

STUDIES OF SUBMERGED FERMENTATION FOR CELLULASE PRODUCTION FROM *ASPERGILLUS FUMIGATUS* (FRESENIUS 1863)

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ABSTRACT

The present work aims to examine a locally isolated fungus, *Aspergillus fumigatus* (Fresenius 1863) which might be capable of producing cellulases effectively and feasibly by utilizing cellulosic wastes in submerged fermentation. Maximum quantity of cellulose (6.91 U/mL) was produced when the strain was grown on culture medium containing sunflower waste powder as substrate after 120 h, temperature 30°C, at initial pH 5.0 and inoculum size 6×10^6 conidia in 50 mL of culture medium. The strain was stable upto pH 9.0 and thermo stable upto 60°C and its product yield coefficient ($Y_{p/s}$) favoured its feasibility for the use in the industries.

Key words: Cellulosic wastes, cellulases, *Aspergillus fumigatus* (Fresenius 1863)

INTRODUCTION

Cellulose is the most produced organic matter by plants on the planet earth. It is a linear polymer of glucose units which are associated together by beta-1, 4-glycosidic linkage (Beguin and Aubert, 1994). A large number of bacteria and fungi can utilize cellulose by converting this insoluble substrate into soluble compounds with the help of group of enzymes, cellulases, which are released by them. The term cellulases means a group of enzymes which includes; Endocellulase (EC 3.2.1.4), it randomly splits internal bonds creating new chain ends; Exocellulase (EC 3.2.1.91), it splits 2-4 units from the ends produced by endocellulase; Cellobiase (EC 3.2.1.21), it splits the exocellulase product into glucose units; Oxidative cellulase (EC 1.1.99.18) depolymerize cellulose by radical reactions; Cellulose phosphorylase (EC 2.4.1.20) depolymerize cellulose using phosphates. They all synergistically convert cellulose into glucose and therefore are used in industries (Bhat, 2000). On a commercial scale, the use of cellulases for the conversion of cellulosic biomass is costly process. Therefore, it is necessary to study a wide range of microorganisms which could be used for cellulose production effectively and feasibly (Wood and Bhat, 1998).

Large quantities of cellulosic wastes are created through agricultural, industrial processes and forestry which accumulate in the environment causing pollution problem. Cellulosic wastes in Pakistan are usually disposed of by environment non-friendly manner. So there may be two objectives which can be achieved by using agricultural wastes as sources of carbon. On one hand they can be used as raw materials for the production of valuable enzymes and other useful products while on the other hand pollution problem can be reduced (Ahmed *et al.*, 2011; 2014; 2015). In the present work specific interest has been focused to the utilization of cellulosic wastes for the production of cellulases from locally isolated strains of *Aspergillus fumigatus* in submerged fermentation.

MATERIALS AND METHODS

Strains

Strain of *Aspergillus fumigatus* (Fresenius 1863) was isolated from the soil of NED University of Engineering & Technology Karachi and culture was maintained following by Dahot (1986). Number of conidia was counted by haemocytometer. Spore suspension was maintained about 4×10^6 conidia and they were added to 50 mL of fermentation media in 250 mL flask.

Enzyme Activity

Cellulase activity was determined by following Wood and Bhat (1998) using carboxymethyl cellulose (Sigma-Aldrich, USA) as a substrate. The reaction mixture contained 0.5 mL of 1 % (w/v) substrate in 0.1 M sodium citrate buffer (pH 4.8) and 0.5 mL of culture broth in a test tube which was then incubated at 40°C for 30 min. The reaction was terminated by adding 1.0 mL of 3, 5-dinitrosalicylic acid (DNSA) and boiled the contents of test tube in water bath for 15 minutes. The absorbance was noted at 540 nm at UV-Visible spectrophotometer. One unit of cellulase

activity was expressed as the amount of enzyme required to release 1 μ mol. reducing sugars per mL per minute under the assay condition by using glucose as a standard curve.

Kinetic parameter

Kinetic parameter, product yield coefficient ($Y_{p/s}$) was determined by the method of Pirt (1975). It is the amount of enzyme which is produced per mg of substrate consumed.

Optimization of Enzyme Production Parameters

All experiments were done in such a way that the parameter optimized in one experiment was fixed in the subsequent experiments for the production of cellulose splitting enzyme. First of all the most suitable culture medium containing various substrates from cellulosic wastes (*Brassica campestris*, *Brassica nigra*, Pomegranate peel, Coconut, Malta peel, Apple peel, sunflower waste, cotton stalk, wheat bran, rice husk, banana peel and sugarcane bagasse) was determined. The composition (in g/L) of culture medium (Burrelet *et al.*, 1966), in which dextrose was replaced by same quantity of cellulosic wastes, was Dextrose 10 g/L, Peptone 5 g/L, Epsom salt 5 g/L, KH_2PO_4 5 g/L, Common salt 2.5, ferrous sulphate hepta hydrate 0.01 g/L, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.002 g/L, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 0.001 g/L and thiamine hydrochloride 0.001 g/L.

