatment for production of mycelial biomass with the highest protein yield and protein content. Furthermore, since the protein yield and protein percent in the biomass was nearly the same after three and five days of incubation, four days incubation period was adopted in the course of further optimization experiments.

4.6.2. Effect of initial pH of growth medium :

The effect of initial pH values of the medium on the biomass and protein yield of M.verrucaria, and T. viride was investigated. Aliquots of modified Czapek medium with 2% treated beet pulp were adjusted to different pH values ranging between 2 and 7 before being inoculated with the fungal cultures and incubated on the shaker. The results were tabulated in Table(13) and graphically presented in Figs (6 and 7). The data indicated that the two fungal culture could grow in a wide range of pH values (2-7). However, the higest values for protein content and protein yield were obtained

Table(13): Effect of initial pH medium on mycelial-protien content biomass and protein yield of Myrothecium Verrucaria and Trichoderma Viride grown on pretreated beet pulp.

Initial pH value	Myrothecium Verrucaria					Tricoderma Viride				
	Biomass	Yield	Protein	Yield	Crude protein	Biomass	Yield	Protein	Yield	Crude protein
	g/L	g/g substrate	g/L	g/g substrate	%	g/L	g/g substrate	g/L	g/g substrate	%
2	10.00	0.500	1.94	0.097	19.44	9.60	0.480	2.19	0.109	22.84
3	11.20	0.560	3.28	0.164	29.40	10.40	0.520	2.70	0.135	26.60
4	9.00	0.450	3.36	0.168	37.75	10.00	0.500	2.80	0.140	28.19
4.5	8.80	0.440	3.84	0.192	43.75	9.80	0.490	2.86	0.143	29.16
5	9.60	0.480	4.30	0.215	44.96	9.60	0.480	2.88	0.144	30.15
6	9.00	0.450	3.88	0.194	43.26	9.40	0.470	3.10	0.155	33.65
7	9.00	0.450	3.48	0.174	38.88	8.80	0.440	2.76	0.138	31.59

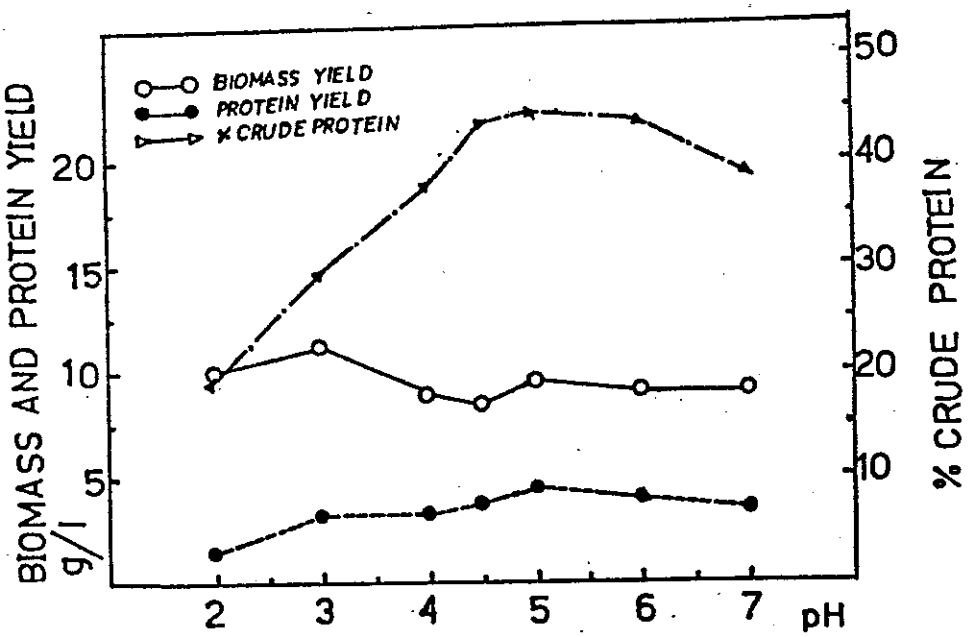


Fig (6): Protein content, biomass and protein yield of M. verrucaria grown on pretreated beet pulp as affected by initial pH of growth medium.

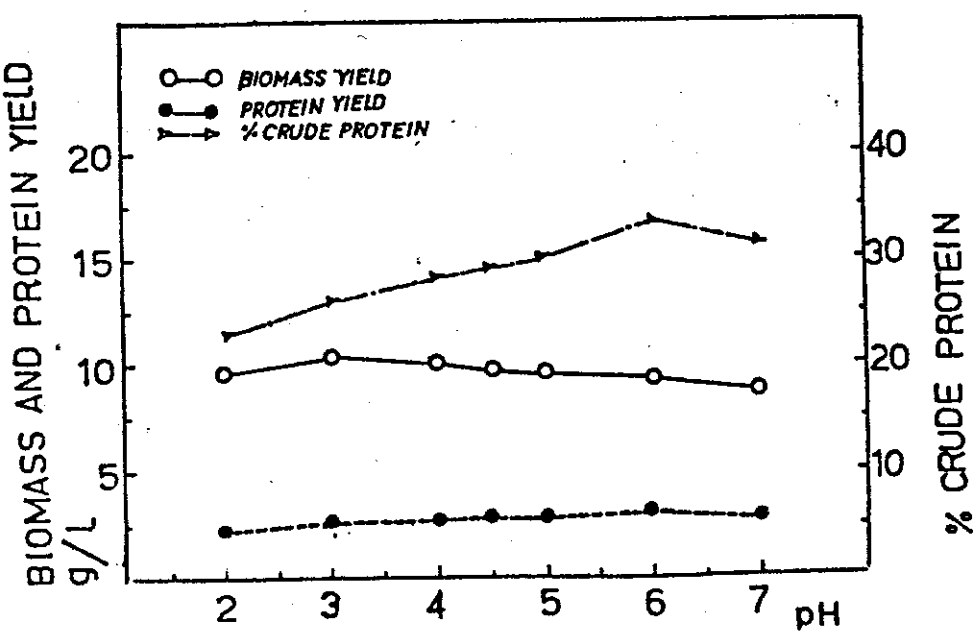


Fig (7): Protein content, biomass and protein yield of T. viride grown on pretreated beet pulp as affected by initial pH of growth medium.

at pH 5-6 for both microorganisms. At pH -2 both M.verrucaria and T.viride yielded their minimal protein content and yield. These results are in accordance with Litchfield (1968) who stated that the most favorable pH range for the growth of most fungi is from 4 to 7.

