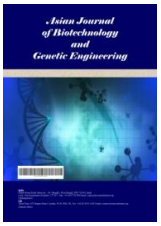


Asian Journal of Biotechnology and Genetic Engineering

4(3): 17-26, 2021; Article no.AJBGE.71663

Optimization Studies on Cellulase Production by *Aspergillus niger* and *Aspergillus fumigatus*

Ogbonna Abigail Ify[1*], Ogbonna Ugoy Sonia Amarachi[2], Onyimba Isaac Amechi[3], Ugwu Eugene Ifeanyi[1], Njoku Andrew Ikechukwu[3], M. Madu Josephine[4], Ogbonna Chike Innocent Chuks[1] and Otafu Esther Ehi[1]

[1] Department of Plant Science and Biotechnology, University of Jos, Nigeria.
[2]Department of Applied Microbiology & Brewing, Nnamdi Azikiwe University, Nigeria.
[3]Department of Science Laboratory Technology, University of Jos, Nigeria.
[4]Department of Environmental Biotechnology and Bio-conservation, National Biotechnology Development Agency (NABDA), Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OAI designed the study and wrote the protocol. Authors OUSA, UEI, NAI and MMJ managed the literature review. Authors UEI and OEE wrote the first draft of the manuscript. Authors OAI and OIA performed the statistical Analysis. Authors OCIC, MMJ and NAI managed the analysis and editing of the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. S. Prabhu, Sri Venkateswara College of Engineering, India.
Reviewers:
(1) A. S. William Arputha Sundar, The Dale View College of Pharmacy & Research Centre, India.
(2) Vinod Kumar Mishra, S. R. Institute of Management and Technology, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/71663

Original Research Article

Received 14 May 2021
Accepted 19 July 2021
Published 23 July 2021

ABSTRACT

Aim: In this study, two fungal species, *Aspergillus niger* and *Aspergillus fumigatus* were screened and optimized for their abilities to degrade cellulose using filter paper and Carboxymethylcellulose (CMC) as substrates.
Materials and Methods: *Aspergillus niger* and *A. fumigatus* procured from the Applied Microbiology Unit of Department of Plant Science and Biotechnology, University of Jos were screened using Whatman No. 1 filter paper and Carboxymethylcellulose as substrates in Petri plates. The fungal species abilities to produce cellulase at varying optimization parameters such as

**Corresponding author: E-mail: ogbonabi@yahoo.co.uk;*

incubation periods (5 days), different incubation temperatures (25-50^{0}C), different pH(3-9) and different substrate concentration (0.25-2%) using Submerged Fermentation (SmF) were also studied.

Results: The plate assay showed that the two species produced cellulases. The highest cellulolytic activity was shown by *A. niger* (23±3.22 mm) as it had larger zones of clearance compared to *A. fumigatus* (13±3.06 mm). However, the organisms grown on filter paper agar showed better hydrolysis compared to the growth on CMC agar. For the Submerged Fermentation (SmF), enzyme activity increased for the first 98 hours of incubation on filter paper recording 2.62 IU/ml for *A. niger* and 2.45 IU/ml for *A. fumigatus* after 48 h and then there was decrease in enzyme activity. For the CMC, the highest enzyme activity was observed at 48 h recording 1.76 U/ml and 1.37 IU/ml for *A. niger* and *A. fumigatus* respectively. Maximum enzyme production was observed at incubation temperature of 30 ^{0}C for *A. niger* and *A. fumigatus* recording 1.05 IU/ml and 1.10 IU/ml on filter paper. Enzyme activity was found to be highest at pH 6 with *A. niger* and *A. fumigatus* recording 2.27 IU/ml and 2.03 IU/ml respectively on CMC broth. The 2% substrate concentration gave the highest enzyme activity of 0.58IU/ml and 0.54IU/ml for *A. niger* and *A. fumigatus* respectively. The increase was linear, the higher the concentration of the substrate, the higher the enzyme activity.

Conclusion: *Aspergillus niger* and *A. fumigatus* have demonstrated potential of synthesizing hydrolytic cellulolytic enzymes and could be employed in the degradation of lignocellulosic wastes. These enzymes could find applications in different industries.

Keywords: Cellulolytic fungi; optimization; filter paper; Aspergillus; Carboxymethyl cellulose; Congo red.

1. INTRODUCTION

Cellulose, a major component of all vegetation is one of the world's most abundant resources [1]. It is constantly replenished by photosynthesis and accounts for nearly one-half of the 18 to 20 metric tons of organic carbon annually fixed by photosynthesis. Cellulose is also a major component of solid waste daily produced in large quantities from such activities as food processing, lumbering operations, paper making, cereal grain harvesting, sugarcane processing, domestic and office activities [2]. It is the main components in the cell walls of plants and is most widely distributed on earth. As the most abundant carbohydrate, cellulose is one of the most important carbon sources in the biosphere [3]. It is a polysaccharide with the formula $[C_6H_5O_{10}]n$, where n ranges from 500 to 5000, depending on the source of the polymer, consisting of a linear chain of several hundred to over ten thousand β-1,4 linked D-glucose units [4]. Due to the strong hydrogen bonds that are found between cellulose chains, through the association of hydrogen bonds, cellulose chains can form cellulose base fibre. Cellulose fibres are composed of long parallel chains of these molecules. The chains are interlinked to each other by hydrogen bonds between the hydroxyl groups of adjacent molecules, leading to the formation of crystalline regions of cellulose [5].

