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Hydrochloric Acid Pretreated Agro Wastes as Carbon Source on CM-Cellulases Production by *Aspergillus Niger*

M. Urs Siyal[1], M. Umar Dahot[2], M. Hanif Noomrio[3]
[1]Department of Biochemistry, Shah Abdul Latif University, Khairpur, Sindh, Pakistan
[2,3]Institute of Biotechnology and Genetic Engineering University of Sindh, Jamshoro Pakistan

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***Corresponding author**
M. Urs Siyal

Email:
urissial@yahoo.com

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Abstract: Main objective of this study is to utilize Agro wastes instead of pure sugars for cellulases productions. It was noted that maximum fungal cell growth was obtained 0.936g/100ml of *Aspergillus niger* was observed at pH 4.11 in hydrolyzed rice husk used as carbon source. *Aspergillus niger* was isolated from the soil of Khairpur. The collection and utilization of suitable Agro wastes used as a carbon source for cellulases production by fungi requirements optimized fermentation process. Five agricultural wastes were measured for cellulolytic enzyme production using pretreatment methods acid. Acid pretreatment was found to be the most efficient and best method for higher enzyme production. Using this cheap and renewable residue, for cellulolytic enzyme production by *Aspergillus niger* boosts its economic value which is not comparable with its current use as animal feed. Agro wastes such as sugarcane peelar bagasse, sugarcane bagasse, banana fruit stalk, sorghum husk and rice husk were hydrolyzed with 0.6N HCl. Rice husk was found good substrate in comparison to other Agro wastes for the growth of *Aspergillus niger* and cellulases production. Maximum activity of cellulases were noted 4.811 (units/ml). The Cellobiase and salicinase maximum production 4.717 and 4.742 units per ml obtained at 240 hours respectively.
Keywords: Cellulase, cellobiase, Salicinase, *Aspergillus niger*, Agro wastes, fermentation.

INTRODUCTION

Cellulase is a multi-component enzyme which hydrolyzes the cellulosic substrates to glucose. Mostly three enzymes are involved endo-β-d-glucanase (EC 3.2.1.4) which catalyzes the arbitrary hydrolysis of soluble and insoluble cellulose chains. Exo-β-d-glucanase (EC 3.2.1.19) aids in liberating cellobiose from reducing and non-reducing ends of cellulose, and hydrolysis of cellobiose to glucose is carried out by β-glucosidase (EC 3.2.1.37) [1].

A number of industrial processes utilize cellulase.The most prominent applications are in textile, paper and pulp, food and animal feed, fuel and chemical industry, waste management, and pharmaceutical industry [2, 3].

Agricultural wastes characterize huge raw materials that can be utilized for production of valuable products. Major components of raw materials, including cellulose (35–50 %), hemicellulose (20–35 %), lignin (15–25 %) and additional compounds give rise residues [4].

Consequently, cellulose being the most abundant polysaccharide constituents of agricultural residues consists of β-1,4 linear polymers of 8000–12,000 glucose units. It is mostly found in crystalline, water-insoluble form, and cannot be easily hydrolyzed by microorganisms [5, 6].

In the present study to determine the finest substrate from various agricultural wastes, this is because among the cellulase producing microorganisms, *Aspergillus niger* has been reported to be effective in the synthesis of all the three cellulolytic enzymes [1].

SOURCES OF CELLULASES

Cellulases are produced by several sources for instance fungi, bacteria, yeast and plants. Seasonal fluctuation hindered a lot in the production of cellulases from plants. Greater amount of cellulases are actually produced by microorganisms. Production of microbial Cellulases can be enhanced numerous times by genetic

and environmental manipulation of microorganisms such as bacteria yeast, and fungi and thus market demand of Cellulases might be accomplished by indigenous means.

Genus *Trichoderma*, that is a filamentous ascomycetes are widely used in industries since it is the paramount cellulase producing strain. Biosynthesis of cellulase was achieved by *T. reesei* QM 9414 using cellulose as carbon source. Production was carried out using the culture of *T. reesei* Rut C-30 and *T. reesei* NG-14. Maximum growth of T. *reseei* C5 and the biosynthesis of cellulase enzyme were obtained using lactose as carbon source. Associated studies on the production of cellulase using Agro wastes were done using *Aspergillus niger* and *T. reesei*. This complex converts crystalline, amorphous, and chemically derived celluloses to glucose.

