AJLS

Asean Journal of Life Sciences, Vol 1(1), 2021, pp 41-49

Accepted date: 28 May 2021
Published date: 21 June 2021

The Effectiveness of Delignification and Juice Clarification using Fungi Xylanase from Rice Straw and Oil Palm Leaf

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Abstract

Microbial enzyme which is xylanase plays a significant part as biocatalyst in many reactions of industrial applications purposes. The xylanase can be produced by *Aspergillus niger* via solid state fermentation system using agricultural waste as substrate. Xylanase is an industrially important class of enzyme that degrades xylan. There are two objectives of this study, firstly, to produce xylanase from rice straw and oil palm leaf via solid state fermentation by *Aspergillus niger* and secondly, to compare its efficacy with commercial xylanase on two different kinds of application, namely, delignification of sugarcane bagasse and clarification of citrus microcarpa juice. The results presented in this study revealed that commercial xylanase was more efficient in both applications. However, xylanase extracted from OPL is capable to have comparatively similar effectiveness with commercial xylanase in bagasse delignification and clarification of citrus microcarpa juice compared to xylanase from rice straw. In consequence, it is suggested that the *Aspergilus niger* xylanase is more effective in respective applications by using oil palm leaf as substrate compared to rice straw.

Keyword: delignification, juice clarification, oil palm leaf, rice straw, solid state fermentation, xylanase

INTRODUCTION

According to Siti & Abdullah (2017), one of the most abundant agricultural residues available in the world is rice straw with the capacity of 20.9 million tons from Africa, 667.6 million tons from Asia, 3.9 million tons from Europe, 37.2 million tons from America and 1.7 million from Oceania. Most farmers are practicing open burning of these materials and this activity is of pollution concerns. On top of that, Norazlina et al. (2015), highlighted among all agricultural wastes, the oil palm industry forms the economic backbone of Malaysia with total contribution

of 49.5% of global production and 64.5% of exports which makes Malaysia is the largest palm oil producer in the world. In general, the agricultural wastes keep on accumulate and causing environmental problem as most of the wastes are disposed by open burning. This practice is a main factor contributing to global warming. The management of these wastes must be given highest priority in the country in ensuring not only in reducing the damaging impact of the waste to the environment, but most prominently in the conversion of these wastes into useful raw material for the production of added value commodities of industrial commercial potentials (Pang et al., 2006).

Xylanase is an industrially important hydrolytic enzymes which catalyses the hydrolysis of xylan to xylo-oligosacharides and pentose sugar (xylose) that has been used in many applications, specifically, energy generation, waste treatment, production of chemicals, clarification of juices, and paper manufacture. The aims of this research are to obtain the fungus xylanase from two different carbon sources which were rice straw and oil palm leaf as cost-effective substrates via solid state fermentation. Next, both crude xylanases obtained from rice straw and oil palm leaves were compared to investigate their efficacy with commercial xylanase in sugarcane bagasse delignification and juice clarification.

The bottleneck in current xylanase synthesis is the high production cost due to the usage of natural xylan as discovered by Sharma & Bajaj (2017), thus reducing the production costs of enzyme was necessary. Therefore, alternatively, the huge amount of rice straw and oil palm leaf wastes generated increasing the biotechnological interest on the usage of the wastes as inexpensive raw materials replacing the natural xylan which economical production of xylanase is of great importance. The bioconversion process of biomass into reactive intermediates and useful products can be achieved through solid state fermentation. Therefore, the objectives of this study were to obtain the fungus xylanase from rice straw and oil palm leaf as cost-effective substrates via solid state fermentation and to compare its efficacy with commercial xylanase on sugarcane bagasse delignification and juice clarification.

