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Optimisation of Xylanase Production from Rice Straw through Solid State Fermentation

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Abstract

The usage of rice straw as a substrate by fungus strains for the enzyme production has been recommended in order to minimise the overall cost enzyme production. Rice straw has a high content of hemicellulose and cellulose that can be potentially utilised for xylanase production. The present work is focused on optimising the production of xylanase using two different types of rice straw, untreated and treated (2.75% NaOH-heat at 121°C, 15 mins) as a substrate to cultivate *Aspergillus oryzae* via solid state fermentation for 48 hours. One-factor at a time technique is used to optimise two parameters, initial moisture content (50%, 60%, 70%, 80%), and carbon and nitrogen amount (01g, 0.2g, 0.3g and 0.4g. The results obtained show that the optimum condition for xylanase production using untreated rice straw is 50% of initial moisture content, and 0.2g of carbon and nitrogen amount meanwhile for treated rice straw is 60% of initial moisture content, and carbon and nitrogen amount at 0.1g for 48 hours in SSF. Under controlled conditions, xylanase activity by *Aspergillus oryzae* is found to be slightly higher when using untreated rice straw, 0.543 ± 0.015 U/ml but there is no significant difference when compared to treated rice straw, 0.445 ± 0.061 U/ml.

Keyword: in vitro propagation; auxin; cytokinin; *Momordica charantia*

INTRODUCTION

Xylanase serves as an alternative in substituting the usage of chemical bleaching agents in deinking process, that has affected on the environmental issue particularly on water pollution, that consequently harming the public health. In addition, xylanase has demonstrated remarkable performance in enhancing the pulp brightness, improving the quality of paper and pulp, increase the purity of dissolving pulp and minimizing the production cost of paper altogether.

In spite of their outstanding performance, the production of xylanase at large scale is still impracticable because of its high budget production and has yield low volume of xylanase. In an effort to reduce the overall cost for enzyme production, the fermentation process can be carried out under solid state fermentation and use of readily available and cheaper agro-industrial residues like rice straw. Generally, rice industries continuously produce huge amounts of wastes annually, including rice husk (20%), rice bran (8%) and rice straw (2.4%) that leads to the accumulation of agro-industrial wastes. Typically, the normal practise of paddy farmers is to discard the rice straw by burning it in an open field after harvest season, which is a cost-effective method for them, but it results in the emission of greenhouse gases (GHG), thus pollutes the air, contributes to global warming and risks the public safety. Besides, rice straw has a high percentage of hemicellulose and cellulose that can be potentially utilised for the bioconversion of fermentable sugar into xylanase. Solid-state fermentation works efficiently to produce hydrolytic enzymes by filamentous fungi. Several factors including optimisation of process parameters, selection of microbial species, and choice of solid substrates are crucial in enhancing the productivity of enzymes.

Therefore, aim of this study is to determine the optimal conditions specifically on initial moisture content, temperature, carbon and nitrogen amount and inoculum level for maximum xylanase activity (U/ml) via one-factor at a time (OFAT) method, as well as to compare the effect of pre-treatment and non-pre-treatment rice straw towards the yield of xylanase production.

MATERIALS AND METHODS

Rice straw

Rice straw was obtained from local paddy farmers at Sekinchan, Selangor. Rice straw was washed and dried in oven at 60°C for 48 hours. Rice straw was cut about 4-5 cm length and grinded into fine powder using laboratory blender. The fine powder was filtered through sieve about 3 mm in size. Then, rice straw was stored in a clean zip-lock bag for further use.

Aspergillus oryzae

The culture of *A. oryzae* were acquired from the MARDI Culture Collection (MCC), Serdang. Strain of *A. oryzae* was cultured on PDA plate using inoculating loop. The PDA plate was sealed with parafilm and incubated at 30°C for 5 days. Sterile distilled water (10 ml) was added to petri dish and the spore was gently scraped with a sterile Hockey stick. The spore suspension was filtered using Whatman No.1 filter paper and the filtrate was stored at 4°C for further use.

Pre-treatment of rice straw

The pre-treated method of rice straw was performed as demonstrated by Maftukhah & Abdullah [1] and Sawisit, Jampatesh, Jantama, & Jantama [2]. The rice straw was pre-treated with 2.75% sodium hydroxide solution at 1:10 (w/v) ratio of rice straw powder. The pre-treated rice straw powder was further treated using an autoclave at 121°C for 15 minutes and filtered by Whatman filter paper. Then, the pre-treated rice straw was washed with tap water until the pH becomes neutral. The washed rice straw was left overnight at 50°C and stored for further use.

