Xylanase Production using Soybean Hulls: Effect of Medium Components

Aliyu Salihu[1,2], and Md. Zahangir Alam[1]

***Abstract*—**This study involves the use of Plackett–Burman design in order to select the important medium components affecting the xylanase production by *Aspergillus niger* AS-1 using soybean hulls as the main substrate. All the nitrogen and mineral sources contributed positively to the overall yield, while the carbon sources (glucose and sucrose) have negative influence on the enzyme production. Thus, urea, Na_2CO_3 and K_2HPO_4 are the most contributing components with 27%, 23%, and 18% respectively. Further experiments using one-factor-at-a-time (OFAT) were used to determine the optimum levels of each of the medium components that showed positive effects. Soybean hulls appeared to be a promising substrate requiring minimum supplementation for xylanase production. However, optimization and determining the synergistic effects of these components require further experimentation involving statistical based designs.

Keywords*—Aspergillus niger, Plackett–Burman, soybean hulls, xylanase.*

I. Introduction

SOYBEAN is widely cultivated across the globe with annual production of more than 259 million tonnes; producing about 20 million tonnes of hulls which represent about 8 – 10% of total grain compositions [1]. Soybean hulls constitute 46–51% cellulose, 16–18% hemicellulose and 1.4–2% lignin [2], which make them attractive residues for production of several value added products including fermentable sugars [3], ethanol [4], animal feed [5], adsorbent [6] and cellulase [7], among others.

Hemicellulose as a heteropolysaccharide contains xylan where D-xylose moiety is β-1,4 linked with different residues including glucuronic acid, acetyl, arabinose, or mannose [8], [9]. Complete hydrolysis of xylan requires a synergistic action of different enzyme systems such as endo-1, 4-β-xylanase, β-D-xylosidase, α-L-arabinofuranosidases, α-glucuronidases and acetyl esterases [10]. The most important among them is endo-1, 4-β-xylanase (EC 3.2.1.8) which randomly hydrolyses the 1, 4 xylopyranosyl bond and depolymerises the xylan molecules into its constituent sugars including xylose, xylobiose, xylotriose, xylotetrose and xylo-oligosaccharides [11]. Xylanase has been found useful in different industrial applications such as biobleaching agent in pulp and paper industry, production of ethanol and xylitol, improvement of animal feed, juice and wine clarification and baking processes [9], [11], [13].

Some of microbial species reported to have high potential for xylanase production include *Aspergillus niger* XY-1 [14], *Penicillium citrinum* xym2 [12], *Trichoderma viride*-IR05 [13], *Streptomyces* sp. Ab 106 [10], *Trichoderma reesei* Rut C-30 [15] and *Geobacillus stearothermophilus* KIBGE-IB29 [9]; in case of agricultural residues, wheat bran, barley, sago, corncob, deoiled jatropha curcas seed cake, rice bran, and brewers spent grains [13] – [17], have been evaluated for the enzyme production through solid state fermentation technique.

Thus, the main bottleneck affecting the wider application of enzyme-based processes is the production cost; thus, attempts to use cheap and available residues like soybean hulls to produce xylanase with considerable yield may contribute greatly in overcoming this challenge. This study intends to evaluate the medium components affecting the production of xylanase by *Aspergillus niger* AS-1 using soybean hulls as the main substrate through the use of plackett-Burman design as well as one-factor-at-a-time (OFAT) methods.

II. Materials And Methods

A. Sample and Inoculum preparation

Soybean hulls were collected from local millers in Zaria, Nigeria. The hulls were milled and sieved to obtain 1 mm particle sizes and kept under laboratory conditions at room temperature.

Potato dextrose agar plate containing *Aspergillus niger* AS-1 was used for inoculum preparation where 25 ml of sterile distilled water was used to wash the plate with the help of bent glass rod. Following the filtration of the suspension through Whatman No.1 filter paper under aseptic conditions; the spores counted using hemocytometer were found to be about 1×10^8 spores/ml.

