Production of Exogenous Enzyme Using CHAETOMIUM THERMOPHILE FUNGUS through fermentation of wheat straw under optimal conditions required for Maximum Enzyme Production

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Abstract— The study was conducted to determine the possibilities of degrading the fibre content of sunflower oil meal (SFOM) by converting complex cellulose and hemicellulose into simple sugars. It was done by treating SFOM with a multi enzyme product from a fungus, *Chaetomium thermophile*, through fermentation of wheat straw under optimal conditions required for maximum enzyme production. Four enzyme activities viz, total cellulase, endo-ß-1, 4-glucanase, ß-glucosidase and xylanase were determined in the multi enzyme. The effect of fermentation duration was determined. Optimum fermentation time found was 96 hours. The thermostability of enzymes was checked at 50, 60, 70 and 80°C. Activities of these enzymes were found to be 1.0, 2.0, 0.95 IU/ml/min. and 12.0mg/ml/min. of Fpase, endoglucanase, ß-glucosidase and Xylanase, respectively. Optimum incubation time was determined as 15 hours at 56°C. Crude fibre contents of SFOM treated and incubated with enzyme was reduced from 24% to 16.0%. The enzyme was also applied to SFOM without incubation

Index Terms— Enzyme Production , Sunflower oil meal, ***CHAETOMIUM THERMOPHILE***

1 INTRODUCTION

Sunflower oil meal (SFOM) is a by-product obtained after the extraction of oil from partially decorticated sunflower seed. Being a good source of protein (29.8-45.5%) the sunflower meal can be developed as a good vegetable protein supplement for poultry rations. However, high level of inclusion of sunflower meal in poultry diets posses certain problems due to its high fibre content (14-18%). The testa of sunflower meal is rich in fibre content, which reduces the digestibility of its meal (Smith, 1968). The fibre content of the seed can be reduced partially by removing the testa through the decortication process (McDonald *et al.,* 1977 and Niazi *et al.,* 1991), but in Pakistan, this method is not practiced. These enzymes can be derived from bacteria and fungi. Commercial enzymes can be a crude mixture of different enzymes of specific activities. These specific activities are strictly limited in their catalytic activities and the environmental conditions under which they function (Simon *et al*., 1996). These enzymes can be applied by two ways either by treating substrate with enzyme and then incubating them under optimum conditions (Aslam, 1999) or simply addition of enzyme to substrate (Abbas *et al*., 1998). Therefore, enzymes are added to facilitate the breakdown of larger molecular structures of the feed ingredients (Ramesh Chander Kuhad et al, 2011) into smaller ones by their specific action and making these nutrients more readily available to the digestive system for better absorption. Successful attempts had been made to eliminate those disadvantages by microbial cellulases and xylanases (Latif *et al*., 1996). Xylanases and cellulases prepared from fungus (*chaetomium thermophile)*, breakdown complex NSP linkages in sunflower oil meal and increase the caloric contents of feeds from 100-200 Kcal/kg feed (Rashid, 1999).

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2 MATERIAL AND METHODS

2.1 Enzyme Assay

Multienzyme (liquid enzyme produced) was analyzed for the activities of different enzymes i.e; cellulases and hemicellulase. There are three main types of enzymes namely endo–ß-1,4-glucanase(EC 3.2,1.4), exo-ß-1,4-glucanase(EC 3.2,1.91) and ß-1,4-glucosidase(EC 3.2,1.21) present in cellulase system. While hemicellulase mainly include xylanase (EC 3.2,1.8). The enzymes were analyzed for the activities of filter paperase (Fpase) endo-ß-1,4-glucanase (CMCase), ß-glucosidase and xylanase. Different concentrations of 10mM glucose solution were taken and diluted to final volume of 1mL . In 1mL standard solution, 2mL 0.1M citrate buffer, pH 4.8 was added. Adding 3mL DNS reagent and boiling for 15 minutes terminated the reaction. Finally 1mL 40% sodium potassium tartarate was added in hot tubes. The tubes were cooled at room temperature and the absorbance was measured at 550nm against blank, in which glucose standard solution was not added.

2.2 ß–Glucosidase and Xylanase

There are not strictly speaking cellulases, but they are nevertheless, very important component of cellulose system, in that they complete the hydrolysis to glucose of short-chain oligosaccharides and cellobiose, which are released by the other enzymes. Xylanase activity was expressed in terms of international units (IU). One IU was the amount of enzyme required to release 1µ mol xylose equivalents in 1mL of enzyme solution in one minute.

