Received: 10 June 2021 | Revised 16 November 2021 | Accepted 16 December 2021

Research Article
Journal of Research and Review in Science,
8-14 Volume 8, December, 2021

DOI:10.36108/jrrslasu/1202.80.0111

ORIGINAL RESEARCH

Isolation of Cellulase Producing Microorganisms From Sugarcane Bagasse Dump Site in Lagos, Nigeria

Bolanle Olaitan Opere, Omotola Fashola, Ahmeed Ashade, Kehinde Adebiyi and Olaide Abiona

Department of Microbiology,
Lagos State University.

Abstract

Introduction: Soil is an important reservoir for diverse group of microorganisms. However, anthropogenic activities affect the quality, composition, and microbial communities in the soil. The polymeric units of compounds in a soil determines the type of microbial activities in the soil.

Aim: This study investigated the cellulose-degrading potential of microorganisms in a sugarcane bagasse dumpsite at Ojo Local Government Area of Lagos State.

Materials and Methods: The samples collected at different soil depths were inoculated on nutrient agar and potato dextrose agar to isolate bacteria and fungi. The cellulose-degrading ability of the isolates were determined by subculturing the isolates into Mandel's medium containing carboxymethyl cellulose (CMC), while the isolates were characterized and identified using biochemical tests.

Results: The physiochemical analysis of the soil samples revealed variations in the parameters such as pH, moisture, nitrogen, organic carbon etc, at different soil depths. A total of four cellulose-degrading bacteria and fungi were isolated. The isolated bacterial species are *Bacillusspp, Serratiaspp, Pseudomonasspp* and *Lactobacillus spp*. *Bacillusspp* had the highest cellulose-degrading potential while *Serratiaspp* showed the lowest. The isolated fungal species are Aspergillus niger, Penicilliumspp, Mucorspp, Neurosporaspp, Microsporiumspp and Aspergillus flavus.

Conclusion: This study demonstrated that soil from sugarcane bagasse dumpsite is a rich source of cellulose and possesses a high rate of activities of cellulolytic bacteria and fungi. *Bacillus* spp and *Mucor* spp are prominent cellulolytic microorganisms with immense potential for industrial applications.

Keywords: Cellulose, Cellulase, Sugarcane bagasse, Solid wastes, Enzymes.

Correspondence
Bolanle Olaitan Opere, Department of Microbiology, Faculty of Science, Lagos State University, Nigeria.
Email:bolanle.opere@gmail.com

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Soil is a major reservoir for several microorganisms. Numerous soil microbes have been screened for the production of antibiotics [1], amylase [2], cellulase [3-4], and other beneficial products. Interestingly, soil filled with solid wastes further possesses a higher reservoir of some polymers that are industrially useful. Solid wastes are made up of about 50% cellulose, 12% hemicelluloses and between 10% to 15% lignin on a dry weight basis [5-6]. These wastes can be leveraged to become assets rather than being liabilities by harnessing their natural reservoir of resources. Microbes which leverage cellulosic materials as the source of their energy could be harnessed for the conversion of these wastes into beneficial products for man's use [7-9].

Cellulose is one of the most abundant renewable polymers on earth. It is naturally organized as microfibrils linked together to form cellulose fibres. Cellulose rarely occurs freely in plant but in complex structure with hemicelluloses and lignin components to form lignocellulosic biomass. It is biologically synthesized by different organisms ranging from higher to lower plants, sea animals and microorganisms including bacteria and fungi [10]. As a major component of plants biomass, yearly production of photosynthetic biomass of cellulose is estimated to be approximately 40 billion tons, making cellulose the most abundant polysaccharide produced in the biosphere [11].

Cellulase is the major enzyme for the conversion of cellulosic materials into simple sugars which can serve as raw materials for the production of different chemicals and fuels for man's usage [12]. Cellulase is of major interest because of its wide array of applications. Cellulases are often used in textile industry for bio-polishing of fabrics and in household detergents for aiding fabric softness. It is also used in the production of biofuels and pharmaceutical applications [13-14]. Cellulolytic enzymes also play an important role in natural biodegradation processes in which plant ligno-cellulosic materials are effectively degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa. Numerous fungi capable of degrading cellulose synthesize large quantities of extracellular cellulases that are more efficient in depolymerising the cellulose substrate [13-14].