The optimum initial pH for protein production was found to be variable depending on both organism and growth medium used in study. Thus, Abd-El-Baky et al (1975) recomended pH 4.0 for maximum protein yield obtained by some species of Rhizopus Mucor and Actinumucor grown on potato media Cahal et al (1979) used wheat straw for SCP production by Cochiobolus specifer. They found that pH 4.5 to be the optimum value for SCP production. On the other hand the optimum pH reported by Chai and Chahal (1979) was 5 using Aspergillus luchuensis. In case of Aspergillus terreus Garg and Neelakantant 1981 reported that pH 4.0 was the optimum pH for bagasse bioconversion to microbial protein . Working with Trichoderma harzianum Ahmed (1983) found that the pH optimum of rice straw medium was 4.6 for obtaining maximum protein yields. Based on the data obtained in the present study initial pH of the medium was adjusted between 5 to 6 in further work.

4.63 Effect of inoculum ratio.

The expenimental flasks (in duplicate) were inoculated with different inoculum size ranging between 1-16% (V/V) of inoculum. Flasks were incubated on a rotary shaker at 28+1°C for four days. Results are presented in Table 15) and graphically illustrated in Figs (8 and 9). The maximum crude protein content (34%) and protein yield (3.8 g/l) of M. verrucaria were obtained with 8% inoculum concentration, while. T.viride showed slight increase of its crude protein content with 2% inoculom volume However, the protein yield slightly increased up to 2.9 g/l with 16% inoculoum size. With inoculum ratio ranged from 1-4% M.verrucaria gave nearly the same protein content and yield that were notably lower than the maximum values obtained with 8% inoculum size. The biomass yield of M.verrucaria showed no significant variations with increasing the inoculum concentration, On the other hand, biomass yield of T.viride was increased with increasing inoculum volume. El-Ashwah et al. (1981) working with fungi imperfecti Spicaria elegans, and Cladosporium. sp used inoculum size ranged from 10 to 30% They found the least protein content and protein yield were obtained at 10% and 20% inoculum size for the two fungal cultures respectively, Garg and Neelakantan,

Table (15) Effect of inoculium size on protien content biomass and protein yield of Myrothecium Verrucaria and Trichoderma Viride grown on pretreated beet pulp.

Inoculium Size	Myrothecium Verrucaria					Tricoderma Viride				
	Biomass	Yield	Protein	Yield	Crude protein	Biomass	Yield	Protein	Yield	Crude protein
% (V/V)	g/L	g/g substrate	g/L	g/g substrate	%	g/L	g/g substrate	g/L	g/g substrate	%
1	11.17	0.558	3.09	0.155	27.56	8.29	0.415	2.49	0.124	30.14
2	11.47	0.574	3.24	0.162	28.19	8.61	0.430	2.67	0.133	31.11
4	10.60	0.530	2.99	0.149	28.19	9.23	0.461	2.68	0.133	29.16
8	11.09	0.555	3.77	0.189	34.02	9.65	0.482	2.79	0.139	29.16
12	11.35	0.567	3.59	0.179	31.59	10.17	0.508	2.87	0.140	28.19
16	11.53	0.577	3.50	0.175	30.38	10.53	0.526	2.92	0.140	27.70

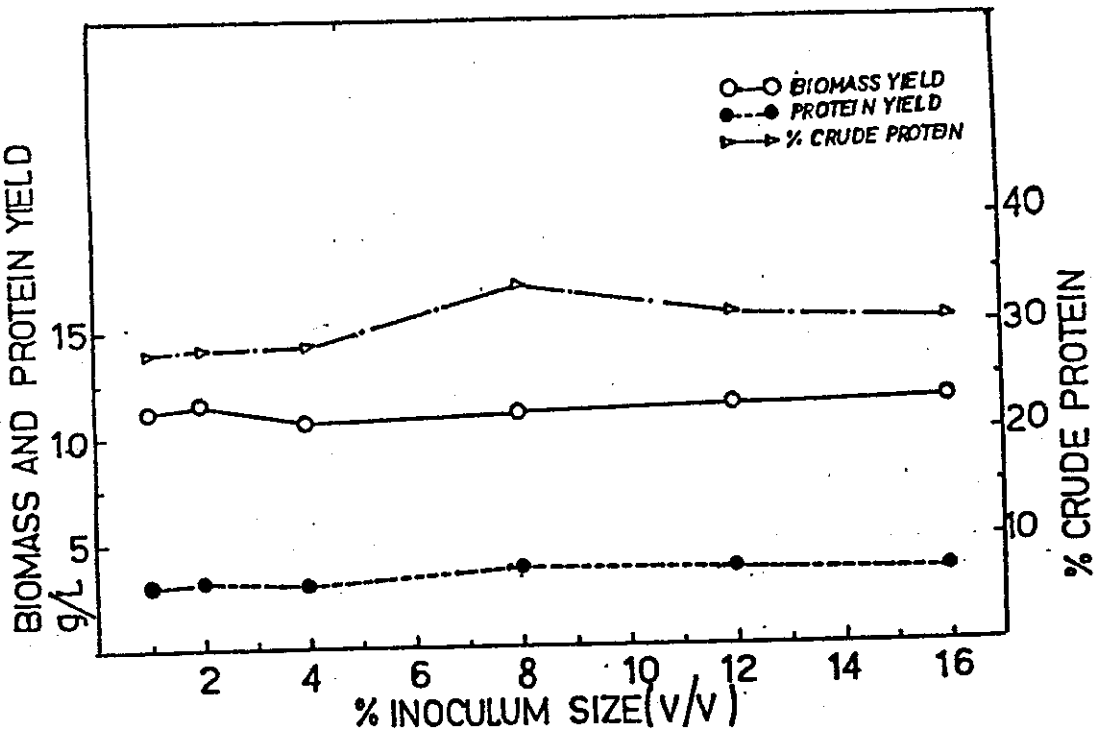


Fig (8): Protein content, biomass and protein yield of M. verruceria grown on pretreated beet pulp as influenced by inoculum size in growth culture.