METHODOLOGY

Materials Preparation

The oil palm leaf (OPL) and rice straw (RS) were collected from oil palm plantation and paddy field, respectively in Selangor. Then, both OPL and RS were washed thoroughly to make them dust-free with tap water, dried and milled using blender and sieved between 1 to 3 mm of fibrous length ground into small particle. Next, they were dried in the oven at 70^{o}C until it kept a constant weight as mentioned by Norazlina, Halim and Shareena (2015).

Physicochemical Characteristics

There were four physicochemical characteristics would be determined. They were moisture content, lignin content, hemicellulose content and ash content. For moisture content, both substrates, rice straw (RS) and oil palm leaf (OPL) were determined by weighing 10g of substrates and placed on aluminium foil. The drying process for both RS and OPL were occurred in hot oven at 100^{o}C for 3 hours. The substrate was then cooled and weighted and re-dried until a constant weight was obtained (AOAC, 2000). The lignin content was determined according to Tappi T222 om-88 with some modifications. About 2 ml of 72% H_2SO_4 (v/v) and 0.2 g of wastes materials was added in 100ml of flask. The mixtures were stirred for 60 minutes at room temperature. Then, 56 ml of distilled water was added into the flask and autoclaved at

120ºC for 15 minutes. After that, the mixtures were filtered by using glass microfiber filter. The residue was washed with hot water until pH 7 and dried at 100℃ overnight. Meanwhile, the hemicellulose content and ash content were measured according to Wise et al., (1946) and Tappi T211 om-93 respectively.

Microorganism and Subculture

The strain of *Aspegillur niger*, is a fungi which was conserved in Microbiology Laboratory. Potato dextrose agar (PDA) agar was prepared by 39 g of PDA powder in 1000 mL of distilled water and autoclaved at 121ºC for 20 minutes. Then, the PDA was cooled to 45-50ºC and poured into sterile petri dishes. The prepared agar was allowed to solidify. The subculture of A. niger was performed by using aseptic technique. The A. niger was subcultured on the PDA plate and incubated for five days at 28ºC. The well growth of *Aspergillus niger* on PDA would be kept in chiller at 4ºC, with regular subculturing every 4 weeks (Kogo et al., (2017).

Inoculum Preparation

After five days of growth of fungi, inoculums were prepared by scraping the sporangium from PDA and suspended in sterilized 25 mL distilled water for 4 petri dishes which next, would make up 100 ml of spore suspension inoculum. Then, the suspension of spores was filtered using Whatman filter paper no.1 and the filtrate was used as inoculum. Next, the inoculum would be kept at 4ºC in chiller (Norazlina, Halim and Shareena 2015).

Solid State Fermentation

The solid-state fermentation was carried out in 250 ml Erlenmeyer flask with size of fermentation weight, 10 g. Firstly, 2.8 g of dried ground rice straw, 0.1 g of sucrose and 0.1 g of yeast were added in 250 ml Erlenmeyer flask. Then, the medium was moistened with 5 ml of distilled water to adjust the final substrate moisture content to 70%. After that, 1 ml of mineral solution was added to the sample as nutrients supplements. The compositions of the mineral solution were 6.48 g of NH_4NO_3, 0.268 g of $CaCl_2$, and 0.00248 $MnSO_4.7H_2O$ in 200 ml beaker. The mixture was firstly, dissolved in 100 ml of distilled water (dH_2O). Then, the mixture was heated and simultaneously stirred on a hot plate with magnetic stirrer until clear solution was obtained (Tony et al., 2010). Next, according to Pal and Khanum (2010), fermentation medium was autoclaved at 120ºC for 15 minutes. After the medium was cooled, the flasks were inoculated with 1 ml of the *Aspergillus niger* inoculum and were incubated for 5 days at 30ºC. The procedures were repeated by replacing the dried ground rice straw with oil palm leaf as the substrate.