After the determination of the most suitable composition of culture medium, incubation time periods (24-240 h), temperatures (20-70°C), pH(3-9), inoculum sizes (4×10^6 - 8×10^6 conidia) and agitation rates (50-300 rpm) were checked in step by step as (Ahmed *et al.*, 2011; 2014; 2015).

RESULTS AND DISCUSSION

Effect of various substrate of culture medium

Effects of various cellulosic substrates (*Brassica campestris*, *Brassica nigra*, pomegranate peel, coconut, orange peel, apple peel, sunflower waste, cotton stalk, wheat bran, rice husk, banana peel and sugarcane bagasse) of culture medium on cellulase production by *A. fumigatus* after 24 h, at 30°C, initial pH 6.0, inoculum size 4×10^6 conidia and agitation rate 50 rpm are presented (Fig. 1). It was observed that enzyme activity is the highest (2.69 U/mL) when sunflower waste was used as cellulosic substrate. Acharya *et al.* (2008) reported saw dust as the good cellulosic substrate for cellulase production from *Aspergillus niger*.

Effect of incubation time period

The effects of incubation time period (24-240 h) on cellulase production by *A. fumigatus* in culture medium containing sunflower waste at 30°C, initial pH 6.0, inoculum size 4×10^6 conidia and agitation rate 50 rpm are plotted in Fig. 2. Activity of cellulase was measured at regular interval of 24 h and it was found that the maximum activity (4.93 U/mL) was observed after 120 h of incubation. On prolonged incubation enzyme activity was decreased, which might be due to denaturing of enzyme or synthesis of inhibiting metabolite (s). Several workers have reported the optimal period of 96 h for the cellulase production from *Trichoderma harzianum*, *Trichoderma sp.* and *Phanerochaete chrysosporium* (Ahmed *et al.*, 2015; Mamma *et al.*, 2008; Khan *et al.* (2007).

Effect of temperature

The effects of incubation temperatures (20-70°C) on cellulase production by *A. fumigatus* after 120 h in culture medium containing sunflower waste at initial pH 6.0, inoculum size 4×10^6 conidia and agitation rate 50 rpm are exhibited (Fig. 3). Activity of cellulase was the highest (4.93 U/mL) at about 30°C. The strain showed thermo stability up to 60°C (0.07 U/mL). Acharya *et al.*, (2008) also reported 30°C as optimum temperature for cellulase production by *Aspergillus niger*.

Effect of initial pH

The effects of initial pH of fermentation medium on cellulase production by *A. fumigatus* after 120 h in culture medium containing sunflower waste at 30°C, inoculum size 6×10^6 conidia and agitation rate 50 rpm are presented (Fig. 4). The range of pH (4.0 to 9.0) was studied and found that initial pH of 5.0 was the best for maximum enzyme production (5.63 U/mL). Various initial pH of fermentation medium reported for cellulase production by different researchers (Acharya *et al.*, 2008; Khan *et al.*, 2007). These differences may be observed even within the same genus of microorganism. (Khan *et al.*, 2007)