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TABLE I
PLACKETT–BURMAN EXPERIMENTAL DESIGN SHOWING THE CODED VALUES FOR XYLANASE PRODUCTION BY A. NIGER SA-1 USING SOYBEAN HULLS AS THE MAIN SUBSTRATE

	[a]Medium components											Xylanase Activity
Run	Glucose	Sucrose	Yeast Extract	Peptone	Urea	$(NH_4)_2SO_4$	$NaNO_3$	K_2HPO_4	$CaCl_2$	Na_2CO_3	$MgSO_4$	(U/g)
1	–1	+1	+1	–1	+1	–1	–1	–1	+1	+1	+1	8.39
2	+1	–1	+1	+1	–1	+1	–1	–1	–1	+1	+1	5.02
3	+1	+1	–1	+1	–1	–1	–1	+1	+1	+1	–1	6.36
4	+1	–1	+1	–1	–1	–1	+1	+1	+1	–1	+1	5.18
5	+1	+1	–1	+1	+1	–1	+1	–1	–1	–1	+1	4.78
6	+1	+1	+1	–1	+1	+1	–1	+1	–1	–1	–1	5.44
7	–1	–1	–1	+1	+1	+1	–1	+1	+1	–1	+1	10.08
8	+1	–1	–1	–1	+1	+1	+1	–1	+1	+1	–1	7.99
9	–1	–1	+1	+1	+1	–1	+1	+1	–1	+1	–1	11.61
10	–1	+1	–1	–1	–1	+1	+1	+1	–1	+1	+1	8.31
11	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	–1	2.51
12	–1	+1	+1	+1	–1	+1	+1	–1	+1	–1	–1	5.77

[a]Selection of medium components as well as their levels was based on literature reports; –1 and +1 indicate the low and high levels respectively

B. Solid state fermentation for xylanase production

Ten gram of Soybean hulls as the main fermentation medium was transferred into a 250 ml Erlenmeyer flask each and then mixed with 10 ml of appropriate nutrient solution based on the PB design matrix as described by Xu et al. [14]. The flasks were autoclaved at 121 °C for 20 min, and then cooled before inoculating the medium with 1 ml of spore suspension (1×10^8 spores/ml). The set ups were incubated at 28 ± 1^0C for 5 days. Following the bioconversion, distilled water was added to each flask and then shaken on a rotatory shaker (180 rpm) for 30 mim at room temperature. The content was centrifuged and the supernatant was used to assay for xylanase activity.

C. Effects of medium components

In order to determine the effects of different medium components on xylanase production using soybean hulls, eleven components were subjected to Plackett–Burman (PB) design. These include carbon sources (glucose and sucrose at 0 – 0.5%); nitrogen sources ((peptone (0 – 0.5%), yeast extract and urea (0 – 0.2%), $(NH_4)_2SO_4$ and $NaNO_3$ (0 – 0.05%)), and inorganic mineral sources (K_2HPO_4 and $CaCl_2$ (0 – 0.05%), Na_2CO_3 and $MgSO_4$ (0 – 0.01%). Table 1 showed the design matrix developed by Design Expert 6.0.8 (Start Ease Inc., Minneapolis, USA) based on 12 experimental runs at two levels (low level (–1) and high level (+1)) together with the xylanase yield as the experimental response. Each experiment was carried out in triplicate. The PB design is described by the first order model:

$$Y = \beta_0 + \sum \beta_i X_i \quad (1)$$

Where, Y is the dependent variable (xylanase activity), β_0 is the model intercept, β_i is the linear coefficient, and X_i is the level of the independent variable. The main effect of each independent parameter is based on difference between the total responses at high and low levels in relation to the number of experiments.

D. Determination of Xylanase activity

Xylan from beechwood was used as a substrate for determining the xylanase activity and the amount of reducing sugar released was measured by dinitrosalicylic acid (DNS) method using D-xylose as the standard [18]. The reaction mixture contains 900µl of 1% (w/v) of the substrate in 0.05 M citrate buffer, pH 5 and 100 µl of the appropriate enzyme solution incubated at 50^0C for 30 min. The reaction was terminated by addition of DNS followed by incubating in boiling water bath for 5 min. The reducing sugar released was measured at 540 nm. One enzyme unit (U) was defined as the amount of enzyme that liberated 1 µmol of xylose per minute under the assay conditions. The results were expressed as U/g soybean hulls.

