IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 9, Issue 1 (Jan. – Feb. 2023), PP 28-33
www.iosrjournals.org

Production of Enzyme Cellulase by some soil fungi causing Biodeterioration of Sugarcane Bagasse

Shubhra Verma[1], Manju Kumari[2], Ramendra Kumar Sinha[3] and Baidyanath Kumar[4]

1& 3. Research Scholar, Department of Botany, T. S. College, Hisua, Nawada (Bihar)
2. Associate Professor, Department of Botany, T. S. College, Hisua, Nawada (Bihar)
4. Corresponding Author: Dr. Baidyanath Kumar, Academic Director, Life Science Research Centre, Digha XTTI, Patna-800011

ABSTRACT: *Sugarcane Bagasse is fibrous structure obtained by crushing the sugarcane stem and extracting juice. It is used in the production of enzymes, amino acids, drugs, ethanol and single cell protein. Sugarcane Bagasse is considered as a pure waste material which can be used to extract cellulose and nanocellulose particles.*

Cellulose is a linear polysaccharide consisting of β-D-glucose chains joined together by β-1, 4-glycosidic linkages with cellobiose residues as the repeating unit at different degree of polymerization.

In the present investigation, production of cellulases viz. Exoglucanase, Endoglucanase and β-glucosidase by Chaetomium globosum, Memnoniella echinata, Aspergillus niger, Penicillium chrysogenum and Cladosporium herbarum was studied. The results revealed that the enzyme activity was highest in Chaetomium globosum, Aspergillus niger and Penicillium chrysogenum. The β-Endoglucanase after seven days of incubation was maximum in Cladosporium herbarum (42.34%) followed by Chaetomium globosum (38.66%), Aspergillus niger (33.25%), Memnoniella echinata (12.15%) and Penicillium chrysogenum (8.35%) in 90 minutes of incubation. The 1, 4-β-Exoglucanase activity (C1) at the end of 7 days of incubation was maximum in Chaetomium globosum followed by, Memnoniella echinata, Cladosporium herbarum, Aspergillus niger and Penicillium chrysogenum. The β-Glucosidase activity in crude enzyme of Chaetomium globosum was maximum, followed by Aspergillus niger, Cladosporium herbarum, Memnoniella echinata and Penicillium chrysogenum.

Microbial decomposition of Sugarcane Bagasse (Cellulose) is brought about by enzyme cellulase. This enzyme complex is secreted by microorganisms only in the presence of cellulose. However, the capacity of producing different components of cellulase differs. In the present investigation, it was found that a single fungal species was not equally efficient in producing all the three components of cellulase.

KEY WORDS: *Sugarcane Bagasse, Cellulase, Biodeterioration, Soil Fungi*

Date of Submission: 14-02-2023 Date of Acceptance: 28-02-2023

I. Introduction

Sugarcane Bagasse is fibrous structure obtained by crushing the sugarcane stem and extracting juice (Michel *et al.*, 2013; Pandey *et al*., 2000) [1, 2]. It is mainly in sugar and alcohol mills, paper and pulp industries, foodstock and biofuel industries (Hern andez-salas *et al*., 2009; Loh *et al*., 2013) [3, 4]. Sugarcane Bagasse is also used in the production of enzymes, amino acids, drugs, ethanol and single cell protein (Eriksson, 1990) [5]. Sugarcane Bagasse is considered as a pure waste material which can be used to extract cellulose and nanocellulose particles (Costa *et al*., 2013; Gilfillan *et al*., 2012; Slavutsky and Bertuzzi, 2014) [6, 7 and 8].

Cellulose is a linear polysaccharide consisting of β-D-glucose chains joined together by β-1, 4-glycosidic linkages with cellobiose residues as the repeating unit at different degree of polymerization. The polymers are packed into microfibrils which are held together by intramolecular hydrogen bonds and van der waals forces.

Sugarcane Bagasses are subjected to microbial attack that causes deterioration by way of decomposition. Under aerobic conditions a wide range of soil saprophytic fungi such as *Chaetomium, Stachybotrys, Memnoniella, Cladosporium, Trichoderma, Alternaria, Aspergillus, Penicillium, Thermoascus* etc colonize the Bagasse.

