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Optimization of Culture Conditions for the Production of Biomass Protein by *Aspergillus niger* NRRL 567

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Abstract: Maximum biomass protein (48.34 mg/ml) was obtained at pH 3.5, temperature 30°C after 96 hours of continuos shaking at 120 rpm by fermentation with *Aspergillus niger* NRRL 567, cultured on corn stover (4%) in optimized medium containing $CaCl_2.2H_20$ (0.02%), $MgSO_4.7H_20$ (0.03%), Urea (0.1%), KH_2PO_4 (0.3%). Cane molasses (0.75%) and Yeast sludge (1%).

Key words: *Apergillus niger* NRRL 567, corn stover, biomass protein, continuos shaking

Introduction

Presently 1.1 thousand million people are suffering from protein malnutrition in the third world. It has been estimated that by the year 2000, the food requirements would be doubled (Dasilva *et al.,* 1987).

Presently our country is seriously facing shortage of conventional food for animals particularly the protein. But on the other hand more than 50 million tonnes of Agricultural waste is available in the country (Azad, 1986). In Pakistan annual production of corn grain in 1284 thousand tonnes and that of corn stover being 20% of corn grain is 256.8 thousand tonnes (Anonymous, 1997). Cycling and recycling of this agricultural waste through microbial fermentation would only reduce the pollutant but also served as potential source of energy for the production of low cost high quality protein. The microbial biomass protein thus produced can be fed not only to human being but also to ruminants and poultry for production of milk, meat and eggs at cheaper rates. Therefore, in this paper culture conditions have been optimized through fermentation technology for the conversion of corn stover into biomass protein using *Aspergillus niger* NRRL 567.

Materials and Methods

Cron stover was obtained from local market. *Aspergillus niger* NRRL 567 was procured from NIBGE Faisalabad after certification. Fungal culture was maintained on sporutation medium consisting of potato dextrose agar (PDA) (pH 4.0 and temp 30°C) for two weeks and subcultured after every fifteen days. Flasks containing different cone of micronutrients and corn stover in 100 ml of medium were incoulated using 5 ml of filterate containing homogenous fungal spores suspension (10^7-10^8 spores per ml) (Zarofonetis, 1959). pH of the medium was adjusted at 4.0. Flasks were incubated on orbital shaker working at 120 rpm for optimum fermentation period. Fermented biomass were filtered and filterate was centrifuged. The supernatant was passed through ultrafiltration using millipore filter and filtrate was assayed for the production of biomass protein. In this way different experiments were performed for the optimization of mode of fermentation, time period, pH, temperature, $CaCl_2.2H_2O$ $MgSO_4.7H_20$, KH_2PO_4 $(NH_4)_2SO_4$/urea, cane mollases and yeast sludge. To find out their optimum value for the production of biomass protein, experiments were carried out in such a way that the concentration of one nutrient which is optimized in one experiment was used in subsequent investigations.

Assay: The biomass protein was determined by the following method.

Biuret method: 1 ml of sample was added in 5 ml biuret solution and allowed it to stand for 15-20 min tot the completion of reaction. The volume was made up to 10 ml after development of violet colouration. The absorbance was noted at 550 nm with the help of spectrophotometer, (Gornall *et al.*, 1949). Standard curve was also obtained by using 10 different concentrations of 1% peptone solution.

Results and Discussion

Study was carried out to improve protein content of corn stover by fermentation with *Aspergillus niger* NRRL 567.

Mode of Fermentation and Time Period: The filterate obtained after 96 hours showed maximum biomass protein (16.43 mg/ml) and it decreased after 120 hour incubation. Similar trend was found in mode of fermentation. Maximum protein production (16.43 mg/ml) was found with continuos shaking after 96 hours of incubation period as given in Table 1. Still culture fermentation gave the lowest protein content proceeded by semi shaking. Similar results has been reported by Moria *et al.* (1978) who produced maximum crude protein 31.3% by Candida Moy 657 with shaking at 30°C for 48 hours under optimum condition (Bajwa *et al.,* 1991) produced maximum biomass protein by Arachniotus sp after 6 day in 0.5% Alkali treated rice straw (Table 1).

pH and Temperature: Maximum protein content were achieved at 30°C (Fig. 1) and pH 3.5 (Fig. 2) after 96 hours period with continuos shaking. Present result for both the conditions are in accordance with the reports from literature. *Candida utilus* gave maximum biomass protein concentration at 30°C (Quierzy *et al.,* 1979). *Aspergillus terrues* were best at pH 4 for biomass protein on alkali treated rice (Garag and Neelakanton, 1982).