Fungi and bacteria are groups of microorganisms that are widespread in the environment, especially in the soil. They are one of the dominant groups that have strong influencing structure and function in the ecosystem, thus they play important roles in several ecological activities [15]. They are also known agents for degrading cellulosic material as part of organic matter [16]. Fungi such as *Trichoderma*spp and *Penicillium chrysosporium*are cellulase producing organisms that have been extensively studied. Also, bacteria such as *Actinomycetes, Cellulomonas and Pseudomonas* are also lignin hydrolyzing bacteria that have also been extensively studied for their cellulolytic properties. However, this paper is focused on the isolation of cellulase producing organisms from sugarcane bagasse dumpsites to avail further knowledge on the cellulolytic properties of microbes from municipal solid wastes site such as sugarcane.

2. MATERIAL AND METHODS

2.1. STUDY AREA

This study was conducted at the sugarcane bagasse dumpsite at Ojo Local Government Area of Lagos State,which is one of the 23 Local Governments of Lagos State under Badagry division. The study area serves as the business hub for Northerners who largely transport sugarcane to Lagos State for consumption in the cosmopolitan city of Lagos. The site harbours heap of sugarcane bagasse because of continuous peel and leftover of the waste material over time.

2.2 SAMPLE COLLECTION

Soil samples were collected from a sugarcane bagasse dumpsite at Ojo, Lagos State for a period of four weeks. Samples collected from the location under study were labelled A, B, and C respectively based on the soil depth. With a sterile hand trowel, soil samples were collected from the top of the soil up to 45cm depth i.e., top, 20cm and 45cm in a universal sample bottle. The samples were immediately transferred to the Microbiology laboratory in Lagos State University for analysis.

2.3 DETERMINATION OF THE PHYSIOCHEMICAL PROPERTIES OF THE SOIL

The physiochemical properties of the soil such as pH, moisture content, electrical conductivity, total nitrogen, total organic carbon, organic matter, average pressure, exchangeable potassium, exchangeable sodium, water holding capacity, cation-exchange capacity (CEC), cobalt, and lead were determined at the three different soil depths.

2.4 BACTERIA ISOLATION AND ENUMERATION

After sample collection and processing, 10g of each soil sample was immersed into 100ml of sterile distilled water and it was stirred to get an even mixture. Each test-tube was filled with 9ml of sterile distilled water and corked with cotton wool wrapped with foil paper. Tenfold serial dilutions of each sample were performed and 0.1ml of the serially diluted sample was spread on the nutrient agar (NA) plates using the spread plate method. The samples inoculated into NA plates were collected from 10^6 to 10^8 while the samples inoculated into the potato dextrose agar (PDA) plates were collected from 10^2 to 10^4. The NA plates were kept at room temperature and observed after 24 h while the PDA plates were observed after 72 h. After incubation, the number of colonies were counted using a colony counter and recorded to determine the bacterial profile for four (4) weeks.

2.5 SCREENING FOR CELLULOSE DEGRADING BACTERIA

The primary criterion for the selection of cellulase-producing microbial isolates was the formation of clear zone around a microbial colony in the Mandels' medium containing Carboxymethyl cellulose (CMC) after the medium was flooded with 1% Congo red solution and was allowed to stand for 15 mins at room temperature [23]. One molar (1 mol/dm^3) of NaCl was thoroughly used to counterstaining the plates. Clear zones which appear around growing bacterial and fungal colonies indicated cellulose hydrolysis. Average diameters of the clear zones were recorded.

Congo red is an indicator and is absorbed by the bonds between polysaccharide chains and the amino groups of the dye, thus providing a basis for rapid and sensitive screening test for cellulose degrading microorganisms.

