Development of xylanase as detergent additive to improve laundry application

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Abstract. The development of kitchen waste from UNISEL’s cafeteria as a substrate in the production of xylanase is an alternative way of converting waste to wealth. The production of xylanase as a detergent or detergent additive capable of removing plant stains compared to limited detergent applications that are unable to remove plant stains. Aspergillus niger was used in this study to degrade hemicellulose in kitchen waste and to secrete xylanase by solid state fermentation (SSF). Optimization of SSF parameters including substrate moisture content, inoculum size and incubation temperature were determined to optimize xylanase production. The finding of maximum activity of xylanase was identified at 80% of the moisture content, 8.0 mL of inoculum size and 30°C of the incubation temperature. The efficacy of xylanase (from optimized SSF parameters) as a plant stain remover is significantly effective compared to the commercial detergent used in this research. However, the mixture of detergent and xylanase can cause the chemical content of the detergent to interfere with the action of the enzyme and its efficacy in the removal of plant stains is less effective. It is also easier to use xylanase with water due to it is capable of producing excellent performance. This study demonstrated the high efficiency of the washing process using xylanase from kitchen waste and can be suggested for the laundry industry.

Keywords: Xylanase, Solid state fermentation, Detergent additive, Laundry application

1. Introduction

In recent years, interest in xylanases has markedly increased mainly due to their usage in the pulp and paper industry [1]. Xylanase is a hemicellulose enzyme produced by beneficial microorganisms such as filamentous fungi like Aspergillus niger. Xylanase shows great prospective for industrial applications mainly for the bioconversion of lignocelluloses to sugar, pulp and paper industry, animal feeds, deinking processes of waste papers and clarification of juices and wines [2]. They are required in bulk amounts and have significant application in paper and pulp industries as hydrolysis of xylan releases lignin from paper pulp and reduce usage of chemical bleaching agents. The application of enzymes like xylanases, cellulases or laccases in paper pulp bleaching is important as they reduce release of pollutants during bleaching and can also enhance the bleaching effect of chemical reagents by affording substantial savings [3].

Currently, the invention relates to the usage of enzymes in cleaning applications, especially in household cleaning applications are namely proteases, lipases, amylases and cellulases. Nonetheless, these enzymes are not able to remove all kinds of dirt, soil or stains present on or in textiles and on kitchenware. Stains from plants, for example grass, vegetables such as spinach, beetroot, carrot, tomatoes, fruits such as all types of cherries and berries, peach, apricot, mango, bananas and grapes as...
well as stains from drinks derived from plant material, such as wine, beer, fruit juices and additionally tomato sauce, are not sufficiently removed by current detergents alone. Pigments in these vegetables or plant materials are associated with the fibrous materials which are a major part of the plant cell walls, either via covalent bonds or via physical binding (“sticking”). Removal of these pigments can be very difficult because in general its compositions are mainly comprising of non-starch polysaccharides; known as cellulose, hemicellulose and pectin. For the most efficient removal of stains enzymes are preferred as these enzymes cut polymeric fibre compounds into smaller pieces and therefore increase the solubilization of the fibre mass with its associated pigments [4]. Normally detergents comprise a bleaching agent and through oxidative reactions, decolourizes the stains, but does not remove them. In addition, these bleaching agents may cause damage to the object to be cleaned, especially when it has to be cleaned often [5], harmful to environment and users. Studies regarding on xylanase activities in cleaning application is very limited. Due to the effectiveness of xylanase as a bio bleaching agent, this study was aimed to the development of xylanase from Aspergillus niger as environmentally friendly detergent additive to improve laundry application.

2. Methodology
2.1 Materials and kitchen waste as substrate
Kitchen waste from UNISEL (Universiti Selangor) Cafetaria was used as a solid substrate fermentation medium (SSF) for the production of xylanase. Organic kitchen waste was sorted by discarding bones and egg shells, with only boneless meat, vegetables, rice and chicken meat remaining. The collected waste was sun-dried during the day and left in the drying oven (80°C) for three days at night. Dry food kitchen waste then blended to produce a fine powder. Aspergillus niger strain was grown on potato dextrose agar (PDA) for experimental purposes and maintained in the Microbiology Laboratory, UNISEL. For the preparation of solid media for culture A. niger, PDA powder (7.8 g) was weighed and dissolved in 200 mL of distilled water. The mixture solution was autoclaved at 121°C for 15 minutes to prevent contamination. After cooling to 44-50°C, the medium was poured into a petri dish and allowed to solidify for 30 minutes following the standard protocol.

