MICROBIAL PRODUCTION OF CELLULASES BY ASPERGILLUS FUMIGATUS USING WHEAT STRAW AS A CARBON SOURCE

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SUMMARY: This study was carried out to utilize wheat straw as a carbon and energy source for the growth of Aspergillus fumigatus and production of cellulases. β-glucosidase and CM-cellulose activities were assayed by using salicin and CM-cellulose as substrate. It was observed from the results that the maximum production of β-glucosidase and CM-cellulase was achieved by Aspergillus fumigatus grown on H_2SO_4 and HCI pretreated wheat straw substrate in comparison to HNO_3 and $HCIO_4$ pretreated wheat straw.

Key Words: Aspergillus fumigatus.

INTRODUCTION

The biodegradation of cellulose to soluble sugar is a process which is only possible after the action of multienzyme system of cellulases produced by cellulolytic microorganisms (5,11). In recent years, more scientific attention is given to the process because of its environmental and economical significance.

The major portion of lower and higher plants is cellulose and it is hydrophilic linear glucose polymer linked with anhydroglucose units bonded by β-1, 4-glucosidic linkage (6). The number of glucose units may vary from 15 to more than 10.000 per molecule of cellulose. The polymer has both crystalline and amorphus. The crystalline region is not easily hydrolyzed by chemical and biochemical reaction but amorphus region is easily hydrolyzed (15). The extracellular cellulase system in fungi have three components such as endoglucanases or CM-cellulases (E.C.3.2.1.4), exoglucanases or cellobiohydrolases (E.C.3.2.1.9) and β-glucosidases (E.C.3.2.1.21) (3).

The endoglucanases or CM-cellulases are capable to hydrolze amorphous cellulose and soluble deriva-

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tives of CM-cellulose. Their attack on amorphous cellulose is to cleave β-glucosidic linkages. The exo- glucanases or cellobiohydrolases degrade amorphous cellulose from nonreducing end to remove cellobiose units. However crystalline cellulose is degraded by the combined action of endo and exoglucanases. β-Glucosidase act on cellobiose and short cello-oligosaccharides from non-reducing end to remove glucose (1).

The main object of present work is to utilize agricultural waste which is produced in large scale in this country as a substrate for the growth of *Aspergillus fumigatus* and production of cellulases.

MATERIALS AND METHODS

Microorganism: The *Aspergillus fumigatus* was isolated and identified in this Laboratory and stock cultures were maintained on Czepaks agar. The sterilized slants were inoculated with *Aspergillus fumigatus.* After inoculation these slants were incubated at 27°C to obtain luxuriant growth.

Chemicals: Carboxymethylcellulose (CMC) and Salicin were purchased from BDH, Sodium potassium tartrate from E. Merc and dinitrosalicylic acid was supplied by Sigma Chemicals. All other reagents used were of analytical grade.

Culture medium: The following ingredients were used for the preparation of culture medium as reported by Burrel *et al.* (2) without changing the chemical composition using g/L of $(NH_4)SO_4$ 2.5g. Fumaric acid 2.0g, KH_2PO_4 1.0g, $MgSO_4.7H_2O$ 0.5g, $(NH_4)Fe(SO_4)_2$ $12H_2O$ 0.2mg, $ZnSO_4.7H_2O$ 0.2mg, $MnSO_4.5H_2O$ 0.1 mg and thiamine hydrochloride 0.1 mg. The pH of the culture medium was adjusted to 6.0.

Preparation of spore suspension: To stock culture 10.0 ml of sterilized water was added and the surface was gently rubbed with sterilized wire loop. The spore suspension was further diluted to 100.00 ml with sterilized water (9).

Hydrolysis of Wheat straw: 10.0 g of wheat straw was hydrolyzed with 800.00 ml of 0.6N H_2SO_4, $HClO_4$, HNO_3 and HCl for two hours on flame, maintaining the level of slurry con-

Table 1: Effect of 0.6 N H_2SO_4 pretreated wheat straw as a carbon source on the production of cellulases by *Aspergillus fumigatus.* Fungus was grown on media with initial pH 6.0 in cooled orbital shaking incubator adjusted at 200 rev/min at 28 ± 2°C.