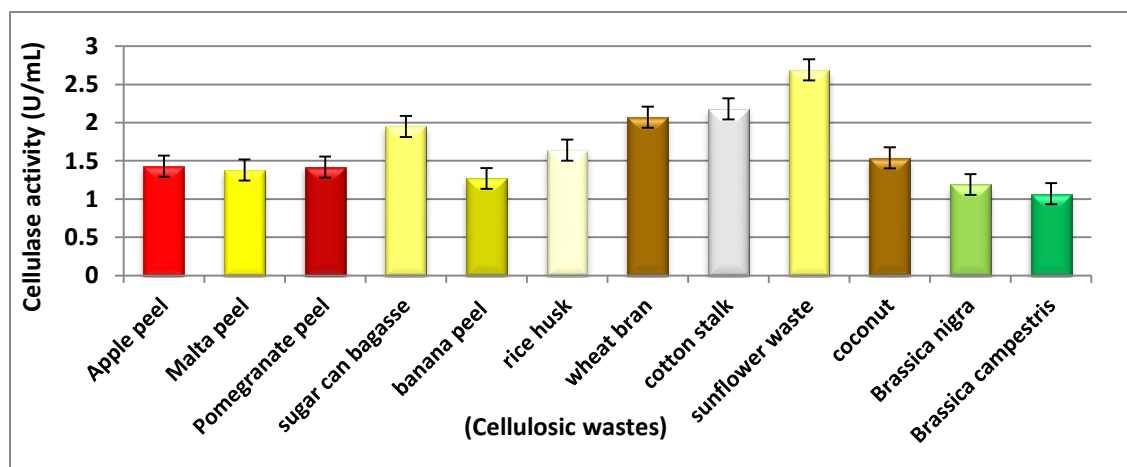


Fig.1.Effects of various cellulosic substrates of culture medium on cellulase production by *Aspergillus fumigatus* (Fresenius 1863) after 24 h, at 30°C, initial pH 6.0, inoculum size 4×10^6 conidia and agitation rate 50 rpm.

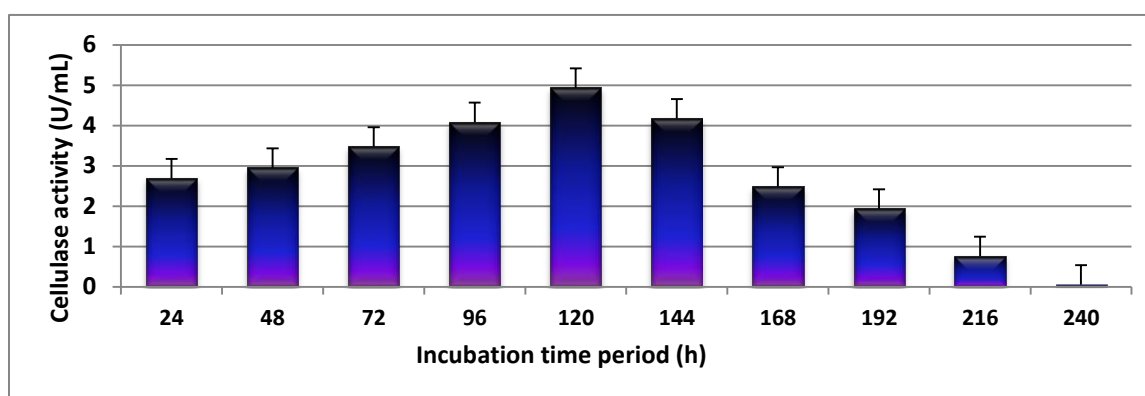


Fig.2. Effects of incubation time periods on cellulase production by *A.fumigatus* in culture medium containing sunflower waste at 30°C, initial pH 6.0, inoculum size 4×10^6 conidia and agitation rate 50 rpm.

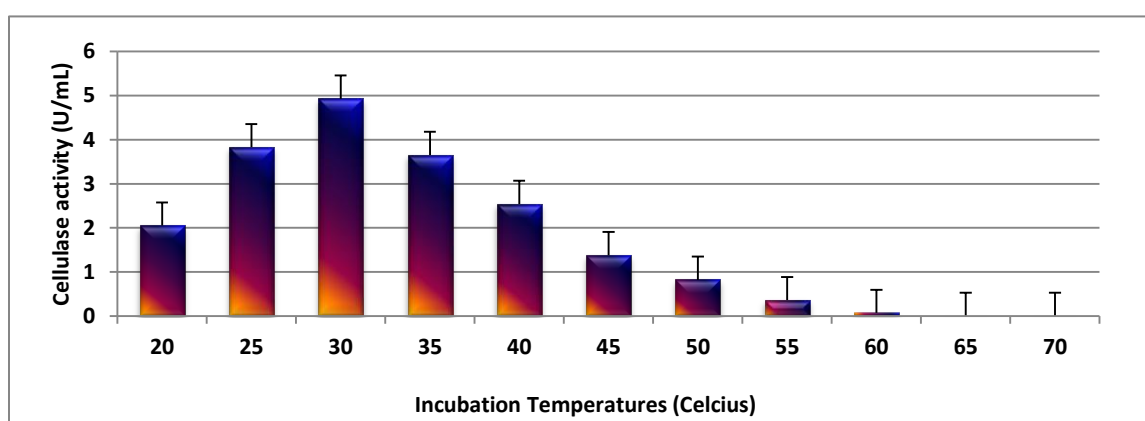


Fig.3. Effects of incubation temperatures on cellulase production by *A.fumigatus* after 120 h in culture medium containing sunflower waste at initial pH 6.0, inoculum size 4×10^6 conidia and agitation rate 50 rpm.