Consumption of cellulolytic enzymes is subject of great interest in the global examining for renewable resources. Industrial process could be one, which indicates to production of fuel, chemicals and feed stocks. Presently growing price of oil demands rising efficiency of cellulase production and utilization. Cellulases will be used to increase digestibility and nutritive value of carrot and coconut by attacking the cell wall [7].

Cellulases might be incorporated in the preparation for rapid digestion in sewage tanks hence resolving the pollution complications [8]. Numerous fungi and bacteria are producing cellulolytic enzymes but cellulolytc enzymes produced by *Aspergillus* sp. have a noble industrial use [9]. Cellulases are also used to improve texture and palatability of poor quality of vegetables. Beside this, Cellulases used for accelerating desiccating of vegetables.

At present, Cellulases are used for the conversion of cellulosic material to glucose. Cellulases produced by microorganisms have a great importance because these enzymes can be used in degeneration of wood and fabrics. They are also effectively used to hydrolyze cellulosic waste to fermentable sugars and these sugars are preferably utilized for the cultivation of microorganisms and synthesis of enzymes, single cell protein etc. Cellulases also used in extraction and clarification of protein isolation from soybeans and processing of fruit juice [10].

1-3--D-Glucan has been used clinically as immunodulating anticancer drug [11]. Alkaline CM-cellulase produced from bacteria are used to improve the efficiency of laundry detergents. Cellulase is used for commercial food processing in coffee. It performs hydrolysis of cellulose during drying of beans. Furthermore, Cellulases widely used in textile industry. Cellulases used in pulp and paper industry for various purposes. Cellulases mostly used for pharmaceutical applications.

Moreover Cellulase is used in the fermentation of biomass into biofuels, though this process is fairly successful in few sugarcane industries in Pakistan. Cellulase used as a treatment for Phytobezoars, a form of cellulose bezoar found in the human stomach. Cellulase digest fiber it help cure digestive such as malabsorption.

Cellulase benefit in the breakdown of plant cell walls cellulose to increase overall efficiency of binding additional cholesterol and cell toxins in the intestine for removal. Cellulase beneficial for food and environmental allergies. Cellulase play important role in drug withdrawal, few examples are cell detox, colon, cleaning and pain syndromes candida yeast infection, gas, bloting accute food allergies, fascial pain or paralysis [12]. Cellulase used in animal health care as feed supplement for the better feed conversion ratio FCR and milk yield enhancer [13].

MATERIALS AND METHODS

Microorganisms

Aspergillus niger was isolated from Soil of Khairpur District and it was identified in the High Technology Research Laboratory, Shah Abdul Latif University Khairpur. Stock culture was maintained on Czepaks agar. Sterilized slants were inoculated with *Aspergillus niger*. After inoculation the slants were incubated at 27C to obtain luxuriant growth.

Isolation of Microorganisms from Soil

Soil is composed with mineral matter, water, air and organic matter. Cellulolytic microorganisms are commonly found in the field soil and forest soils. Isolation and maintenance of pure culture of cellulolytic microbes was done from soil sampling as reported by [14].

Cultural Methods for Soil Microorganisms

Soil is an ecosystem which contains a variety of microbial population bacteria and fungi. Fungi are chemoorganotroph and use organic compound as a source of carbon and energy. Microbial community in soil is important because of its relationship to soil fertility and biogeochemical cycling of elements and potential use of specific industrial applications. Enumeration of soil microorganisms may be accomplished by the plate count technique, Most Probable Number MPN technique and spread plate count [15].

Isolation of Fungi from the Soil Sample

Isolation of fungi from the soil sample was done by the Dilution Plate Technique [15]. One gram of the soil was added into 9 ml of sterilized distilled water to make the 1:10 dilution and shaked for 60 minutes. Then a series of 1:50, 1:100, 1:1000 dilution were prepared.

One ml of each dilution was inoculated on the surface of three replicates of Czepaks Dox agar in petri dishes. Inoculated petri dishes were incubated at 29 C for seven days. After incubation the grown colonies were counted and separated. All fungal cultures isolated during investigation were maintained on Czepaks Dox agar medium at 25 C.

