CELLULASE PRODUCTION BY *TRICHODERMA REESEI* AND ITS APPLICATION IN HYDROLYZING OIL PALM EMPTY FRUIT BUNCHES

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This study aimed to optimize cellulase production from *Trichoderma reesei* and apply it for the hydrolysis of oil palm empty fruit bunches (OPEFB). The effects of substrate, pH, nutrient, incubation period, and temperature on cellulase production were investigated using the solid-state fermentation method. OPEFB hydrolysis involved varying enzyme loadings (5, 10, 15, and 20 U/g substrate). The results indicated that the highest CMCase activity (1.02±0.008 U/mL) was achieved under optimal conditions, which included using rice bran as the substrate at 30 °C, pH 6.5, without nutrient addition, and an incubation period of 6 days. In OPEFB hydrolysis, the highest concentration of reducing sugars, 2.395 mg/mL, was observed with a 10 U/g enzyme loading after 48 hours of hydrolysis. FTIR results revealed that the characteristic absorption band at 1205 cm^{-1}, representing the C1-O-C4 glycosidic bond of cellulose, was not observed in the sample hydrolyzed at the 10 U/g enzyme loading. This suggests the capability of the enzyme to hydrolyze OPEFB.

Keywords: cellulase, *Trichoderma reesei*, solid-state fermentation, hydrolysis, oil palm empty fruit bunches

INTRODUCTION

Cellulase is an enzyme that plays a crucial role in the breakdown of cellulose, a complex carbohydrate found in plant cell walls.[1] Cellulase has significant industrial applications, particularly in the production of biofuels and in the textile and paper industries. In the textile and paper industries, cellulase is employed to modify or remove cellulose fibers, improving the texture and appearance of fabrics and enhancing the quality of paper products.[2,3] In biofuel production, cellulase is used to convert cellulose-rich biomass (*e.g.*, agricultural residues) into sugars, which can then be fermented into ethanol or other biofuels.[4]

Cellulase is produced by various microorganisms, including bacteria, fungi, and archaea.[5] These enzymes work by catalyzing the hydrolysis of the β-1,4-glycosidic bonds that link individual glucose molecules in cellulose chains. As a result, cellulose is converted into simpler sugars like glucose.[6] There are different types of cellulase, including endoglucanases, exoglucanases (cellobiohydrolases), and beta-glucosidases, each with specific functions in cellulose degradation.[7] These enzymes often work

synergistically to efficiently break down cellulose into its constituent sugars. Cellulase research and applications continue to evolve, with ongoing efforts to improve enzyme efficiency, lower production costs, and expand their use in various industries, including bioenergy and waste management.[4]

Cellulase produced by the fungus *Trichoderma reesei*, often referred to as *Trichoderma reesei* cellulase or simply *Trichoderma* cellulase, is one of the most widely used cellulases in various industrial applications.[8,9] Optimization of cellulase production was conducted by Rosyida *et al*. using liquid fermentation on rice straw substrate with the *T. reesei* FNCC 6012. However, the enzyme activity obtained was still low, at 0.041 U/mL.[10]

Therefore, the objective of this study has been to enhance cellulase production from *T. reesei* FNCC 6012 by employing the solid-state fermentation (SSF) method, with the final goal of increasing enzyme activity. Solid-state fermentation offers advantages for cellulase production, such as the use of low-cost agricultural residues and reduced water consumption. This study involved the optimization of various parameters, which included the selection of the solid substrate (media), pH, nutrient supplementation, temperature, and the duration of the incubation period. Subsequently, cellulase obtained from the optimized production will be used as a biocatalyst in the hydrolysis of oil palm empty fruit bunches (OPEFB). The valorization of OPEFB is directed towards managing the waste generated in Indonesia, which accounts for 20-23% of fresh fruit bunches (FFBs) production.[11] Hydrolyzing OPEFB using cellulase obtains reducing sugars, with glucose being the primary component. This sugar can then be utilized as a valuable raw material for synthesizing various chemicals, including bioethanol and organic acids.