2.6 CHARACTERIZATION AND IDENTIFICATION OF ORGANISMS

Pure cultures of isolates were subjected to ten (10) biochemical tests in accordance with standard procedures to identify the bacteria isolates which showed cellulolytic potential. These tests include: Catalase, Citrate, Oxidase, Motility, Indole production, Urease test and Sugar fermentation. Fungal isolates were identified based on colonial morphology and microscopic morphology which shows the level at which the contaminant plume moves with the groundwater flow at a direction of North to South.

3. RESULTS

This work investigated the microbial profile of the soil samples from Sugarcane bagasse dumpsite at Ojo local government, Lagos. The isolates were screened for cellulase production with a view to selecting bacteria and fungi having potential for cellulase production. Table 1 represents findings on the physical, chemical and biological properties of experimental soil examined.Table 2 shows the bacterial profile of the dumpsite from week 1 to week 4. There was substantial growth of microbes all through the four weeks across different levels. However, the highest microbial count was recorded for the deepest soil level of 45 cm across the weeks.

Four (4) different bacterial species and Seven (7) different fungal species were isolated and screened. Tables 3 and 4 shows the clearance zone of the cellulolytic bacteria and fungi isolated across the four weeks of isolation.

Table 1. Physical, chemical and biological properties of experimental soil (0-20 cm)

PARAMETERS	TOP	20Cm	45Cm
pH	7.78	8.33	8.90
Electrical conductivity	311.00	419.00	192.30
Total Nitrogen	0.08	0.14	0.09
Total organic carbon	0.67	1.70	0.88
Organic matter	1.15	2.92	1.51
Av. P	31.97	21.97	10.13
Exchangeable Potassium	0.62	0.38	0.28
Exchangeable Sodium	0.51	0.45	0.54
CEC	9.52	10.38	18.19
Moisture content	31.22	16.75	12.69
Water holding capacity	259.20	394.80	183.40
Cobalt	17.91	18.76	34.34
Lead	29.71	55.02	48.06

Table 2: Bacterial profile of samples across four weeks

Dilution Factor	*Week 1*			*Week 2*			*Week 3*			*Week 4*		
	A(Top)	B (20cm)	C(45cm)	A (Top)	B (20cm)	C (45cm)	A (Top)	B (20cm)	C (45cm)	A (Top)	B (20cm)	C (45cm)
10^6	$8.7x10^7$	$7.1xl0^7$	$12.2x10^7$	$7.9x10^7$	$6.9x10^7$	$7.5x10^7$	$10.6x10^7$	$11.2x10^7$	$15.2x10^7$	$9.3x10^7$	$10.1x10^7$	$12.2x10^7$
10^7	$5.1x10^8$	$4.7x10^8$	$10.9x10^8$	$5.3x10^8$	$4.9x10^8$	$7.8x10^8$	$8.3x10^8$	$7.4x10^8$	$10.2x10^8$	$7.2x10^8$	$6.9x10^8$	$8.3x10^8$
10^8	$4.7x10^9$	$5.3x10^9$	$9.8x10^9$	$4.2x10^9$	$5.2x10^9$	$6.2x10^9$	$6.4x10^9$	$6.9x10^9$	$7.8x10^9$	$5.7x10^9$	$7.1x10^9$	$7.5x10^9$

After counting the colonies, the Bacteria and Fungi were screened for cellulolytic potential by sub-culturing into NA-CMC Mandels' medium plates. The plates were kept at room temperature for 48 hours. After 48 hours, the plates that had growths were flooded with 1% Congo-red dye and counterstained with NaCl. The clearance zones were measured with a ruler (cm). This was done across the four weeks. The results are as follows:

Table 3: Average clearance zones for cellulolytic Bacteria across four (4) weeks (CZ – Clearance Zone)