Cellulases are a group of hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer, cellulose to smaller sugar components. Cellulases have enormous potential in industries and are used in food, beverages, textile, laundry, paper and pulp industries [6,7,8,9]. Cellulolytic enzymes also play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa. However, fungi are well known agents of decomposition of organic matter in general and of cellulosic substrate in particular [1,10]. As lytic enzymes, they are also of major importance in the protoplast production for tissue culture and plant metabolites production [11,12,13]. Researches on cellulases have progressed very rapidly over the last five decades. There have been much researches aimed at obtaining novel microorganisms producing cellulases with higher specific activities and greater efficiency. Major impediments to exploit the commercial potential of cellulase are the yield, stability, and cost of cellulase production in the economics of the process, which hinder their application in industries. A number of approaches have been adopted, aiming towards reducing the cost of enzyme production which includes the use of different lignocellulosic wastes such as sawdust, corn cob, wheat straw, rice straw and wheat bran [14,15]. Since it is already a known fact that fungi are involved in the degradation of materials, also keeping in view the importance and application of the cellulases in broad areas, this study was therefore designed to screen two fungal strains

of *Aspergillus niger* and *Aspergillus fumigatus* as hyper-producers of cellulase using CaboxymethylCellulose (CMC) and filter paper as substrates.

2. MATERIALS AND METHODS

2.1 Isolation of Fungi

Preserved fungi samples (*Aspergillus niger and Aspergillus fumigatus)*were obtained from the Applied Microbiology Culture Room in Department of Plant Science and Biotechnology, University of Jos. The bottles containing the organisms were flooded with distilled water to harvest the spores. Using pour plate method, the fungi were inoculated and grown on Sabouraud Dextrose Agar (SDA) medium and kept in an incubator at 25±2^{o}C for 5days. Fungal colonies were observed after 5days of incubation. Macroscopic and microscopic examination were carried out for the authentication of the two fungal species, *Aspergillus niger and Aspergillus fumigatus.* Identification of each of the fungal isolates was done using identification scheme of [16,17].

2.2 Screening of the Test Fungi for Cellulolytic Activity

The fungal isolates were grown on basal salt agar medium as originally defined by Mandels *et al*. [12], (g/l KH_4PO_4, 1.4; NH_4NO_3, 1.0; KCl, 0.5; $MgSO_4.7H_20$, 0.1; $FeSO_4$. $7H_2O$, 0.01; H_2O, 1000L, agar powder, 10) supplemented with 1% Carboxymethylcellulose (CMC) powder. The Petri plates were incubated at 25^{o}C for 5 days. The plates were removed after 5days. At the end of the incubation, to visualize the zones of hydrolysis, the agar medium was flooded with aqueous solution of Congo red (1%) for 15minutes. The Congo red was then poured out and the plates were left to stand for 24hours at room temperature. Clear zones of hydrolysis were observed and the diameters of the zones were measured (mm) in triplicates and recorded. Similar experiment was carried out using 1% Whatman (No 1) filter paper powder as substrate.

2.3 Effects of Incubation Period of Five (5) Days on Cellulase Production

The fungal isolates were grown in separate conical flasks on basal salt solution using Submerged Fermentation (SmF) as defined by Mandels *et al*. [12], (g/l KH_4PO_4, 1.4; NH_4NO_3, 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.1; $FeSO_4$. $7H_2O$, 0.01; H_2O, 1000L) supplemented with 1% Carboxymethylcellulose (CMC) powder. The conical flasks were properly plugged, labeled and were incubated at 25^{o}C for 5days. Aliquots (2ml) of the culture filtrate from the incubated flasks were harvested at 24 hour interval and were centrifuged at 6000rpm for 15minutes. Cell-free supernatants were obtained and served as the enzymes source. A volume of 0.5ml of each of the enzymes source was measured and put into four separate test tubes. A volume of 0.5ml of 0.05ml citrate buffer of pH 4.8 was added into the four (4) test tubes containing 0.5ml of enzymes solutions each. The fifth test tube that contained only distilled water served as the blank. A volume of 0.5ml of 1% CMC was added into the four (4) test tubes and mixed properly. The 5 test tubes were then placed in water bath at 30^{o}C for 30minutes. The reaction was stopped by the addition of 1ml of 3, 5 -Dinitrosalicylic acid (DNSA) reagent into 5 test tubes and mixed properly. It was afterwards boiled for 10minutes and cooled in ice water for colour stabilization. Reading of absorbencies was done in triplicates using Jen way spectrophotometer at 540nm wavelength to ascertain the enzyme activity. The CMCase activity was measured using a calibration curve of glucose. One (1) unit of CMCase was defined by the amount of enzyme that released 1µmol of glucose per minute. Similar experiment was carried out using 1% Whatman (No 1) filter paper powder as substrate.

