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Optimization of solid-state fermentation for production of cellulase from agricultural residues

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Abstract

Agricultural residues such as rice straw, wheat bran, and sugarcane bagasse accumulate in vast quantities each harvest season and pose disposal problems when burned in the open. Converting these lignocellulosic wastes into a substrate for microbial enzyme production is one route to add value while reducing environmental harm. This research optimised solid-state fermentation (SSF) conditions for cellulase production by *Aspergillus niger* MTCC 2196 grown on three agricultural residues at the Federal University of Agriculture, Abeokuta, Ogun State, during August-December 2023. Wheat bran supported the highest cellulase activity (18.3 IU g⁻¹ dry substrate) at 72 h, pH 5.5, 30 °C, and 65% initial moisture content, followed by rice straw (14.6 IU g⁻¹) and sugarcane bagasse (12.1 IU g⁻¹). A one-factor-at-a-time approach established that pH 5.5, 30 °C, and a moisture level of 65% were the most favourable conditions. Supplementation with 1% yeast extract further raised cellulase yield on wheat bran to 21.7 IU g⁻¹. The findings indicate that wheat bran, an inexpensive agro-residue, can serve as an effective substrate for on-farm cellulase production applicable to bioethanol pre-treatment and animal feed processing.

Keywords: Solid-state fermentation, cellulase, agricultural residues, enzyme production, bioprocessing, *Aspergillus niger*, wheat bran, lignocellulose, optimisation, substrate moisture

Introduction

The enzymatic breakdown of cellulose into fermentable sugars sits at the heart of second-generation bioethanol production, yet the high cost of commercial cellulase preparations remains a barrier to economic viability ^[1]. Solid-state fermentation (SSF) offers a cost-effective alternative to submerged fermentation for cellulase production because it uses cheap lignocellulosic residues as both the carbon source and the physical support for fungal growth, and it operates at lower water volumes, reducing downstream processing costs ^[2]. Nigeria generates over 160 million tonnes of crop residues annually; cassava peels and rice straw together account for roughly 60 million tonnes, much of which is burned in the field ^[3]. Wheat bran and sugarcane bagasse are likewise produced in surplus by milling industries. Filamentous fungi—particularly *Aspergillus niger* and *Trichoderma reesei*—are the most widely used cellulase producers under SSF because they secrete a complete enzyme system (endoglucanase, exoglucanase, β-glucosidase) and tolerate low water activity ^[4]. However, enzyme yields are sensitive to fermentation variables such as pH, temperature, moisture content, and nitrogen supplementation, and optimum conditions differ with both the organism and the substrate ^[5].

Earlier reports optimised SSF for cellulase using individual substrates, but comparative data across multiple residues under identical conditions are less common, especially for the humid tropical climate of southwestern Nigeria ^[6]. This research therefore compared rice straw, wheat bran, and sugarcane bagasse as substrates for cellulase production by *A. niger* MTCC 2196 under SSF and optimised key process parameters to maximise enzyme yield.

Material and Methods

Material

Aspergillus niger MTCC 2196 was obtained from the National Collection of Industrial Microorganisms, Lagos, and maintained on potato dextrose agar slants at 4 °C.

Rice straw was collected from the university farm, wheat bran from a local flour mill in Abeokuta, and sugarcane bagasse from a nearby sugar factory in Ogun State, all in Abeokuta district. Substrates were oven-dried at 60 °C, ground to pass a 1 mm sieve, and autoclaved (121 °C, 20 min) before use. Chemicals (carboxymethylcellulose, DNS reagent, yeast extract) were of analytical grade (Oxoid, UK) [7].

Methods

SSF was carried out in 250 mL Erlenmeyer flasks containing 10 g of each substrate moistened with Mandels' mineral salt solution to the desired moisture level and inoculated with 1 mL spore suspension (1×10^7 spores mL⁻¹).

