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Bioconversion of Filter Press Cake (Mud) of Sugar-cane to Biomass Protein and its Biological Evaluation

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Abstract: Filter Press Cake (Mud) was enriched with biomass protein using *Arachniotus* sp. as fermentative organism. The Mud was dewaxed for increased protein production before fermentation. Proximate analysis of native and biomass was done. It was found that after fermentation ash contents decreased from 17.50 to 16.00 % and fiber was decreased from 28 to 2%. Ether extract remained the same. Prior to the production of biomass protein certain conditions like fermentation period (72 h), substrate to water ratio (6%), $(NH_4)_2SO_4$(0.1%), $CaCl_2.2H_2O$ (0.025%), $MgSO_4.7H_2O$ (0.015%), KH_2PO_4 (0.1%), Cane molasses (1.5%), and Corn Steep Liquor (2.0%) were optimized. These conditions were then applied to produce biomass protein on large scale. The biomass thus produced contained 26.25% crude protein, 13.12% true protein, 6.73% ether extract, 16% ash and 2% crude fiber. Amino acid analysis of biomass revealed the presence of 15 amino acids. The chemical score of protein was '0' and Leucine and Valine were first and second limiting amino acids respectively. The protein quality of biomass was tested in terms of digestibility that showed an average digestibility of 80.917%.

Key words: Bioconversion, sugar-cane, biomass, proteins, filter press case, mud, biological evaluation

Introduction

Protein is an essential part of human and animal diet as it is of great importance to all living matter. With the outset of exploding population, protein deficiency is an important nutrient problem in most developing countries. Cereals, which were thought to be the major nutrient elements for both poultry and livestock, lack good quality proteins and essential amino acids like lysine, arginine and threonine (Saima, 1996).

Supplementation of vegetable proteins with that of animal protein in practice results in higher feeding costs (Dasilva *et al.*, 1987). It is therefore, imperative to produce economically good quality proteins from non-conventional sources. A number of organizations all over the world are actively investigating the process using variety of agricultural waste/residue as feed stock for biomass protein production using either fungi/bacteria or yeast.

Filter press cake (Mud) is an agro-industrial waste of the cane sugar mills. In Pakistan 15617606 metric tones of cane is crushed and about 468528.18 metric tones of filter cake is disposed off annually and not being utilized properly. It contains wax, protein and some nutrient elements like Na, K, Ca, phosphorus and sucrose content. Filter press cake (Mud) from sugar-cane may be used as a potential feed of the animals especially in poultry feed. In the manufacture of cane sugars, the precipitated impurities contained in the cane juice, after removed by filtration, form a cake of varying moisture content called filter cake also called scum's and cachaza. The amount of filter mud present in cane and its decomposition varies greatly with the locality, variety of cane, milling efficiency and method of clarification (Jamil, 1989).

It is essential to enrich filter press cake with biomass protein before supplementation in poultry rations and livestock. Fermenting it with *Arachniotus* sp. under optimum conditions can do enrichment of this filter press cake with protein. A project was therefore planned to develop a fermentation process by utilizing dewaxed filter press cake as a substrate for the production of protein enriched biomass with the *Arachniotus* sp. and to evaluate chemically and biologically.

Materials and Methods

This project was initiated in September 1999 and was completed in December 2000. The filter press cake (Mud) obtained from Crescent Sugar Mills Ltd. Faisalabad, was dried in oven at 100°C up to constant weight. It was analyzed for its proximate composition (Moisture, Ether extract, Ash, Crude fiber, Crude protein) before utilization as fermentative substrate (AOAC, 1984). For dewaxation dried and ground material was taken in benzene. It was shaken, allowed to stand and decanted thrice. Wax was obtained from benzene by distillation. The remaining residue was dried to 100°C in oven and was used as fermentative substrate.

Pure culture of filamentous fungal organism *Arachniotus* sp procured from Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan was raised on nutrient agar slants medium.