E. Determination of optimum levels of important medium components

Based on the results of PB design, the positively contributing components were subjected to one-factor-at-a-time (OFAT) so as to determine the possible optimum range of these components.

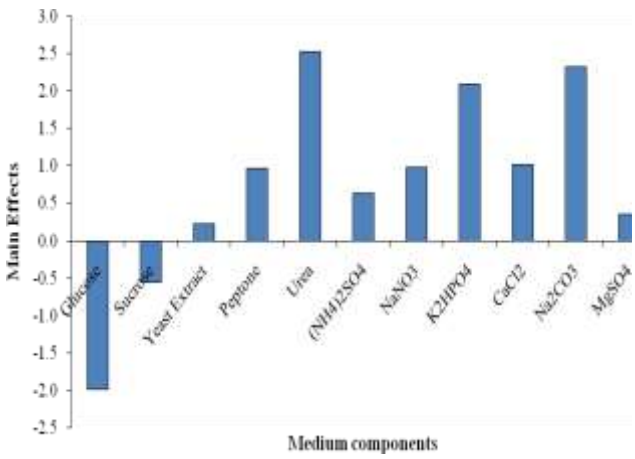

Fig. 1 Main effects of the medium components based on Plackett–Burman experimental results

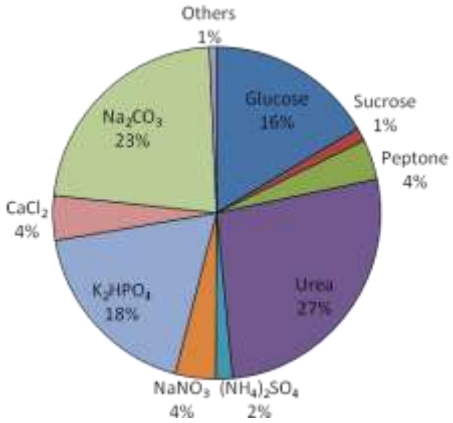

Fig. 2 Pie chart representing the percentage of contribution of the selected medium components

III. Results And Discussion

A. Screening of medium components for xylanase production

This study involves the use of PB design which has been reported to be useful in identifying the important components affecting the production processes through a minimum number of experiments [19]. Eleven medium components were screened through 12 experimental runs as indicated in Table 1. From the table, Xylanase activity (U/g) was found to be highest in run 9 (11.61 U/g) followed by run 7 (10.08 U/g), this high yield could be attributed to the absence of carbon sources (glucose and sucrose) in both runs. While the lowest production was recorded in run 11 (2.51 U/g) where exogenous addition of medium components was not added; this indicated that soybean hulls on its own contain some residual nutrients that favored the growth of *A. niger* AS-1 for xylanase production.

Main effect which relates the experimental runs with total responses at high and low levels was used to show the contribution of each component on the response (Fig. 1). This showed that all the components contributed in enhancing the xylanase production with the exception of carbon sources (glucose and sucrose); which have negative effect on the overall yield. In terms of contribution, urea, Na_2CO_3 and K_2HPO_4 are the most positively contributing components with 27%, 23%, and 18% respectively (Fig. 2). From this figure, glucose accounted for about 16% in terms of contribution but its main effect showed that this contribution is inhibitory to the overall yield. This is in agreement with what was reported by Xu et al. [20] where the presence of carbon sources including glucose, sucrose, starch and cellobiose enhanced the growth of *Pseudomonas* sp.WLUN024 at the expense of enzyme production.