Chemicals

Carboxymethyl cellulose CMC Salicin and cellobiose were purchased from BDH, Sodium Potassium tartrate from E Merck and 3, 5-dinitrosalicylic acid was supplied by Sigma Chemicals. Other reagents used were of analytical grade.

Culture Medium

Following ingredients were used for the preparation of culture medium as reported by [16] without changing the chemical composition using g/L of (NH_4) SO_4 2 .5 g/L; fumaric acid 2 .0 g/L; KH_2 PO_4 1.0 g/L; Mg SO_4. $7H_20$; 0 .5 g/L; (NH_4) $_2$ Fe $(SO_4)_2$. $12H_2O$; 0.2mg/L: $ZnSO_4$ $7H_20$ 0.2 mg/L; $MnSO_4$, $5H_2$, 0.1mg/L and thiamine hydrochloride 0.1 mg/L. The pH of the culture medium was adjusted to 6.0.

Preparation of Spore Suspension

In stock culture *Aspergillus niger*, 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 ml with sterilized water [17].

Hydrolysis of Agriculture wastes

10.0 g of each agricultural waste such as sugarcane peelar bagasse, sugarcane bagasse, banana fruit stalk, sorghum husk and rice husk were hydrolyzed with 800 ml of 0.6N HCl for two hours on flame, maintaining the level of slurry constant. Digested slurry was autoclaved for 30 minutes at 1.5 kg / cm. Slurry was filtered through whatman No.1 filter paper after cooling at room temperature. The filtrate of solubilized agricultural waste was incorporated into mineral medium as a carbon source. The loss in weight of agricultural waste was determined after drying at 105C to constant weight [18].

Cultivation Condition

50ml of solubilized agricultural waste incorporated with mineral medium was taken in 250 ml conical flasks plugged with cotton wool and autoclaved at 1.5kg / cm^2 for 20 minute. Sterilized media cooled at room temperature, inoculated with 1.0 ml of *Aspergillus niger spore*s. Flasks were incubated in cooled orbital shaking incubator at 28 2C adjusted at 200 revolutionary per minute. The culture broth was separated from mycelia after an interval of 24 hours incubation period by filtration through whatman No.1 filter paper. The enzyme activities of CM–cellulase, - glucosidase and salicinase were examined in the culture broth. The mycelium was dried at 105C in an oven to constant weight [17].

Assay of CM- Cellulase activity

CM-cellulase activity was determined as reported method by [19]. 1.0 ml of enzyme sample (culture broth) was mixed with 1.0 ml of 1% CM – cellulose and 2. 0 ml of sodium acetate buffer pH 4.6. The reaction was carried out at 35C for one hour. Reducing sugar released was estimated by the dinitrosalicylic acid method CM-cellulase activity is calculated from Glucose standard. One unit of CM–cellulase activity is defined as the amount of the enzyme that liberates one mg/ml of reducing sugar as glucose from CM cellulose under the assay conditions.

Assay of - glucosidase (cellobiase and Salicinase) activity

- glucosidase and salicinase activities were determined by the method of [20] 1.0 ml of enzyme sample (culture broth) was mixed with 1.0 ml of 1% cellobiose (for cellobioase) or Salicin (for Salicinase) and 2.0 ml of Sodium acetate buffer pH 4.6.The reaction was carried out at 35 C for One hour. The reducing sugars produced were estimated by dinitrosalicylic acid method with glucose as a standard.

One unit of Cellobiase and Salicinase activities are defined as the amount of the enzyme that liberate one mg/ml of reducing sugar as glucose from cellobiose or salicin under the standard assay condition.

Determination of reducing sugars

Concentration of reducing sugars in the hydrolysate of Agro wastes and culture broth was determined by dinitrosalicylic acid (DNS) method [21] and results were calculated from glucose as a standard.

Determination of protein

Protein content of culture broth was determined by Lowry *et al*. method [22] and the results were calculated from bovine serum albumin as a standard.

Determination of total carbohydrate

Concentration of carbohydrate in the agricultural wastes hydrolysate and culture broth was measured by phenolsulphuric acid method [23] and the results were calculated from standard curve of glucose.

STATISTICAL ANALYSIS

The data is presented as means ± SD. Analysis of the data was done by one- way ANOVA.

RESULTS AND DISCUSSION

The production of cellulases by fermentation has been thoroughly investigated and it is affected by a variety of physiochemical factors.