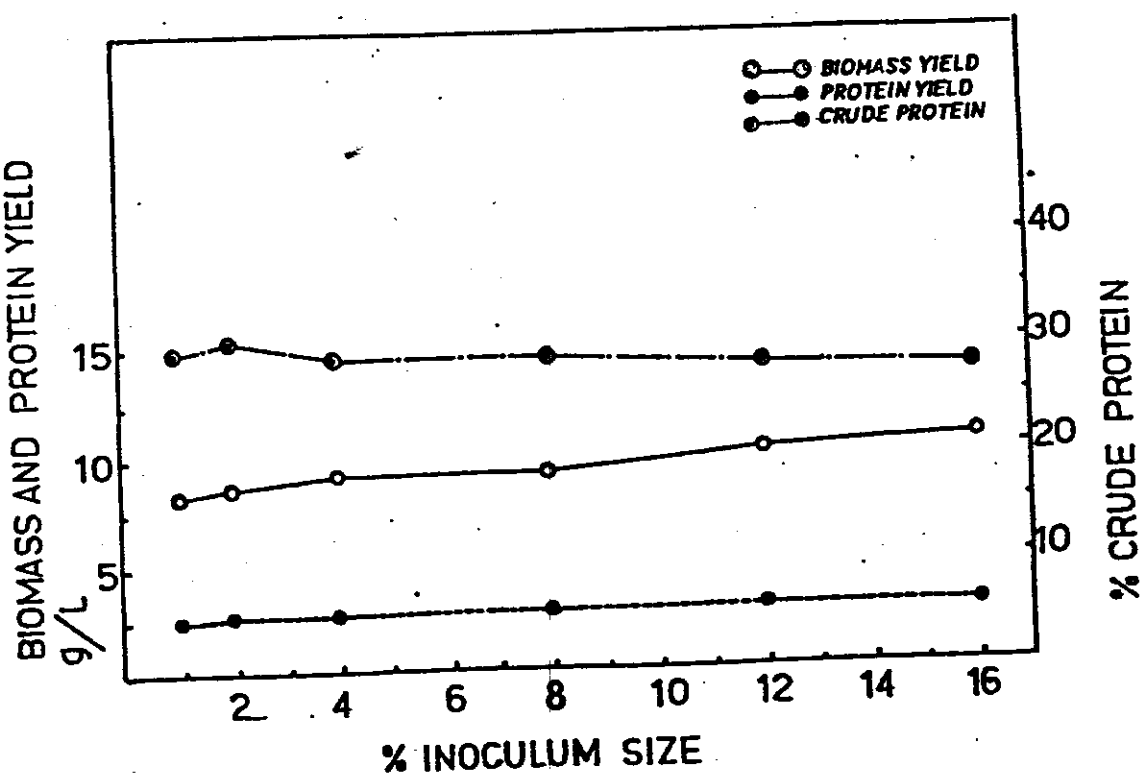


Fig (9): Protein content, biomass and protein yield of T. viride grown on pretreated beet pulp as influenced by inoculum size in growth culture.

1981 studied the effect of the inoculum size of Aspergillus terreusCN1 on protein production, stated that there was an increase in protein content and protein recovery from 1 to 6% inoculum volume .

From the previous data, 8% and 2% inoculum ratio were slelcted for conducting further studies on M. verrucaria and T.viride, respectively.

4.6.4 Effect of nitrogen source :

The fungal protein production is not only affected by the amount of nitrogen source but it is also affected by the nature of nitrogen source supplied in the growth medium, (Singh and Chahal, 1974; Ghai and Chahal, 1979)Therefore, the effect of type and amount of nitrogen source in the beet pulp medium were investigated with respect to their effect on protein yield produced byM.verrucaria and T.viride

4.6.4.1 Effect of the type of nitrogen source :

The effect of the type of nitrogen source on biomass and protein content of M.verrucaria and T.viride was investigated taking into account all the optimum growth conditions obtained from the former experiments. Thus, four inorganic salts (ammonium sulphate, ammonium hydrogen phosphate, ammonium chloride, and sodium nitrate) in addition to three

Table (16): Effect of nitrogen source on protein content biomass and protein yield of Myrothecium verrucaria and Trichoderma viride grown on pretreated beet pulp.

Nitrogen Source	Myrothecium Verrucaria					Tricoderma Viride				
	Biomass	Yield	Protein	Yield	Crude protein	Biomass	Yield	Protein	Yield	Crude protein
	g/L	g/g substrate	g/L	g/g substrate	%	g/L	g/g substrate	g/L	g/g substrate	%
Urea	10.80	0.540	3.95	0.197	36.59	8.40	0.420	3.30	0.165	39.27
Ammonium sulphate	11.75	0.587	3.72	0.186	31.68	9.64	0.482	3.26	0.163	33.91
Ammonium hydrogen phosphate	11.87	0.593	3.73	0.186	31.46	9.78	0.489	3.30	0.165	33.69
Ammonium chloride	11.75	0.587	3.72	0.186	31.68	9.66	0.483	2.86	0.143	29.67
Sodium nitrate	10.63	0.527	3.48	0.173	33.02	8.80	0.441	2.90	0.145	33.02
Yeast extract	13.80	0.690	3.86	0.194	28.11	10.50	0.525	3.26	0.163	31.01
Peptone	13.20	0.660	4.09	0.207	31.46	10.34	0.517	3.50	0.175	33.91
Control	15.70	0.785	2.04	0.106	13.61	11.98	0.699	1.68	0.084	14.05