EXPERIMENTAL

Materials

For cellulase production, three distinct agro-industrial residues were employed as substrates: rice bran, wheat bran and wheat pollard. These substrates were sourced from a local market. Additionally, oil palm empty fruit bunches (OPEFB) obtained from a palm oil plantation on Sumatra Island were used as a substrate in the hydrolysis process. These OPEFBs underwent alkali pretreatment using a 10% NaOH solution with a solid-to-liquid ratio of 1:5, at a temperature of 150 ºC for 30 minutes. The chemical composition of pretreated-OPEFB revealed 65.82% cellulose, 4.66% hemicelluloses, and 15.40% lignin. *T. reesei* FNCC 6012 strain utilized in this study was procured from the Center for Food and Nutrition Studies at Gadjah Mada University. All chemicals used were of analytical grade.

Methods

Inoculum preparation

A total of 1 colony of *T. reesei* FNCC 6012 culture was transferred to an agar slant and incubated at 30 ºC for five days. The resulting culture was supplemented with 10 mL of sterile aquadest, and spores growing on the surface of the slant agar were collected using an inoculation loop until all spores were dissolved in aquadest. This solution was used as the inoculum to be added to the fermentation media.

Enzyme production

Enzyme production was carried out under solid-state fermentation (SSF). The fermentation media, containing 10 g of substrate and 10 mL of aquadest, were prepared in Erlenmeyer flasks (250 mL) and inoculated with fungal inoculums. The media were sterilized at 121 ºC for 15 minutes and then cooled to room temperature. Then, 1 mL of inoculum was added to the fermentation media and mixed using a spatula. The media were incubated at 30 ºC for 5 days.

Extraction of enzymes

After 5 days of fungal growth, the media were extracted by adding 50 mL of aquadest and stirring until homogenous. Then, the extract was stored in a refrigerator at 4 ºC for 2 hours to stop fungal growth. After 2 hours, the homogenous solution was filtered using cheesecloth. The filtrate was centrifuged at 4 ºC, 8000 rpm, for 10 minutes. The supernatant obtained from the centrifugation was referred to as crude enzyme. The crude enzyme from each substrate selection sample was analyzed for enzyme activity.

Optimization of cellulase production

Effect of different agro-based waste materials as a substrate on cellulase production

Three substrates (rice bran, wheat bran, and wheat pollard) were used in cellulase production through solid-state fermentation (SSF). The crude enzyme from each substrate selection sample was analyzed for enzyme activity. The substrate with the highest enzyme activity was used for pH optimization.

Effect of different pH on cellulase production

Variation of pH (pH 4, 4.5, 5, 5.5, 6, 6.5, and 7) on SSF was used with one substrate selected. The crude enzyme from each pH condition sample was analyzed for enzyme activity and, then, the optimum pH condition was used in the next experiment for the effect of nutrient addition.

Effect of adding nutrients on cellulase production

Addition of nutrients in the form of Mandels' mineral salts (0.25-peptone; 0.1-yeast extract; 2.0-KH_2PO_4; 0.3-$CaCl_2 \cdot 2H_2O$; 1.4-$(NH_4)2SO_4$; 0.005-$FeSO_4 \cdot 7H_2O$; 0.3-$MgSO_4 \cdot 7H_2O$; 0.0016-$MnSO_4 \cdot H_2O$; 0.0014-$ZnSO_4 \cdot 7H_2O$; 0.002 $CoCl_2 \cdot 6H_2O$ (g/L)) to the buffer solution was examined for cellulase production.

Effect of different incubation periods on cellulase production

The optimization method for the incubation period involves assessing the impact of nutrient addition. The fermentation incubation period was varied at 3, 4, 5, 6, 7, and 8 days. The determined optimum incubation time was then employed in the subsequent optimization of incubation temperature.

Effect of different incubation temperatures on cellulase production

The incubation temperature optimization method refers to the incubation time optimization method. The adjustments involved varying the fermentation incubation temperature at 25 ºC, 30 ºC, 35 ºC, and 40 ºC. Optimal conditions for each optimization parameter were then applied for the optimized production of enzymes.

Enzyme production under optimized conditions

Enzyme production involved replicating cellulase production under optimal conditions in six Erlenmeyer flasks. The crude enzyme obtained from each Erlenmeyer flask was combined for protein content analysis and specific activity analysis and then used for enzymatic hydrolysis.