Collection and utilization of suitable Agro wastes as a carbon source for cellulase production *Aspergillus niger* optimized fermentation process. Agro wastes such as sugarcane peelar bagasse, sugarcane bagasse, banana fruit stalk, sorghum husk and rice husk contains different ingredients. Agro wastes materials are to a certain extent variable from source to source not in cellulose, hemicellulose and lignin, but also in other ingredients such as lipid, mineral matter ash and Nitogen. By way of a result each natural substrate would be predicted to have unique set up of procedure conditions optimized for glucose production as well as minimized secondary product contamination. So as to reduce, lignocellulosic waste commonly has to be hydrolyzed before utilized as a substrate or commonly called as media for the growth of microorganism for desired product. Many techniques are available for hydrolysis for example physical grinding to fine powder by ball milling, attritor milling and two roll compression milling, chemical acid and base and enzymatic cellulase, cellobiase and salicinase. It is proposed by various workers that hydrolysis of cellulosic wastes by enzymatic treatment possess several advantages however major hindrance is its high rate for application. By using acid treatment technique hemicellulose and cellulose are hydrolyzed to certain level increasing pentose and hexoses.

Additionally, dilute acids are utilized to degrade hemicellulose, cellulose and other non-crystalline polymer to simple sugars such as glucose. Chemical pretreatment technique is cheap and highly effective. Attempts were made in this study to hydrolyze Agro waste to fermentable sugars by chemical acid technique and findings are presented in Table 1. It is quite evident from this table that sugarcane bagasse solubilized more with 0.6N HCL. Total sugar mentions to all sugars dissolved in liquid and it is determined through converting all sugars to monomers. Reducing sugar refers for all sugar with a free reducing end group. Ratio of total sugar or reducing sugar reveals average degree of polymerization of sugar moieties in solution. An acid hydrolysates of Agro-wastes were enhanced with mineral medium for the growth of *Aspergillus niger* and cellulolytic enzymes production

Tables 2-3 showed the growth pattern and cellulolytic enzyme synthesis by *Aspergillus niger*, grown on 0.6N HCL pretreated sugarcane peelar bagasse and industrial sugarcane bagasse.

It is observed from the Tables 2 and 3 that the greater amount of CM-Cellulase, cellobiase and salicinase were produced by *Aspergillus niger* in case of sugarcane peelar bagasse at 240 hours respectively. It was 0.943, 1.488 and 1.906 units/ml. while in case of sugarcane bagasse, the time period was noted at 240 hours respectively. It was noted maximum yield of cellulases was 240 hours it was 0.219, 1.922 and 0.498 units/ml.

Final pH of the medium increased during fermentation in both cases. The maximum amount of fungal biomass was obtained at 240 hours, when *Aspergillus niger* grown in acid pretreated sugarcane peelar bagasse and sugarcane bagasse. The concentration of total sugar, reducing sugar and total protein decreases with the increase of growth period of *Aspergillus niger* as shown in tables 2-3 *Aspergillus niger* was grown on 0.6N HCl pretreated banana fruit stalk and sorghum husk mineral medium for the production of cellulases.

It is observed from the Tables 4-5 that maximum production of CM-Cellulase, cellobiase and salicinase was attained at 240 and 240 hours respectively 2.076, 2.14, 2.093 and 0.871, 1.49, 0.319 units/ml when 0.6N HCl pretreated banana fruit stalk and sorghum husk were used as carbon source. Final pH of the culture broth was found in acidic medium and remained less than initial pH values throughout incubation time, but in case of sorghum husk final pH values increasing in order. Biomass was found maximum at 240 hours, when *Aspergillus niger* was grown on 0.6N HCl pretreated rice husk and sugarcane peelar bagasse. It was noted that the concentration of total sugar, reducing sugar and total protein were found decreasing in order.

It was observed in Table 6 that cellulases secretion increases till 240 hours. Total sugar, reducing sugar and total protein constantly decreasing in order because the growth was increasing and an organism was utilizing reducing sugar as a carbon source of energy. Whereas change in pH towards acidic was detected with increase in time of incubation may be due to some organic acids production. Fungal biomass was increasing in order throughout fermentation. Maximum cellulases production was noted 4.811, 4.717 and 4.742 units/ml from rice husk.