Cellulose is hydrolyzed by enzyme cellulose. It is a combination of three types of enzymatic activities viz. Cellobiohydrolase (CBH or 1, 4-β-D-Glucan cellobiohydrolase, EC 3.2.1.91), Endo-β-1, 4-Glucanase (EG or endo-1, 4-β-D-Glucane-4-glucanohydrolase, EC 3.2.14) or CX and β-Glucosidase (BG, EC 3.2.1.21). The complete cellulose system comprising CBH, EG and BG components synergistically act to convert crystalline cellulose to β-D-Glucose. The exocellobiohydrolases and endoglucanases act together to hydrolyze cellulose to

small cello-oligosaccharides. The cello-oligosaccharides (Cellobiose) are then subsequently hydrolyzed to β-D-Glucose by β-glucosidase.

Though the production of cellulose by soil fungi has been extensively studied (Chapman *et al.,* 1975; Forbes and Dickenson, 1977; Upreti and Joshi, 1984; Baidyanath and Prasad, 2004) [9, 10, 11 and 12]. Very little information is available on the production of cellulose by soil fungi causing biodeterioration of Sugarcane Bagasse. Hence in the present investigation, production of cellulases viz. Exoglucanase, Endoglucanase and β-glucosidase by *Chaetomium globosum*, *Memnoniella echinata*, *Aspergillus niger*, *Penicillium chrysogenum* and *Cladosporium herbarum* was studied.

II. Materials And Methods

In order to study the surface mycoflora of Sugarcane Bagasse, the Bagasse fibres were placed on moist blotting paper in Petri dishes at 25 $\pm 2^0$C. The surface mycoflora were observed after seven days. *Chaetomium globosum*, *Memnoniella echinata*, *Aspergillus niger*, *Penicillium chrysogenum* and *Cladosporium herbarum* were isolated in seven days of plating.

In order to determine the capacity of enzyme production by these fungi, Czapek Dox liquid medium supplemented with 2% carboxy methyl cellulose (CMC) was prepared. pH of the medium was adjusted to 5.0. Fifteen days old cultures were used as the inoculum. A loopful of culture was mixed, homogenized with sterile distilled water with the help of glass rod to make thorough suspension and added 1 ml to the flask. Streptopenicillin was used to check the bacterial growth. The flasks were incubated at 20 $\pm 2^0$C and 90% relative humidity. After fifteen days cultures were filtered through Buchner funnel using Whatman Nunber-1 filter paper. The filtrate was centrifuged at 2000 rpm for 40 minutes to remove the spores. The supernatant fluid containing the crude enzyme was transferred to a conical flask and stored at 4^0C after adding a few drops of toluene as a preservative.

The endoglucanase (CX) activity of the above cellulolytic fungi was assayed by viscometer as suggested by Reese (1950) [13] and Reese *et al*., (1950) [14]. 0.2 ml of test solution was added to 10 ml of 1% CMC and the time was immediately noted. The mixture was stirred and 10 ml of it was transferred to "Oswald" viscometer and its viscosity was determined at regular intervals of 30 minutes. The test was carried out at 30^0C in a water bath. The percentage of CX activity was calculated by following formula:

Percent of CX (endoglucanase) = B X 100/ A

Where, A indicates the assumed total fall of viscosity from initial reading to that of distilled water and B is the actual fall of viscosity in 30 minutes in that particular set of experiments.

C1 (Exoglucanase) activity was assayed by the method suggested by Miller (1959) [15]. 50 mg of absorbent cotton was placed in the test tubes containing 5 ml of filtrate (crude enzyme) and incubated at 30^0C for 48 hours. Dissolution of cotton indicated the presence of C1 enzyme (Exoglucanase) secreted by fungi. β-Glucosidase activity was assayed by the method suggested by Mendel et al., (1976). 0.5 ml crude enzyme solution and 0.5 ml of salicin in citrate buffer was added in culture filtrate. The mixture was kept in an 18 mm test tube and mixed well in vortex mixture. The tube was then incubated at 50 ± 5^0C for 30 minutes. The reducing sugar of the mixture was estimated by DNS (Dinitro salicylic acid) method as suggested by Miller (1959; Mendel *et al*., 1976) [15, 16]. The results obtained have been presented in Table-1, 2 and 3 Figure-1, 2 and 3.

Table-1: Extracellular enzyme secretion in µg/ml in Czapek Dox liquid medium by five fungal isolates.