OPEFB hydrolysis

The OPEFB hydrolysis was performed according to the method describe in previous research by Dahnum *et al.*[12] A substrate loading of 50 g/L was employed for hydrolysis in a total volume of 20 mL. Pretreated OPEFB (1 g) was distributed among four Erlenmeyer flasks and mixed with 15 mL, 10 mL, 5 mL, or 0 mL of 0.05M citrate buffer at pH 4.8 to induce variations in enzyme activity (5 U/g, 10 U/g, 15 U/g, 20 U/g). After sterilization at 121 ºC for 15 minutes, crude enzyme (5 mL, 10 mL, 15 mL, 20 mL) was added to each flask for each enzyme activity variation. The hydrolysis process was carried out in an incubator shaker at 50 ºC and 120 rpm. Hydrolysate sampling was conducted every 24 hours for reducing sugar analysis.

Enzyme assay

Enzyme activity was determined using the CMCase method.[13] The materials prepared for enzyme activity analysis included 2% CMC substrate, DNS reagent, crude cellulase enzyme, and 0.05M citrate buffer at pH 4.8. Sample analysis was done by reacting 0.5 mL of buffer, 0.5 mL of enzyme, and 0.5 mL of CMC. Control analysis was performed by reacting 0.5 mL of buffer and 0.5 mL of enzyme. The blank tube only contained 0.5 mL of buffer solution. All three tubes were incubated in a water bath at 50 ºC for 10 minutes. Then, 0.5 mL of DNS reagent was added, and they were placed in a boiling water bath for 5 minutes. Afterward, 6.5 mL of distilled water was added to each tube, and the absorbance was measured at a wavelength of 540 nm using a visible spectrophotometer. A glucose standard solution was prepared at concentrations of 0-1 mg/mL. One unit of the activity was defined as the amount of enzyme that released 1 µmol equivalent of glucose per minute under assay conditions.

Protein content determination of enzyme

Protein content was determined using the Lowry method.[14] The crude enzyme (0.1 mL) was mixed with 3.9 mL of distilled water, 5.5 mL of Lowry C, and 0.5 mL of solution D. The mixture was homogenized and left at room temperature for 30 minutes. The absorbance was measured at a wavelength of 660 nm. A bovine serum albumin (BSA) standard solution was prepared at concentrations of 0-2 mg/mL.

Reducing sugar analysis

Reducing sugars from OPEFB-hydrolysate were assessed using the DNS method.[15] A specified quantity of hydrolysate (1 mL) was mixed with 1 mL of DNS reagent, and the tubes were placed in a boiling water bath for 5 minutes, followed by adding 3 mL of distilled water. Absorbance was measured at a wavelength of 540 nm. A glucose standard solution was prepared at concentrations of 0-0.7 mg/mL.

Characterization of solid residues from OPEFB hydrolysis by FTIR

The characteristization of the lignocellulosic biomass by FTIR was performed by the method reported in previous research by Triwahyuni *et al.*[16] Solid residues from OPEFB hydrolysis were washed with 50 mL of aquadest and then dried overnight at 50

ºC. OPEFB samples were characterized using a Shimadzu FTIR spectrometer and analyzed based on glycosidic bond cleavage.

Statistical analysis

All experiments were carried out in duplicate. Microsoft Excel was used to calculate data means and standard deviations. The effects of varying process conditions, *i.e.* substrate, pH, incubation period, and incubation temperature, on cellulase activity were determined by one-way ANOVA, followed by the least significant difference (LSD) test in ANOVA to assess the significant differences ($P<0.05$). Meanwhile, the cellulase activity with and without adding nutrients was analyzed by the Independent Samples Test. All data were analyzed by the Statistical Package for Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

Effect of different substrates on cellulase production

Nutrients in the substrate are utilized by fungi to secrete extracellular enzymes, which break down complex macromolecules into simpler compounds that can be utilized for growth.[17] Nutrients serve as a source of carbon, a source of nitrogen, and an inducer. In this study, rice bran, wheat bran, and wheat pollard were lignocellulosic substrates used as nutrients for *T. reesei* to secrete cellulase.[18] The cellulase secreted by *T. reesei* breaks down the cellulose in the substrates into smaller molecules that can be utilized by the fungus for growth. Figure 1 shows the CMCase activity on the three selected substrates. Among the three selected substrates, *T. reesei* FNCC 6012 exhibited the highest enzyme activity on rice bran, so further optimization focused on using rice bran as the substrate.