2.4 Optimization Studies On Cellulase Production

2.4.1 Effects of incubation temperature on cellulase production

To determine the optimum temperature for cellulase production, the reaction mixture was incubated at different temperatures of 25^{o}C, 30^{o}C, 40^{o}C and 50^{o}C for 30 minutes after mixing one (1) ml of crude enzyme, one (1) ml of 0.05 M sodium citrate buffer (pH 4.8) and one (1) ml of 1% CMC respectively. After incubation at the different temperatures, DNSA reagents were added to stop the reaction and the mixtures were boiled for 10minutes and cooled in ice water afterwards. Boiled enzyme extract from the control flask served as blank and D-glucose served as standard. The absorbencies of the mixtures were read in triplicates at 540nm wavelength using a spectrophotometer as

described by Jeffries [18]. Enzyme activity expressed in international units (IU) is defined as micromoles (µmol) of glucose released per min per ml of culture filtrate. Similar experiment was carried out using 1% Whatman (No 1) filter paper powder as substrate.

2.4.2 Effects of different pH on cellulase production

To determine the optimum pH for cellulase production, 1 ml 0.05 M sodium citrate buffer) was adjusted to pH 3, 4, 5, 6, 7, 8 and 9. One (1) ml of the crude enzyme was then mixed with one (1) ml of 1% CMC in test tubes and incubated at 30^{o}C for 30 minutes. DNSA reagent was then added to stop the reaction and the resultant mixture was boiled for 10 minutes and then cooled in ice water. Boiled enzyme extract from the control flask served as blank and D-glucose served as standard. After cooling, the absorbencies of the mixtures were obtained in triplicates at 540nm wavelength using a spectrophotometer [18]. Enzyme activity expressed in international units (IU) is defined as micromoles (µmol) of glucose released per min per ml of culture filtrate. Similar experiment was carried out using 1% Whatman (No 1) filter paper powder as substrate.

2.4.3 Effects of substrate concentration on cellulase production

Modified method of Jaafaru and Fagade [19] was used for the effects of Substrate concentration on cellulase production. This was done to ascertain which substrate concentration was the optimum for the production of cellulase. This was done in 16 test tubes that were properly labeled. Different concentrations of the substrate (CMC), 0.25%, 0.5%, 1%, 1.5% and 2% were put into appropriate labeled tubes. One ml of the crude enzyme was mixed with 1ml of 0.05 M citrate buffer (pH 4.8) which were then added to the tubes and mixed properly to obtain a homogenous mixture. The test tubes were then incubated in a water bath at 30^{o}C for 30mins after which 1ml of DNSA reagent was dropped into each of the test tubes and mixed properly to stop the reaction. Boiled enzyme extract from the control flask served as blank and D-glucose served as standard. The mixtures were then boiled for 10minutes to ensure colour stabilization and then cooled immediately in the ice water and their absorbances were taken in triplicates [18]. Enzyme activity expressed in international units (IU) is defined as micromoles (µmol) of glucose released per min per ml of culture filtrate. Similar experiment was carried out using 1% Whatman (No 1) filter paper powder as substrate.

2.5 Statistical Analysis

Statistical analysis was carried out by subjecting data to Analysis of Variance (ANOVA) followed by a Tukey-Kramer comparison test. Results were represented as mean ± SEM and at $P < 0.05$, the results were considered statistically significant.

3. RESULTS

The fungi identification showed the test organisms as Aspergillus *niger* and *Aspergillus fumigatus* respectively with their distinct features as observed under the microscope. The cultural characteristics and the structure of the test organisms are shown in Plates 1 and 2.

The results of the cellulolytic activity of the fungal strains and their growth in broth media is presented in Table 1. The diameters of the clearance zones indicated the cellulose degrading ability of the fungi and thus, their cellulose production capability. The highest cellulolytic activity was shown by *A. niger* (23±3.22 and 13±3.06 mm) for filter paper and CMC respectively as it had larger zones of clearance compared to *A. fumigatus* (14±1.53 and 8±1.00 mm) for filter paper and CMC respectively. The growth of the organisms was evident after 48 hours of inoculation as the broth became cloudy with time. Also, the pH of the organisms in the different broths containing different substrates increased from its acidic pH towards neutral and gradually towards basic.