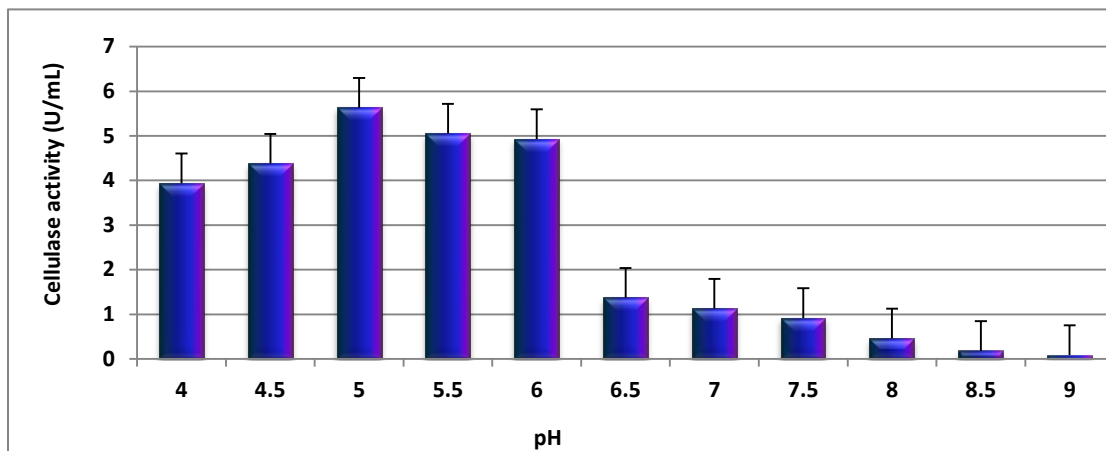


Fig.4.Effects of initial pH of fermentation medium on cellulase production by *A.fumigatusa*,after 120 h in culture medium containing sunflower waste at 30° C, inoculum size 6×10^6 conidia and agitation rate 50 rpm.

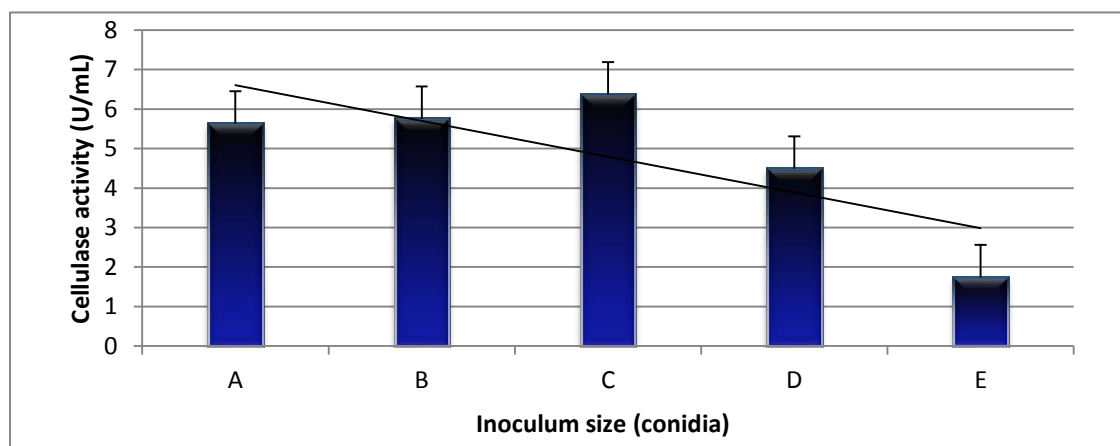


Fig.5.Effects of inoculum sizes on cellulase production by *A.fumigatusa* ,after 120 h in culture medium containing sunflower waste at 30°C, initial pH 5.0 and agitation rate 50 rpm Where A= 4×10^6 , B= 5×10^6 C= 6×10^6 D= 7×10^6 E= 8×10^6