Substrata Level: Effect of varying levels of corn stover as a substrate i.e. 1, 2, 3, 4, 5, 6% in the fermentation after 96 hours were studied as shown in (Table 2). The maximum production of biomass protein 16.7 mg/ml was recorded in 4% substrate level. However drastic reduction in protein content was found at 5% and 6% substrate levels. Our findings are in the line with Lee *et al.* (1979) who fermented 8% bamboo short husk for maximum protein 25.6% production by cellumonass species.

$CaCl_2.2H_2O$: Six different levels of $CaCl_2.2H_2O$ were used to produced maximum biomass protein as shown in Table 2.

Table 1: Productions of biomass proteins (mg/ml) under different mode of fermentation and time period of corn stover with *Aspergllus niger* NRRL 667

Incubation Period in hours	Continuous Shaking (mg/ml)	Semi shaking (mg/ml)	Stiller culture (mg/ml)
24	9.2	7.4	5.7
48	9.5	9.5	6.43
72	10.40	9.2	7,73
96	16.43	10.6	8.17
120	14.60	9.7	6.95

The protein content increased upto 17.8 mg/ml with 0.02% $CaCl_2.2H_20$. However it decreased with further addition of $CaCl_2.2H_20$ and reached 13.20 mg/ml of protein at 0.06% $CaCl_2.2H_20$. Results are also in accordance with those of Mahmood *et al.* (1991) and Chahal *et al.* (1987). The former two scientists reported 0.1% $CaCl_2.2H_2O$ as the optimum concentration for biomass protein production but later produced maximum biomass protein 40% from corn stover by *Pleurotus sajoreaju* in the presence of 0.03% $CaCl_2.2H_2O$

Moo-Young *et al*. (1992) used 0.02% $CaCl_2.2H_2O$ for the production of maximum biomass protein by *Neurospora gdruphilla* and chaetomiurn cellulolyticum.

$MgSO_4.7H_20$: The results are given in the Table 2. The maximum protein was obtained using different concentrations of $MgSO_4.7H_2O$. 0.03% $MgSO_4.7H_20$ yielded 20.20 mg/ml protein content. The results are supported by Kageaki *et al*. (1977), Moria *et al.* (1978), Peter *et al.* (1981) and Endriquez and Rodriguez (1983) all produced maximum biomass protein with 0.25% $MgSO_4.7H_20$ by different fermentative organisms in the growth media of different substrates.

$(NH_4)_2SO_4$/Urea: Nitrogen is necessary to meet the growth requirement of microorganism. Two different Nitrogen sources i.e. $(NH_4)_2SO_4$ and urea were used in this case. Following concentration of $(NH_4)_2SO_4$ i.e. 0.2, 0.3 and 0.4% were used while that of urea 0.1, 0.15, 0.20% were used. Maximum protein yield by $(NH_4)_2SO_4$ 0.2% was 14.70 mg/ml. while that of urea 0.1 was 22.6 mg/ml. So better source for protein production is urea. The results of present study are in contradiction with the earlier reports (Moria *et al.,* 1978). Quierzy *et al*. (1979), Tolysbaev (1980) and Kageaki *et al.* (1977), who reported increase in biomass production with the addition of $(NH_4)_2SO_4$. However Moo-Young *et al*. (1992) and Chahal *et al*. (1987) also observed increase in protein content with the addition of urea.

KH_2PO_4: Protein production increases by the addition of KH_2PO_4. The maximum biomass protein (23.15 mg/ml) was obtained with 0.3% KH_2PO_4 as given in the Table 2 and decreased with the further addition of salt. The results are

Table 2: Effect of varying concentration of substrate level, $CaCl_2$ $2H_2O$, $MgSO_4.7H_2O$, $(NH_4)_2SO_4$/Urea, KH_2PO_4, cane molasses, yeast sludge, for the production of biomass protein by *Aspergullus niger* NRRL 567