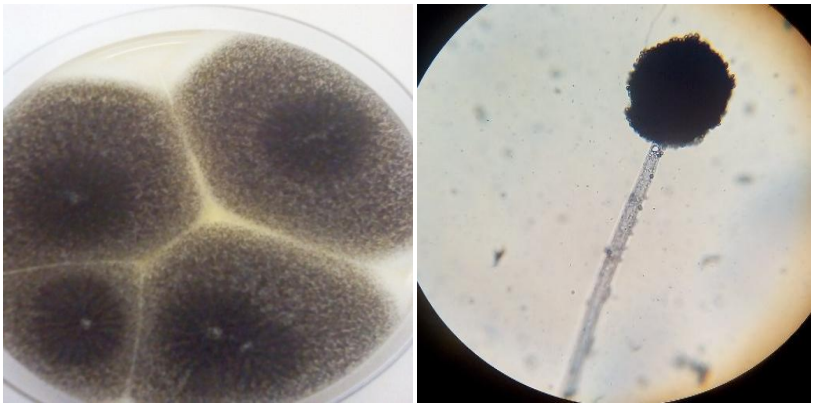
The effects of the incubation period of 5 days on the cellulase production are shown in Fig. 1.The results revealed that enzyme activity was at its maximum at 72 hours (3 days) after inoculation. *Aspergillus niger* and *A. fumigatus* had the highest peak of activity of cellulase production of 2.63 and 2.45 IU/ml respectively for filter paper. For the CMC, *A. niger* with had the highest enzyme activity of 1.76 IU/ml, followed by *A. fumigatus* that produced 1.37 IU/ml.

The effects of varying temperatures showed that *Aspergillus fumigatus* and *A. niger* recorded the highest enzyme activity of 1.10 IU/ml and 1.06 IU/ml respectively at incubation temperature of 25^{0}C for filter paper. For the CMC, also

A.fumigatus also had the highest enzyme production of 0.54 IU/ml at temperature of 40^0C. *Aspergillus niger* recorded enzyme production of 0.29 IU/ml at incubation temperature of 30^0C. The details of the effects of varying temperatures on enzyme production are presented in Fig. 2.

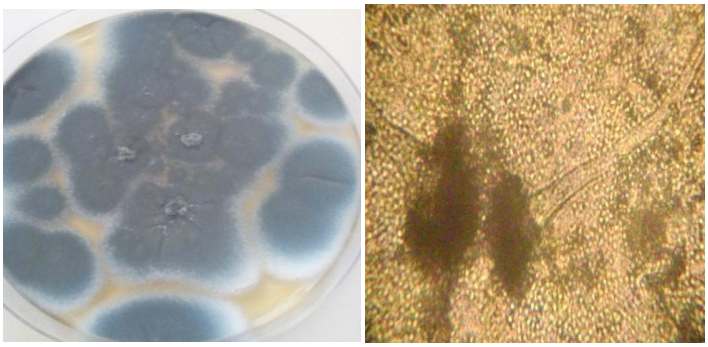
The results of the effects of varying pH on cellulase production are presented in Fig. 3. Different pH values also had effects on the enzyme production among the two fungal species. Optimum pH recorded for the *Aspergillus fumigatus* and *A. niger* was pH 3 with peak production of 2.92 IU/ml and 2.67 IU/ml for filter paper activity. However, pH 6 was the optimum for CMC activity with highest production of 2.22 IU/ml and 2.02 IU/ml respectively for *Aspergillus niger* and *A. fumigatus* respectively.

The different concentrations of the substrate used in the study also had effects on enzyme production and activity. It was observed that For CMC, *Aspergillus niger* and *A. fumigatus* recorded the highest enzyme production peak of 0.58 IU/ml and 0.54 IU/ml respectively at substrate concentration of 2%. *Aspergillus fumigatus* and *A. niger* recorded the highest enzyme production peak of 0.027 IU/ml and 0.021 IU/ml respectively at the highest concentration of 2% of filter paper during hydrolysis. The lowest concentration of 0.001 IU/ml and 0.002 IU/ml was recorded for the two fungi. The details of the results of the effects of substrate concentrations on enzyme production are presented in Fig. 4.



(a) (b)

Plate 1. (a) Culture of *Aspergillus niger* (b) Photomicrograph of *Aspergillus niger*



(a) (b)

Plate 2. (a) Culture of *Aspergillus fumigatus* (b) Photomicrograph of *Aspergillus fumigatus*

Table 1. Cellulolytic activity of the fungal isolates grown on different substrates

Organism	Substrate (mm)	
	Filter paper	**CMC**
A. niger	23±3.22	13±3.06
A. fumigatus	14±1.53	8±1.00
LSD	1.96	