Week 1		**Week 2**		**Week 3**		**Week 4**	
Bacterial Isolate	**CZ (cm)**	**Bacterial Isolate**	**CZ (cm)**	**Bacterial Isolate**	**CZ (cm)**	**Bacterial Isolate**	**CZ (cm)**
Serratia Sp	1.6	*Pseudomonas Sp*	1.8	*Bacillus Sp*	2.8	*Serratia Spp*	1.5
Bacillus Sp	1.8	*Lactobacillus Sp*	2.0	*Lactobacillus Sp*	1.7	*Bacillus Spp*	2.2
Lactobacillus Sp	2.8	*Serratia Sp*	1.3	*Serratia Spp*	1.6	*Pseudomonas Spp*	1.8
Bacillus Spp	2.2			*Pseudomonas Spp*	1.9	*Bacillus Spp*	3.1
				Bacillus Spp	2.2	*Lactobacillus Spp*	2.0
				Serratia Spp	2.7		

Table 4: Average clearance zones for cellulolytic Fungi across four (4) weeks (CZ – Clearance Zone)

Week 1		*Week 2*		*Week 3*		*Week 4*	
Fungal Isolate	**CZ (cm)**	**Fungal Isolate**	**CZ (cm)**	**Fungal Isolate**	**CZ (cm)**	**Fungal Isolate**	**CZ (cm)**
Aspergillus niger	4.8	*Mucor Spp*	73	*Aspergillus flavus*	5.5	*Neurospora Spp*	5.3
Penicillium Spp	4.2	*Penicillium Spp*	5.1	*Penicillium Spp*	5.0	*Penicillium Spp*	4.7
Mucor Spp	6.5	*Aspergillus flavus*	6.0	*Neurospora Spp*	5.2	*Aspergillus niger*	6.0
Neurospora Spp	4.6	*Neurospora Spp*	5.3	*Mucor Spp*	7.3	*Fusarium Spp*	5.6
Microsporium Spp	6.1			*Aspergillus niger*	5.7	*Mucor Spp*	7.1
Aspergillus flavus	4.5			*Microsporium Spp*	5.5		
				Aspergillus niger	5.5		

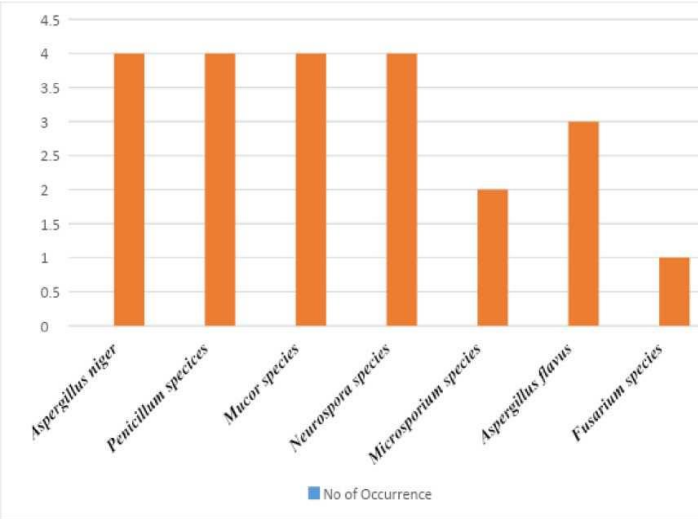

Figure 1: Frequency of occurrence of cellulolytic fungi across the four weeks of isolation

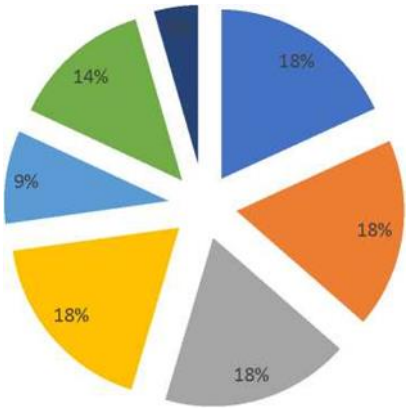

Figure 2: Percentage occurrence of cellulolytic fungi.