Sr No.	Substrate level (%)	$CaCl_2.2H_2O$ (%)	$MgSO_4.7H_2O$ (%)	$(NH_4)_2SO_4$/urea (%)	KH_2PO_4 (%)	Cane molasses (%)	Yeast sluge (%)	Biomass protein mg/ml (%)
T1	1							7.20
T2	2							9 02
T3	3							10.0
74	4							16.20
T5	5							8.96
T6	6							8.62
T1		0.01						17.3
72	4	0.02						17 8
T3		0.03						17.3
T4		0.04						15.2
T5		0,05						13.9
T6		0.05						13.3
T1			0.01					15.6
T2			0.02					18.20
T3			0.03					20.20
T4			0.04					17.39
T5			0.05					17.04
T6			0.06					16.81
T1				0.2				14.70
T2				0.3				12.15
T3				0.4				8.56
T4	4	0.02	0.03	0.01				22.62
T5				0.15				16.34
T6				0.20				13 52
T1					0.1			21 30
72					0.2			21.70
T3	4	0.02	0.03	0.01	0.3			23.15
T4					0.4			17.50
T5					0.5			14,95
T6					0.6			14.50
T1						0.025		34.13
T2						0.050		36.52
T3	4	0.02	0.03	0.01		0.075		36.63
T4						0.10		26.34
T5						0.125		25.32
T6						0.150		24.80
T1						0.075	1.0	48.34
T2							2.0	41 73
T3							3.0	34.70
T4							4.0	34.39
T5							5.0	33.7
T6							6.0	31.25

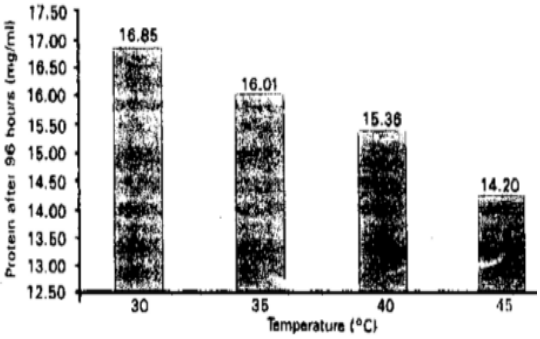

Fig. 1: Effect of varying temperature on production of biomass protein (mg/mil by fermenting corn stover with *Aspergillus niger* NRRL 567

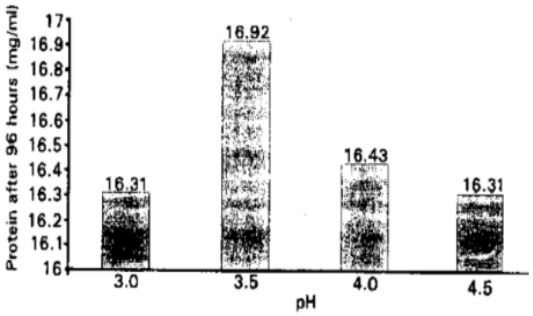

Fig. 2: Influence of varying pH on production of biomass protein (mg/mil by fermenting corn stover with *Aspergillus niger* NRRL 567

close to the finding of other researchers (Moria *et al.,* 1978) and Kageaki *et al.* (1977) found 0.4, 0.2 and 0.5% KH_2PO_4 respectively as optimum levels for the production of Microbial protein.

Cane Molasses: Six concentrations of cane mollases i.e. 0.025 to 0.15% as given in Table 2 were added in the medium. Significant rise in protein biomass (36.63 mg/ml) was recorded at 0.075% cane molasses. However it decreased with further addition of cane molasses. It was due to fact that cane molasses is a good source of carbon and mineral nutrient plus trace elements (Moo-Young *et al*., 1992). The results are also supported by El-Eshwah *et al.* (1978) who obtained 34.38% biomass protein from shaked medium based on mollases, sweet potatoes and corn steep using six different culture of fungi. Hussain (1993) used molasses for the production of high quality single cell protein.

Yeast Sludge: Addition of yeast sludge increases the protein content of biomass protein upto significant level, Yeast sludge enhanced the protein content from 36.63 mg/ml to 48 mg/ml (Table 2). However, further addition of yeast decreased the biomass protein sharply at 33.7 mg/ml concentration with 5% yeast sludge. In this case enhanced promotion of protein biomass is due to fact that yeast sludge promotes the growth of microorganism because it contain a mixture of vitamin B complex and some essential amino acids. The results of present study are in line with that of (Ghanem *et al.,* 1991) who obtained maximum protein 34% in the optimum fermentation medium of milled beet pulp containing 0.01% yeast extract produced by trichoderma ressi. Hongpattarakere and H-Kittikun (1995) carried out optimization of single cell protein from Cassova starch and reported (0.01%) yeast extract as optimum for Schwannoyes cestklli.

Statistical analysis: The results were statistically analyzed by using ANOVA and DMR which were found highly significant (Steel and Torrie, 1982).

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