The growth medium for culturing *Arachniotus* sp. for the production of higher protein was developed and its conditions were optimized by conducting various experiments. Different culture conditions including varying fermentation periods (24-120 h), substrate to water ratio (1-7%), varying ionic concentrations, [$(NH4)_2SO_4$(0.05, 0.10, 0.15, 0.20, 0.25%), $CaCl_2.2H_2O$(0.010, 0.015, 0.020, 0.025, 0.030%), $MgSO_4.7H_2O$(0.010, 0.015, 0.020, 0.025, 0.030%), KH_2PO_4(with 0.05, 0.10, 0.15, 0.20, 0.25, 0.30%)] Cane molasses (0.5-2.5%) and corn steep liquor(0.5-2.5%) were optimized. Preoptimized pH 4 and 30± 2°C temperature was used for fermentation (Bajwa *et al.*, 1991). Enrichment of sample with protein was achieved by fermenting it under optimum conditions.

Biomass produced under optimum conditions was dried at 100°C under vacuum. The dried biomass was analyzed for its proximate composition (AOAC, 1984), true protein (Munro and Fleck, 1966) amino acid profile (Moore and Stein, 1954) and increased biomass protein (Hiller *et al.*, 1948).

The protein quality of biomass was tested in terms of digestibility using three-enzyme method (Pedersen and Eggum, 1981). Chemical score of biomass protein was calculated using the method of Anonymous (1957).

Results

The primary objective of the study was to upgrade the filter press cake with respect to protein. Chemical composition of native biomass was done (Table 1).

The following conditions were optimized for the enrichment of biomass protein with *Arachniotus* sp. For fermentation periods it was observed that production of biomass protein increased with increase in fermentation period from 24 to 72 h, while it decreased, with further increase in fermentation period (Fig. 1). The maximum production (15.20%) was obtained at 72 h. This may be due to the fact that *Arachniotus* sp. utilized all nutrients from the medium after 72 h and then it starts using the fermentation product for its growth.

There was an increase in production of biomass protein from 1 to 7% substrate to water ratio. A decrease in biomass protein was

Table 1: Chemical composition of filter press cake (Native)

Component (on dry basis)	Dry matter	Ash	Ether extract	Crude fiber	Crude protein	NFE	Moisture	Wax
Composition (%)	74.86	17.50	6.73	28.00	10.35	8.88	25.14	3.40

Table 2: Crude protein content (%) of biomass produced by *Arachniotus* sp. with varying concentrations of cane molasses and corn steep liquor under optimum conditions

	Concentrations (%)				
Material used	0.5	1.0	1.5	2.0	2.5
Cane molasses (%)	23.33	23.10	24.75	23.95	23.85
Corn steep liquor (%)	24.23	25.96	25.75	26.25	25.67

Substrate 6%; $(NH_4)_2SO_4$, 0.1%; $CaCl_2.2H_2O$, 0.025%; $MgSO_4.7H_2O$ 0.015%; KH_2PO_4, 0.1%; pH 4 and 30± 2°C

Table 3: Amino acid profile of biomass produced using *Arachniotus* sp.

Amino acids	Percent at protein basis (g/100 g)
Aspartic acid	1.738
Threonine	0.689
Glutamic acid	1.963
Alanine	0.979
Cystine	1.529
Valine	0.211
Methionine	0.634
Isoleucine	0.931
Leucine	0.000
Tyrosine	0.716
Phenylalanine	0.401
Lysine	29.265
Histidine	0.537
Arginine	0.335
Proline	3.192

Table 4: Chemical score of biomass protein using FAO (1957) method

Amino acids	FAO amino acids pattern (mg/g)	Biomass amino acids (mg/g)	Available amino acids (%)
Isoleucine	42.0	9.31	22.166
Leucine	48.0	0.00	0.000
Lysine	42.0	292.65	696.785
Methionine	22.0	6.34	28.818
Phenylalanine	28.0	4.01	14.321
Threonine	28.0	6.89	24.607
Valine	42.0	2.11	5.023
Chemical score			0.00
Limiting amino acids			
1st			Leucine
2nd			Valine