According to Dang and Thava[19] and Gloria *et al*.,[20] the composition of rice bran mainly includes fiber (20.5%–33.3%), starch (16.1%–26.7%), ashes (9.2%–13.9%), protein (13.2%–18.6%), and lipids (9.5%–22.9%). This might be one of the factors that influence the fungal growth and cellulase production when it is used as a substrate for fermentation. However, the one-way ANOVA, followed by the LSD test, indicated no significant difference ($p= 0.124 > 0.05$) between and within groups of different substrates on cellulase production. Since there is no significant difference among them, all three materials could potentially serve as substrates for cellulase production. Still, this study chose rice bran as a substrate considering its relatively higher enzyme activity and its abundant availability in Indonesia, compared to wheat bran and wheat pollard.

Effect of pH on cellulase production

The pH of the media plays a role in changing the morphology, physiology, and metabolism of microorganisms, thereby affecting enzyme secretion.[21] The highest CMCase activity was observed at pH 6.5, with an activity value of 1.183±0.096 U/mL (Fig. 2). There was a significant effect of pH ($p=0.000$) on cellulase enzyme production. Different results were reported by Kalsoom *et al*., where *T. reesei* isolates showed the highest enzyme activity of 1.165 IU/mL at pH 6. Fungi grow and produce cellulase well under slightly acidic conditions.[22] Similar results to this study were reported by Jampala *et al*., where the production of cellulase with high activity was observed in the pH range of 6-7. The highest cellulase activity by *T. reesei* NCIM 1186 in that study was observed at pH 6.5.[23]

Effect of adding nutrients on cellulase production

Nutrient addition only increased activity by 10.31% from 1.183 U/mL to 1.305 U/mL (Fig. 3), so it can be concluded that the growth of the fungus and enzyme production on rice bran were sufficient without the supplementary addition of nutrients. According to the Independent Samples Test, sig. (2-tailed) obtained was $0.063 > 0.05$, thus, there was no significant difference between the results obtained with or without addition of nutrients. Based on Bhosale's research, in 100 grams of rice bran, there are 17.5 g of protein, 13.1 g of fat, 52.33 g of carbohydrates, 52.1 mg of Ca, 28.1 mg of Fe, 6.02 mg of Zn, and 1.185 mg of P.[24] With such composition, rice bran becomes a good substrate, providing enough carbon and nitrogen for fungal growth and enzyme production. Therefore, optimization of time and temperature was continued without the supplementary addition of nutrients.

Effect of incubation period on cellulase production

Cellulase is produced by fungi during their

growth, and enzyme activity is determined through enzyme activity analysis. During its growth process, fungi go through four phases: lag, exponential, stationary, and death phases.[25] Fungal growth and enzyme activity were observed from day 3 to day 8. In this study, we observed the exponential and stationary phases of fungal growth. The exponential phase was characterized by a very high enzyme activity on days 3 to 4, while the stationary phase showed relatively constant enzyme activity from days 4 to 8. The lag phase was not observed because enzyme activity on days 1 and 2 was not measured, while the death phase was not observed because enzyme measurement was stopped on day 8, which was still in the stationary phase of the fungus.