Solid state fermentation and extraction

The fermentation process was performed as reported by Norazlina, Ku Halim, Abd Manaf, & Abu Bakar [3] with some modification using 250 mL Erlenmeyer flask containing 10g of total working substrate. The medium was moistened with distilled water One ml of mineral stock

solution was added to the sample. Both yeast extract and sucrose were added as nitrogen and carbon sources to boost the fermentation process. The (C&N) amount was varied at 0.1g to 0.4g. The initial pH was maintained at 5.0. Then, the flask was sterilized using autoclave for 15 minutes at 121°C. After cooled, one ml of varying levels of one week-old inoculum (1%-5%) of *A. oryzae* was added and inoculated in the flask. Later, the inoculated flasks were incubated, and the incubation time was constantly set for 2 days. The procedure was repeated by replacing with treated rice straw. Xylanase was extracted using 30 mL of citrate buffer (0.1 M) at pH 4.8. Then, the mixture solution was left to homogenise at 200 rpm using rotary shaker for 2 hours. The homogenised xylanase was further separated from solid through filtration using Whatman No.1 filter paper. The filtered xylanase was centrifuged at 10,000 rpm for 10 minutes to get a clear supernatant. The clear supernatant was stored at 4°C in chiller for xylanase assay.

Optimisation of process parameters

The experiment was carried out following the OFAT method. This demonstrates that one variable was changed over time while the others remained constant. Various process parameters such as moisture content (50%, 60%, 70% and 80%), and carbon and nitrogen amount (0.1g, 0.2g, 0.3g, 0.4g) were tested and adjusted in the production of xylanase using untreated rice straw and treated rice straw.

Statistical analysis

The maximal xylanase production was tabulated and analysed using Microsoft Excel. Two-way analysis of variance (ANOVA) was used to analyse the effects of selected process parameters on the yield of xylanase production using two different types of rice straw with Minitab 20.3 software where $p < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Effect of initial moisture content on xylanase production

Moisture content is a vital element affecting the enzyme production under SSF condition. Various range of initial moisture content (50-80%) were tested for xylanase production by *A. oryzae* in SSF for 48 hours. Results (Figure 1) indicated that *A. oryzae* produced 8.190 U/ml and 5.553 U/ml of xylanase using untreated rice straw and treated rice straw at 50% and 60% initial moisture content respectively.

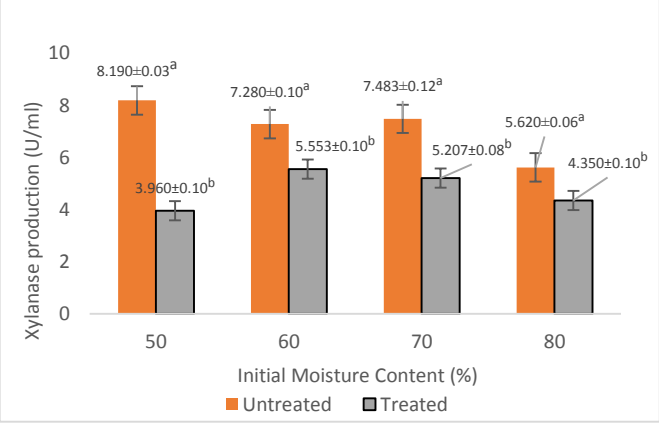

Figure 1: Effect of initial moisture content on xylanase production (U/ml) by *A. oryzae* using untreated and treated rice straw in SSF for 48 hours of incubation period, 0.1g of C&N amount, temperature at 31°C, and 1% of inoculum level.

Then, the xylanase production reduced gradually for both samples. Results of two-way variance analysis showed that the initial moisture content is not significantly influence on xylanase production at ($p > 0.05$), meanwhile different types of rice straw is significantly influence on xylanase production at ($p < 0.05$).

According to Gautam et al. [4], moisture content encourages the substrate to swell and renders the substrate favourable for the growth of microorganisms. The optimum moisture content leads in the accelerated growth of microorganisms and immediate initiation of enzyme production. However, the optimum moisture content for the enzyme production differs based on the type of microorganisms and the physical structure of solid substrates used during the fermentation process. Higher moisture levels may influence the structure of the particles, lowers volume of gas, reduces rate of diffusion, and lowers permeability, leading to decreased rate of oxygen transfer. In contrast, lower moisture levels result in a decreased swelling, nutrients insolubility in substrate and a high tension of water The decline of xylanase production possibly due to the reduction of nutrient solubility in fermentation medium, consequently, eventually affects the microbial growth and activity [5].