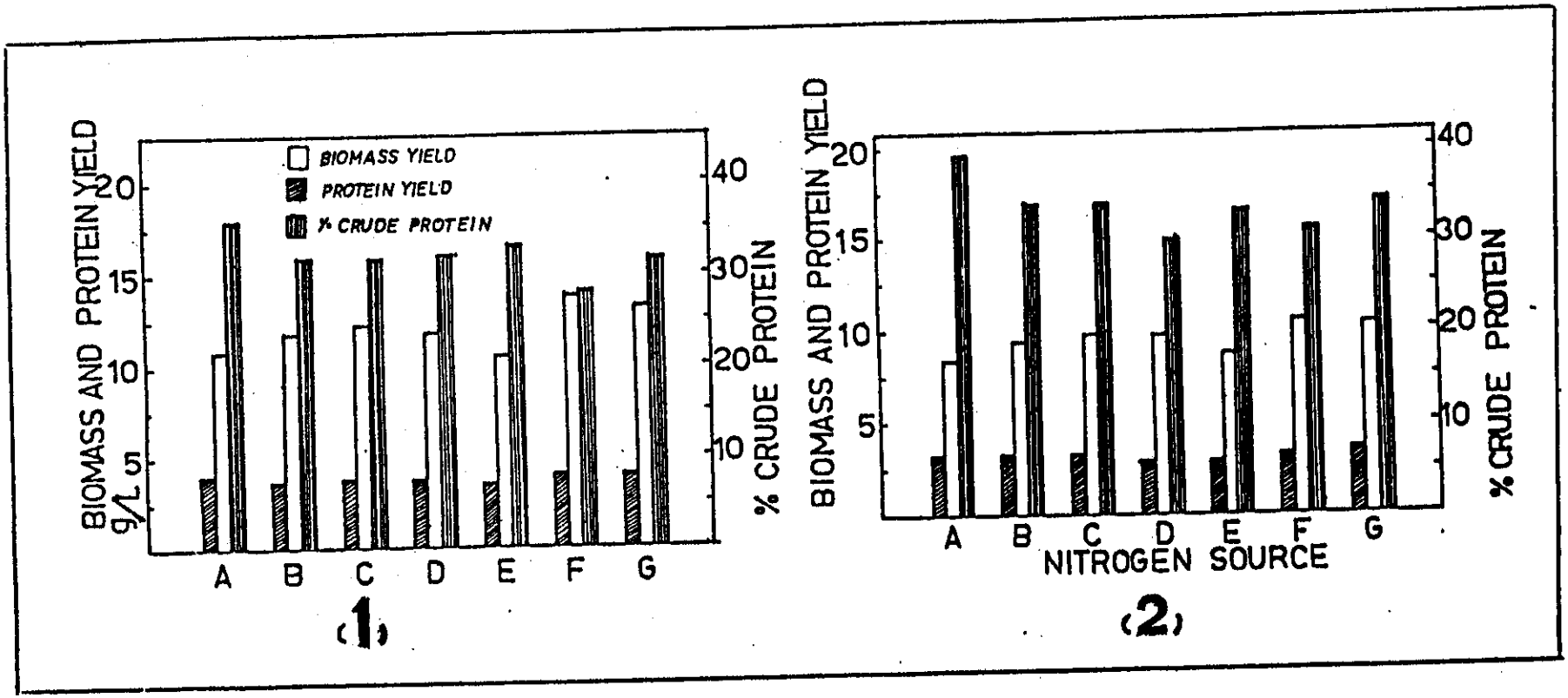


Fig (10): Protein content, biomass and protein yield of M. verrucaria (1) and T. viride (2) grown on pretreated beet pulp as a function of nitrogen source. (A urea; B ammonium sulphate; C amm. hydrogen phosphate; D amm. chloride; E sodium nitrate; F yeast extract; G peotone).

organic sources (urea, yeast extract, and peptone) were evaluated.

The amount of each nitrogen source previously mentioned was calculceted so as to contain a quantity of nitrogen equivalent to that present in 0.3% of ammonium sulphate (636 mg nitrogen liter) Accordingly, the calculated amount of each nitrogen source was incorporated to replace the original nitrogen source (Sodium nitrate) of the modified Czapek medium. Final concentration of acid-treated beet pulp of 2% was used. In this experiment neutralization of the acid treated beet pulp was carried out using sodium hydroxide instead of ammonium hydroxide. Results are tabulated in Table 16), and graphically illustrated in Fig.(10). It was obovious from the results that the maximum protein contents yielded by M.verrucaria (36.59%) and T.viride (39.27%) were obtained with using urea as nitrogen source. On the other hand, the maximum biomass yields (4 g/l and 3.5 g/l), and maximum protein yields (13.20 g/l and 10.34 g/l) were obtained with peptone as nitrogen source with M.verrucaria and T.viride, respectively . Urea gave nearly the same values for protein yield as those obtained using peptone by both microorganisms. Tesults show that the addition of nitrogen source to the beet pulp medium was of significant importance for increasing both protein content and protein yield.

The higest biomass yield with lowest protein content of the control medium reflects a lower biodegradability of beet pulp in absence of nitrogen source, Garg and Neelakantan 1981 concluded that in general the biomass dry matter yield of Aspergillus terreus was low with higar crude protein content of biomass. Conversely, with higher dry matter yield the crude protein content was low.

An interpretation of this converse relation between amount of protein content and biomass yield was discussed by Imrie and Righelato (1976). They stated that calculation of the substrate mass balance for protein production by molds indicated that most of nitrogen is retained in microbial biomass yet neaely half of the carbohydrate substrate is lost as carbon dioxide for the synthesis of highly ordered biological macromolecules. Hence, with higher crude protein content of biomass a lower dry matter yield is obtained.