Table–1: Effect of 0.6N HCl on hydrolysis of agricultural wastes and the yield of percentage of hydrolysis total protein, total Carbohydrate and reducing sugar

Parameters	Sugarcanepeelar bagasse	Sugarcane bagasse	Banana fruit stalk	Sorghum husk	Rice husk
Initial weight of sample grams	10.00	10.00	10.00	10.00	10.00
Final weight of sample grams after hydrolysis	7.12	6.34	8.61	7.80	8.55
Loss of weight grams	2.88	3.66	1.39	2.20	1.45
% of hydrolysis	28.8	36.6	13.9	22.0	14.5
Total protein mg/ml soluble filtrate	2.41	2.31	2.22	2.14	2.17
Total carbohydrate mg/ml soluble filtrate	3.22	3.31	3.16	3.11	3.12
Reducingsugar mg/mlsoluble filtrate	2.11	2.55	2.12	2.14	2.91

Table-2: Effect of 1% sugarcane peelar bagasse waste hydrolyzed with 0.6N HCl on cellulases production by *Aspergillus niger* when incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28 ± 2C

Time Period Hours	Final pH	Weight of mycelia g/100ml	Total sugar mg/ml	Reducing Sugar mg/ml	Total Protein mg/ml	Enzyme activity units/ml		
						C1	C2	C3
24	5.22	0.056	490	426	628	0.159	1.045	0.317
			±1.455	±0.883	±0.578	±0.001	±0.001	±0.001
48	5.41	0.081	462	374	566	0.325	1.082	0.817
			±1.734	±1.156	±0.883	±0.002	±0.002	±0.002
72	5.55	0.097	385	341	535	0.476	1.222	1.053
			±1.203	±1.456	±1.203	±0.003	±0.003	±0.004
96	6.31	0.108	378	314	473	0.823	1.253	1.669
			±1.766	±1.766	±1.529	±0.004	±0.007	±0.001
120	6.48	0.121	372	270	418	0.872	1.284	1.707
			±2.030	±2.030	±1.858	±0.005	±0.004	±0.002
144	6.87	0.125	347	242	392	0.901	1.299	1.765
			±2.336	±2.084	±2.188	±0.006	±0.006	±0.003
168	7.11	0.133	311	230	360	0.915	1.318	1.802
			±2.607	±1.203	±2.520	±0.008	±0.009	±0.004
192	7.14	0.145	300	195	345	0.918	1.367	1.871
			±0.883	±1.734	±2.851	±0.007	±0.008	±0.005
216	7.21	0.152	296	163	327	0.924	1.467	1.882
			±1.156	±2.407	±2.966	±0.009	±0.010	±0.006
240	7.41	0.162	284	138	307	0.943	1.488	1.906
			±2.649	±2.336	±2.655	±0.010	±0.011	±0.007

C1= CM-cellulase, C2=Cellobiase, C3= Salicinase, ±= error of standard deviation

Table-3: Effect of 1% sugarcane bagasse waste hydrolyzed with 0.6N HCl on cellulases production by *Aspergillus niger* when incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28 ± 2C

Time Period Hours	Final pH	Weight of mycelia g/100ml	Total sugar mg/ml	Reducing Sugar mg/ml	Total Protein mg/ml	Enzyme activity units/ml		
						C1	C2	C3
24	4.21	0.22	486	433	490	0.162	0.42	0.151
			±0.578	±0.883	±1.455	±0.001	±0.002	±0.152
48	4.22	0.35	456	414	462	0.185	0.317	0.153
			±1.156	±1.455	±1.734	±0.002	±0.001	±0.002
72	4.23	0.42	450	371	385	0.194	0.394	0.161
			±0.883	±2.084	±1.203	±0.003	±0.011	±0.004
96	4.24	0.48	416	370	378	0.197	0.749	0.292
			±1.455	±1.156	±1.766	±0.004	±0.003	±0.010
120	4.28	0.55	378	330	372	0.201	0.817	0.412
			±1.203	±2.336	±2.030	±0.005	±0.012	±0.008
144	4.33	0.63	342	324	347	0.202	1.053	0.483
			±1.529	±1.766	±2.336	±0.006	±0.004	±0.006
168	4.35	0.68	294	304	325	0.211	1.122	0.485
			±1.766	±2.909	±2.607	±0.007	±0.004	±0.007
192	4.38	0.75	252	296	300	0.213	1.517	0.489
			±2.030	±1.156	±0.883	±0.008	±0.021	±0.008
216	4.42	0.79	112	284	296	0.216	1.775	0.491
			±2.407	±2.649	±1.156	±0.009	±0.003	±0.009
240	4.48	0.82	109	230	284	0.219	1.922	0.498
			±1.073		±2.649	±0.010	±0.005	±0.022