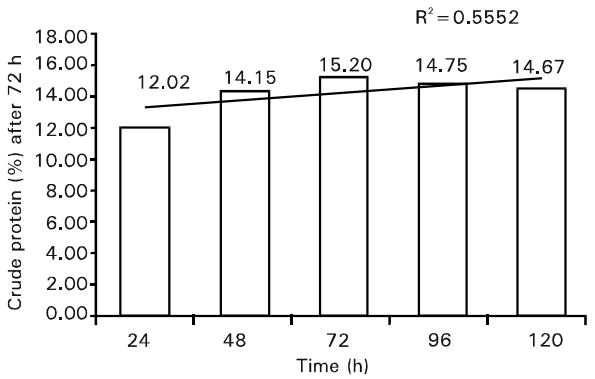

Fig. 1: Effect of different fermentation periods on biomass protein production by *Arachniotus* sp.

observed with higher substrate to water ratio (Fig. 2). The maximum production (16.39%) was obtained at 6% substrate to water ratio.

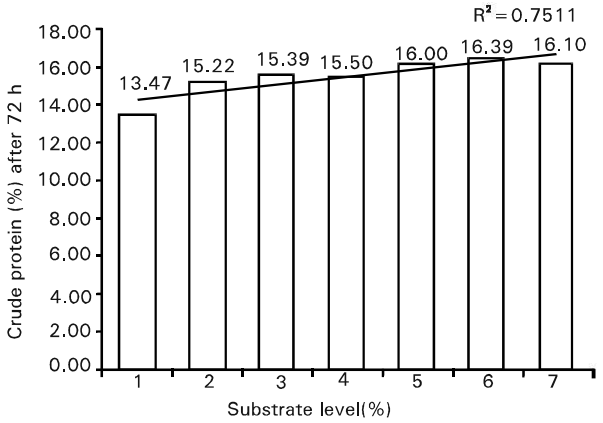

Fig. 2: Effect of various levels of dewaxed filter press cake on biomass protein production by *Arachniotus* sp.

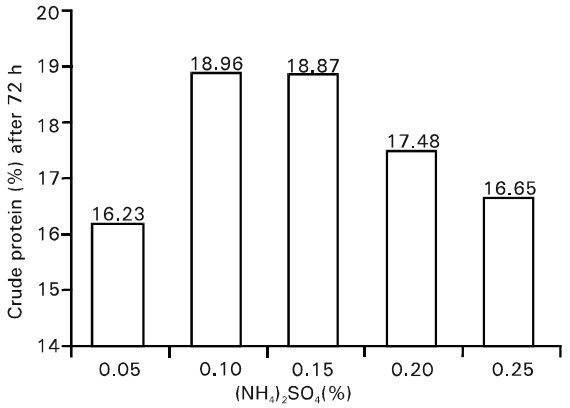

Fig. 3: Effect of varying concentrations of $(NH_4)_2SO_4$ on biomass protein production by *Arachniotus* sp.

For establishing the optimum ionic concentrations in growth medium, different experiments were conducted with varying levels of various ions. Experiments were carried out in such a way that concentration of the nutrient optimized in one experiment was maintained in the subsequent studies.

Addition of $(NH4)_2SO_4$ resulted in increased biomass protein production from 0.05% to maximum (18.96%) at 0.10% and then decreased with further increase in $(NH_4)_2SO_4$ (Fig. 3).

For $CaCl_2.2H_2O$ biomass protein production increased from 0.010% to maximum (19.98%) at 0.025% and then decreased with further increase in $CaCl_2.2H_2O$ (Fig. 4)

Biomass protein production was increased with an addition of

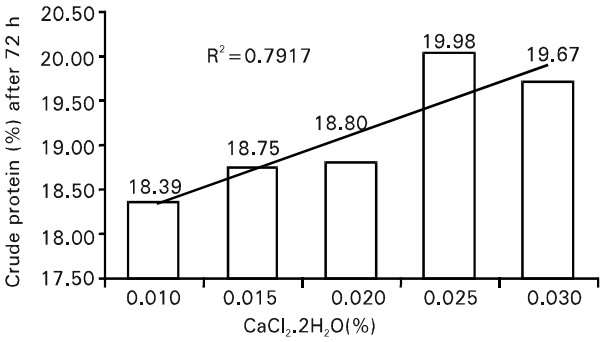

Fig. 4: Effect of varying concentrations of $CaCl_2.2H_2O$ on biomass protein production by *Arachniotus* sp.