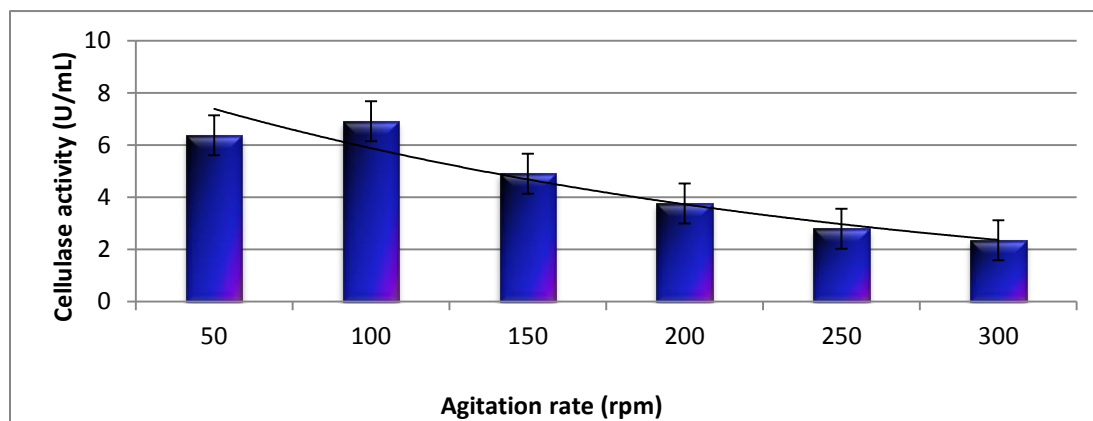


Fig.6. The effects of agitation rates on cellulase production by *A.fumigatusa*after 120 h in culture medium containing sunflower waste at 30°C, at initial pH 5.0 and inoculum size 6×10^6 conidia.

Table 1. Product yield coefficient ($Y_{p/s}$) at various stages of submerged fermentation process.

Incubation Time (h)	$Y_{p/s}$ (U/mg)	T (Celsius)	$Y_{p/s}$ (U/mg)	pH	$Y_{p/s}$ (U/mg)	Inoculum Size (Conidia)	$Y_{p/s}$ (U/mg)	Agitation rate (rpm)	$Y_{p/s}$ (U/mg)
24	0.03	20	0.47	4	0.85	4×10^6	2.19	50	3.05
48	0.15	25	0.64	4.5	1.37	5×10^6	2.89	100	3.64
72	0.24	30	1.28	5	2.19	6×10^6	3.05	150	2.56
96	0.79	35	1.05	5.5	2.06	7×10^6	2.03	200	1.32
120	1.28	40	0.83	6	1.28	8×10^6	1.01	250	0.47
144	1.02	45	0.46	6.5	0.74			300	0.08
168	0.83	50	0.19	7	0.26				
192	0.73	55	0.01	7.5	0.01				
216	0.41	60	0.00	8	0.01				

Effect of inoculum size

The effects of inoculum sizes on cellulase production by *A.fumigatus* after 120 h in culture medium containing sunflower waste at 30°C, initial pH 5.0 and agitation rate 50 rpm are plotted (Fig. 5). Flasks were added with 4×10^6 - 8×10^6 conidia and maximum cellulose activity (6.37 U/mL) was observed when 6×10^6 conidia were added to the medium. Dominguesa *et al.*, (2000) also reported 10^6 spores as the optimized inoculum size for cellulose production from *Trichoderma reesei*. Literature survey revealed that researchers used various inoculum sizes (Acharya *et al.* 2008; Ahmed *et al.*, 2011; 2014; 2015; Dominguesa *et al.* 2000; Khan *et al.*, 2007). Large inoculum size caused overgrowth and nutritional imbalance resulting less production of enzyme (Ahmed *et al.*, 2015; Archana and Satyanarayana, 2011; Mamma *et al.*, 2008).

Effect of agitation rate

The effects of agitation rates on cellulase production by *A.fumigatus* after 120 h in culture medium containing sunflower waste at 30°C, at initial pH 5.0 and inoculum size 6×10^6 conidia are given (Fig. 6). The fermentation medium was agitated at 50, 100, 150, 200, 250 and 300 rpm. Activity of cellulase was maximum (6.91 U/mL) at 50 rpm. Literature survey revealed that researchers reported various agitation rates (100-200 rev/min) for enzymes production by different microorganisms (Acharya *et al.*, 2008; Ahmed *et al.*, 2011; 2014; 2015; Dominguesa *et al.*, 2000; Khan *et al.*, 2007).

Kinetic parameter

Kinetic parameter, product yield coefficient ($Y_{p/s}$) of cellulase production throughout the fermentation process is summarised in Table 1. It can be observed that values are favourable (3.64 U/mg) with the optimized conditions. Similar types of values of product yield coefficient ($Y_{p/s}$) were also reported by Kiran *et al.* (2004).

CONCLUSION

From the study it was concluded that the strain, *Aspergillus fumigatus* produced maximum quantity of cellulase (6.91 U/mL) after 120 h in culture medium containing sunflower cellulosic waste at 30°C, at initial pH 5.0 and inoculum size 6×10^6 conidia. It was a pH and thermostable strain whose product yield coefficient ($Y_{p/s}$) is 3.64 U/mg which is a favourable value for optimized condition.

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(Accepted for publication September 2015)