Data are means ±standard deviation

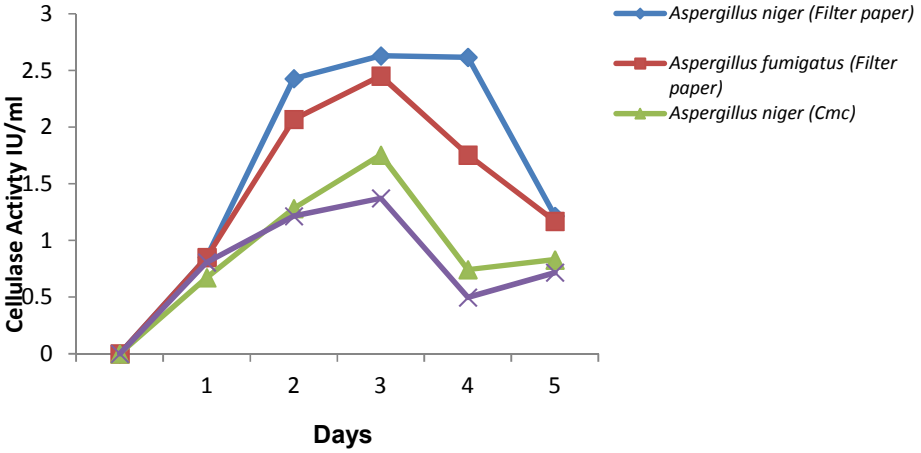


Fig. 1. Effects of incubation period on cellulase production

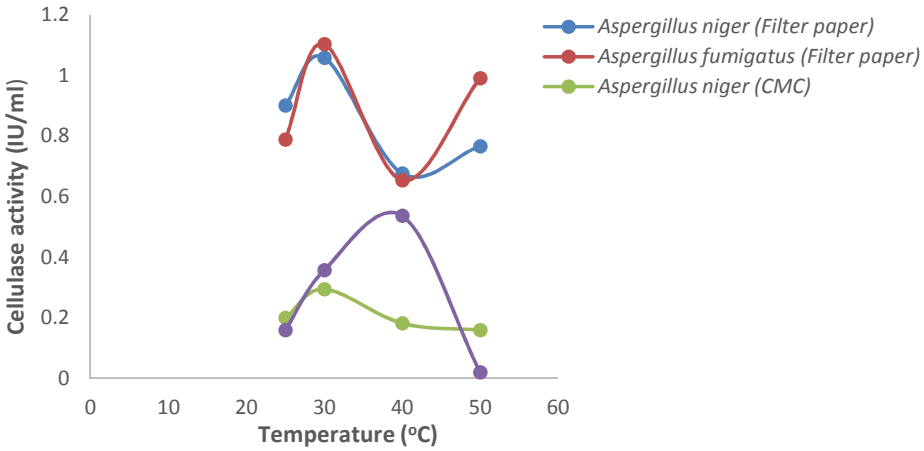


Fig. 2. Effect of temperature on cellulase production

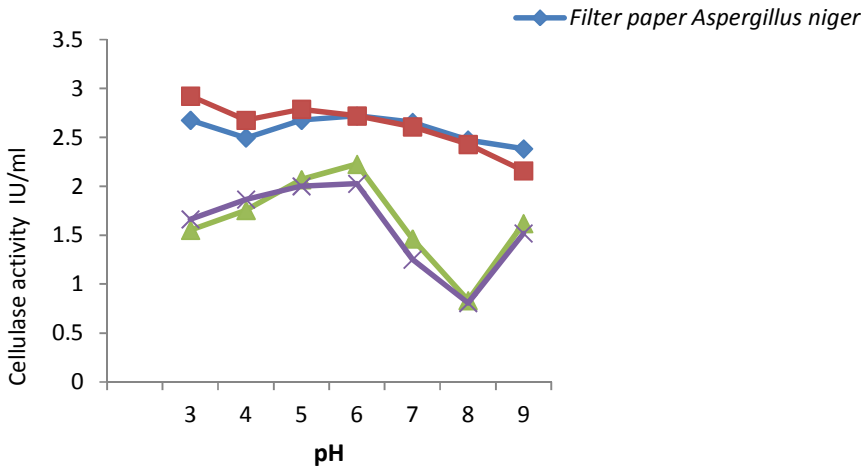


Fig. 3. Effects of varying pH on enzyme activity

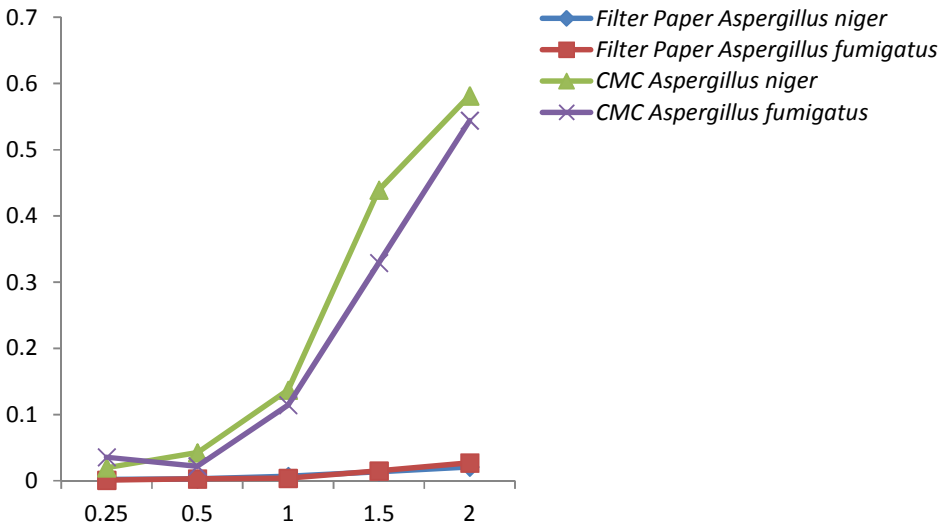


Fig. 4. Effects of substrate concentration on enzyme production

4. DISCUSSION

The cellulolytic activity of two fungal strains, *Aspergillus niger* and *A. fumigatus* were studied using two cellulose substrates including Carboxymethylcellulose and Whatman No.1 filter paper as sole carbon sources. Generally, production of fungal cellulase and hemicellulases has been shown to be inducible and was affected by the nature of the substrates used [20,21]. Therefore, the choice of appropriate inducing substrate for maximum enzyme production was put into consideration. Among the two substrate used, filter paper gave the highest cellulase production. This explains that filter paper showed better cellulase activity than CMC when fermented with both *A. niger* and *A. fumigatus.* This could be as a result of the fact that filter paper is 100% cellulose more than Carboxymethycellulose. However, considerable amount of enzyme production was observed on Carboxymethycellulose (CMC). Mandal and Ghosh [22] reported CMCase production on banana peels waste through conventional method and discovered CMCase activity 54 U/mg in Solid State Fermentation. It has been reported in researches that inexpensive substrates such as wheat bran, wheat fiber, rice straw, and molasses are agro wastes that are successful for an adequate amount of construction and development of different enzyme [23]. In another study, the CMCase was produced maximally through *Trichoderma* sp. on apple pomace which was recorded at 32 ºC [24].

The incubation period showed that there was an exponential growth which showed increase in enzyme production linearly. The maximum yield of CMCase activity was detected at 72 hours (3 days) after inoculation. *Aspergillus niger* and *A. fumigatus* had the highest peak of activity of cellulase production of 2.63 and 2.45 IU/ml respectively for filter paper. For the CMC, *A. niger* with had the highest enzyme activity of 1.76 IU/ml, followed by *A. fumigatus* that produced 1.37 IU/ml. However, further increase in the incubation time reduced enzyme production (Fig. 1). This could be due to depletion of micro and macro nutrients in the fermentation medium with the lapse in time, which stressed the fungal physiology resulting in the inactivation of secreting machinery of the enzymes [25]. Short incubation periods for enzyme production offer the potential for inexpensive production of enzymes [26]. This was in line with the studies done by Devanathan *et al.* [27,28,29].