Effect of carbon and nitrogen amount on xylanase production

One of the main factors influencing the enzyme production and activity is the carbon and nitrogen source employed in the fermentation medium. In this research, carbon source used is sucrose, while nitrogen source used is yeast extract. The amount is differing from 0.1g, 0.2g, 0.3g and 0.4g. Figure 2 illustrates the effect of carbon and nitrogen amount using untreated rice straw and treated rice straw at amount of 0.2g and 0.1g was the best enhancer for the xylanase production using *A. oryzae* in SSF for 48 hours, respectively. The highest production of xylanase using untreated rice straw, 7.317 U/ml at amount of 0.2g of carbon and nitrogen sources, whereas treated rice straw, 8.053 U/ml at 0.1g of carbon and nitrogen amount. According to two-way ANOVA results, both C&N amount and different types of rice straw evaluated did not significant influence on xylanase production by *A. oryzae* at ($p > 0.05$).

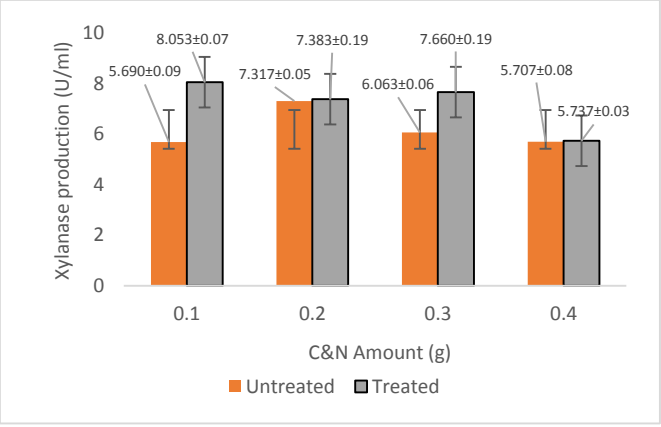

Figure 2: Effect of carbon and nitrogen amount on xylanase production (U/ml) by *A. oryza*e using untreated and treated rice straw in SSF for 48 hours of incubation period, temperature at 31°C, and 1% of inoculum level. Untreated rice straw (initial moisture content (50%). Treated rice straw (initial moisture content (60%)).

A previous study by Norazlina et al. [3] on the effect of carbon and nitrogen ratio on the xylanase production by *A. niger* under SSF, where C/N ratio at value of 0.4 produced maximal titre xylanase (16.0461 U/ml) using sucrose and peptone as carbon and nitrogen sources. According to Selim et al. [5], the addition of carbon sources and nitrogen sources such as xylan, starch and cellulose, ammonium

phosphate and peptone increased the xylanase production by *A. niger* in SSF with value of 1081.47, 946.59, 950.45, 1196.49 and 1169.22 IU/gdw, respectively.

Controlled conditions for xylanase production

Table 1: Controlled conditions for xylanase production by untreated rice straw and treated rice straw.

Process parameters/ Rice straw	Untreated	Treated
Initial moisture content (%)	50	60
C&N amount (g)	0.2g	0.1g

Xylanase assay

Results from xylanase activity (Table 2) shows that the untreated rice straw obtained the highest amount of xylanase activity (0.543 ± 0.015 U/ml) compared to treated rice straw with value of 0.446 ± 0.061 U/ml. Based on 2 Sample T test (Table 3), the p-value > 0.05, hence concludes that the xylanase production for the two types of rice straw is not differ.

Table 2: Xylanase activity by crude xylanase obtained from untreated rice straw and treated rice straw.

Samples	**Xylanase Activity (U/ml)**
Untreated Rice Straw	0.543 ± 0.015
Treated Rice Straw	0.446 ± 0.061

Table 3: 2 Sample T test between xylanase production for the two types of rice straw.

T-Value	**DF**	**P-Value**
-1.63	4	0.178

CONCLUSION

The controlled conditions for untreated rice straw were 50% of initial moisture content and 0.2g of carbon and nitrogen amount while treated rice straw were 60% of initial moisture content and 0.1g of carbon and nitrogen amount for 48 hours in SSF. It can be concluded that untreated rice straw produces slightly higher of xylanase activity, 0.543 ± 0.015 U/ml but there is no significant difference when compared to treated rice straw, 0.445 ± 0.061 U/ml.

ACKNOWLEDGEMENT

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