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Comparative Production of Cellulase from Chemical and Biological Pretreated Groundnut Husk

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Authors' contributions

This work was carried out in collaboration between all authors. Author NA designed the study and wrote the protocol. Author HK wrote the first draft of the manuscript and managed the literature searches. Authors ZA, HA and RJA performed and managed the experimental process and result analyses. All authors read and approved the final manuscript.

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ABSTRACT

Lignocellulose biomass was bioconverted with *Aspergillus niger* and *Trichoderma viride* to produce cellulase. Groundnut husk is a cheap and readily available substrate with potential for bioconversion. Cellulase production from alkali and biological pretreated groundnut husk was compared in this study. Rapid production of cellulase following FPase & CMCase derived from both organisms were observed. Enzymatic activities in all the pretreated GH increased steadily from day one and peaked at day seven. Biological pretreated GH showed significant ($p<0.05$) increase in total cellulase and endoglucanase yield (activity) than alkali pretreated GH.

Keywords: Cellulase; groundnut husk; endoglucanase; Aspergillus niger; Trichoderma viride.

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1. INTRODUCTION

Lignocellulosic materials such as crop residues, grasses, wood chips, sawdust and animal waste (solid) can be considered as a potential source for large amount of low-cost utilizable products. Many physicochemical, structural and compositional factors hinder the hydrolysis of cellulose present in biomass to sugars and other organic compounds that can later be converted to feeds. Enzymatic hydrolysis is hindered by the following substrate-related factors: cellulose contains highly resistant crystalline structure, lignin and hemicellulose surrounding cellulose form a physical barrier and sites available for enzymatic attacks are limited [1]. Pretreatment, an important tool for practical lignocellulose conversion processes is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzymes [2]. The goal of pretreatment is to make the cellulose accessible to hydrolysis for conversion to simple sugars and can be achieved by physical, chemical, physicochemical and biological processes.

In nature, degradation of cellulosic biomass is performed by mixtures of hydrolytic enzymes collectively known as cellulases. Enzymes are among the most important bio-products and are being utilized in a large number of processes in the areas of industrial, environmental and food biotechnology. Filamentous fungi are preferred for commercially important enzymes production, because the level of the enzymes produced by these cultures is higher than those obtained from yeast and bacteria [3]. *Aspergillus* and *Trichoderma* are the most important and safe microorganisms for industrial use and are more potent producers of cellulose [4]. The enzymatic degradation of waste cellulose by fungal cellulases has been suggested as a feasible alternate for the conversion of lignocellulosics into fermentable sugars and fuel ethanol [5]. The cellulase system in fungi is considered to comprise of three hydrolytic enzymes: endo-1, 4- β -D- glucanase [carboxymethyl cellulase (EC.3.2.1.4)], which cleaves β-linkage randomly in the amorphous parts of cellulose; exo-1, 4- β -D-glucanase [cellobiohydrolase (EC.3.2.1.91)], which hydrolyzes cellobiose from either the nonreducing or the reducing end, generally from the crystalline parts of cellulose and β –glucosidase [cellobiase (EC.3.2.1.21)], which releases glucose from cellobiose and short chain cellooligosaccharides (cellodextrin) [6]. Although β-glucosidase has no direct action on cellulose but it is regarded as a component of cellulose system because it completes cellulose hydrolysis [7] as shown in Fig. 1. *Trichoderma* produces an extracellular, stable, and efficient cellulase enzyme system. *Trichoderma* is a partially catabolite repressed, hypercellulolytic mutant strain, widely studied, with improved enzyme production capabilities, when compared to the wild-type and some other strains [8]. Microbial consortium consisting of two or more different microorganisms is known to be largely responsible for many biotransformations in natural environment. Mixed culture fermentations are widely used in biotechnology for many processes including the production of antibiotics, enzymes, fermented food, composting, dairy fermentation, bioconversion of apple distillery and domestic wastewater sludge [9].