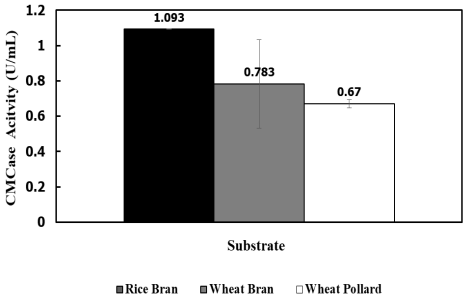

Figure 1: Effect of substrate on cellulase production

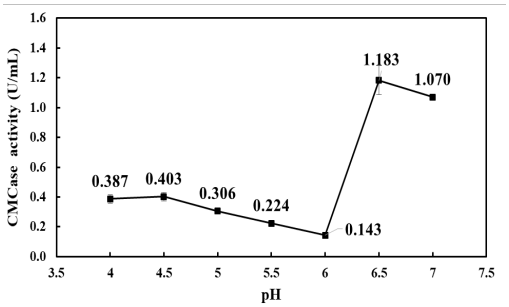

Figure 2: Effect of pH on cellulase production

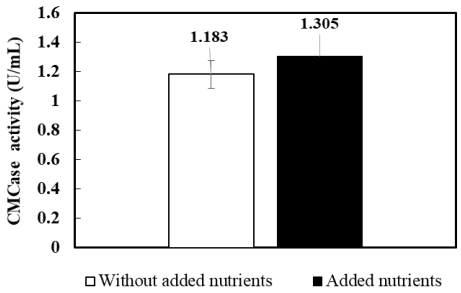

Figure 3: Effect of nutrient addition on cellulase production

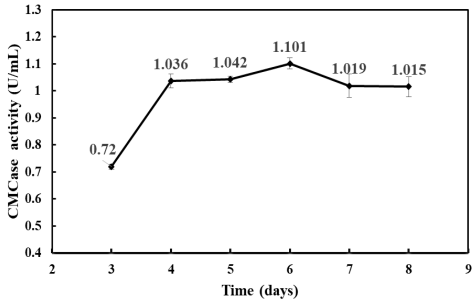

Figure 4: Effect of ıncubation time on cellulase production

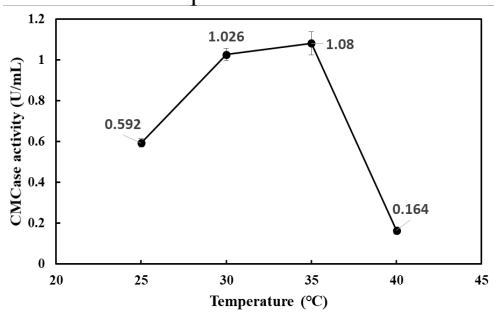

Figure 5: Effect of temperature on cellulase production

The highest cellulase activity was observed on the sixth day, with an activity value of 1.101±0.02 U/mL (Fig. 4). There was also a significant effect of the incubation period (p=0.000) on cellulase enzyme production. Similar results were reported in the study by Lodha *et al*. regarding the optimization of cellulase production from co-cultured *T. reesei* NCIM 1186 and *Penicillium citrinum* NCIM 768, which showed the highest cellulase activity on the sixth day, with an activity value of 2.38 FPU/g.[26] However, this result differs from the findings of the study by Darabzadeh *et al*., on *T. reesei* CECT 2414, which showed cellulase activity of 1.317±0.018 U/g after 4 days

of incubation.[27]

Effect of incubation temperature on cellulase production

Enzyme activity increased with increasing incubation temperature, but after surpassing 35 °C, enzyme activity decreased (Fig. 5). There was a statistically significant effect of incubation temperature on cellulase production ($p=0.000 < 0.05$). Incubation at 30 °C showed lower activity than at 35 °C, with an activity of 1.026±0.029 U/mL. Increasing the temperature by 5 °C from 30 °C to 35 °C only increased enzyme activity by 0.054 U/mL, thus, a temperature of 30 °C was considered more efficient and was chosen as the optimum incubation temperature. An incubation temperature of 30 °C was also reported by Kalsoom *et al*. using *T. reesei* isolates from soil and leaf litter, and the cellulase activity value was 1.165 U/mL.[22] In Darabzadeh's study, the highest enzyme activity of *T. reesei* CECT 2414 was also observed at 30 °C with an activity value of 1.16±0.03 U/g.[27]

Enzyme production under optimized conditions

From the enzyme production process, the optimum conditions were obtained, namely, using rice bran as the substrate at 30 °C, pH 6.5, without nutrient addition, and incubation for 6 days. The enzyme optimization method employed in this research followed a conventional approach, involving a number of experiments to systematically vary process conditions and gather empirical data for each variation.