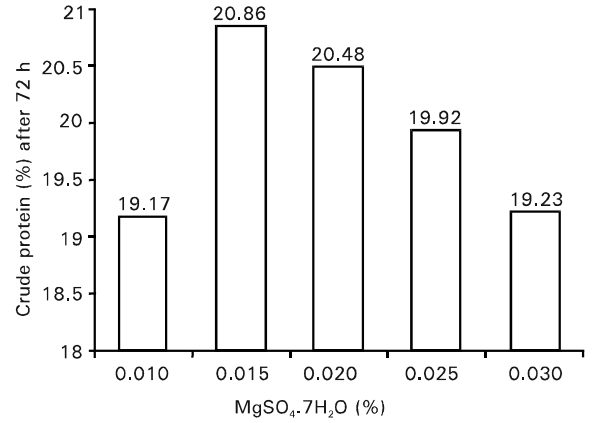

Fig. 5: Effect of varying concentrations of $MgSO_4.7H_2O$ on biomass protein production by *Arachniotus* sp.

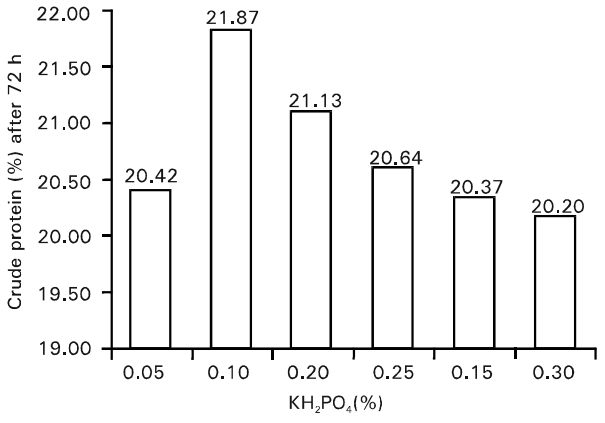

Fig. 6: Effect of varying concentrations of KH_2PO_4 on biomass protein production by *Arachniotus* sp.

0.010 to 0.015% $MgSO_4.7H_2O$ and decreased with further increase in $MgSO_4.7H_2O$ (Fig. 5). Moreover there was an increase in biomass protein production with the addition of KH_2PO_4 from 0.05 to 0.10% and decrease with further addition of salt (Fig. 6). The production of protein was increased with an addition of 0.5 to 1.5% molasses. It decreased with further higher molasses concentration (Table 2). There was an initial increase in the production of protein from 0.5 to 2.0% corn steep liquor, which decreased thereafter (Table 2). The average digestibility of microbial protein was found to be (80.917%).

Amino acid profile revealed that only 15 amino acids were present in biomass (Table 3). The chemical score of biomass protein was '0'% (Table 4). Leucine was the first limiting amino acid and second was Valine. It showed that isolated protein cannot replace the protein feed ingredients utilized in the formulation of animal feed or human feed.

Finally ash content of biomass decreased from 17.5 to 16% on fermentation using *Arachniotus* sp. under optimal conditions. Crude fiber also decreased from 28 to 2%, because *Arachniotus* sp. utilized fiber as an energy source. Ether extract remained the same. The biomass increased from 10.35 to 26.25% which was the main objective.

Discussion

For fermentation periods the maximum production (15.20%) was obtained at 72 h. Present results are in line with Akram *et al.* (1991) who fermented wheat bran roots with *Aspergillus terreus* and recovered final product containing 23.77% crude protein after 72 h of incubation. Similarly Alam (1986) produced maximum biomass protein from rice polishing by *Arachniotus* sp. after 72 h of incubation at pH 4 and 30°C.