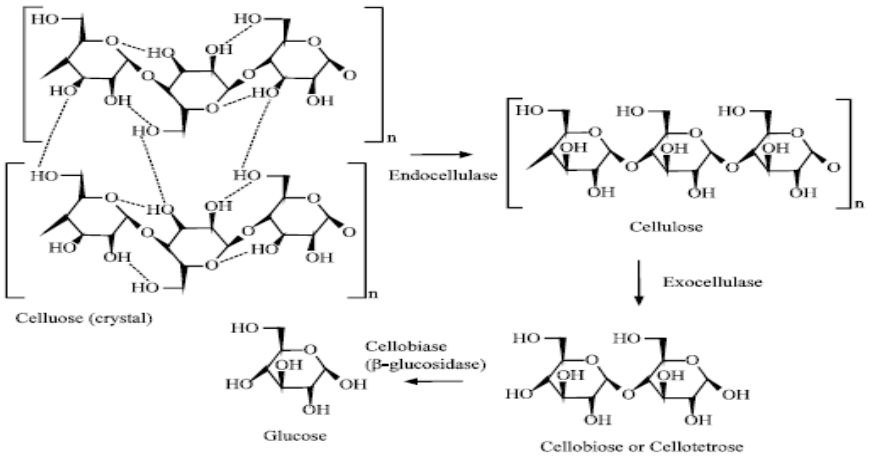

Fig. 1. Mode of action of various components of cellulase [10]

2. MATERIALS AND METHODS

2.1 Reagents

Hydrochloric acid, nitric acid, sodium hydroxide, sodium citrate, citric acid, magnesium sulphate, zinc chloride, potassium nitrate, iron chloride, calcium chloride. All chemicals used were of analytical grade.

2.2 Microorganisms

Aspergillus niger was isolated from groundnut seed and *T. viride* was isolated from cultivated soil. The sources were suspended in 10 mL distilled water after which it was serially diluted in 9 mL distilled water. 0.5 mL of the solution was pour plated on PDA plates, incubated at 28°C for 72hr. Both organism morphology and structure as described in Fungal Atlas [11] were used to identify the organisms, these were sub-cultured on other plates and slants to obtain pure strain. They were maintained on PDA medium and stored at 4°C.

2.3 Preparation and Solid State Fermentation of GH

Groundnut shell was collected from field, milled and sieved to 0.2 mm particle size. This was pretreated with 500 mL 2M sodium hydroxide for 1 hour, washed until pH is neutral, then dried in oven at 60°C for 7 hours. 10 g was weighed into 250 mL Erlenmeyer flask, moistened with 40 mL Basal medium, autoclaved at 121°C for 15 min and ready for use [12]. Actively growing fungi (in form of flocs), 10 ml each were inoculated directly on moistened GH in a sterile environment by opening a small part of the flask (mixed fermentation). Twenty milliliter (20 mL) of each fungus was inoculated in different flasks (monoculture fermentation). Each treatment was carried out in duplicate and flasks were incubated at 28°C for 14 days [13].

2.4 Bioassay of Cellulase

Samples from all three sets were incubated at 28°C in an incubator for 14 days. After 24 hours of inoculation, an aliquot of fermenting GH was taken from each of the flasks for enzyme extraction. Citrate buffer 0.05M (3 mL) was added and vigorously shaken on the rotary shaker at 200 rev/min for 30 minutes. The solid biomass was separated from the suspension by centrifuging at 3000 rpm for 5minutes. The supernatant was used as the source of enzyme. The procedure was repeated with each batch of organism after every 24 hours to assay for the presence of cellulase enzymes for the period of 14 days [14]. Cellulase assays were carried out as described by Ghose [15].

2.4.1 Filter paper assay (FPase)

Total cellulase activity was carried out based on the method of filter paper assay for saccharifying cellulase. 1 mL of 0.05M citrate buffer was added to the test tube containing one Whatman No.1 filter paper strip (1 cm x 6 cm). After that, 0.5 mL of sample solution (supernatant) also prewarmed to 40°C was added to the citrate buffer solution. The resulting solution was mixed thoroughly and then transferred to a water-bath maintained at 50°C. After 60 min (reaction step) the test tubes were removed from the water bath, and 3 mL of 3, 5-dinitrosalicylic acid (DNS) solution was added and mixed thoroughly to stop the enzymatic reaction. Tubes were covered and placed in a boiling water bath for 5 minutes. All the tubes were cooled to room temperature in a cooling water bath. The absorbance was taken at 540 nm against water blank. Enzyme activity was expressed as FPU/ml (Amount of reducing sugar released per ml of filtrate per minute).