Other studies have utilized statistical methods to optimize enzyme production, which have the advantage of minimizing the number of experiments, resulting in operational cost savings. Furthermore, these models offer the opportunity to explore potential synergistic interactions among fermentative parameters established in the process. Statistical techniques such as central composite design, Box-Behnken, Doehlert, mixture planning, and artificial neural networks (ANN) have been reported to optimize enzyme production.[28,31] For example, a comprehensive investigation into the production of xylanase via solid-state fermentation (SSF) by *Aspergillus oryzae* ATCC 10124 using two statistical approaches: centroid simplex and Box-Behnken experimental design, found a remarkable increase of up to 165% in enzymatic activity, compared to non-optimized production.[29] In another study, carboxymethyl cellulase was produced via submerged fermentation and characterized, with saccharification optimized using the Box-Behnken design. The optimal pH and temperature for the enzyme produced by *Trichoderma viride* were determined to be 5 and 50 °C, respectively.[30]

In this study, enzyme multiplication was performed by repeating the production process in 6 separate Erlenmeyer flasks under the obtained optimum conditions. The cellulase activity obtained was 1.02±0.008 U/mL. The enzyme activity in this study was higher than the results reported by Rosyida *et al*. with the same strain.[10] During its growth process, fungi produce not only cellulase, but also other proteins. In this study, the protein content wasfound to be 1.299 mg/mL. The dividing of the cellulase activity obtained by the protein content resulted in specific activity of the enzyme. Specific activity is used to determine the purity level of the enzyme. If the specific activity is high, then the purity of the cellulase enzyme produced by the fungus is high. Conversely, if the specific activity is low, other protein products that bind to the enzyme are more abundant, resulting in low enzyme purity.[32] The specific activity of the enzyme was 0.784 U/mg. The low specific activity value indicated that the cellulase enzyme obtained was not purified. Hence, future research aimed at augmenting enzyme activity levels must be conducted. This could involve genetic engineering techniques, as well as refining crude enzymes to enhance their purity.

Hydrolysis of OPEFB

Figure 6 illustrates the concentration of reducing sugars produced through hydrolysis at different enzyme loadings. The hydrolysis at an enzyme loading of 10 U/g substrate achieved the highest concentration of reducing sugars after a 48-hour process. Conversely, the lowest concentration of reducing sugars was observed in the hydrolysis with a 20 U/g substrate loading. Moreover, based on the observations made during sampling, at 24-72 h, there were still many OPEFB fibers in the hydrolysis solution. This may be due to the low activity of the cellulase

enzyme used, resulting in incomplete conversion of cellulose into reducing sugars. In this study, the OPEFB substrate concentration used for hydrolysis was 5%. The total volume of the hydrolysis solution, including buffer and enzyme, was kept constant to maintain the substrate concentration. The addition of more enzymes resulted in less buffer being added. This affected the pH conditions of each hydrolysis solution. The pH conditions for each enzyme variation of 5 U/g, 10 U/g, 15 U/g, and 20 U/g were 5.14, 5.21, 5.40, and 6.27, respectively. The enzyme addition of 10 U/g at pH 5.21 showed the best hydrolysis activity because it had the highest concentration of reducing sugars, making it the optimal pH for hydrolysis. These findings are consistent with the pH range recommended by Sigma-Aldrich (Novozyme), which is pH 5-5.5 for enzymatic hydrolysis of lignocellulosic biomass substrates.[33]