The most suitable nitrogen source for SCP production seems to differ according to the organism and substrate used in the growth medium. Singh and Gahal (1974) found that Aspergillus terreus, Cladosprium and Fusarium equiste produced maximum protein percent

when ammonium nitrate was used as nitrogen source. However, Lizak, (1975) reported that KNO_3 and $(NH_4)_2 HPO_4$ were the best sources of inorganic N in the formation of cellulolytic enzymes by dark coulored fungi. Also Kalunyants et al., (1976) found that NH_4NO_3 increased the cellulase activity of T.viride than other forms of inorganic nitrogen on the other hand urea was the best nitrogen source for SCP production by Sporotrichum pulvorulonum as reported Erikson and larson (1975). Morefore, Lee and Hsie (1977) found that the addetion of urea increased cell mass production of a mixed microbial culture from 9.9 to 12.6 g/L grown on bagasse pretreated with 2% NaOH at 120 °C for 15 minutes. Recently Ahmed., (1983) reported that urea was the best nitrogen source for Trichodrma harzianum grown on rice straw media .

From data obtained in the present work, it could be noted that urea may be the best nitrogen source for SCP production from beet pulp using M.verrucaria and T.viride. Thus, in the next experiment urea was selected for investigating the effect of the amount of nitrogen source on biomass and protein yield of M.verrucaria and T.viride.

4.6.4.2 Effect of urea concentration :

A study was made using different concentrations of urea as nitrogen source in the acid treated beet pulp medium. Furthermore, in the same experiment the acid treated beet pulp followed by ammonium hydroxide (treatment NO.8) or sodium hydroxide neutralization with and witout addition 0.3% sodium nitrate were also compared. Due to the relative sensitivity of T.viride to higher urea concentrations(shown by a preliminary experiment), the final concentrations tested ranged between 0.0175 to 0.28%. The results are presented in tables(17)and(18)and illustrated in Figs (11 and 12).

It is clear from the data that the highest protein content (37.5%) and highest protein yield (3.46 g/l) produced by M.verrucaria were obtained with 0.14% urea. In case of T.viride a maximum of 36% crude protein and 3.36 g/l protein yield were obtained using 0.07% urea as final concentration.

As mentioned before the protein content and protein yields of T.viride decreased as the concentration of urea increased over 0.07% final concentration. Thus the protein content sharply decreased down to 2.39 g/l as the concentration of urea increased up to 0.28%. M.verrucaria showed a lesser sensitivity

Table (17): Effect of different concentrations of urea on protein content, biomass and protein yield of Myrothecium verrucaria.

Treatment and nitrogen source	Biomass yield		Protein yield		Crude protein
	g/L	g/g sub	g/L	g/g sub	%
Urea (0.07%)	10.36	0.518	3.08	0.154	29.75
Urea (0.14%)	9.24	0.462	3.46	0.173	37.50
Urea (0.28%)	8.00	0.400	2.63	0.131	32.90
Pretreatment (No.8)	11.80	0.590	2.91	0.145	24.67
Pretreated beet pulp + 0.3% sodium nitrate	6.83	0.341	2.04	0.102	30.00
Pretreated beet pulp with sodium hydroxide neutralization	13.20	0.660	1.58	0.079	12.09

Table (18): Effect of different concentration of urea on biomass and protein yield of Trichoderma viride.

Treatment and nitrogen source	Biomass yield		Protein yield		Crude protein %
	g/L	g/gsub	g/L	g/gsub	
Urea (0.0175%)	9.84	0.490	2.19	0.109	22.25
Urea (0.0350%)	9.07	0.453	2.21	0.112	24.68
Urea (0.0700%)	9.36	0.468	3.36	0.168	36.05
Urea (0.1400%	8.27	0.444	2.80	0.140	33.87
Urea (0.2800%)	12.11	0.605	2.34	0.117	19.35
Pretreated beet pulp	7.44	0.372	2.23	0.112	30.24
Pretreated beet pulp +0.3% sodium nitrate	9.36	0.468	2.78	0.139	29.75
Pretreated beet pulp with sodium hydroxid neutralization	11.84	0.592	1.66	0.082	14.03

even to the higher concentration of urea over 0.14% final concentration. Accordigly, it might be concluded that different fungal cultures may exhibit different responses to different nitrogen source concentrations which could be reflected in their biomass protein yield and protein content. This may be due to the different C:N ratios suitable for each fungal culture, Lichfield (1968) reported that the highest protein concentration and lowest fat contents in the mycelium are obtained at C:N ratios from 5:1 to 25:1. He added that above this latter value fat content became excessive and protein yields decreased significantly.

M.verrucaria showed a less protein content when grown on beet pulp treated with acid and neutralized with ammonium hydroxide than that obtained with 0.14% urea. A lesser protein content was obtained when sodium nitrate was omitted from the growth medium. On the other hand, T.viride showed a different response since it gave nearly the same protein content irrespective to the presence or absence of sodium nitrate in the growth medium. This means that the added sodium nitrate to the growth medium was not utilized and the acid treated beet pulp followed by ammonium hydroxide neutralization contained a sufficient amount of nitrogen for growth of T.viride. It

could be noted also that the highest biomass yields given by both fungal cultures were concomitant with the lowest protein content.

Based on the results obtained in these experiments concentrations of urea equal to 0.14 and 0.07% were adopted and added to the growth medium as nitrogen source for M.verrucaria, and T.viride, respectively, in further work.

4.6.5 Effect of aeration :

This experiment aimed to study the effect of aeration on growth and protein content of M.verrucaria and T.viride grown on beet pulp medium. The aeration level was adjusted through the control of air : medium ratio using 250 ml conical flaskes. The shaking speed of the shaker was set at constant speed 150 r.p.m. All the appropriate growth conditions chosen from the previous work were adopted here. The air to medium ratios in the experimental flasks varied between (20 :1) to (5:3) to obtain different aeration levels.