Time period hours	Final pH	Weight of mycelia G/L	T. sugar mg/ml broth	R. sugar mg/ml broth	T. protein mg/ml broth	μ mol / ml broth Enzyme activity C_1	C_2
24	6.03	0.051	0.595	0.525	0.415	0.0063	0.225
48	5.91	0.144	0.545	0.440	0.410	0.0090	0.017
72	6.31	0.152	0.495	0.240	0.405	0.0126	0.014
96	7.24	0.180	0.425	0.150	0.400	0.0333	0.011
120	8.07	0.204	0.250	0.085	0.380	0.0369	0.009
144	8.18	0.216	0.215	0.075	0.375	0.0459	0.007
168	8.24	0.242	0.210	0.070	0.360	0.1320	0.005
192	8.25	0.207	0.203	0.077	0.350	0.0855	0.005
216	8.26	0.182	0.195	0.080	0.340	0.0680	0.004
240	8.00	0.140	0.180	0.081	0.328	0.0432	0.002

C_1 = β-Glucosidase, C_2 = CM-cellulase.

Table 2: Effect of 0.6N $HClO_4$ pretreated wheat straw as a carbon source on the production of cellulases by *Aspergillus fumigatus*. Fungus was grown on media with initial pH 6.0 in cooled orbital shaking incubator adjusted at 200 rev/min at 28±2°C.

Time period hours	Final pH	Weight of mycelia G/L	T. sugar mg/ml broth	R. sugar mg/ml broth	T. protein mg/ml broth	μ mol / ml broth Enzyme activity C_1	C_2
24	5.85	0.152	2.89	1.92	0.570	0.0216	0.021
48	5.80	0.279	0.41	0.22	0.380	0.0171	0.015
72	6.20	0.258	0.38	0.21	0.360	0.0126	0.014
96	7.32	0.185	0.16	0.18	0.360	0.0111	0.012
120	7.43	0.184	0.46	0.14	0.380	0.0090	0.011
144	7.52	0.154	1.32	0.08	0.810	0.0183	0.010
168	7.51	0.214	1.48	0.15	0.680	0.0306	0.007
192	7.54	0.228	4.20	0.18	0.665	0.0270	0.006
216	7.56	0.212	3.30	0.13	0.650	0.0261	0.005
240	7.55	0.208	3.00	0.12	0.630	0.0024	0.004

C_1 = β-Glucosidase, C_2 = CM-cellulase.

stant. The digested slurry was autoclaved for 30 minutes at 1.5 kg/cm^2. The slurry was filtered through Whatman number 1 filter paper after cooling at room temperature. The filtrate of solubilized wheat straw was incorporated into mineral medium as a carbon source. The loss in weight of wheat straw was determined after drying at 105°C to constant weight.

Cultivation condition: 50.0 ml of solubilized wheat straw incorporated with culture medium was taken in 250 ml conical flasks plugged with cotton wool and autoclaved at 1.5 kg/cm^2 for 20 minutes. The sterilized media cooled at room temperature was inoculated with 1.0 ml of *Aspergillus fumigatus* spores. The flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at 28 ± 2°C adjusted at 200 rev min^{-1}. The culture broth was separated from mycelium after an interval of 24 hours incubation period by filtration through Whatman number 1 filter paper. The enzyme activities of CM-Cellulase and β-Glucosidase were examined in the culture broth. The mycelia was dried at 105°C in an oven to constant weight.

Determination of total carbohydrate: The carbohydrate content of digested wheat straw and culture broth was measured by phenol sulphuric acid method (4) with glucose as standard.

Determination of reducing sugars: Reducing sugars in digested wheat straw and culture broth were determined by dinitrosalicylic acid (DNS) method (14) with glucose as standard.

Determination of protein: The protein content of culture broth was determined by Lowry *et al.* method (12) with bovine serum albumin as standard.

Assay of CM-Cellulase activity: CM-Cellulase activity was determined as reported by Mandels *et al.* (13).

One unit of CM-cellulase activity was defined as the amount of the enzyme that liberated one micro mole of glucose per ml culture broth from CM-cellulose under the assay conditions.

Table 3: Effect of 0.6N HCl pretreated wheat straw as a carbon source on the production of cellulases by *Aspergillus fumigatus*. Fungus was grown on media with initial pH 6.0 in cooled orbital shaking incubator adjusted at 200 rev/min at 28 ± 2°C.