2.2 Inoculum and fermentation medium preparation
Spores of A. niger which were grown on PDA for 5-6 days (30°C) were transferred to 25 mL of sterile distilled water for each 6-petri dish. Suspension consisting of 150 mL of spore for further use as a stock suspension. The suspended cultures were filtered using the Whatman no.1 filter paper. The filtrate was used as an inoculum. The fermentation medium composition consists of 20% kitchen waste, 10% inoculum, 3% sucrose, 7% peptone, 50% distilled water and 10% mineral solution. The composition of the mineral salt solution as follows (in g/L), NH4NO3: 16.2; CaCL2:0.67; MnSO4.H2O: 0.062. The contents were thoroughly mixed and autoclaved at 121°C for 15 minutes to prevent contamination. After cooling to 44-50°C, the medium was poured into a petri dish and allowed to solidify for 30 minutes following the standard protocol.

2.3 Solid-state fermentation
The cultivation of A. niger was conducted using SSF with a total fermentation weight of 10 g. The kitchen waste powder of 2.8 g was placed as a substrate in 250 mL of Erlenmeyer flasks. Furthermore, the mineral solution of 1 mL was added to the samples. The medium was moisture with 5 mL of distilled water to adjust the final moisture content of the substrate to 70%. Manipulating distilled water in a similar manner was performed to achieve a moisture content of 50%, 60%, 80% and 90% of the final substrate. After the moisture content was achieved, the medium was autoclaved at 120°C for 15 minutes. After the medium cooled, the flask was inoculated at various inoculum volumes (5mL, 6mL, 7mL 8mL and 9mL) and incubated at different incubation temperatures (25°C, 30°C, 32°C, 37°C and 40°C) for seven days. The three parameters were evaluated, consisting of moisture content, inoculum size and incubation temperature, to produce the optimum yield of xylanase.
2.4 Xylanase enzyme extraction

The fermented substrate was extracted with 70 mL of distilled water. These extraction conditions were optimized in the previous study by Tony et al.[6]. The solution was intensely homogenized at 180 rpm for 30 minutes and the solid biomass residues were removed from the suspension by filtration via Whatman filter paper No. 1. The resulting filtrate was centrifuged at 12000 x g for 20 min to eliminate cell and residual waste [7]. The cell-free supernatant was used as a fresh enzyme for xylanase assay.

2.5 Xylanase assay and standard curve

Xylanase activity was measured by taking 1.5 mL of the reaction mixture consisting 0.5 mL of supernatant and 1 mL of 1% (w/v) birchwood xylan in 0.05 M citrate buffer (pH 5.3) [6]. At 50 °C for 20 min, the mixture was incubated in a water bath. Approximately 3 mL of DNS was added to the mixture. Furthermore, the mixture was centrifuged at 12000 rpm for 15 min. The enzyme reaction was accomplished by boiling the mixture for 5 min at 100 °C. The samples were analyzed at 540 nm by using spectrophotometer. The reduced sugar content was measured with D-xylose as standard at various concentrations. The xylanase assay and the D-xylose standard were performed synchronously to measure the enzyme activity. A standard curve was generated based on different concentrations of D-xylose in combination with an Absorbance reading at 540 nm. The production of xylanase was calculated based of the linear regression of the standard curve using xylanase extraction of the fermentation product samples and the value must be within the range. As a consequence, one unit of enzyme activity is the amount of enzyme that releases 1 μmol of D-xylose in 1 minute under the assay condition.