Results are presented in Tables (19). The maximum protein content given by M.verrucaria was 36.3% with (10:1) air : medium ratio while T.viride gave its maximum protein content 33,9% with 5:2 (air : medium ratio) On the other hand, the minimal

Table (9): Effect of aeration on protein contents biomass and protein yield Myrothecium Verrucaria and Trichoderma Viride grown on beet pulp.

Aeration level (Air:Medium ratios)	Myrothecium Verrucaria					Tricoderma Viride				
	Biomass	Yield	Protein	Yield	Crude protein	Biomass	Yield	Protein	Yield	Crude protein
	g/L	g/g substrate	g/L	g/g substrate	%	g/L	g/g substrate	g/L	g/g substrate	%
20 : 1	11.20	0.560	2.98	0.149	26.61	9.60	0.494	2.96	0.153	30.97
10 : 1	9.94	0.447	3.24	0.162	36.29	8.70	0.435	2.65	0.132	30.48
5 : 1	8.55	0.428	2.85	0.142	33.39	8.00	0.400	2.51	0.125	31.45
5 : 2	9.50	0.475	3.21	0.160	33.87	8.17	0.408	2.76	0.138	33.87
5 : 3	11.47	0.574	4.05	0.202	35.32	8.46	0.634	2.37	0.178	28.06

* The aeration level was adjusted through control of air : medium ratio in the culture medium.

velues for protein content (26.6 / and 28%). were obtained with the use of air : medium ratios of 20:1 and 5:3 in cultures of M.verrucaria and T.viride, respectively. At air : medium ratio of (5:3) M. verrucaria yielded its maximal biomass and protein yield concomitant with high protein content (35.3%) On the other hand, T.viride showed a little variation in its biomass yield with respect to the aeration ratios indicating insensitivity of the organism to variations of aeration level used in the present experiment .

With reference to these results it could be concluded that, the lowest protein content of M verrucaria obtained upon using a high aeration ratio (20:1, air : medium ratio) may be attributed to the pattern of growth resulting under these conditions. In this case the organism tend to form filamentous growth (other than the uniform pellet) that adhered to the flask walls and consequently have undergone sporulation Conversely, T.viride showed a pellet type of growth at this level of aeration. On the other hand, the higher protein content given by M.verrucaria with the lowest aeration may indicate that this level of aeration was not growth limiting for this organism. This may be also applicable to T.viride up to the

penultimate aeration ratio (5 : 2).

At lower aeration level (5 : 3, air : medium ratio)the aeration level became growth limiting as was indicated by the low protein content in the latter organism. Garg and Neelakantan (1981) working with Aspergillus terreus grown on bagasse medium adopted three aeration levels (5:1, 5:2, 5:3, air medium ratios) using 250 ml fermentation flasks They stated that the maximum protein content of biomass (19.4%) was botained at 5:1, air : medium ratio, while the maximum protein recovery (9.1 g/100g bagasse) was obtained at 5:2, air : medium ratio.

Based on the results obtained from the aeration experiment air : medium ratios of 5:2 and 5:3 were selected for conducting further work with M.verrucaria and T.viride, respectively,

4.6.6. Effect of beet pulp concentration :

Different concentrations of beet pulp ranged from 2.5 to 40 g/liter were used for investigating the effect of beet pulp concentration on biomass and protein content of M.verrucaria, and T.viride.

The beet pulp treated with acid and neutralized with sodium hydroxide was used as applied in the last two experiments. Urea was added to the growth medium as nitrogen source in the approporate concentrations adopted from previous experiments. Duplicate flaskes were used, the amount of medium in each were 150 ml and 100 ml for M.verrucaria, and T.viride, respectively. After inoculation, flasks were incubated at 28 ± 1°C and 150 r.p.m in rotary shaker.

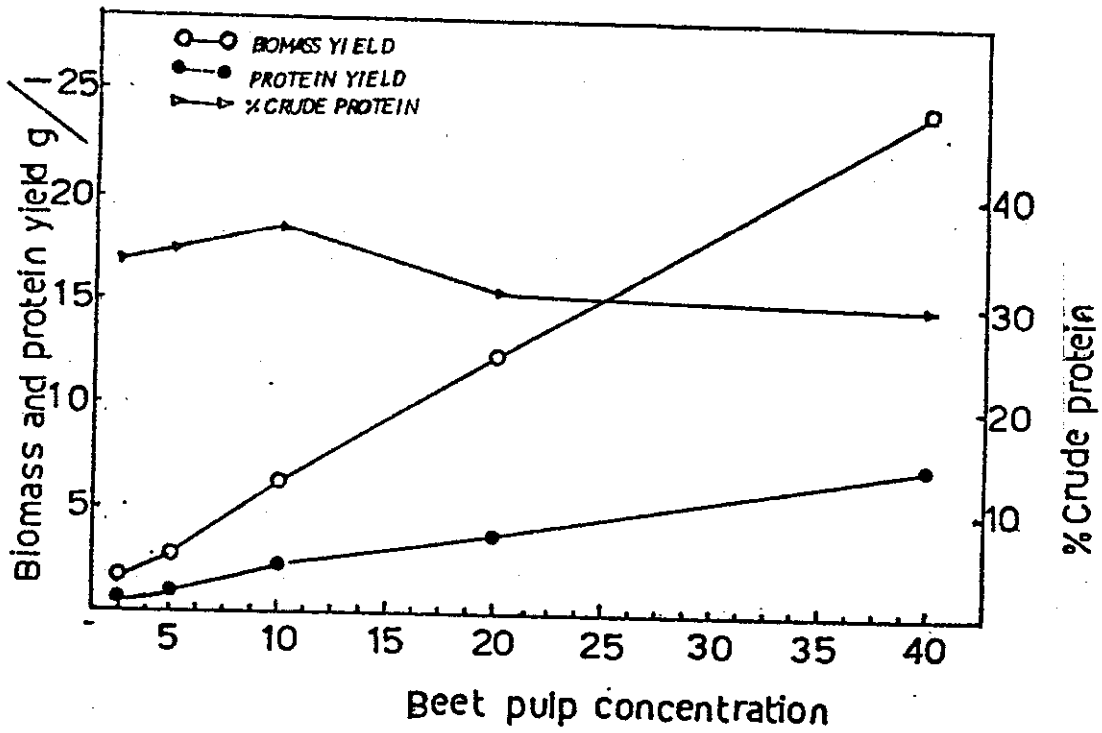
The results are tabulated in Table (. 20 .), and illustrated in Figs(13,14)Results reveal that values for protein content were progressively increased to a maximum value of 37.15% for M.verrucaria, at 10 g/l beet pulp concentration while the corresponding value of T. viride was 39.80% at 20 g/l beet pulp concentration. With increasing beet pulp concentration, the protein content was notably decreased in both microorganisms. Thus, protein content of T. viride was lower by about 25% when the concentration of beet pulp was increased from 20 to 40 g/l.