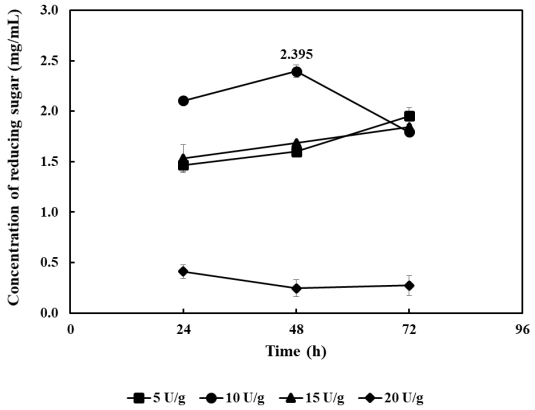

Figure 6: Concentration of reducing sugar after hydrolysis

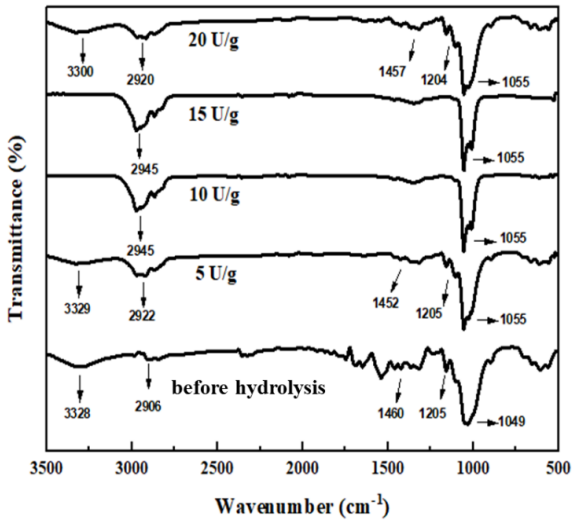

Figure 7: FTIR spectra of solid OPEFB before and after hydrolysis

Similar pH conditions were reported in the study by Torres *et al.*, where hydrolysis of water hyacinth substrate by cellulase from *T. harzianum* PBLA was conducted at pH 5.2.[34] Different pH conditions were also investigated in the study by Astolfi *et al*., who reported optimum results in the hydrolysis of soybean husk substrate by cellulase from *T. reesei* NRRL 3652 at pH 5.3.[35] The sugar concentration decreased after 48 hours, which may be due to fungal spores consuming the sugar in the hydrolysate.[36] The results obtained in this study were higher compared to the study by Sugiwati *et al*.[37] In that study, the NaOH pretreated OPEFB substrate was hydrolyzed by cellulase produced by *Aspergillus niger* and the highest reducing sugar concentration (1.16 mg/mL) was obtained after 72 hours of hydrolysis.

FTIR analysis of hydrolyzed OPEFB solid residue

Figure 7 displays the FTIR spectra of residual solids from OPEFB after hydrolysis. The absorption at 3600-3200 cm^{-1} indicates O-H stretching of cellulose hydroxyl groups. The absorption range of 2960-2830 cm^{-1} indicates C-H stretching of alkyl groups in cellulose, hemicelluloses and lignin.[38] The absorption band at 1454 cm^{-1} indicates O-H bending of cellulose and hemicelluloses. The absorption at 1205 cm^{-1} indicates C1-O-C4 symmetric stretching in cellulose and hemicelluloses.[39] The absorption at 1055 cm^{-1} indicates C-O-C ring pyranose bonds.[40]

Based on Figure 7, it can be seen that there is a similarity in the spectra between the addition of 5 U/g and 20 U/g of enzyme. The spectra in both enzymes show the characteristic absorption bands of O-H and glycosidic bonds in cellulose. The FTIR spectrum of OPEFB before hydrolysis was used as a reference, and it was observed that the characteristic absorption bands that appeared were identical to those of 5 U/g and 20 U/g. Therefore, based on the FTIR spectrum, it can be concluded that enzyme loadings of 5 U/g and 20 U/g exhibited poor hydrolysis capability. The similarity in spectra was also observed for the addition of 10 U/g and 15 U/g of enzyme. The spectra for both enzymes showed that the absorption bands for O-H and glycosidic bonds in cellulose were no longer observed. Based on the FTIR spectrum, it can be concluded that enzyme loadings of 10 U/g and 15 U/g exhibited good hydrolysis results.

The FTIR analysis is consistent with the reducing sugar data analyzed in the hydrolysate. The highest reducing sugar concentration of 2.395 mg/mL was obtained with the addition of 10 U/g of enzyme. FTIR characterization results of solid residue from hydrolyzed OPEFB indicate that cellulose has been hydrolyzed by the enzyme.

CONCLUSION

The optimal conditions for cellulase production using *T. reesei* FNCC 6012 via the SSF method were determined to be utilizing rice bran as the substrate at 30 °C, pH 6.5, without nutrient addition, and with an incubation period of 6 days. The resulting cellulase activity was measured at 1.02±0.008 U/mL, with a protein content of 1.299 mg/mL and a specific enzyme activity of 0.784 U/mg. In OPEFB hydrolysis, the highest concentration of reducing sugars, 2.395 mg/mL, was achieved with a 10 U/g enzyme activity loading after 48 hours of hydrolysis. FTIR results revealed that the characteristic absorption band at 1205 cm^{-1}, representing the C1-O-C4 glycosidic bond of cellulose, was not observed in the sample corresponding to the 10 U/g enzyme loading. This indicates the capability of the enzyme to hydrolyze OPEFB. Further research is recommended for optimizing cellulase production using statistical methods and cellulase purification to enhance the ability to hydrolyze OPEFB for obtaining higher product concentration.

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