Fungi	Enzyme production in µg/ml		
	Days of incubation		
	7	14	21
Chaetomium globosum	3.25 ±0.21	5.55 ±0.11	16.75 ±0.21
Memnoniella echinata	3.0 ±0.11	3.45 ±0.10	3.85 ±0.16
Aspergillus niger	3.15 ±0.12	4.25 ±0.21	14.65 ±0.11
Penicillium chrysogenum	3.14 ±0.12	4.13 ±0.15	13.75 ±0.21
Cladosporium herbarum	3.25 ±0.25	3.45 ±0.31	4.15 ±0.25

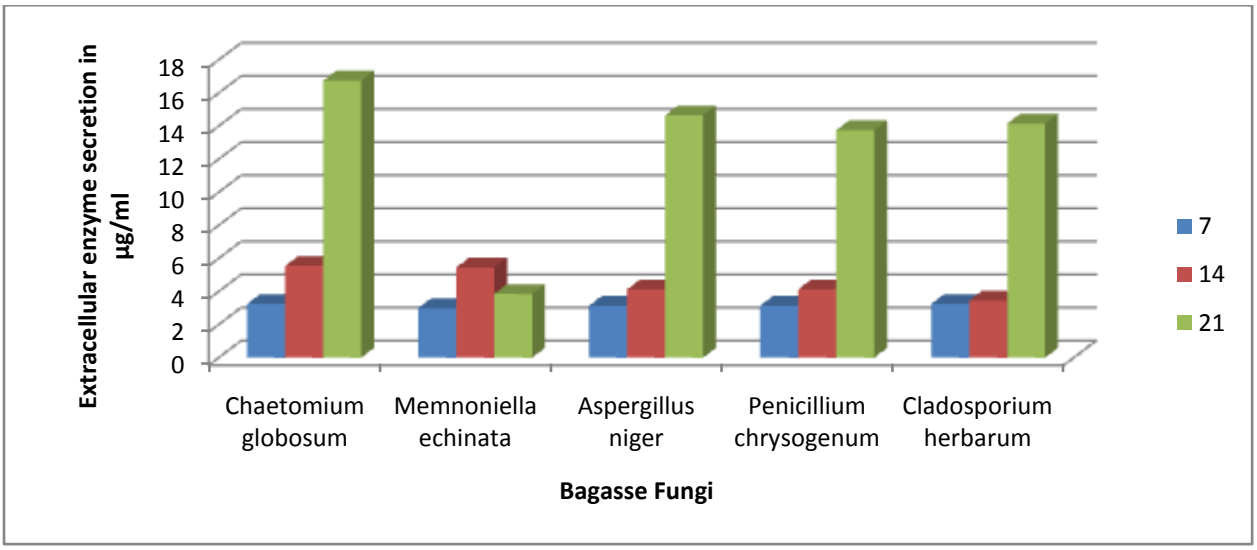

Figure-1: Extracellular enzyme production by Bagasse fungi after 7, 14 and 21 days of incubation.

Table-2: Percentage loss in viscosity of CMC by crude enzyme (CX) produced by Bagasse fungi

Bagasse Fungi	Time interval in minutes	Percentage loss in viscosity at different time intervals Days of incubation		
		7	14	21
Chaetomium globosum	30	18.65 ±0.25	42.55 ±0.21	51.45 ±0.21
	60	32.65 ±0.31	47.85 ±0.15	50.25 ±0.31
	90	38.66 ±0.26	61.35 ±0.15	67.65 ±0.23
Memnoniella echinata	30	4.25 ±0.16	42.35 ±0.25	46.27 ±0.30
	60	8.65±0.22	58.90 ±0.15	51.35 ±0.14
	90	12.15 ±0.16	66.55 ±0.17	53.55 ±0.17
Aspergillus niger	30	9.45 ±0.11	35.62 ±0.13	40.75 ±0.35
	60	16.15 ±0.22	59.25 ±0.21	60.45 ±0.41
	90	33.25 ±0.16	60.17 ±0.21	61.25 ±0.26
Penicillium chrysogenum	30	Nil	21.45 ±0.21	24.45 ±0.17
	60	Nil	32.15 ±0.16	35.15 ±0.21
	90	8.34 ±0.12	36.75 ±0.30	40.16 ±0.26
Cladosporium herbarum	30	17.65 ±0.21	46.75 ±0.22	40.15 ±0.11
	60	35.85 ±0.19	60.45 ±0.16	67.68 ±0.14
	90	42.34 ±0.21	63.43 ±0.17	66.65 ±0.65