2.4.2 Carboxymethyl cellulase (CMC-ase)

Carboxymethyl cellulose, 2%, in 0.05M Sodium citrate buffer, pH 4.8 was used as the substrate for endoglucanase activity assay. Enzyme extract (supernatant from centrifugation of aliquot from each set of fermentation) (0.5 ml) was diluted in 0.5 ml 0.05M citrate buffer in a 25 mL capacity test tube and temperate to 50°C. Carboxymethyl cellulose, 0.5 mL, was added, mixed well and incubated at 50°C for 30 minutes. Dinitrosalicylic acid (DNS), 3 mL, was added and mixed. These were transferred to a rack on the table, after which the test tubes (24) were boiled in vigorously boiling water for exactly 5 minutes. All samples, enzyme blanks, glucose standards and spectro zero were boiled together. After boiling, they were immediately transferred to a cold water bath and 20 mL distilled water was added, mixed by completely inverting the tube so that the solution leaves the bottom of the tube. The colour formed was measured against the spectro zero at 540 nm. Enzyme activity was expressed as IU/ml that is one unit of endoglucanase is the amount of enzyme that released one micromole of reducing sugars per minute under the assay conditions (pH 4.8, 50°C).

2.5 Statistical Analysis

SPSS statistical package version 20 was used to analyse data. Results were expressed as mean ± standard deviation (SD) and data was analysed by one-way analysis of variance (ANOVA). The difference between fermentations was computed using DUNCAN range multiple test, p value less than 0.05 was considered significant ($p<0.05$).

3. RESULTS

Total cellulase and endoglucanase activities at different incubation time (day: 1, 7 and 13) during fermentation of pretreated GH are shown in the tables below. Filter Paper Unit (FPU) (FP-ase) and Carboxymethyl cellulase (CMC) (CMC-ase) assays were respectively used for the assays above. For biological pretreated GH ($_{B}$GH), total cellulase activity when *A. niger* was inoculated ($_{B}$GH *A. niger*) was observed to increase significantly ($p<0.05$). *T. viride* activity was observed to fluctuate however the activity increased significantly for co-culture as shown in Table 1. For chemical pretreatment, total cellulase activity of flask inoculated with *A. niger* (cGH *A. niger*) was observed to decrease significantly ($p<0.05$), however the activity in co-culture fermentation fluctuate with significant decrease at the end of the fermentation period.

A. niger fermenting biologically pretreated GH was observe to have the best activity trend for total cellulase activity.

The Endoglucanase activity of biological pretreated GH on which *T. viride* ($_{B}$GH *T. viride*) and co-culture spores ($_{B}$GH cc) were inoculated was observed to significantly ($p<0.05$) decrease (0.19 to 0.03 IU/ml/min) and increase (0.02 to 0.06 IU/ml/min) respectively, however the activity of *A. niger* increase rapidly within 6 days (0.04 to 0.17 IU/ml/min) after which it remained relatively constant (Table 2).

In comparison with the latter assay, activity of *A. niger* in chemical pretreated GH ($_{C}$GH) decrease significantly ($p<0.05$) with time, activities in *T. viride* and co-culture treatments increased with *T. viride* activity increasing drastically.

4. DISCUSSION

It was observed that activity profile for all fermentation of both pre-treatments increased within the first day, then gradually decreased for total cellulase activity. Both pretreatments had highest activity after day seven, 0.111IU for biological pretreatment and 0.084IU for chemical pretreated GH. This might be due to structural changes in cellulose as reported by Subramaniam [16], suggesting that these changes will increase the substrate in susceptibility to microbe and enzyme attack. Changes in cellulose structure involve pore structure, particle size, lignin and hemicellulose association, crystallinity and degree of polymerization. Similar report was made by Damisa [17] using *A. niger* on 2M NaOH pretreated bagasse, corn cob and corn straw which had 0.067, 0.049 and 0.504 IU enzyme activities respectively. Another study by Reddi and Narasimha [18], reported the total cellulolytic activity of *A. niger* GNEM7 on 1N HCl pretreated pea seed husk to be 9.13IU/ml.

Endoglucanase activity was observed to be highest (0.191 IU/ml/min) in the biological pretreated sample when compared to the chemical pretreated sample (0.026 IU/ml/min). These occurred at day one (1) and seven (7) respectively after inoculation of *T. viride* indicating that cellulose content of biological pretreated GH is readily available for assay than that of chemical pretreated GH hence the high activity. This may also be attributed to increase in surface area and less crystalline structure of biological pretreated GH. Endoglucanase activity of $_{C}$GH *T. viride* was not significantly different for days 7 and 13. This might be due to the absence of β–glucosidase repression mechanism present in *A. niger* that stops further breakdown of cellobiose to simple sugars as a result of excess glucose. This study is in accordance with reports by Kocher [19], who had maximum cellulase production (FPase 0.09 and CMCase 0.12 IU/ml/min) after 8 days by *T. harzianum*8230 using rice straw as carbon source. Abd El-Zahel and Fadel [20] reported maximum activity (1.17 IU/g) on rice straw pretreated with 1% NaOH using *T. reesei* F418.