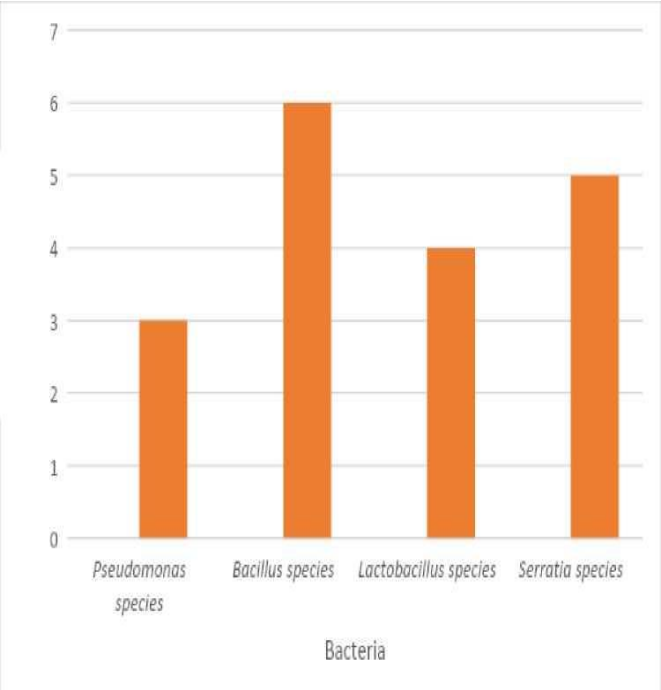

Figure 3: Frequency of occurrence of cellulolytic bacterial isolates across the four weeks of isolation.

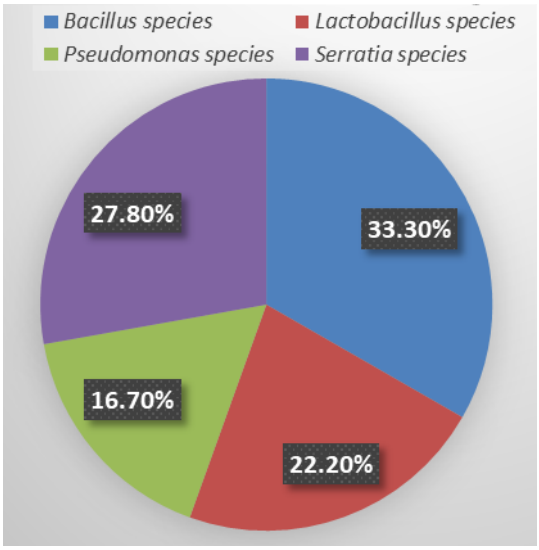

Figure 4: Percentage occurrence of cellulolytic bacteria.

4. DISCUSSION

This study demonstrated that soil from sugarcane bagasse dumpsite is a rich source of cellulose and possesses a high rate of activities of cellulolytic bacteria and fungi. The bacterial isolates include: *Bacillus species, Lactobacillus species, Pseudomonas species* and *Serratia species.* The fungal species isolated were: *Aspergillus niger, Penicillium species, Mucor species, Neurospora species, Microsporium species, Aspergillus flavus* and *Fusarium* species. Similar isolations have been made by Bishnu et al. [4], Saranraj et al. [17]] where they were all isolated from various soil samples including sugarcane bagasse, yam peels, cassava and rice straws dumping sites. All of the organisms were not present for all four weeks of the experiment; they all had their week of absence. This suggests that they might have been affected by environmental factors, mainly water activity, which is a corroboration of some of the environmental factors which affect growth of organisms that has been discussed by AgriInfo, [19]. Water is needed to dilute the soil's high acidity (pH 3.4) and therefore as shown in Table 1, the rainfall which characterized the first, third and fourth sampling weeks might have raised the humidity and the water activity of the soil, hence the high microbial count and quality observed in the three weeks. There was no rainfall in the second sampling week, hence the low microbial count and low quality of cellulolytic bacteria observed in the second week of sampling.

Bacillus species had the highest occurrence across the four weeks (with six isolates) than other bacterial isolates probablybecause*Bacillus* is one of the most common cellulolytic bacteria [20].

The clearance zone across the four weeks of isolation shows that *Bacillus* species is the most prominent cellulolytic bacteria amongst the bacteria isolated across the four weeks of isolation. The highest clearance zone of *Bacillus* species recorded is 31.0 mm. *Bacillus* species has the highest clearance zone across the three weeks in which it was present. It was absent only in the second week of isolation. This is

corroborative of the work of Kim et al. [21], where *Bacillus*sp was isolated as the leading cellulolytic bacteria from 176 samples of agricultural waste sites.

