Examination the Hydrolysis Feasibility of OPEFB Biomass Using Aspergillus niger as Cellulase Enzyme-producing Fungus

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Abstract: The objective of this study was to obtain optimization results from the biological hydrolysis of Oil Palm Empty Fruit Bunches (OPEFB) using Aspergillus niger (A. niger) BIOTROP 2173 isolated from grain. Optimized hydrolysis parameters include temperature, pH and time. The hydrolysis process was carried out by growing A. niger on OPEFB powder (± 30 mesh) through two schemes, namely hydrolysis on OPEFB pretreatment with 10% NaOH and hydrolysis on OPEFB non-pretreatment. The optimization results show that the best hydrolysis process of A. niger BIOTROP 2173 occurs in OPEFB pretreatment. The optimum conditions for temperature, pH and time obtained are 40°C, 6 and 24 hours, respectively. Although the amount of reducing sugar produced was lower than the OPEFB non-pretreatment, the performance of the cellulase enzyme during the hydrolysis process of OPEFB pretreatment was very good, with a fast hydrolysis rate. These results indicate that the performance of A. niger BIOTROP 2173 in the hydrolysis process is influenced by the pretreatment stage. The optimum conditions obtained then became a reference in the production of reducing sugar based on A. niger BIOTROP 2173. The amount of reducing sugar produced from OPEFB pretreatment was 0.94 mg.mL⁻¹, while for OPEFB non-pretreatment was 15.83 mg.mL⁻¹.

Key words: Aspergillus niger BIOTROP 2173, grain, OPEFB, hydrolysis, reducing sugar

1 Introduction
Nowadays, the biggest challenge of many countries is the problem of energy crisis which is a priority for people’s survival1-2. The projected demand for energy in 2050 almost triples due to an increase in accordance with human development. It seems that the energy problem will still be the main topic that must be solved together3-5. Efforts to obtain alternative energy have long been explored to reduce dependence on fossil energy resources6-7. In 2018, energy demand in Indonesia is very dependent on fossil fuels, this is evidenced by the total final energy consumption(without traditional biomass) of 114 million tons of oil equivalent (MTOE) derived from 40% of transportation, 36% of the industry, 16% of households, 6% of the commercial sector and 2% of other sectors8. Limited fossil energy sources cause the need for renewable energy development and energy conservation9-11. The risk of global warming due to fossil fuel combustion emissions and the increasingly reduced petroleum reserves, increasingly encouraging the development of efforts to produce and use biofuels as alternative energy that is more environmentally friendly and sustainable12. Biofuel can be defined as fuel derived from biomass and produces relatively little CO₂ gas so it is more environmentally friendly13-15. One such biofuel is bioethanol derived from lignocellulosic biomass waste as a very attractive raw material for researchers to continue to explore and optimize the production process as an alternative energy source that

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can be renewable and sustainable development\cite{16-18}. Cellulose which has been separated from lignocellulosic biomass can be converted to glucose through the hydrolysis process and then converted to bioethanol through the saccharification process. OPEFB is palm oil waste with abundant lignocellulosic content\cite{19,20}. In our previous study, we have implemented the OPEFB biomass pretreatment process to produce bioethanol even though quantitative bioethanol production is still low\cite{21,22-25}. We are pleased to develop OPEFB biomass waste because it is abundant biomass waste and every 1 ton of CPO production produces OPEFB lignocellulose waste which reaches 1.1 tons\cite{26,27}. The application of renewable biofuel energy sources needs to be increased given the limited sources of fossil fuels and expensive fuels. OPEFB lignocellulose waste is abundant biomass material that can be applied to obtain biofuels that are cheaper, environmentally friendly, and as a renewable and sustainable material\cite{28-30}. In the production of bioethanol, we need to go through several steps such as pretreatment, hydrolysis, fermentation and product separation\cite{31,32}. So in this study, we focus on the optimization of hydrolysis under biological science related to utilizing $\beta$-glucosidase enzyme derived from $A. niger$ before implementing in the saccharification process (fermentation).