It was also observed that the total cellulase and endoglucanase activities produced by simultaneous co-culture fermentation were slightly low when compared with the values of monoculture fermentations. This may be attributed to lack of synergy between both organisms due to fast growth of *T. viride* over *A. niger* as suggested by Ikram-ul-Haq [3]. Moses [21] reported that a high level of β-glucosidase is necessary to avoid the accumulation of cellobiose, which is a strong inhibitor of endoglucanase and exoglucanase.

Table 1. Total cellulase (FPU) activities on pretreated GH

Treatment	Assay	Biological pretreated GH			Chemical pretreated GH		
		Day 1	Day 7	Day 13	Day 1	Day 7	Day 13
A. niger	FPU	0.09±0.001^{b}	0.11±0.001^{b}	0.10±0.001^{c}	0.07±0.001^{b}	0.08±0.001^{b}	0.03±0.00^{a}
T. viride	(IU/ml/min)	0.19±0.003^{d}	0.09±0.001^{a}	0.08±0.002^{b}	0.14±0.001^{c}	0.07±0.001^{a}	0.06±0.00^{c}
Co-culture		0.16±0.002^{c}	0.09±0.001^{a}	0.12±0.001^{d}	0.05±0.001^{a}	0.08±0.001^{b}	0.04±0.00^{b}
Control		0.08±0.002^{a}	0.09±0.003^{a}	0.04±0.002^{a}	0.07±0.001^{b}	0.08±0.001^{b}	0.04±0.00^{b}

Mean ± SD. Values in the same column in different treatment with different superscript are significantly different (p<0.05). A. niger- GH on which A. niger fermented; T. viride- GH on which T. viride fermented; Co-culture- GH on which A. niger and T. viride acted; Control- GH on which no organism was inoculated

Table 2. Endoglucanase (CMC) activities on pretreated GH

Treatment	Assay	Biological pretreated GH			Chemical pretreated GH		
		Day 1	Day 7	Day 13	Day 1	Day 7	Day 13
A. niger	CMC	0.04±0.002^{b}	0.17±0.001^{d}	0.08±0.001^{d}	0.034±0.001^{c}	0.028±0.001^{c}	0.01±0.00^{a}
T. viride	(IU/ml/min)	0.19±0.001^{c}	0.09±0.002^{b}	0.03±0.002^{b}	0.01±0.001^{a}	0.03±0.001^{bc}	0.03±0.00^{b}
Co-culture		0.02±0.003^{a}	0.10±0.000^{c}	0.06±0.001^{c}	0.03±0.001^{b}	0.02±0.001^{a}	0.03±0.00^{b}
Control		0.02±0.001^{a}	0.02±0.001^{a}	0.02±0.003^{a}	0.03±0.001^{b}	0.02±0.001^{a}	0.01±0.00^{a}

Mean ± SD. Values in the same column in different treatment with different superscript are significantly different (p<0.05). A. niger- GH on which A. niger fermented; T. viride- GH on which T. viride fermented; Co-culture- GH on which A. niger and T. viride acted; Control- GH on which no organism was inoculated

In order to overcome the deficiency of β-glucosidase in a *Trichoderma sp.* derived enzyme system, the fungus can be co-cultured with another fungus, *Aspergillus* species, which is a good source of β-glucosidase. This was observed in the present study for the co-culture sets, where there was a gradual increase in total cellulase activity and a dwindling value in Endoglucanase activity from high to low and then high thus indicating a boost in activity by the co-cultured organism (*A. niger*). Moses [21] reported maximum cellulase activity on the 6th day (18.5 IU/ml) with β-glucosidase activity of 21.8 IU/ml. It was postulated that cellulases from different microorganisms are closely related to each other and that endoglucanase enzyme component from one fungal species can operate with exoglucanase of another at least when both fungi have the "endoglucanase-exoglucanase" system.