Several studies showed the contribution of different medium components on Xylanase activity; urea was reported to be the best nitrogen source for xylanase production for *Alternaria mali* ND-16 in the presence of wheat bran at 1.26 g/L [21]. Similarly, *A. niger* B03 required urea (0.9 g/L) and $(NH_4)_2HPO_4$ (2.6 g/L) for maximum xylanase production using agricultural residues [22]. Singh et al. [23] obtained maximum xylanase activity by *A. niger* ITCC 7678 in alkali treated rice husk supplemented with $NaNO_3$ after 5 days of incubation under controlled temperature of 32.5^0C and pH of 5.5. The optimum medium components that led to xylanase activity of 14374.6 U/g by *A. niger* XY-1 using wheat bran were found to be urea, Na_2CO_3 and $MgSO_4$ at 41.63 g/L, 2.64 g/L and 10.68 g/L respectively [14]. The findings of Irfan et al. [13] showed that supplementation of sugarcane bagasse with organic and inorganic nitrogen sources (urea, tryptone, yeast extract, $NaNO_3$ and $(NH_4)_2SO_4$) resulted in enhancement of xylanase activity by *Trichoderma viride*-IR05.

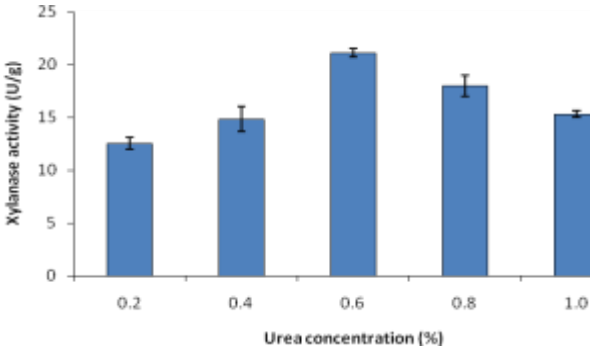

Fig. 3 Effect of different concentrations of urea on xylanase production

Based on this, the medium components that have negative influence (glucose and sucrose) on xylanase production were excluded and the remaining factors that positively contributed to the production i.e. urea, Na_2CO_3, K_2HPO_4, $CaCl_2$, peptone, $NaNO_3$ and $(NH_4)_2SO_4$ were further analyzed using one-factor-at-a-time (OFAT) experiments.

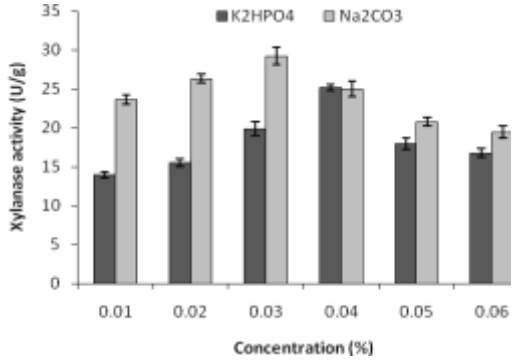

Fig. 4 Effect of different concentrations of K_2HPO_4 and Na_2CO_3 on xylanase production

Clear trends were observed in Fig. 3 and 4 which showed that there were increments in xylanase activity with increase in concentrations of urea, Na_2CO_3 and K_2HPO_4 up to a certain limit; while the rest of the components (Data not shown) were found to reach their optimum at the selected upper levels used in PB experiments. In this study, the optimum concentrations obtained for urea and Na_2CO_3 were found to be lower than what were reported by Xu et al. [14] where 4% and 0.3% respectively led to maximum production of xylanase by *A. niger* XY-1. In case of K_2HPO_4, 0.04% was found to be sufficient compared to 0.4% reported for *Pseudomonas* sp. WLUN024 [20].

Thus, soybean hulls contain some residual nutrients that can serve as carbon source and inducer for microbial growth as well as xylanase production. The hemicellulose content (16 – 18%) and the fact that soybean hulls are abundant and cheap could contribute in developing a cost-effective medium for industrial production of xylanase.

IV. Conclusion

Soybean hulls showed potential to be utilized as raw material for xylanase production by *A. niger* AS-1. The use of PB design aids in the selection of medium components through its unbiased and accurate estimation of linear effects. Among all the components, three components (urea, Na_2CO_3 and K_2HPO_4) were found to be the most contributory based on OFAT analysis. Thus utilization of soybean hulls as inexpensive and renewable residues for xylanase production boosts their industrial relevance when compared with their current usage.

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