On the other hand, T.viride gave nearly the same protein content with 10 and 20 g/l beet pulp concentration.

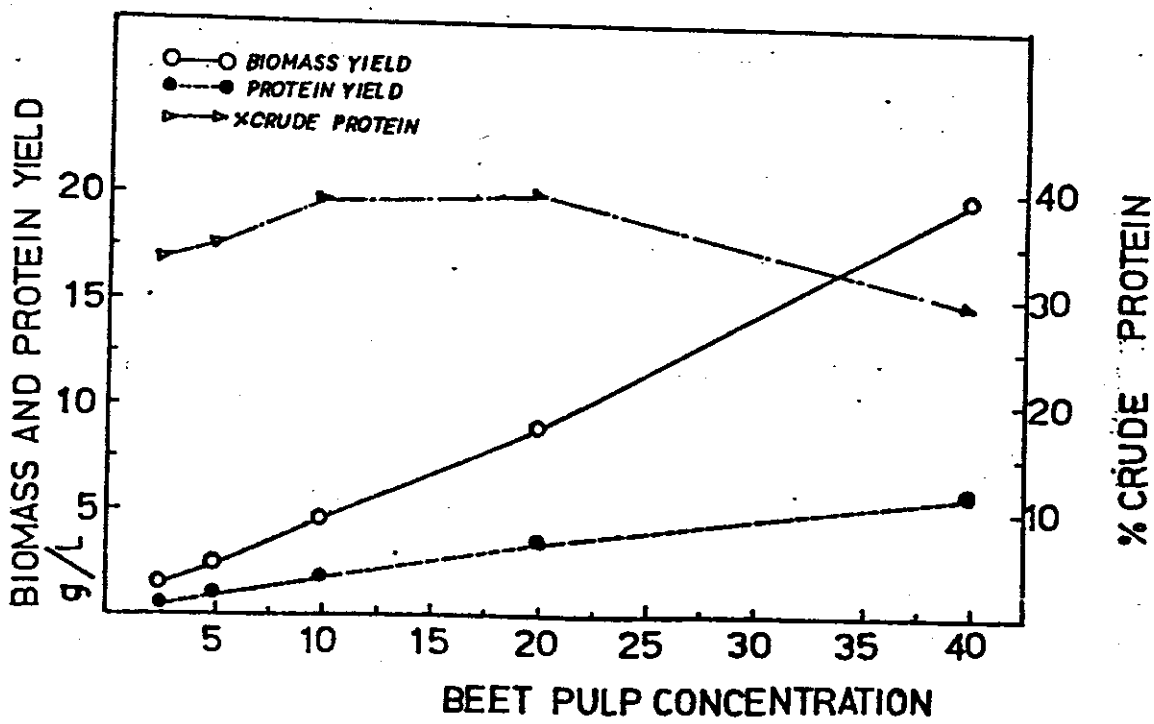
The highest protein contents of M.verrucaria and T.viride (37% and 39.8%) were obtained with

Table(20): Effect of beet pulp concentration (g/L) on protein content biomass and protein yield of Myrothecium Verrucaria and Trichoderma Viride.

Beet Pulp concentration	Myrothecium Verrucaria					Tricoderma Viride				
	Biomass	Yield	Protein	Yield	Crude protein	Biomass	Yield	Protein	Yield	Crude protein
	g/L	g/g substrate	g/L	g/g substrate	%	g/L	g/g substrate	g/L	g/g substrate	%
2.5	1.94	0.775	0.65	0.259	33.43	1.54	0.616	0.51	0.202	32.90
5	2.81	0.562	0.98	0.196	35.03	2.69	0.537	0.94	0.188	35.02
10	6.29	0.629	2.33	0.233	37.15	4.58	0.459	1.80	0.180	39.27
20	12.42	0.621	3.81	0.191	30.78	8.87	0.436	3.53	0.137	39.80
40	24.17	0.604	7.17	0.179	29.72	19.69	0.492	5.75	0.143	29.19



Fig(13): Protein content, biomass and protein yield of M. verrucaria as affected by concentration of pretreated beet pulp in the growth medium.



Fig(14): Protein content, biomass and protein yield of T. viride as affected by concentration of pretreated beet pulp in the growth medium.

using 10 and 20 g/l beet pulp concentrations respectively. Data also show that both biomass and protein yield increased as the beet pulp concentration increased. The highest values for biomass and protein yield obtained with M. verrucaria and T. viride were (24 and 19.7 g biomass /L) and (7 g and 5.75 g protein L), respectively, with using 40 g/L of beet pulp. Menezes et al (1976) found that the maximum protein yield given by M.verrucaria, T. viride and Geotrichum sp.was obtained using 10 g bagasse/l of the medium. They added that the average productivity increased with increasing bagasse concentration up to 20g/L The optimum concentrations of cellulosic substrates seems to differ accordding to the type of the by-product and also depending on the microorganism used.

More recently Ahmed (1983) recommended the use of 20-25 g/L of rice straw to obtain the highest protein yield using Trichoderma harzianum.