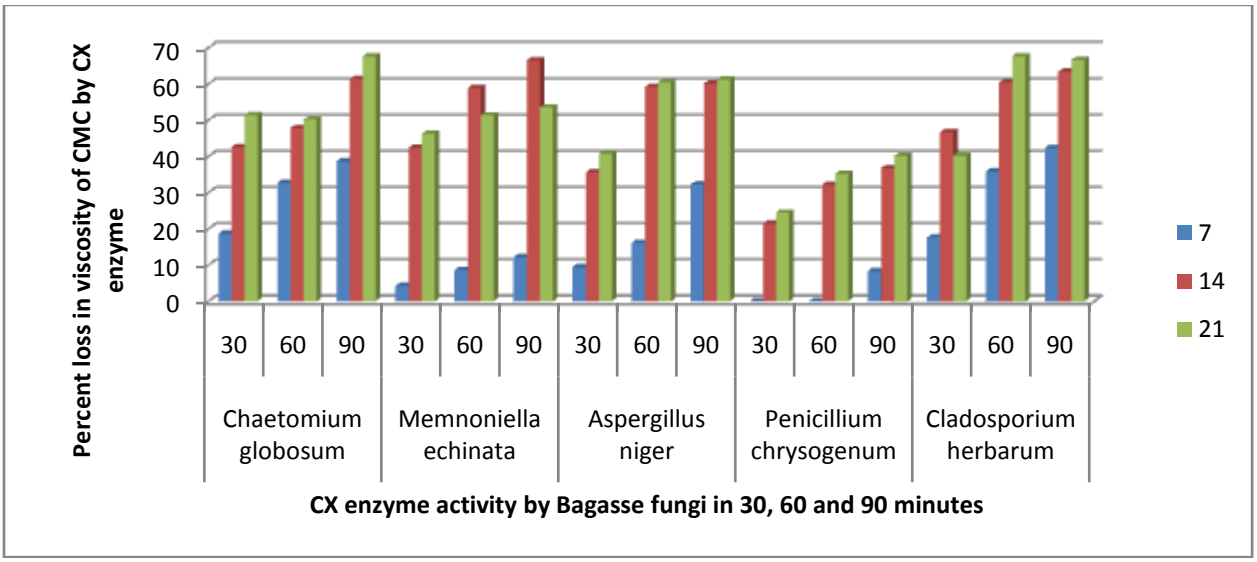

Figure-2: Percent loss in viscosity of CMC by CX endoglucanase by Bagasse fungi after 7, 14 and 21 days of incubation

Table-3: 1, 4-β-Glucanase (C1 or Exoglucanase) and β-Glucosidase activities of Bagasse fungi

Bagasse fungi	Days of incubation	C1 activity	β-Glucosidase activity
		Reducing sugar in mg/ml of culture filtrate	
Chaetomium globosum	7	0.955	0.99
	14	1.955	1.95
	21	2.957	1.80
Memnoniella echinata	7	0.651	0.55
	14	0.855	1.05
	21	0.935	0.97
Aspergillus niger	7	0.456	0.85
	14	0.701	0.75
	21	0.855	0.41
Penicillium chrysogenum	7	0.415	0.041
	14	0.565	0.55
	21	0.625	0.50
Cladosporium herbarum	7	0.635	0.65
	14	0.715	0.75
	21	0.812	0.75