2.3 Production of Cellulases and Xylanases by *C.THERMOPHILE* In 20 liter Bio Reactor

Enzyme production was carried out in 20 litre fermenter (B. Braun) using in-vivo sterilization. The conditions set for the production of enzymes were as follows: A working volume of 12 litres Eggins and Pugh medium with 20% (w/v) wheat straw was used as carbon source. An aeration rate of flow of 3 l/m. The agitation of the bioreactor was maintained at 300 rpm. The pH of the medium was allowed to fluctuate from 5 to neutral, the dissolved oxygen pO_2 was kept at 100%. Enzyme activities of FP-ase 2 U/ml, ß-Glucosidase 2 U/ml, CMC-ase 6 U/ml and Xylanase 80 U/ml were obtained at increasing rates in four days. The daily enzyme activities were determined. The wheat straw was found to be a highly suitable inducer of these enzymes, and is highly cheap thus reducing the cost of the enzyme production. Moreover, urea and corn steep liquor of commercial grade were used instead of α-asparagine and yeast extract. These parameters have immense effect on the up scaling process, since these enzyme have to be produced at higher scale for application in poultry feed.

2.4 Enzyme Harvesting And Processing

After the production of enzymes at the fermenter level the enzymes were harvested from the fermenter. The crude enzyme broth was first filtered through a nylon cloth into a suitable container. The broth was concentrated for application in poultry feed. Since the enzymes were required in appreciable amounts so 50 liters of crude enzyme was concentrated in large laboratory incubators at 60°C which resulted in the concentration of enzymes to five fold their original concentration. This concentration resulted in reduction of moisture content which could lead to fungal contamination in feed. The enzyme activities of concentrated enzymes increased many folds (Table-1). After concentrating the enzymes propionic acid was added 5ml in 5 liters as a preservative, also tetracycline was added 1ml (25mg/ml) in 1 litre of enzymes. Then enzymes were placed in cold room set at 4°C for use in poultry feed.

2.5 Thermostability and pH Stability Profile of *C. THERMOPHILE* Enzymes

As these enzymes are being recommended for poultry feed industry therefore, their thermostability and pH profile were investigated and showed encouraging results (Table-6 and 7). The enzyme were subjected to temperatures of 70, 80, 90 and 100°C. It showed 100% relative activity at 70°C, whereas at 80°C some activity was partially lost after 5 min. of treatments, however at 90°C enzyme activity was reduced to 60% of the original after 5 min. At 100°C the activity showed a steep drop to 15% (Table-8). Thus these enzymes showed significant thermostability upto 80-90°C for 5 min. required during pelleting of the poultry feed. Similarly the enzymes were found to be tolerant to wide ranges of pH. The enzymes were subjected to pH ranging from 7 to 3 and were found to be tolerant at pH range of 7 to 4. However, at pH 2, 30% of activity was lost, however, ß-glucosidase lost 50% of its activity at 3 pH. But at pH 2, almost 80% enzyme activity was lost. Thus pH profile of *C. thermophile* was also found to be highly encouraging (Table-2).

TABLE-1

ENZYME ACTIVITIES OF *C. THERMOPHILE* (U/ml)

Time (hours)	FP-ase	CMC-ase	Xylanase	ß-Glucosidase
24	0.8	1	20	0.5

48	1	2.5	45	1.2
72	1.6	3.8	62	1.8
96	2	6	80	2

TABLE-2

THERMOSTABILITY OF *C. THERMOPHILE* ENZYMES (%)

Temperature	Time (m)	FP-ase	CMC-ase	Xylanase	ß-Glucosidase
70°C	5	100	100	100	100
80°C	5	90	90	90	80
90°C	5	70	70	75	50
100°C	5	10	10	15	10

TABLE-3

pH STABILITY OF *C. THERMOPHILE* ENZYMES (%)

pH	FP-ase	CMC-ase	Xylanase	ß-Glucosidase
7	100	100	100	100
6	100	100	100	100
5	100	100	100	100
4	90	90	90	80
3	70	70	75	50
2	20	20	30	10

TABLE-4

CONCENTRATION OF CRUDE *C. THERMOPHILE* ENZYMES AT 60°C IN INCUBATION

Enzymes	Crude Enzymes	Conc. Enzyme	Concentration
FP-ase	0.31	1.5	4.8 fold
CMC-ase	1.40	0.72	4.8 fold
ß-Glucosidase	0.2	0.4	2.0 fold
Xylanase	60	150	2.5 fold
Ext. Protein	0.675	3.15	4.66 fold

3 RESULTS AND DISCUSSION

This study was aimed to utilize more fibre contents of SFOM by converting it into simple sugar with the help of cellulolytic enzymes. A wild strain fungus, *Chaetomium thermophile*, was used on wheat straw as substrate cultured. The sunflower oil meal (SFOM) required to be added in the rations was treated with the enzyme. A performance trial on broiler chicks was conducted to determine the efficiency of cellulolytic enzymes, in their basal rations.