The maximum production (16.39%) was obtained at 6% substrate to water ratio. Our findings are in accordance with Lee *et al.* (1979), who fermented 6.0% bamboo shoot husk for maximum protein (25.6%) production by *Cellulomonas* sp. Furthermore Sana (1992) used wheat bran for biomass protein production and reported that 6% was the optimum substrate to water ratio for maximum crude protein production.

In current study the addition of $(NH_4)_2SO_4$ resulted in increased biomass protein production from 0.05% to maximum (18.96%) at 0.10%. Results are in line with those of Chahal *et al.* (1987), who produced maximum fungal biomass protein from corn stover through fermentation with Pleurotus sajor-cajo in the presence of 0.14% $(NH_4)_2SO_4$ as optimum nitrogen source and Mahasneh (1997) who reported that addition of ammonia into the fermentation medium increased the single cell protein production by five strains of *Chlorella* sp. from 15.7 to 41.8%.

For $CaCl_2.2H_2O$ biomass protein production increased from 0.010 to maximum (19.98%) at 0.025%. Results are in accordance with those of Chahal *et al.* (1987), who produced maximum biomass protein (40%) from corn stover by Pleurotus sajor-caju in the presence of 0.03% $CaCl_2.2H_2O$ and optimum concentrations of other micro-nutrients. Our results are not in line with Mahmood *et al.* (1991), who reported 0.01% $CaCl_2.2H_2O$ as the optimum concentration of this salt. This variation in results may be due to difference in substrates.

Biomass protein production was increased with an addition of 0.010 to 0.015 $MgSO_4.7H_2O$ and decreased with further increase in $MgSO_4.7H_2O$. The results strongly support findings of Bajwa *et al.* (1991) and Mahmood *et al.* (1991) who reported 0.01% $MgSO_4.7H_2O$ as the optimum level for biomass protein production by *Arachniotus* sp. using alkali treated rice straw and rice polishing as substrates respectively.

Moreover in present study there was an increase in biomass protein production with the addition of KH_2PO_4 from 0.05 to 0.10%. These results are in line with those of Bajwa *et al.* (1991), who reported 0.2% KH_2PO_4 as an optimum for maximum single cell protein production (15.17%) by *Arachniotus* sp. using alkali treated rice straw as substrate.

The production of protein was increased with an addition of 0.5 to 1.5% cane molasses. The findings of present study supports the results of Bajwa *et al.* (1991), who fermented alkali treated rice straw with *Arachniotus* sp. for 6 days and reported that addition of 1.0% cane molasses resulted in maximum (15.97%) production of biomass protein.

There was an increase in the production of protein from 0.5 to 2.0% corn steep liquor. This fact is in contradiction with the findings of Khan (1990), who fermented defatted rice polishes with *Candida utilis* and reported that addition of 5% corn steep liquor resulted in maximum biomass protein. This difference in results is due to different substrates and organisms.

The average digestibility of microbial protein was found to be

80.917%. Results are in accordance with Guo and Guo (1989) who produced biomass protein containing (22.82%) crude protein, which showed *in vitro* digestibility of 83%.

Finally we conclude that for maximum biomass production using *Arachinotus* sp. the fermentation medium should contain 6% dewaxed filter press cake, 0.1% $(NH_4)_2SO_4$, 0.025% $CaCl_2.2H_2O$, 0.015% $MgSO_4.7H_2O$, 0.1% KH_2PO_4, 1.5% cane molasses and 2% corn steep liquor. All the inorganic nutrients, cane molasses and corn steep liquor had a positive effect on crude and true protein contents of the biomass. It was found that protein increased and crude fiber decreased from 28 to 2% because *Arachinotus* sp. used it as an energy source. The biomass produced after fermentation contains fifteen amino acids but it lacks the essential amino acids Valine and Leucine. Therefore *Arachinotus* sp. under optimal conditions can be used for utilizing dewaxed filter press cake as a substrate for the production of protein enriched biomass. *Arachinotus* sp. can also be tested for bioconversion of other agricultural waste/residue in to biomass protein.

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