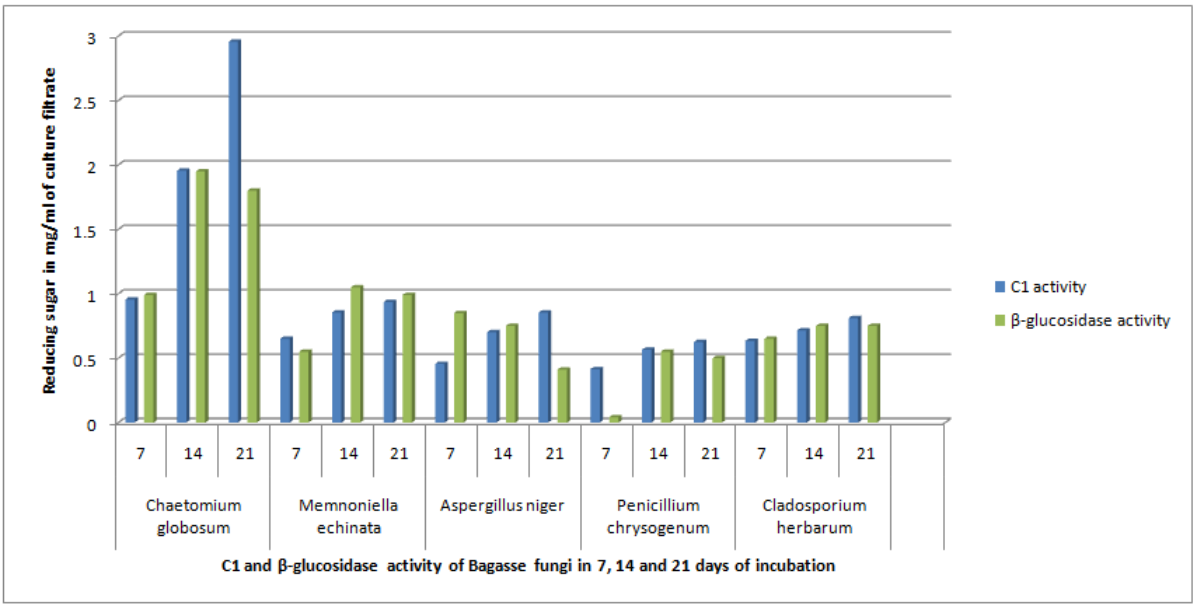

Figure-3: 1, 4-β-Glucanase (C1) and β-glucosidase activity of Bagasse fungi after 7, 14 and 21 days of incubation (Reducing sugar in mg/ml of culture filtrate)

III. Results

In course of present investigation, five mycoflora viz. *Chaetomium globosum*, *Memnoniella echinata*, *Aspergillus niger*, *Penicillium chrysogenum* and *Cladosporium herbarium* were isolated from sugarcane Bagasse after seven days of plating. These mycoflora were assayed for their ability to produce extracellular cellulose in liquid culture media. After 7, 14 and 21 days of incubation, culture filtrates were used for enzyme estimation.

From the results (Table-1; Figure-1) it is evident that out of five fungal species screened for their ability to secrete enzyme, the enzyme activity was highest in *Chaetomium globosum*, *Aspergillus niger* and *Penicillium chrysogenum* which produced 16.75 µg/ml, 14.65 µg/ml and 13.75 µg/ml of extracellular enzyme after twenty one days of incubation. *Memnoniella echinata* and *Cladosporium herbarum* were more or less similar in enzyme production that produced 3.85 µg/ml and 4.15 µg/ml of enzyme respectively after 21 days of incubation. After 7 days of incubation, all these Bagasse fungi produced lowest concentration of extracellular cellulase in the range of 3.0 µg/ml to 3.25 µg/ml. Increase in enzyme production was noticed on increasing the incubation period.

From Table-2; Figure-2 it is clear that the β-Endoglucanase activity (in terms of % loss in viscosity of CMC) after seven days of incubation was maximum in *Cladosporium herbarum* (42.34%) followed by *Chaetomium globosum* (38.66%), *Aspergillus niger* (33.25%), *Memnoniella echinata* (12.15%) and *Penicillium chrysogenum* (8.35%) in 90 minutes. With increase in incubation period there was enhanced β-Endoglucanase activity which was more rapid up to 14 days of incubation. At the end of incubation period there was a loss in viscosity up to 67.65%, 66.65%, 61.55%, 61.25% and 40.16% by crude enzyme obtained from the culture

filtrates of *Chaetomium globosum, Cladosporium herbarum, Aspergillus niger, Memnoniella echinata* and *Penicillium chrysogenum* respectively.