2.6 Washing performance evaluation

Clean cotton cloth (6cmx6cm) was colored with dragon fruit. The stained clothes were washed in separate flasks of 100 mL using four cleaning agents that including 1) water containing commercial detergent (1g/100 mL) and 5mL of xylanase (50µg/mL); 2) water containing commercial detergent (1g/100 mL); 3) water containing 15mL of xylanase (50µg/mL) and 4) water containing 10mL of xylanase (50µg/mL). Each flask was incubated at 30°C for 30 min under agitation (120 rpm). After incubation, the absorbance of fabric soaking water was measured by spectrophotometer at 540nm. The efficacy of the cleaning agent was determined by the removal of contaminated fabric stain by an absorbance reading resulting from enzyme activity. The high reading absorbance refers to the high content of the particles due to the removal of the stain by a cleaning agent. As a result, the enzyme activity in the removal of stained clothing water from each cleaning agent was measured in order to determine the best efficiency of the cleaning agent.

2.7 Statistical analysis

Data on the production of xylanase with relation to moisture content, inoculum size and incubation temperature were expressed as mean and standard deviation of mean. The wash performance assessment of the cleaning agent was also measured by mean and standard deviation of mean via an enzyme activity assessment. All mean of dependent variables were compared by a one-way ANOVA followed by Tukey post hoc test analysis to determine the differences between the multi-group mean. The level of significance selected was p<0.05.

3. Result and Discussion

The optimization of xylanase production was observed for seven days on different moisture content, incubation temperature and inoculum level. The presence of xylanase enzyme in seven days with 80% moisture content, 30°C incubation temperature and 8.0 ml inoculum size were found as the best result for the xylanase production by A. niger. Furthermore, xylanase activity was analyzed by the standard curve resulted from xylanase assay with different concentrations of D-xylose toward absorbance at 540 nm. The production of xylanase was calculated based of the linear regression of the standard curve using xylanase extraction of the fermentation product samples as shown in Figure 1.
Figure 1. Standard curve with different concentrations of D-xylose toward absorbance at 540 nm.

Xylanase production mostly influenced by the initial substrate moisture content in the medium culture. Several agricultural waste like kitchen waste contains high carbon sources which suitable for the growth of \textit{A. niger} as well as for xylanase production. The finding in this study revealed that 80\% substrate moisture content had the highest enzyme production with 69.3 \textmu g/ml as shown in Figure 2. Surprisingly, the xylanase production decreased with the increasing of substrate moisture content. This may due to the decrease of the nutrients in the substrate which affected the growth of the microbial agent and led to the poor production of the enzyme. Previous study stated that growth of fungal strain increased maximumly on seven days incubation, hence secreted total protein and sugar were found to be maximum after 6 days of incubation [8].

Figure 2. Effect of initial moisture content on xylanase production by \textit{A. niger}. Bar represents the mean ± standard deviation. Bars showing similar superscript letters are not significantly different at \(p > .05\).

Substrate were inoculated with different volume of inoculum for seven days. The finding shows that the highest enzyme production was obtained at 8.0 ml of an inoculum level with 66.3\textmu g/ml of xylanase production. The enzyme production decreased with an increasing volume of inoculum size. The reduction may due to the imbalanced ratio of nutrients in the substrate to the amount of biomass. The
higher the inoculum size means there will be a large amount of volume of initial biomass which will compete for nutrients and oxygen in the substrate.

![Graph showing effect of inoculum size on xylanase production by A. niger.](image)

**Figure 3.** Effect of inoculum size on xylanase production by *A. niger*. Bar represents the mean ± standard deviation. Bars showing similar superscript letters are not significantly different at $p > .05$.

Incubation temperature is one of the important parameters that determine the enzyme production under solid-state fermentation. Figure 4 below shows that 30°C is the optimum incubation temperature with 70.0 ug/ml of xylanase production. This finding is in line with previous study which stated that 30°C was the optimum temperature for xylanase production [9]. Above and below than the optimum temperature fungal growth was inhibited and the xylanase production decreased sharply.