Several hydrolysis processes have been widely studied by researchers using enzymatic, acid and base conditions\cite{33,34}. They have advantages like short time duration, low-cost, and simple preparation. In fact, the loss is to damage environmental pollution, where acid and base solutions can damage the aquatic environment and threaten the life of aquatic biota. Meanwhile, major impediments to exploiting the commercial potential of cellulose materials through enzymatic degradation pathway are slow reaction rates, lack of an ideal reactor system and complexity of interfacial heterogenous hydrolysis influenced by various factors (e.g., structure and composition of cellulosic materials, cellulase adsorption and desorption, enzyme inhibition by cellobiose and glucose)\cite{35,36}. However, enzymatic hydrolysis makes for a safer environment because it is prone to denaturation. Thus, the hydrolysis process is very important to study because lignin content in lignocellulose may hamper the hydrolysis process. Hydrophobic of lignin can disturb the hydrolysis by hindering the enzyme to reach the cellulose and by binding to the enzyme and acting as inhibitors. In a previous study by\cite{27} reported that the hydrolysis process was carried out by growing $A. niger$ on OPEFB which functions to produce cellulase and increase the level of sugar consumption. This is reasonable considering that sugar is the main source of microorganisms\cite{38,39}. In addition, cellulase from $A. niger$ has high $\beta$-glucosidase activity. This is in agreement with\cite{38} where cellulase enzyme from $A. niger$ has the $\beta$-glucosidase activity of 168 U/mL which was ten-fold as compared to that in cellulase 1.5 litres.\cite{39}

In this work, optimization of the OPEFB hydrolysis process by using $A. niger$ as a producer of cellulase enzymes was studied. In general, we went through several steps, namely the pretreatment process under alkaline conditions (NaOH) and then hydrolysis process using $A. niger$ (Fig. 1). Study of temperature changes, pH, time, and cellulase enzyme activity were evaluated to review the effectiveness of the hydrolysis process using OPEFB to produce glucose components. The method we use in the production of cellulase enzymes is Solid-State Fermentation. Although this method is the same as reported by\cite{41-44}, however we use different sources. The source of the cellulase enzyme that we use is grain residue. This residue is waste that can pollute the environment. So this condition makes our study more economical. Nevertheless, the results obtained from this study are able to provide the

![Fig. 1 Schematic diagram of the hydrolysis process by comparing non-pretreatment and pretreatment of OPEFB using $A. niger$, (1) physical pretreatment, (2) pretreatment process, (3) 10% NaOH solution prepared, (4) separation of black liquor and residue, (5) purification of residue, (6) hydrolysis process using $A. niger$, (7) glucose analysis using UV-Vis spectrophotometer.](image-url)
2 Methods
2.1 OPEFB pretreatment
Firstly, physical treatment has been carried out by destructing OPEFB into small fibrous, dried, and sifted to 30 mesh filter. This condition we call as non-pretreatment (unpretreated OPEFB) because it is not yet included in the pretreatment process (Fig. 1). Subsequently, the pretreatment of OPEFB has applied by weighing as much 500 g non-pretreatment OPEFB and adding a 10% NaOH solution by using a chemical explosive reactor (CHEMEX) with a temperature of 150°C for 3 hours. After that, it is filtered to separate the OPEFB residue and black liquor by using separation pump then the residual OPEFB pretreatment is washed by distilled water until pH = 7 and pressed with a hydraulic press to reduce the water content <10%. It was analyzed to observe the chemical constituents such as lignin, cellulose, and hemicellulose compounds by using high-performance liquid chromatography (HPLC) refers to the National Renewable Energy Laboratory (NREL) procedure.

Where the OPEFB residue (1 mm diameter and water content <10%) was weighed as much as 0.3 g and added into glass tube. Then, it added with 72% H₂SO₄ and hydrolyzed for 2 hours at a temperature of 20°C. Every 15 min., it is homogeneous using a vortex tool and transferred into a 100 mL Schott bottle which containing 42 mL distilled water and re-hydrolyzed in an autoclave for 60 min. at 121°C. Afterwards, the sample was cooled to ambient temperature and filtered with a Buchner filter and 0.45 µm filter paper. The filtrate is neutralized with CaCO₃ until the pH is 7.8 mm and covered with aluminium foil which aims to maintain the safety of liquid from spilling when the centrifuge process. Finally, we determine the cellulase enzyme activity of the OPEFB sample using a UV-Vis spectrophotometer, in which the sample was added the 3.0 mL DNS solution and heated on warm water at a 30°C for 30 minutes to increase the rate reaction. Absorbance is determined based on linearity curves for various concentrations of glucose standards.