Extraction of Xylanase

The extraction was done according to Kogo et al. (2017) with some modifications where the crude xylanase was extracted from fermented substrates with 70 mL of distilled water. The mixture was vigorously homogenized on rotary shaker for 30 minutes at 180 rpm. The solid biomass residues were separated from the suspension by filtration through Whatman no.1 filter paper. Subsequently, the residues went through centrifuged at 12 000 x g, 4ºC, 20 minutes. The cell free supernatant was used as the source of the crude xylanase preparation.

Xylanase Assay

Xylanase activity was assayed and incubated in water bath at 50°C, in 0.05 M of citrate buffer at pH 5.3 for 20 minutes. Enzyme yield was expressed as U/g of dry bagasse. Xylanase activity was determined using 1% of xylan birchwood functioned as substrate in the enzyme reaction. The reducing sugar was determined using dinitrosalicylic acid method (DNS) with D-xylose

as standard reference at 540 nm through spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme which releases1 μmol of D-xylose in 1 minute under the assay condition (Norazlina, 2016).

Lignin Content Determination

Two milliliters of 72% H_2SO_4 (v/v) and 0.2 g of dried sugarcane bagasse that was treated with enzyme was added in 100 ml of flask. The mixtures were stirred for 60 minutes at room temperature. Then, 56 ml of distilled water was added into the flask and autoclaved at 120ºC for 15 minutes. After that, the mixtures were filtered using glass microfiber filter. The residue was washed with hot water until pH reached 7 and dried at 100ºC overnight (TAPPI).

Juice Clarification

The juice clarification process was carried out by mixing 10 ml of juice and 0.1% of crude xylanase enzyme from rice straw at different concentration as 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, 2.5 g/L and 3.0 g/L. Then, the mixtures were incubated in water bath at 40ºC for 120 minutes. The juice pH was maintained in the range of 4.6 to 4.8. At the final stage of enzyme reaction, the mixture was heated up at 90ºC for 5 minutes to inactivate the enzyme reaction. Then, the treated juices went through a centrifuge process at 3000 g for 10 minutes. The supernatant was collected and was filtered through a Whatman no.1 filter paper. The clarity was determined by taking the absorbance at 660 nm using UV spectrophotometer. For reference purpose, distilled water was used and the same process was repeatedly using crude xylanase from oil palm leaf and commercial xylanase. After the delignification of sugarcane bagasse and juice clarification, reducing sugar was estimated as xylose equivalent by dinitrosalicylic acid (DNS) method (Gangwar, Prakash and Prakash, 2014).

RESULTS AND DISCUSSION

Delignification of Sugarcane Bagasse

The delignification process was conducted by hydrolysing the sugarcane bagasse with sufficient amount of crude xylanase from different sources (rice straw and oil palm leaf) and commercial xylanase in different concentration such as 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, 2.5 g/L and 3.0 g/L.

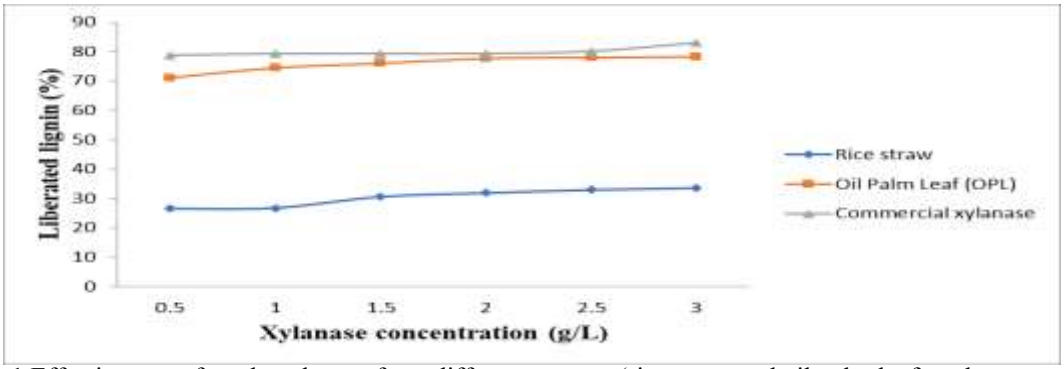

Figure 1 Effectiveness of crude xylanase from different sources (rice straw and oil palm leaf) and commercial xylanase on sugarcane bagasse delignification.