5. CONCLUSION

The results of this study indicate that action of microorganisms on pretreated GH is suitable and effective in cellulase production. Cellulase production from groundnut husk with fungi like *Trichoderma viride* through solid state fermentation is effective in endoglucanase production. This is an important enzyme required for breakdown of polysaccharides into monosaccharide, which further help to produce utilizable products and can be converted into ethanol and other organic products. Cellulase has a lot of industrial applications including production of food and medicines and help to breakdown the waste plants materials to clean up the environment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kim SB, Um BH, Park SC. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology. 2001;96:673-686.
2. Mosier NS, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch MR. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology. 2005;96:673–686.
3. Ikram-ul-Haq I, Muhammad MJ, Tehmina SK. An innovative approach for hyperproduction of cellulolytic and hemicellulolytic enzymes by consortium of *Aspergillus niger* MSK-7 and *Trichoderma viride* MSK-10. Afri J. of Biotechnology. 2006;5(8):609-614.
4. Camacho NA, Aguilar OG. Production, purification and characterization of a low molecular mass xylanase from *Aspergillus species* and its application in baking. Applied Biotechnology. 2003;104(3):159-172.
5. Elad Y. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection Journal. 2000;19:709-714.
6. Rajoka MI, Durrani IS, Khalid AM. Kinetics of improved production and thermostability of an intracellular β-glucosidase from a mutant- derivative of *Cellulomonas biozotea*. Biotechnology Letter. 2004;26: 281-285.
7. Chen J, Stites WE. Increasing the thermostability of staphylococcal nuclease: Implications for the origin of protein thermostability. J. Molecular Biology. 2001; 303:125-130.
8. Juhász T, Egyhazi A, Réczey K. β-Glucosidase production by *Trichoderma reesei*. Applied Biochemistry and Biotechnology. 2005;121:243-254.
9. Alam MZ, Fakhru'l-Razi SA, Abd-Aziz A. Bioconversion of wastewater sludge by immobilized microbial treatment. In: Proceedings of International Water Association (IWA) Conf. on Water and Waste water Management for Developing Countries, Kauala-Lampur, Malaysia. 2001;344-353.
10. Carcieri S. Analyzing the ability of modified yeast to ferment zylose to ethanol. A project Submitted to Worcester Polytechnic Institute. 2010;5-12.
11. Robert AS, Ellen SH, Jens CF. Introduction to food and airborne fungi. Ponsen and Looyen, Wageningen, Netherlands. 2004; 361.

12. Onilude AA, Oso BA. Effect of fungi enzyme mixture supplementation of various dietary fibres contains diets fed broiler chicks 1 performance and carcass characteristic. World Journal of Microbiology and Biotechnology. 1999;15: 17-19.
13. Sadasivam S, Manickam A. In: Biochemical methods for agricultural sciences. Willey Eastern Limited, New Delhi. 1992;5-11.
14. Grace J, Jaronski S. Solid substrate fermentation of *Beauveria bassiana* and *Metarhizium anisopliae.* USDA-ARS Northern Plains, Agricultural Research Laboratory. 2005;1-7.
15. Ghose TK. Measurement of cellulase activities. Pure and Applied Chemistry. 1987;59(2):257-268.
16. Subramaniam S. Production of cellulase by *Trichoderma oblingosporum* using pre-treated paddy husk as a substrate. B. Sc Dissertation from University of Technology Malaysia. 2007;18-20.
17. Damisa D, Ameh JB, Umoh VJ. Effect of chemical pretreatment of some lignocellulosic wastes on the recovery of cellulase from *Aspergillus niger* AH3 mutant. African Journal of Biotechnology. 2008;7(14):2444-2450.
18. Reddi M, Narasimha G. Utilization of Pea husk as a substrate for cellulase production by mutant *Aspergillus niger.* Biotechnology. 2011;1:17-22.
19. Kocher G, Kalra K, Banta G. Optimization of cellulase production by submerged fermentation of rice straw by *Trichoderma harzianum* Rut-C 8230. The Internet Journal of Microbiology. 2008;5:2.
20. Abd El-Zahel F, Fadel M. Production of bioethanol via enzymatic saccharification of rice straw by cellulase produced by *Trichoderma reesei* under solid state fermentation. New York Science Journal. 2010;72-78.
21. Moses R, Leelavathy R, Lekew WA. Optimization of Solid State Fermentation conditions for the production of cellulase by *Trichoderma reesei.* European Journal of Applied Engineering and Scientific Research. 2012;1(4):196-200.

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