Time period hours	Final pH	Weight of mycelia G/L	T. sugar mg/ml broth	R. sugar mg/ml broth	T. protein mg/ml broth	μ mol / ml broth Enzyme activity C_1	C_2
24	5.81	0.148	3.63	1.80	0.540	0.0315	0.027
48	5.94	0.136	0.58	1.00	0.435	0.0264	0.019
72	6.56	0.120	0.52	0.58	0.400	0.0216	0.017
96	7.72	0.124	0.48	0.12	0.360	0.0198	0.015
120	8.03	0.126	0.43	0.05	0.360	0.0135	0.013
144	8.05	0.130	0.38	0.03	0.330	0.0108	0.011
168	8.16	0.150	1.23	0.07	0.810	0.0144	0.017
192	8.41	0.170	1.76	0.11	0.810	0.0405	0.050
216	7.61	0.164	2.33	0.50	0.675	0.0639	0.063
240	5.98	0.132	2.85	0.85	0.650	0.0477	0.037

C_1 = β-Glucosidase, C_2 = CM-cellulase.

Assay of β-Glucosidase activity: β-glucosidase activity was determined by the method of Sternberg *et al.* (17).

One unit of β-glucosidase activity was defined as the amount of enzyme that liberated one micro mole of glucose per ml culture broth from cellobiose under the standard assay conditions.

RESULTS AND DISCUSSION

The digestion of wheat straw with acids such as H_2SO_4, $HClO_4$, HCl and HNO_3 to simple sugars was carried out to select a suitable pretreatment method. Simple sugars produced from wheat straw with acid treatment were used as a carbon and energy source for the growth of microorganism and cellulase production which needs optimized fermantation process. Several pretreatment methods are reported in the literature such as physical chemical and enzymatic but sulphuric acid of about 0.5% is usually used at 150-185°C (16). An agricultural waste hydrolyzed with sulphuric acid produces variety of sugars and their degradation products. It is reported that the release of fermentation sugars varies from acids to acids used in hydrolysis process (7,8). A comparison of the quantity of cellulases production and growth pattern of *Aspergillus fumigatus* in various acid pretreated wheat straw mineral medium was made and results are presented in Tables 1, 2, 3 and 4. It was observed from Table 1 and 3 that the rate of β-glucosidase and CM-cellulase production by *Aspergillus fumigatus* in 0.6N H_2SO_4 and HCl pretreated wheat straw mineral medium reached maximum at 168 and 216 hours and 24 and 216 hours respectively. Whereas an appreciable amount of cellulases was not produced when fungus was grown on 0.6N $HClO_4$ and HNO_3 ¯ pretreated wheat straw mineral medium. However, fluctuations were recorded in case of total sugar, reducing sugar, protein and final pH during the growth of *Aspergillus fumigatus* for production of cellulases as shown in Tables 1, 2, 3 and 4. Evidence in the literature suggests that there is no definite relationship between time of growth and cellulases production but the nature of simple sugar, pH of

Table 4: Effect of 0.6N HNO_3 pretreated wheat straw as a carbon source on the production of cellulases by *Aspergillus fumigatus*. Fungus was grown on media with initial pH 6.0 in cooled orbital shaking incubator adjusted at 200 rev/min at 28 ± 2°C.

Time period hours	Final pH	Weight of mycelia G/L	T. sugar mg/ml broth	R. sugar mg/ml broth	T. protein mg/ml broth	μ mol / ml broth Enzyme activity C_1	C_2
24	5.92	0.120	3.78	1.60	0.400	0.0477	0.020
48	6.29	0.202	10.56	1.90	0.250	0.0515	0.015
72	6.87	0.170	8.91	1.52	0.230	0.0531	0.012
96	7.26	0.124	7.24	0.55	0.190	0.0585	0.010
120	8.04	0.154	4.74	0.12	0.240	0.0470	0.009
144	8.16	0.160	0.99	0.09	0.325	0.0369	0.007
168	8.37	0.169	0.41	0.05	0.325	0.0338	0.006
192	8.56	0.174	0.49	0.05	0.232	0.0264	0.004
216	8.81	0.172	0.60	0.04	0.140	0.0423	0.008
240	8.70	0.172	0.60	0.04	0.130	0.0508	0.009

C_1 = β-Glucosidase, C_2 = CM-cellulase.

the media composition play a strong role in cellulases production.

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