The optimization studies showed that environmental factors such as pH, temperatures and substrate concentrations had effects on enzyme production for *A. niger* and *A. fumigatus* during the degradation of filter paper and CMC which were assessed using Liquid State Fermentation. On the effects of incubation temperature on enzyme production, it was observed that cellulase production gradually increased from 25^{o}C for both organisms on filter paper and CMC substrates and reached their

maximum at 30°C except for *A. fumigatus* that had its maximum production at 40°C on CMC medium (Fig. 2). This result is in line with the work done by Hanif *et al*. [30], who also reported an increase in cellulase production by *A. niger* up to 30°C and thereafter, the production declined. Deswal *et al*. [31] optimized physiological parameters for cellulase production by *Fomitopsis* sp under Solid State Fermentation and show that cellulase production was in its maximum at 30°C. Irshad et al. [32] also studied that if the temperature of incubation is changed, the activity of enzyme also changes in a valuable amount.

Optimum pH for maximum cellulase production for *A. fumigatus* and *A*. *niger* grown on filter paper was pH 3 while the optimum pH for maximum cellulase production for *A. fumigatus* and *A. niger* grown on CMC was pH 6 (Fig. 3). The pH values were within the ranges that support fungal growth in culture. Dutta et al. [33] carried out a research on the optimization of pH along with other parameters and found out that the optimal pH for the production of cellulases among different species of fungi varies but lies mostly in the optimum range values between pH 3.0 to 9.0. Among physiological parameters, pH of the growth media plays important roles by inducing morphological changes in the microorganisms and enzyme secretion. The pH change observed during the growth of microorganisms also affects production stability in the medium [34]. Similar observation was made for cellulase production by *Aspergillus terreus* QTC in Submerged Fermentation (SMF) by Ali *et al.* [35] and *Trichoderma reesei* in Solid State Fermentation (SSF) by Doppelbauer *et al.* [36]. The pH 7 value was reported by Kirshna [37] for the production of bacterial cellulases by using banana waste in Solid State Fermentation. The results obtained in this research were in line with those reported for other fungal cellulases produced by *Aspergillus aculeatus* [38] and *A. niger* [39].

From the results on the effects of substrate concentration on enzyme production, the results obtained for the two fungal species for both filter paper and CarboxymethylCellulose revealed optimum level of substrate concentration at 2% (Fig. 4). These results were in slightly different from other works like those of Sarkar and Aikat [40] that had theirs at 1% and Amaeze et al. [41] who had theirs at 3%. This could be attributed to other factors such as the type of substrate used in the study and incubation temperature.