The 1, 4-β-Exoglucanase activities (C1) at the end of 7 days of incubation was maximum in *Chaetomium globosum* followed by, *Memnoniella echinata, Cladosporium herbarum, Aspergillus niger* and *Penicillium chrysogenum*. These molds produced 0.955 mg/ml, 0.635 mg/ml, 0.651 mg/ml, 0.456 mg/ml and 0.415 m,g/ml of reducing sugar after seven days of incubation. The C1 activity increased with increase in incubation period. After 21 days of incubation *Chaetomium globosum, Memnoniella echinata, Aspergillus niger, Cladosporium herbarum* and *Penicillium chrysogenum* produced 2.957 mg/ml, 9.35 mg/ml, 0.855 mg/ml, 0.813 mg/ml and 0.625 mg/ml of reducing sugar in crude enzyme extract (Table-3; Figure-3).

The β-Glucosidase activity in crude enzyme of *Chaetomium globosum* was maximum, followed by *Aspergillus niger, Cladosporium herbarum, Memnoniella echinata* and *Penicillium chrysogenum*. These molds produced 0.99 mg/ml, 0.85 mg/ml, 0.65 mg/ml, 0.55 mg/ml and 0.041 mg/ml of reducing sugar respectively after 7 days of incubation. The β-glucosidase activity increased with increase in incubation period. After 21 days of incubation these molds produced 1.80 mg/ml, 0.41mg/ml, 0.75 mg/ml, 0.97 mg/ml and 0.50 mg/ml of reducing sugar respectively (Table-3; Figure-3).

IV. Discussion

Microbial decomposition of Sugarcane Bagasse (Cellulose) is brought about by enzyme cellulase. This enzyme complex is secreted by microorganisms only in the presence of cellulose (Desai and Pandey, 1971; Baidyanath and Prasad, 2004) [17 and 12]. However, the capacity of producing different components of cellulase differs. In the present investigation, it was found that a single fungal species was not equally efficient in producing all the three components of cellulase.

The extracellular secretion of enzyme protein in liquid culture media was maximum in Chaetonium globosum. The CX endoglucanase activity was maximum in *Chaetomium globosum, Cladosporium herbarum, Aspergillus niger* and *Memnoniella echinata*. This revealed the inefficiency of these fungi in producing other components of cellulose complex. C1 exoglucanase activity was maximum in *Chaetomium globosum*. Bagool and Wani (2004) [18] studied 12 fungi for their C1 exoglucanase and CX endoglucanase activities and reported that most of the moulds including *Penicillium citrinum, Chaetomium venezuelensis, Sphaeronema allahabadens* and *Penicillium islandicum* were not uniform in their C1 and CX activities. B-glucosidase activity was highest in *Chaetomium globosum* and *Memnoniella echinata*. This enzyme is an important component of the cellulose complex. B-Glucosidase activity results in the production of β-D-Glucose as a product of complete hydrolysis of Sugarcane Bagasse. This might make these fungi a suitable organism for industrial saccharification of cellulosic waste (Sugarcane Bagasse). Banik *et al*., (1989) [19] in their investigation reported *Aspergillus fumigatus,* a potential organism for industrial saccharification of cellulosic wastes. The present investigation brings an addition of the bagasse fungi viz. *Chaetomium globosum, Memnoniella echinata, Aspergillus niger, Cladosporium herbarum* and *Penicillium chrysogenum* to be employed for industrial saccharification of Sugarcane Bagasse and other plant wastes.

V. Conclusion:

The extent of degradation of Bagasse varies. It may be ascribed to the variation in the number and types of fungi colonizing the Bagasse fibres if other factors are not limiting. It may be noted that individually the fungus is not as effective in degradation of Bagasse as in combination. The first decomposer fungi release simple forms nutrition. These simpler forms are utilized by weak cellulolytic colonizers which persist for more time. When the substrate is again depleted off of the simpler nutrition, the weak cellulolytic forms disappear. At this stage few more potent cellulose decomposing fungi appear and continue the decomposition of cellulose. The uniform distribution of some fungi is due to their highly saprophytic ability to degrade cellulose and greater tolerance to varying environmental conditions. Moreover, their ability to produce toxic substances helps them in their survival under most competitive situation.

ACKNOWLEDGEMENT: Authors are thankful to Dr. Baidyanath Kumar, Academic Director, Life Science Research Center Patna for providing necessary laboratory facilities and support.