4.7 Nucleic acid content of M.verrucaria and T.viride:

In the presnet study the two organisms namely M.verrucaria and T.viride were grown on media containing beet pulp treated with two different procedures that were found to give the highest protein content

Table 2 Biomass yield, protein yield, crude protein, and nucleic acid content of M. verrucaria and I.viride grown on beet pulp treated with two selected pretreatments. under the optimum conditions dotaind in the present study.

Substrate.	Biomass yield		Protein yield		%Crude protein	%Nucleic acids.	Nucleic acids g/100g protein.
	g/L	g/g substrate	g/L	g/g substrate.			
			M. verruaria				
Beet pulp treated with acid, followed by nautralization with ammonium hydroxide.	10.53	0.526	4.19	0.209	40.47	0.856	2.115
Beet pulp treated with acid, followed by neutralization with sodium hydroxide, and using urea as nitrogen source	10.33	0.516	3.11	0.155	30.08	0.863	2.869
			T. Viride				
Beet pulp treated with acid followed by neutralization with ammonium hydroxide.	8.86	0.443	2.91	0.145	32.81	0.705	2.148
Beet pulp treated with acid, followed by neutralization with sodium hydroxide, and using urea as nitr en source.	7.10	0.355	2.56	0.128	36.09	0.604	1.673

as compared with the other pretreatments. The obtained mycelial biomasses were analyzed for both protein yields, and their nucleic acid contents. summarized in tables (21).

Results indicate that the nucleic acid content of both fungal cultures tested did not exceed 1% (calculated per 100 g dry weight of biomass). Schellart (1975) reported that nucleic acids content of different microorganisms depends on both growth rate and growth conditions. Furthermore, the nucleic acid content would also differ according to the specific organism under study.

The presence of high nucleic acid content in SCP for human consumption should be avoided since that would lead to the accumulation of urate formed as a result of purines degradation, thus causing gout and renal calculi.

However, this factor is of less importance for use of SCP in animal feed, In the present study the, biomasses produced by M.verrucaria and T.viride have already low nucleic acid content. probably due to the presence of appreciable part of indigested beet pulp along with the fungal mycelia.

Thus, the nucleic acid content may not be a source of harzard if the fungal biomass produced here is used for human consumption or animal feed.

4.8 Amino acid composition of M. verruaria and T.viride grown on beet pulp medium.

In the present experiment the two fungal cultures namely, M.verrucaria and T.viride were grown on pretreated beet pulp media under standard conditions. The two different pretreatments adopted in the present experiment were selected since they gave the highest protein content upon comparison with other pretreatments tested in the experiments .

The amino acids composition of M.verrucaria and T.viride grown on pretreated beet pulp is summarized in Table(22). The acid hydrolyzates of both fungi revealed the presence of at least 17 amino acids namely aspartic acid, threonine serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, tyrosine, phenylalanine, histidine, lycine and arginine, proline and traces of tryptophan. Appreciable amounts of ammonia were also detected. The results show that the amino acids varied greately in their quentities in the hydrolyzates of the two fungal biomass under study.

Table Amino Acid composition of *M. verrucaria* and *T. viride* grown on beet pulp treated with two treatments and utilizing two different nitrogen sources.

Amino acid	*M. verrucaria*					*T. viride*				
	Beet pulp treated with acid and then neutralised with NH_4OH-(Nitorgen source was$NaNO_3$).		Beet pulp treated with acid and then neutralised with NaOH(Nitorgen source was urea)		Average g/100g dry wt.	Beet pulp treated with acid and then neutralised with Nh_4OH(Nitrogen source was $NaNO_3$).		Beet pulp treated with acid and then neutralised with NaOH(Nitrogen source was urea).		Average g/100g dry wt.
	g/100g dry wt,.	g/16g N	g/100g dry wt.	g/16g N		g/100g dry wt.	g/16 g N	g/100g dry wt.	g/16g N	
Aspartic acid	1.50	3.72	1.5	5.00	1.50	4.51	13.75	2.12	5.87	3.31
Threonine	0.51	1.27	1.13	3.76	0.82	0.62	1.89	1.17	3.24	0.90
Serine	0.67	1.66	0.88	2.94	0.77	1.23	3.75	1.15	3.19	1.19
Proline	0.23	0.57	1.65	5.48	0.94	1.54	4.96	0.90	2.50	1.22
Glutamic acid	1.34	3.30	6.79	16.76	4.06	1.90	5.79	1.76	3.19	1.83
Glycine	0.74	1.83	1.65	3.12	1.19	0.74	2.25	2.09	5.80	1.41
Alanine	0.80	1.97	0.94	6.69	0.87	4.07	12.40	1.92	5.30	3.00
Valine	1.08	2.66	0.85	2.82	0.96	0.28	0.83	0.30	0.84	0.29
Methionine	0.26	0.63	0.88	0.27	0.26	0.04	0.11	0.07	0.18	0.05
Cysteine	-	-	-	-	-	-	-	-	-	-
Isoleucine	0.75	1.85	0.30	0.99	0.52	0.29	0.89	0.90	2.50	0.60
Leucine	1.12	2.77	0.90	2.98	1.01	1.51	4.59	1.64	4.53	1.57
Tyrosine	0.21	0.53	0.14	0.47	0.17	0.07	0.22	0.12	0.33	0.09
Phenylalanine	0.49	1.22	0.28	0.92	0.38	0.38	1.15	0.28	0.78	0.33
Histidine	0.44	1.09	0.30	0.99	0.37	0.75	2.29	0.34	0.93	0.54
Lysine	0.60	1.48	0.73	2.43	0.66	1.06	3.23	0.29	0.81	0,67
Arginine	0.22	0.53	0.38	1.25	0.30	0.63	1.91	0.38	1.05	0.50
Tryptophan(lessthan)	0.0003	0.0007	0.0003	0.0007	0.0003	0.0003	0.0007	0.0003	0.0007	0.0003