5. CONCLUSION

In conclusion, the research mainly compared how *A. niger and A. fumigatus* hydrolyzed cellulose when grown on filter paper and CarboxymethylCellulose and the effects of some optimization parameters like incubation period, temperature, pH and substrate concentration on enzyme activity and production. At the end of the study, both test fungi showed abilities to degrade filter paper and CarboxymethylCellulose. Filter paper showed better production of cellulases than CarboxymethylCellulose. However, *Aspergillus niger* showed higher production of cellulase on both of the substrate used than *A. fumigatus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lynd LR, Wiener PJ, Van-Zyl WH, Pretorious IS. Microbial Cellulose utilization: Fundamentals and biotechnology. Microbial Molecular Biology Reviews. 2002; 66: 506-522.
2. Abu EA, Onyenekwe PC, Ameh DA, Agbaji AS, Ado SA. Cellulase (EC 3.2.1.3) production from sorghum bran by *Aspergillus niger* SL1: An assessment of pre-treatment methods. Proceedings of the International Conference on Biotechnology: Commercialization and Food Security, Abuja, Nigeria, 2000;153-157.
3. Zhang YH, Himmel ME, Mielenz JR. Outlook for cellulase improvement: Screening and Selection Strategies. Biotechnol. Adv. 2006; 24: 452-481.
4. Spence KL, Venditti RA, Habibi Y, Rojas OJ, Pawlak JJ. The effect of chemical composition on microfibrillar cellulose films from wood pulps: mechanical processing and physical properties. Bioresour. Technol. 2010;101:5961-5968.
5. Filson PB, Dawson-Andoh BE Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials. Bioresour. Technol. 2009;100: 2259-2264.
6. Jahangeer Sadaf, Khan Nazia, Jahangeer Saman, Sohail Muhammad, Shahzad Saleem, Ahmad Aqeel, Khan Ahmed Shakeel. Screening and Characterization

of Fungal Cellulases Isolated from the Native Environmental Source. Pak. J. Bot. 2005; 37(3): 739-748.

7. Miettinen-Oinonen A, Londesborough J, Joutsjoki V, Lantto R, Vehmaanperä J. Three cellulases from Melanocarpus albomyces with applications in the textile industry. Enz Microbial Technol. 2004; 34: 332 341.
8. Cavaco-Paulo A, Gübitz G. Catalysis and processing. In: Cavaco-Paulo A, Gübitz G. (ed.). Textile Processing with Enzymes. England, Woodhead Publishing Ltd: 2003; 86-119.
9. Walsh G. Industrial enzymes: proteases and carbohydrases. In: Proteins; Biochemistry and Biotechnology. John Wiley and Sons. Ltd.; 2002.
10. Peciulyte D. Isolation of Cellulolytic fungi from waste paper gradual recycling materials. Ekologija 2007; 53: 11-18.
11. Davis B. Factors influencing protoplast isolation. In: Peberdy J. F., Ferenizy L. (eds.). Fungal Protoplasts: Applications in Biochemistry and Genetics. Marcel Dekker, New York: 1985; p356.
12. Mandels ML, Hontz L, Nistrom J. Enzymatic hydrolysis of waste cellulose. Biotechnol Bioengineering. 1974; 16: 471–493.
13. Bhat MK. Cellulases and related enzymes in biotechnology. Biotechnol Adv. 2000; 18(5):355-83.
DOI: 10.1016/s0734-9750(00)00041-0.
PMID: 14538100.
14. Lo C, Zhang Q, Lee P, Ju L. Cellulase production by *Trichoderma reesei* using sawdust hydrolysate. Applied Biochem. Biotechnol. 2005; 121-124: 561-573.
15. Betini JHA, Michelin M, Peixoto-Noggueira SC, Jorge JA, Terenzi HF, Polizeli MLTM.. Xylanases from *Aspergillus nige*r, *Aspergilus niveus* and *Aspergillus ochraceus* Produced under Solid State Fermentation and their Application in Cellulose pulp bleaching. Bio proc. Biosyst. Eng, 2009; 32: 819-824.
16. Nagamani A, Kunwar IK, Manoharachary C. *Handbook of soil fungi*. IK International Pvt. Ltd., New Delhi. 2006;475.
17. Domsch KH, Gams W, Anderson TH. Compendium of Soil Fungi. London, New York, Torroute, Sydney, San Francisco: Academic Press. 1980; 869pp.
18. Jeffries TW. Manual on production and applications of cellulose laboratory Procedures . 1996;1-10. Available:http://www2.biotech.wisc.edu/jeffries/cellulases/mandels.html
19. Ja'afaru MI, Fagade OE. Optimization studies on cellulase enzyme production by an isolated strain of *Aspergillus niger* YL128. African Journal of Microbiology Research2010; (24): 2635-2639.
20. Corel G, Anikan B, Unaldi MN, Guvenmez H. Some properties of crude Carboxymethylcellulase of *Aspergillus niger* Z10 wild type strain TUBITAK. 2002; 26: 206-213.
21. Ojumu TV, Solomon OB Betiku E, Layokun SK Amigun B. Cellulase production by *Aspergillus flavus* Linn. isolate NSPR 101 fermented in sawdust, bagasse and corncob. Afr. J. Biotechnol. 2003; 2: 150-152.
22. Mandal M, Ghosh U. Value Addition to Horticultural Solid Waste by Applying It in Biosynthesis of Industrially Important Enzyme: Cellulase. In Utilization and Management of Bioresources 2018; pp. 279-289.
23. Iqbal HMN, Ahmed I, Zia MA, Irfan M. Purification and characterization of the kinetic parameters of cellulase produced from wheat straw by *Trichoderma viride* under SSF and its detergent compatibility. Adv. Biosci. Biotechnol. 2011; 2: 149-56.
24. Sun H, Ge X, Hao Z, Peng M. Cellulase production by *Trichoderma* sp. on apple pomace under solid state fermentation. Afr. J. Biotechnol 2010; 9(2): 163-166.
25. Nochure SV, Roberts MF Demain AL. True cellulase production by *Clostridium thermocellum* grown on different carbon sources. Biotech. Lett. 1993;15(6):641-646.
26. Sonjoy SB, Bex K, Honston H. Cellulose Activity of *Trichoderma reesei* (rut 30) on Municipal Solid Waste. Applied Biochemistry and Biotechnology. 1995; 51-52(1): 145-153.
27. Devanathan G, Shanmugan A, Balsibramanian T, Manivannan, S. (2007). Cellulase Production by *Aspergillus niger* Isolated from coastal Mangrove Debris. Trends in Applied Science Resources, 2: 23-27.
28. Acharya PB, Acharya DK, Modi HA. Optimization for cellulose production by *Apsergillus niger* using sawdust as substrate. African Journal of Biotechnology, 2008; 7(22): 4146-4152.
29. Sherif AA, El-Tanash AB, Atia, N. Cellulase production by *Aspergillus*