Conflict of Interest: Authors declare no conflict of interest directly or indirectly

REFERENCES

[1]. Michel, D, B. Bachelier, J.-Y. Drean, O. Harzallah (2013): Preparation of cellulosic fibers from sugarcane for textile use, in: Conference Papers in Materials Science, Guimare aes.

[2]. Pandey, A, C.R. Soccol, P. Nigam, V.T. Soccol (2000): Biotechnological potential of agroindustrial residues. I: sugarcane bagasse, **Biores. Technol**. **74:** 69–80.

[3]. Hern_andez-Salas, J, M.S. Villa-Ramı_rez, J.S. Veloz-Rend_on, K.N. Rivera-Hern_andez, R.A. Gonz_alez-C_esar, M.A. Plascencia-Espinosa, S.R. Trejo-Estrada (2009) : Comparative hydrolysis and fermentation of sugarcane and agave bagasse, **Biores. Technol**. **100:** 1238–1245.

[4]. Loh, Y. R, D. Sujan, M.E. Rahman, C.A. Das (2013): Sugarcane bagasse-the future composite material: a literature review, **Res. Cons. Recycl**. **75:** 14–22.
[5]. Eriksson, K. E (1990): Biotechnology in the pulp and paper industry. **Wood Sci. Technol**, **24:** 79-101.
[6]. Costa, S. M, P.G. Mazzola, J.C.A.R. Silva, R. Pahl, A. Pessoa Jr., S.A. Costa (2013): Use of sugar cane straw as a source of cellulose for textile fiber production, **Ind. Crops Prod**. **42**: 189–194.
[7]. Gilfillan, W. N, D.M.T. Nguyen, P.A. Sopade, W.O.S. Doherty (2012): Preparation and characterisation of composites from starch and sugar cane fibre, **Ind. Crops Prod**. **40**: 45–54.
[8]. Slavutsky, A. M, M.A. Bertuzzi (2014): Water barrier properties of starch films reinforced with cellulose nanocrystals obtained from sugarcane bagasse, **Carbohydr. Polym**.**110**: 53–61.
[9]. Chapman, E. S., Evans, E., Jacobelli, M. C. and Logan, A. A. (1975): the cellulolytic and amylolytic activity of Populospora thermophilla, **Mycologia, 67:** 608-615.
[10]. Forbes, R. S. and Dickinson, G. H. (1977): Effect of temperature, pH and nitrogen on cellulolytic activity of Fusarium avaenaceum, **Trans. Br. Mycol. Soc**. **68:** 199-235.
[11]. Upreti, J. C. and Joshi, M. C. (1984): Cellulolytic activity of mesophilic and thermophilic fungi isolated from mushroom compost. **Indian Phytopath**, **37:** 473-476.
[12]. Baidyanath Kumar and J. S. Prasad (2004): Production of cellulases by some paper rotting fungi, **Journal of L. N. Mithila University**, **Vol (1), No. 2:** 1-4.
[13]. Reese, E. T. (1950): A microbiological process report. Enzymatic hydrolysis of cellulose, **App. Microbiology**, **4:** 39-45.
[14]. Reese, E. T., Siu, R. G. H. and Levinson, H. S. (1950): the biological degradation of soluble cellulose derivatives**. Journal. Bacteriol**, **59:** 485-497.
[15]. Miller, G. L. (1959): Use of Dinitrosalicylic acid reagent for determination of reducing sugars, **Analyt. Chem, 31:** 426-428.
[16]. Mendel, M., Andreotti, R. and Roche, C. (1976): Measurement of saccharification of cellulose, **Biotech. Bioeng. Symp**. **6:** 21-23.
[17]. Desai, A., J. Pandey, S. N. (1971): Microbial degradation of cellulosic textiles, **J. Sci and Ind. Res**. **30:** 598-606.
[18]. Bagool, R. G. and Wani, D. D. (1986): Studies on cellulolytic fungi from Maharashtra State (India), Ind. Journal of Microbiology, **26:** 131-137.
[19]. Banik, B. Mishra, A. K. and Geeta, N. (1989): Partial purification and properties of Extracellular cellulose from Aspergillus fumigatus, **Trans. of Bose res. Ins, 52 (2-3):** 43-55.

Subhra Verma, et. al. "Production of Enzyme Cellulase by some soil fungi causing Biodeterioration of Sugarcane Bagasse."*IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), 9*(1), (2023): pp. 28-33.