![Graph showing effect of incubation temperature on xylanase production by A. niger.](image)

**Figure 4.** Effect of incubation temperature on xylanase production by *A. niger*. Bar represents the mean ± standard deviation. Bars showing similar superscript letters are not significantly different at $p > .05$. 
3.1 Washing performance evaluation

For detergent application, the presence of xylanase enzyme with 80% moisture content was tested. Figure 5 shows the result of wash efficiency evaluation of cleaning agent types through enzyme activity assessment. Based on the figure 5, the best efficiency of xylanase activity for detergent application was found when water added with xylanase (15ml) which gives 0.475 U/Min reading.

![Figure 5](image)

**Figure 5.** Wash efficiency evaluation of cleaning agent types through enzyme activity assessment. Bar represents the mean ± standard deviation. Bars showing similar superscript letters are not significantly different at $p > .05$.

Enzyme-based detergents usually used in small quantity as compared to synthetic chemicals. They can work at very low temperature, environment-friendly and completely biodegradable rather than removal of proteins by non-enzymatic detergents which can result in permanent stains due to oxidation and denaturing caused by bleaching and drying. The effectiveness of xylanase from *A. niger* as a bio bleaching agent was confirmed when this enzyme was tested for the bleaching cellulose pulp where a significant reduction in Kappa number and a gain in brightness were observed [10]. Reduction in kappa number, brightness increases and release of reducing sugars showed the potentiality of this fungal strain and its crude xylanase effect in paper and pulp industry [81].

Result above revealed new findings which shown that xylanase able to act as plant stain removal or bleaching agent without added with detergent. Xylanase itself with water able to work efficiently to remove plant dyes. These findings in line with the previous study which stated that xylanase hydrolyse the direct polysaccharide beta-1,4-xylan into xylose, in this manner separating hemicellulose, one of the significant parts of the plant cell wall. These compounds arise mainly from the reactions between residual lignin present in wood fibres, causing the brown colour of unbleached pulp, and the chlorine used for bleaching [11]. However, the mixture of detergent and xylanase can cause the chemical content of the detergent to interfere with the action of the enzyme and its efficacy in the removal of plant stains is less effective.

4. Conclusion

In this study, kitchen waste was used as a substrate for the processing of valuable product, xylanase, and can be used in various industrial and biotechnological sectors. The suitability of xylanase used as a cleaning agent was evaluated. Three parameters, such as moisture content, inoculum size and incubation temperature, were studied in order to achieve optimum xylanase production. It shows that 80% of the
moisture content, 8mL of inoculum size and incubation temperature at 30°C produced high yields of xylanase. As a consequence, xylanase activity was recorded at 0.475 U/min in a cleaning agent consisting of a mixture of water and 750 μg/15 mL of xylanase. It is suggested that the quantity of 750μg/15mL of xylanase is sufficient to remove plant stain. Since xylanase is derived from kitchen waste, the cost of production can be minimized and made available to customers at lower prices compared to cleaning agents on the market. Re-use of waste kitchen not only reduces the cost of detergent, but can also help in reducing land and water pollution.

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Reference
[1] Souza M C O, Roberto I C, Milagres A M F 2014 52, 768–772.
[2] Pang P K and Ibrahim C O 2005 Songklanakarin Journal Science Technology, 27, 325-336.
[3] Valls C and Roncero M B 2009 Bioresour Technol, 100, 2032–2039
[4] Roelck A M A H, Cuperus A J J, Ooijen V, Dick J V S 1999 United States Patent.
[5] Alazard D and Raimbault M E J 2013 Appl. Microbiol. Biotechnol,199-209.
[6] Tony J F, Bo-Chin L and Shu-Chih L 2010 N Biotechnol, 27, 25-32.
[7] Ghanem N B, Yusef H H and Mahrouse H K 2000 Bioresource Technolg,73, 113-121
[8] Sridevi A, Sandhiya A, Ramanjaneyulu G, Narasimha G and Suvarnalatha D P 2016 springer link, 6 165
[9] Sharma T, Dassani S and Richhariya J 2020 J Adv Sci Res, 2, 45-51
[10] Polizeli MLTM., Rizzatti ACS, Monti R., Jorge J.A., Amorim D S 2006 Appl Microbiol Biotech, 577-591
[11] Ellaiah P, Adinarayana K, Bhavani Y, Padmaja P and Srinivasulu, Biochemistry, 2002, pp. 615-620