Figure 1 shows the effect of crude xylanase from different sources (rice straw and oil palm leaf) and commercial xylanase on delignification of sugarcane bagasse. The enzyme concentration was the main contribution in this process because the effectiveness of the

enzymes was increased if the concentration is increased. In most instances, commercial xylanase was more efficient than the crude enzymes because the higher percentage of lignin liberated from bagasse was achieved by the commercial xylanase. In comparison between the crude enzymes, xylanase from oil palm leaf (OPL) was more efficient than the xylanase from rice straw.

The concept behind the delignification process was to reduce the usage of bleaching chemicals. In the pulp and paper industries, residual lignin from pulp was liberated using chemicals. This involves many problems including amount of energy consumed, requirement of large volume of chemicals and environmental polluting. Therefore, xylanase was used in bleaching process because bleaching with xylanase has already proven its potential as an environmentally friendly bleaching technology. The use of xylanase as a bio-bleaching process allows the savings of chemicals (Gangwar, Prakash and Prakash, 2014).

Content of Reducing Sugar (Xylose)

After hydrolysed process, the mixture was filtered to separate the residue from the solution. The reduced sugar content of filtrate was estimated as xylose equivalent by dinitrosalicylic acid (DNS).

Regarding to this matter, the application of crude xylanase from rice straw and oil palm leaf on hydrolysing the sugarcane bagasse has been compared with commercial xylanase. Figure 2 shows that the effectiveness of enzymes on this application was increased as concentration of enzymes increased. The absorbance reading in the graph indicates the measurements for reducing sugar determination. In comparison, commercial xylanase was highly effective on hydrolysed the bagasse than the crude enzymes. Based on the graph, crude xylanase from OPL was more efficient in hydrolysing the sugarcane bagasse than the crude xylanase from rice straw.

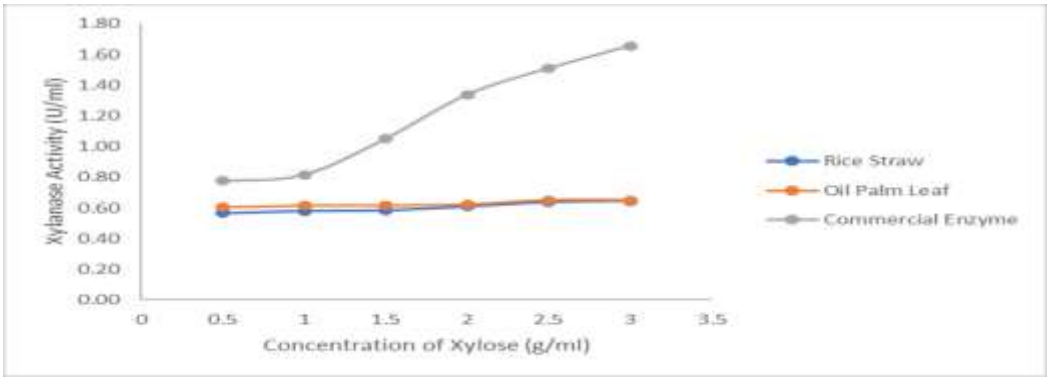

Figure 2 Effectiveness of crude xylanase from rice straw, oil palm leaf and commercial xylanase on hydrolysis of sugarcane bagasse (xylose absorbance).