Lactobacillus species is the second most prominent cellulolytic bacterial isolate. The highest clearance zone observed from these isolates is 28.0mm which was observed in the third week of isolation. The lowest clearance zone observed is 17.0mm which was observed in the first week of isolation. This range of cellulolytic activities is also similar to the range of cellulolytic activities for *Lactobacillus* species in the study of Ajijolakewu et al., [18], *Pseudomonas* species is the third most prominent cellulolytic bacterial isolate. The highest clearance zone of *Pseudomonas* species isolated across the four weeks is 19.0 mm which was observed in the third week of isolation while the lowest clearance zone is 18.0 mm which was observed in the second and fourth weeks of isolation. In contrast to our study, Datta et al. [22] isolated *Pseudomonas* spp as the leading cellulase-producing bacteria, having higher cellulolytic potential than *Bacillus*spp got from the same sample. *Serratia* species has the lowest cellulolytic potential amongst the four isolates. The highest clearance zone observed is 17.00 mm which was observed in the third week of isolation while the lowest clearance zone observed is 13.0 mm which was observed in the second week of isolation. This is coherent with the works of Ajilolakewu et al. [18], where *Serratia* species isolated from sugarcane bagasse dump sites.

For the fungal isolates, it was observed that *Aspergillus niger*, *Penicillium* species, *Mucor* species, *Neurospora* species appeared more than the other organisms. *Fusarium* species was seen to appear only once, *Penicillium* species, *Mucor* species and Neurospora species were isolated in all the weeks. It was observed that the 45cm depth had the highest number of isolates which was 9, followed by 20cm which had 8 isolates, then top which had 5 isolates.

Mucor species was observed to have the highest clearance zone with the average of 70.5mm and *Penicillium* species had the lowest clearance zone with the average of 47.5mm across all the 4 weeks.

The fungal isolates showed more cellulolytic activities than the bacterial isolates. *Mucor* species has the highest of all the bacterial and fungal isolates. The least cellulolytic fungal isolate – *Penicillium* species – with a clearance zone of 42mm produce more cellulases than the bacterial isolate – *Bacillus* species – with the highest clearance zone (31 mm). The fungal isolates produce more cellulases because of their abilities to survive in harsh environmental conditions, which are major characteristics of lignocellulosic sites such as the sugarcane bagasse dump sites. These findings were similar to the study of Ajilolakewu et al. [18].

5.CONCLUSION

Several microorganisms are widely spread in the environment, including the soil, where they contribute to the structure and function of the ecosystem. Majority of these soil bacteria and fungi are known to play a major role in degradation organic and inorganic waste products and have been screened for the production of important products such as antibiotics, amylase, cellulase, and other numerous beneficial products. In this work, we demonstrated successfully that soil from sugarcane bagasse dumpsite is a rich source of cellulose and possesses a high rate of activities of cellulolytic bacteria and fungi. The major deduction from this work is that *Bacillus* species and *Mucor* species are prominent cellulolytic microorganisms and have immense potential for industrial applications.

Contributions

Bolanle Opere designed the study, performed the laboratory bench work, wrote the protocol, and the first draft of the manuscript. Muibat Fashola and Ahmed Ashade managed the analyses of the study. Kehinde Adebiyi and OlaideAbiona managed the literature searches and references. All authorsread and approved the final manuscript.