3.1 Production of Enzyme Using *CHAETOMIUM THERMOPHILE*

Predetermined conditions (Latif *et al*., 1996) & (Sharada et al 2013) such as substrate level (2%), inorganic nutrient concentrations, temperature (45°C), pH (6.5 to 6.7) and nitrogen level (Urea, 0.05%) were used for the production of enzyme in 20L Bioreactor. The aeration was set at 0.5vvm and pH was allowed to fluctuate. The temperature was kept at 45°C. The quantity of enzyme extract was 10 liter.

3.2 Effect of duration of Fermentation

A fermentation profile was obtained for enzyme production in the bioreactor upto 120 hours. The sample of enzyme was drawn after every 24 hours for enzyme activity. It was found that the optimum fermentation time was 96 hours at which maximum activities of cellulases and xylanases were obtained. Maximal Filter Paperase activity (FP-ase activity) of 1U/ml was obtained at 96 hours fermentation. A gradual increase of activity was noted upto 120 hours of fermentation with an increase of 25% at 96 hours.

β-glucosidase activity was maximum after 120 hours of fermentation. There was a gradual increase in the activity with time, reaching the maximum at 120 hours. The increase in activity was 19% at 120 hours over the 72 hours . The best ratio of β-glucosidase and FP-ase for saccharification was said to be 1:1 (Chalal, 1993). Keeping in view, the time for harvesting the enzyme was thus adjusted after 96 hours of fermentation, as the ratio of FP-ase and β-glucosidase was near to 1:1.

3.3 Thermostability of Enzyme

The enzymes produced by *Chaetomium thermophile* are reported to be heat stable as compared with enzymes from mesophile organisms (Latif *et al*., 1995). The heat tolerance of the enzyme was thus studied. The enzymes were subjected to heat treatment at 50, 60, 70 and 80°C temperatures for 24 hours. The samples were collected and enzyme activities were assayed to determine the extent of loss in the enzyme activity. There was a gradual loss of enzyme activity with the increase in temperature .

3.4 Enzyme Activity

Findings of this study were in accordance with Latif *et al*. (1995) who produced cellulase from thermophile fungi by growing it on Kallar grass. They screened seven indigenous thermophilic fungi for cellulase and xylanase production. The highest activities produced were 0.4, 0.5, 3.5 and 0.14 μ/ml of filter paper cellulose, CMCase, xylanase and β-glucosidase, respectively. Later these activities were improved 5-8 fold. Singh *et al*. (1990) used *Chaetomium.thermphilic* for cellulolytic enzyme production and determined the enzyme activities. The

highest activities were 0.33, 0.14 and 1.02 1U/ml for exoglucanase, endoglucanase and β-glucosidase, respectively.

3.5 Enzyme Treatment of Sunflower Oil Meal Incubation Period

Predetermined conditions for enzyme treatments as temperature 56°C, pH 6.4 (Latif *et al*., 1996) and enzyme: susbtate, 1:1 (Aslam, 1999) & (Raza et al 2009) was used to determine the optimum incubation time required for maximum saccharification of fibre present in SFOM . Enzyme and SFOM was incubated at 56°C for different periods of time (3-30 hours).

The results revealed that fibre content decreased with increase in incubation time upto 27 hours. Uptill 18 hours the rate of saccharification was high. Beyond this time the rate of saccharification decreased with the increase in incubation time. It may be due to increase in concentration of sugars in the system which cause catabolite repression (Trehn, 1993; Birk *et al*., 1997). There was non-significant difference in the reduction of fibre at 15, 18, 21 and 30 hours. Mishra *et al*. (1984) and Aslam (1999) reported that the rate of saccharification showed a very significant drop for the prolonged enzyme substrate interaction during 48 hours.

4 Conclusion

Experiment was conducted to determine the possibilities of degrading the fibre content of sunflower oil meal (SFOM) by converting complex cellulose and hemicellulose into simple sugars. It was done by treating SFOM with a multi enzyme product from a fungus, *Chaetomium thermophile,* through fermentation of wheat straw under optimal conditions required for maximum enzyme production. Four enzyme activities viz, total cellulase, endo-ß-1, 4-glucanase, ß-glucosidase and xylanase were determined in the multi enzyme. Optimum fermentation time found was 96 hours. The thermostability of enzymes was checked at 50, 60, 70 and 80°C. Activities of these enzymes were found to be 1.0, 2.0, 0.95 IU/ml/min. and 12.0mg/ml/min. of Fpase, endoglucanase, ß-glucosidase and Xylanase, respectively. Optimum incubation time was determined as 15 hours at 56°C. Crude fibre contents of SFOM treated and incubated with enzyme was reduced from 24% to 16.0%. The enzyme was also applied to SFOM without incubation.

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