C1= CM-cellulase, C2= Cellobiase, C3= Salicinase, ±= Error of standard deviation

Table-4: Effect of 1% banana fruit stalk waste hydrolyzed with 0.6N HCl on cellulases production by *Aspergillus niger* when incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28 ± 2C

Time Period Hours	Final pH	Weight of mycelia g/100ml	Total sugar mg/ml	Reducing Sugar mg/ml	Total Protein mg/ml	Enzyme activity units/ml		
						C1	C2	C3
24	5.76	0.068	426	379	418	0.212	0.763	0.286
			±0.883	±1.058	±1.858	±0.001	±0.007	±0.003
48	5.84	0.079	473	367	392	0.285	0.807	0.291
			±1.529	±2.084	±2.188	±0.002	±0.009	±0.004
72	6.15	0.112	487	353	360	0.286	0.928	0.298
			±1.529	±1.455	±2.520	±0.003	±0.008	±0.006
96	6.41	0.148	490	352	345	0.291	1.143	1.321
			±1.455	±1.766	±2.851	±0.004	±0.024	±0.010
120	6.88	0.197	527	295	327	0.298	1.221	1.473
			±1.203	±0.883	±2.966	±0.006	±0.010	±0.009
144	7.28	0.204	529	278	307	1.321	1.771	1.802
			±1.205	±1.734	±2.655	±0.010	±0.005	±0.004
168	7.71	0.208	532	270	242	1.473	1.785	1.871
			±0.205	±2.030	±2.084	±0.009	±0.008	±0.005
192	7.81	0.211	542	195	230	1.786	1.923	1.883
			±0.882	±1.734	±1.203	±0.008	±0.004	±0.006
216	7.92	0.215	549	163	195	1.918	1.932	1.906
			±0.880	±2.407	±1.734	±0.007	±0.014	±0.007
240	7.96	0.219	562	138	163	2.076	2.14	2.093
			±1.888	±2.336	±2.407	±0.011	±0.097	±0.008

C1= CM-cellulase, C2= Cellobiase, C3= Salicinase, ±= Error of standard deviation

Table-5: Effect of 1% sorghum husk hydrolyzed with 0.6N HCl on cellulases production by *Aspergillus niger* when incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28 ± 2C

Time Period Hours	FinalpH	Weight of mycelia g/100ml	Total sugar mg/ml	Reducing Sugar mg/ml	Total Protein mg/ml	Enzyme activity units/ml		
						C1	C2	C3
24	4.88	0.081	575	341	580	0.127	1.23	0.102
			±0.578	±1.456	±0.883	±0.017	±0.001	±0.003
48	4.92	0.096	545	314	566	0.201	1.41	0.108
			±0.883	±1.766	±1.203	±0.005	±0.002	±0.001
72	4.98	0.123	473	270	464	0.234	1.42	0.157
			±1.455	±2.030	±1.529	±0.038	±0.003	±0.002
96	5.22	0.169	462	242	428	0.384	1.43	0.169
			±1.734	±2.084	±1.766	±0.007	±0.004	±0.001
120	5.31	0.172	406	230	390	0.455	1.44	0.185
			±1.766	±1.203	±2.407	±0.009	±0.005	±0.004
144	6.32	0.175	379	195	362	0.477	1.45	0.201
			±1.058	±1.734	±1.858	±0.035	±0.006	±0.006
168	6.41	0.178	367	182	354	0.531	1.46	0.212
			±2.084	±0.578	±2.084	±0.012	±0.007	±0.005
192	7.28	0.176	353	163	317	0.665	1.47	0.264
			±1.455	±2.407	±1.156	±0.016	±0.008	±0.010
216	7.31	0.174	351	153	296	0.718	1.48	0.308
			±1.762	±2.007	±2.407	±0.027	±0.009	±0.001
240	7.61	0.171	295	138	289	0.871	1.49	0.319
			±0.880	±2.336	±2.851	±0.0066	±0.010	±0.009