fumigatus grown on mixed substrate of rice straw and wheat Bran. Research Journal of Microbiology 2010; 5(3): 199-211.
30. Hanif A, Tasmeen A, Rajoka MI. Induction, Production, Repression and Expression of exoglucanase synthesis in *Aspergillus niger.* Bioresource Technology. 2004; 94 (3): 311-319.
31. Deswal D, Khasa TP, Kuhad RC. Optimization of Cellulase Production by a brown not fungus *fomitopsis* sp. RCK 2010 under solid state fermentation. Bioresoruce Technology. 2011; 102: 6065-6072.
32. Irshad M, Asgher M, Sheikh MA, Nawaz H. Purification and characterization of lactase produced by *Schyzophylum commune* IBL-06 in solid state culture of banana stalks. Bio Res 2011; 6: 2861-2873.
33. Dutta T, Sahoo R, Sengupta R, Ray SS, Bhattacharjee A, Ghosh S. Novel cellulases from an extremophilic filamentous fungi *Penicillium citrinum*: production and characterization. J. ind. Microbial. Biotechnol. 2008; 35:275-282.
34. Gupta R, Gigras P, Mohepata H, Goswani VK, Chuahan B. Microbial Amylases: A biotechnological perspective. Process Biochemistry 2003; 38 (11): 1599-1616.
35. Ali S, Sayed A, Sarker RI, Alam R. Factors Affecting Cellulose Production by *Aspergillus terreus* using water hyacinth. World Journal of Microbiology and Biotechnology. 1991; 7 (1): 62-66.
36. Doppelbauer R, Esterbauer H, Steiner W, Lafferty RM, Steinmuller H. The use of lignocellulosic wastes for production of cellulase by *Trichoderma reesei.* Appl. Environ. Microbiol. 1987; 26(5): 485-494.
37. Krishna C. Production of bacterial cellulases by solid state bioprocessing of banana wastes. Bioresour. Technol 1999; 69(3), 231-239.
38. Murao S, Sakamoto R, Arai M. Cellulase of *Aspergillus aculeatus*. In: Methods in Enzymology, Wood, W.A. and S.T. Kellog (Eds.). Vol. 160, Academic Press Inc., London. 1988;275-284.
39. Jecu L. Solid State Fermentation of agricultural wastes for endoglucanase production. 1999;11:1-5
40. Sarkar N, Aikat K. *Aspergillus fumigatus* NITDGPKA3 Provides for Increased Cellulase Production. International Journal of Chemical Engineering Volume 2014, Article ID 959845, 9 pages http://dx.doi.org/10.1155/2014/959845
41. Amaeze NJ, Okoliegbe IN, Francis ME. Cellulase production by *Aspergillus niger* and *Saccharomyces cerevisiae* using fruit wastes as substrates.International Journal of Applied Microbiology and Biotechnology Research. 2015;3: 36-44.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/71663