Flasks were incubated at 30 °C (baseline) for 12-96 h. Enzyme extraction used 50 mL of 0.05 M citrate buffer (pH 4.8) with agitation at 150 rpm for 30 min, followed by filtration and centrifugation ($10,000 \times g$, 15 min). Cellulase (filter paper activity, FPase) was assayed by the method of Ghose [8] and expressed as IU g⁻¹ dry substrate. Optimisation followed a one-factor-at-a-time approach: pH (4.0-6.5), temperature (25-40 °C), moisture (45-80%), and nitrogen supplement (yeast extract 0.5-2.0%). All experiments were run in triplicate. Data were analysed by one-way ANOVA and Tukey's test ($p < 0.05$) using R v 4.3.1.

Results

Table 1: Cellulase activity (IU g⁻¹ ds) on different substrates over fermentation time

Substrate	24 h	48 h	60 h	72 h	84 h	96 h
Rice straw	2.1	8.3	11.2	14.6	13.9	12.8
Wheat bran	3.4	10.7	14.8	18.3	17.6	16.1
S.cane bagasse	1.7	6.4	9.1	12.1	11.8	10.9
CD (p=0.05)	0.6	1.2	1.4	1.8	1.7	1.5

ds = dry substrate.

Table 2: Effect of nitrogen supplementation on cellulase yield (IU g⁻¹ ds) using wheat bran at 72 h

Yeast extract (%)	Cellulase activity	Protein (mg g ⁻¹)
0 (control)	18.3 ^b	24.1
0.5	19.8 ^b	27.6
1.0	21.7 ^a	31.4
1.5	21.2 ^a	33.8
2.0	19.6 ^b	36.2

Means with different superscripts differ at $p < 0.05$.

All three substrates showed a characteristic rise-and-fall pattern in cellulase activity, peaking at 72 h and declining thereafter. Wheat bran consistently outperformed the other residues at every time point, reaching 18.3 IU g⁻¹ at 72 h

under baseline conditions (pH 5.5, 30 °C, 65% moisture). Rice straw peaked at 14.6 IU g⁻¹, and sugarcane bagasse at 12.1 IU g⁻¹.