C1= CM-cellulase, C2= Cellobiase, C3= Salicinase, ±= Error of standard deviation

Table-6: Effect of 1% rice husk hydrolyzed with 0.6N HCl on cellulases production by *Aspergillus niger* when incubated in cooled orbital shaking incubator adjusted at 200 rev/min with initial pH 6.0 at 28 ± 2C

Time Period Hours	Final pH	Weight of mycelia g/100ml	Total sugar mg/ml	Reducing Sugar mg/ml	Total Protein mg/ml	Enzyme activity units/ml		
						C1	C2	C3
24	4.11	0.936	561	304	535	0.811	0.246	0.136
			±0.707	±0.001	±0.707	±0.040	±0.003	±0.008
48	4.19	0.914	526	260	497	0.847	0.807	0.413
			±0.707	±1.414	±1.414	±0.024	±0.009	±0.004
72	4.25	0.841	520	252	455	0.921	0.817	0.485
			±3.536	±0.001	±1.414	±0.058	±0.002	±0.003
96	5.22	0.661	472	242	388	1.097	0.872	0.49
			±2.121	±2.121	±1.414	±0.009	±0.005	±1.156
120	5.32	0.561	453	236	343	1.984	0.901	0.551
			±2.121	±0.707	±1.414	±0.003	±0.006	±0.005
144	6.38	0.522	448	227	332	2.871	0.915	0.677
			±1.414	±2.121	±5.657	±0.006	±0.008	±0.002
168	6.41	0.441	405	221	327	3.381	0.928	0.735
			±3.536	±0.707	±2.121	±0.036	±0.007	±0.002
192	6.44	0.438	388	214	293	3.391	1.045	0.758
			±1.414	±2.828	±4.950	±0.333	±0.024	±0.001
216	6.51	0.391	371	200	278	4.141	1.053	0.902
			±0.707	±3.536	±5.657	±0.097	±0.004	±0.019
240	6.55	0.367	353	189	243	4.811	4.717	4.742
			±1.414	±0.707	±1.414	±0.004	±0.010	±0.077

C1= CM-cellulase, C2= Cellobiase , C3= Salicinase, ±= Error of standard deviation

DISCUSSION

Current agricultural wastes are generated in large quantities in various countries and many of them are underutilized and considered as waste particularly in developing countries. Substantial efforts have been made by several researchers in converting these agricultural wastes to valuable products including biofuels, animal feed, biofertilizer, and enzymes [24, 25]. These processes help in controlling some of the environmental challenges associated with their disposal. The polymeric constituents of agricultural wastes used in this study in terms of cellulose, hemicellulose and lignin. It is important for the support of the growth of microorganisms for valuable product formation.

Normally dilute acids are utilized to degrade hemicellulose, cellulose and other non-crystalline polymer to simple sugars such as glucose. Acid hydrolysis produces slight decomposition of monosaccharide and conventional neutralization is not necessary. Chemical pretreatment method is less expensive and more effective. Ratio of total sugar or reducing sugar reveals the average degree of polymerization of sugar in solution.This is important that this ratio is near to 1.0. [26, 27] reported that higher amount of cellulase production achieved by indigenous strains. In present study confirm that the maximum production (4.811 units/ml) of CM-cellulase was obtained at 240 hours of incubation when rice husk hydrolysate was used as a carbon source. However, cellobiase and salicinase maximum production 4.717 and 4.742 units/ml were obtained at 240 hours respectively when rice husk hydrolysate used as a carbon source by indigenous strain of *Aspergillus niger* which was isolated from Khairpur Sindh.

CONCLUSION

Consequently, an agricultural waste in the form of cellulose that is the richest renewable biomass in the biosphere has been exhibited to be used in the production of valuable products by microorganism. Sugarcane peealar bagasse, sugarcane bagasse, banana fruit stalk, sorghum husk and rice husk that are few of these agricultural wastes used in this work as fermentation substrate produced a large amount of cellulase enzymes by *Aspergillus niger*. The results highlight industrial potentials of the substrates as possible raw materials for cellulase enzyme production by *Aspergillus niger*.

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