REFERENCES

1. Ahmed R, Sani A, Ajijolakewu A, Alamu F. Soil screening for antibiotic-producing microorganisms. Adv EnvtBiology. 2013;71:7-11.
2. Ajijolakewu A, Sani A. Amylase production by *Penicillium chrysogenumin* a submerged fermentation of cassava powder. In: 25th Annual Conference of Biotechnology Society of Nigeria. 2012; pp. 161-167.
3. Chand P, Aruna A, Maqsood A, Rao L. Novel mutation method for increased cellulase production. Journal of Applied Microbiology. 2005;98:318-323.
4. Bishnu J, Megh R, Dinita S, Jarina J, Rajani M et al. Lignocellulosic ethanol production: Current practices and recent developments. Biotech Mol Bio Rev. 2011;68:172-182.
5. Rani D, Nand K. Production of thermostable cellulase-free xylanase by *Clostridium* absonum CFR-702. Process Biochemistry. 2000;36: 355-362. 10.1016/S0032-9592(00)00224-
6. Gautam P, Bundela A, Pandey M, Sarsaiya S. Screening of Cellulolytic Fungi for Management Of Municipal Solid Waste. J App Sci in Envt Sanitation.2010;5(4): 391-395.
7. Belewu M, Banjo N (1999). Biodelignification of rice husk and sorghum stover by edible mushroom (PleurotussajorCoju). Trop. Animal Sci J. 1999;1: 137 – 142.
8. Banjo N, Kuboye A. Comparison of the effectiveness of some common Agro-industrial wastes in growing three tropical edible mushrooms. In: Internal Conference on Biotechnology: Commercialization and Food Safety, Nigeria. 2000; pp. 161- 165.
9. Belewu M, Afolabi O. Biochemical degradation of Corn cobs and Aboria saw-dust by Oyester Mushroom. In: International conference on Biotechnology: Commercialization and Food Security, Abuja, Nigeria. 2000; pp. 169 – 173.

10. Gilberto S, Julien B, Alain D. Cellulosic Bionanocomposites: A review of preparation, properties and applications. Polymers. 2010;2(4):728-765.
11. Hatami S, Alikhani H, Besharati H, Salehra N. Investigation on Aerobic Cellulolytic Bacteria in Some of North Forest and Farming Soils. American-Eurasian Journal of Agricultural & Environmental Sciences. 2008;3(5):713-716.
12. Ryu D, Mandels M. Cellulases: biosynthesis and applications. Enzyme Microb Tech. 1980;2:91–102.
13. Abdelnasser S, Ahmed I (2007). Isolation and Identification of new cellulases producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme. Australian Journal of Basic and Applied Sciences. 2007;1(4):473-478.
14. Cherry J, Fidantsef A (2003). Directed evolution of industrial enzymes: an update. CurrOpin Biotech. 2003;14:438-443.
15. Orgiazzi A, Lumini E, Nilsson R, Girlanda M, Vizzini A (2012). Unravelling Soil Fungal Communities from Different Mediterranean Land- Use Backgrounds. PLoS ONE, 2012;7(4):34 – 47.
16. Ahmed M, El-Zayat S, El-Sayed M.A. Cellulolytic activity of cellulose-decomposing fungi isolated from Aswan hot desert soil. Unit of Environmental Studies and Development, Aswan university. 2018;1(2):35-48.
17. Saranraj P, Stella D, Reetha D. Microbial Cellulases and Its Applications: A Review. International Journal of Biochemistry and Biotechnology. 2012;1:1-12.
18. Ajijolakewu K, Sani A, Oyeyiola G, Ahmed R, ArekemaseM .Odebisi-Omakanye M, Laba S, Cellulase Production Potentials of the Microbial Profile of Some Sugarcane Bagasse Dumping Sites in Ilorin, Nigeria. Notulae Scientia Biologicae. 2013;5(4):445 -449.
19. AgriInfo: Factors affecting distribution, activity and population of soil microorganisms. 2011; http://agriinfo.in/?page=to pic&superid=5&topicid=152. (Assessed on 30/05/2013).
20. Sukumaran R, Singhania R, Pandey A. Microbial Cellulases- Production, applications and challenges. Journal of Scientific and Industrial Research. 2005; 64:832-844.
21. Kim Y, Lee S, Cho Y, Oh H, Ko Y. Isolation of Cellulolytic *Bacillus subtilis* Strains from Agricultural Environments. International Scholarly Research Notices. (2011).
22. Datta A, Gupta B, and Gupta S (2013). Optimization of Cellulase Production from Bacteria Isolated from Soil. International Scholarly Research Notices.