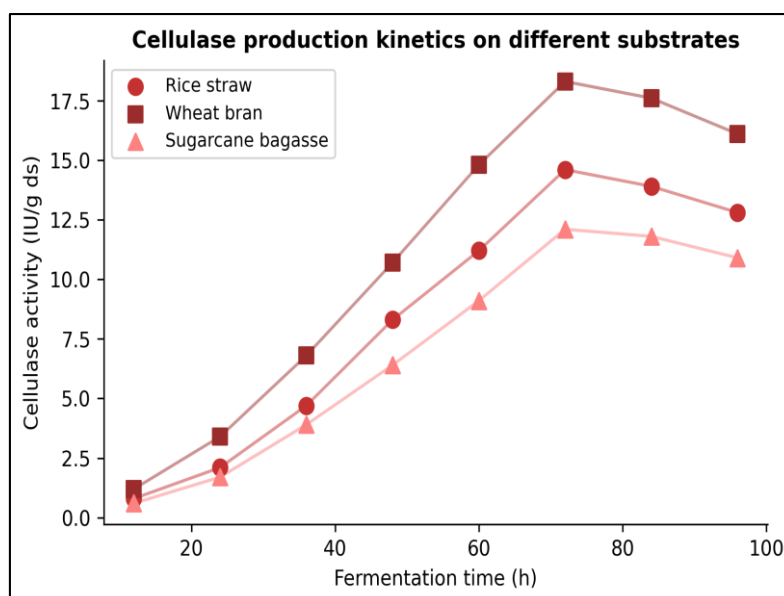


Fig 1: Cellulase production kinetics on three agricultural residues during SSF

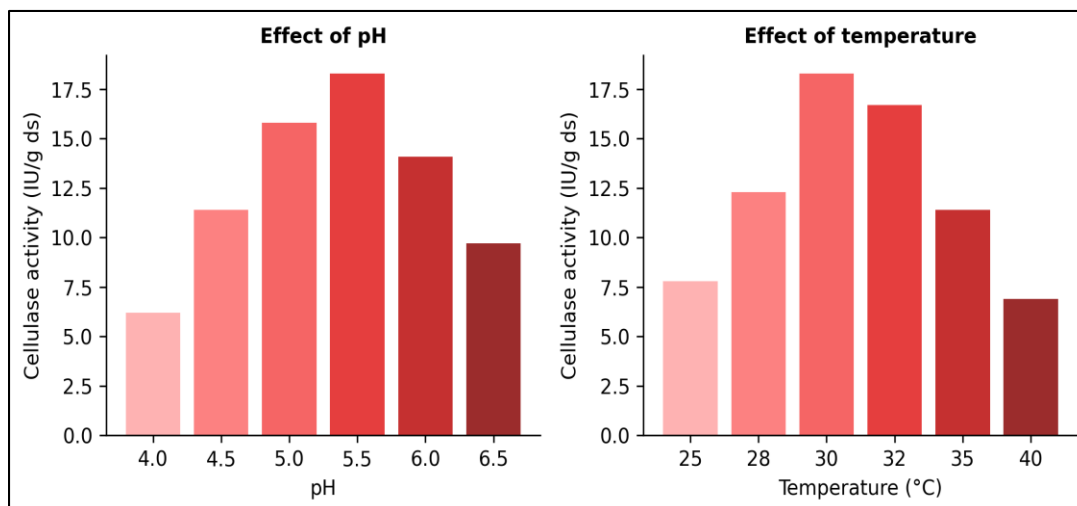


Fig 2: Effect of pH and temperature on cellulase activity using wheat bran at 72 h

Comprehensive Interpretation

The superiority of wheat bran reflects its balanced composition of cellulose, hemicellulose, starch, and protein, which provides both inducer and readily metabolisable nutrients for *A. niger*. Optimum pH (5.5) and temperature (30 °C) fall within the range typical for fungal cellulases. Nitrogen supplementation with 1% yeast extract boosted activity by 18.6% over the unsupplemented control, though higher concentrations (2%) led to excessive mycelial growth at the expense of enzyme secretion.

Discussion

Wheat bran's superiority as an SSF substrate for cellulase production is consistent with reports by Deswal *et al.* [9], who recorded 15.2 IU g⁻¹ with *Fomitopsis* sp. on wheat bran, and by Bansal *et al.* [4], who achieved 16.8 IU g⁻¹ with *A. niger* on the same substrate. The somewhat higher yield in the present research (18.3 IU g⁻¹) may relate to the higher ambient humidity of the southwestern Nigeria region, which helps maintain substrate moisture during extended incubation [10]. The decline in activity after 72 h coincides with depletion of readily accessible cellulose and accumulation of glucose, a known repressor of cellulase gene expression via carbon catabolite repression [11].

Rice straw, despite its abundance, yielded lower cellulase than wheat bran. This is likely due to the higher silica content of rice straw (12-15% SiO₂), which encrusts cellulose fibrils and physically impedes fungal penetration [3]. Pre-treatment with dilute alkali could potentially remove silica and improve enzyme yields, though it would add processing cost [12]. Sugarcane bagasse, with its higher lignin fraction (22-25%), presented a similar access barrier [13].

From an application standpoint, a cellulase titre of 18-22 IU g⁻¹ is adequate for on-farm saccharification of crop residues into fermentable sugars, provided enzyme loading is scaled appropriately [14]. Coupling SSF cellulase production with small-scale bioethanol fermentation could create a closed-loop system for residue valorisation in rural settings.

Conclusion

The present research set out to compare three agricultural residues as substrates and to optimise SSF conditions for cellulase production by *A. niger* MTCC 2196. Wheat bran emerged as the best substrate, yielding 18.3 IU g⁻¹ ds at 72 h

under optimum conditions of pH 5.5, 30 °C, and 65% moisture. Addition of 1% yeast extract raised the yield to 21.7 IU g⁻¹. Rice straw and sugarcane bagasse, though less productive, still generated usable cellulase levels and represent low-cost alternatives where wheat bran is scarce. From a practical standpoint, the optimised SSF protocol is simple enough for adoption in small-scale bioprocessing units. Future work should explore combinatorial substrates, response surface methodology for multi-factor optimisation, and downstream concentration of the crude enzyme for pilot-scale saccharification trials.

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