Juice Clarification

Citrus microcarpa juice was clarified by using crude xylanase extracted from rice straw and oil palm leaf. The process also was done by using commercial xylanase for comparative purpose. The enzyme was used at difference concentration as 0.5 g/L, 1.0 g/L, 1.5 g/L, 2.0 g/L, 2.5 g/L and 3.0 g/L at incubation at 400^{o}C for 120 minutes. Figure 3 shows a comparison of xylanase effectiveness from sources (rice straw, oil palm leaf and commercial xylanase) in juice clarification. The clarity value was obtained by subtracting the absorbance value of juice without clarification from the absorbance value of juice after clarified. Therefore, the higher absorbance value indicated a clearer juice. The clarity mainly depends on the enzyme

concentration and Figure 3 was the evident because it shows that the clarity was increased when concentration of the enzyme from three difference sources were increased. In comparison, the different sources of enzymes were showed positive results where it was effectively function on the clarification of juice.

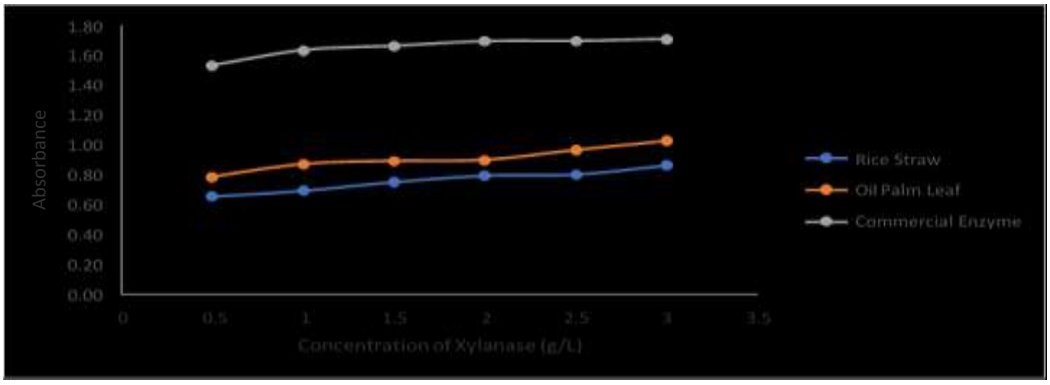

Figure 3 Comparison of effectiveness of xylanase from rice straw, oil palm leaf and commercial xylanase on juice clarification

Based on Figure 3, the commercial xylanase showed the high effectiveness on clarification of the juice if compared with other two different sources of xylanase. The xylanase from oil palm leaf (OPL) was in second rank on effectively clarified the juice and the xylanase extracted from rice straw showed the lowest effectiveness on clarification citrus microcarpa juice. The commercial xylanase showed the highest effectiveness on these applications because it is-purified enzyme and therefore able to degrade and breakdown all the colloid suspension to produce clearer juice. In contrast, the xylanase from rice straw and oil palm leaf (OPL) were also showed the positive results in clarification fruit juice but less effective if compared to commercial enzyme. These crude enzymes were not purified and contain other enzyme or substance which may inhibit the clarification process. These is one of the reasons to show crude enzymes are not effective as commercial enzyme.

CONCLUSION

The production of xylanase from OPL and RS by solid state fermentation (SSF) was the alternative way for better usage of waste biomass due to its inexpensive and readily available materials. The application of crude xylanase extracted from OPL and RS on delignification of sugarcane bagasse and juice clarification was compared with commercial xylanase. By contrast, commercial xylanase was more efficient in all the applications compared to other crude xylanases. This was because the commercial xylanase was highly pure whereas the crude xylanase that extracted from OPL and RS were not purified and may contain other substances or enzymes. Interestingly, crude xylanase from OPL was more efficient compared to crude xylanase from RS. This may due to the less lignin content in OPL than the RS. Lignin is an undesirable polymer, and its removal required high energy. If lignin content is low, xylan in the substrate tend to be easily degraded by *Aspergillus niger* and produced high xylanase activity.

ACKNOWLEDGEMENT

The authors acknowledge the research grant provided by the Universiti Selangor under the Bestari Research Grant (GPB-02/UNISEL17/ST007).

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