2.2 OPEFB hydrolysis using Aspergillus niger
In this study, we use of A. niger isolated from grain which is obtained from Phytopathology Laboratory, Southeast Asian Regional Center for Tropical Biology (SEAMEO BIOTROP), Bogor. In this step, OPEFB residue from the pretreatment process was weighed 250 g then inserted into 8 Erlenmeyers and added 0.05 M citrate buffer. It is inserted into an autoclave for ±1 hour with a temperature of 120°C. Subsequently, it is put into a laminar airflow system to apply the hydrolysis process using A. niger. It is entered into the incubator shaker at a temperature (30°C, 35°C, 40°C, 45°C) and the time variation of 24, 48, and 72 hours. These steps also apply for non-pretreatment OPEFB (Fig. 1 (6)). After that, the decantation process was applied to separate the water content from OPEFB to the glass tube and covered with aluminium foil which aims to maintain the safety of liquid from spilling when the centrifuge process.

| Composition (% | No pretreatment | Alkaline pretreatment (10% NaOH) |
|----------------|----------------|----------------------------------|
| Cellulose      | 36.59          | 54.24                            |
| Lignin         | 26.53          | 17.42                            |
| Hemicellulose  | 24.97          | 18.13                            |
| Others         | 11.91          | 10.21                            |

Table 1 The chemical constituents of OPEFB before pretreatment.

3 Results and Discussion
3.1 Chemical constituents of OPEFB pretreatment process
In the initial stage, we determined the chemical content of OPEFB biomass before committing of the pretreatment process. The purpose of this stage is to represent the chemical constituents contained in the OPEFB biomass waste. Table 1 shows the chemical constituents of OPEFB waste before going through the pretreatment process. The high cellulose content of 36.59% explains that the largest chemical constituents in OPEFB are cellulose, lignin, and hemicellulose. The same result was shown by that before pretreatment, chemical contents such as cellulose, hemicellulose, and lignin were also examined with values of 35.2%, 17.9%, and 24.1%, respectively. According to also reported that in OPEFB biomass waste contains high lignin and cellulose compounds, this of course provides the advantage to be converted into renewable bioenergy materials. Unfortunately, the lignin content in lignocellulose can also inhibit the hydrolysis process. Therefore, chemical pretreatment must be carried out to increase the cellulose content and increase swelling, increase the internal surface of the cellulose and decrease the degree of crystallinity and further increase lignin disruption. Lignin has hydrophobic properties so that it interferes with the hydrolysis process.
by inhibiting the enzyme to react cellulose and by binding to the enzyme and acting as an inhibitor. In addition, it can also reduce cellulose hydrolysis by passively binding to cellulolytic enzymes. Analysis of ash content as complementary data representing undetectable chemical constituents in OPEFB biomass.

The mechanism of enzymatic hydrolysis of OPEFB into glucose (reducing sugar) is a complicated process because the cellulase enzyme consists of at least endoglycanase (EG), cellobiohydrolase (CBH), and β-glucosidase (βG). However, this complex process can be simplified into two stages as shown in Fig. 2. The first stage is a heterogeneous reaction between the insoluble substrate (cellulose) and the dissolved enzyme. This stage produces oligosaccharides which are dissolved through the synergistic action of CBH and EG which is considered as the control step for the whole hydrolysis. The second stage is a homogeneous reaction to the breakdown of oligosaccharides into glucose which is catalyzed by βG.

3.2 Optimization of the hydrolysis process

3.2.1 Standard curve to analyze of reducing sugar

Determination of the standard curve to obtain reducing sugar content in each variation is to vary the concentration of 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mg.mL$^{-1}$ from a standard solution of glucose 100 mg.L$^{-1}$. The purpose of this step is to determine the level of reducing sugar in each variation by plotting linear regression to the absorbance obtained from the UV-Vis spectrophotometer analysis. Based on Fig. 3, it can be seen that linear regression is obtained with the equation $y = 0.1629x - 0.1665$ and a correlation coefficient of 0.998. This study applies the Somogyi-Nelson method, which is a method for determining reducing sugar levels. In principle, reducing sugars will be reduced by Cu$^{2+}$ to Cu$^{+}$ ions, then it will reduce arsenomolybdate compounds to form greenish-blue complexes. The Somogyi-Nelson method is more specific if it is used to determine reducing sugar levels in samples that have a mixture of sugar compounds in it, compared to the anthrone-sulfate method$^{46}$.

3.2.2 Temperature optimization

In the hydrolysis process under A. niger, temperature variations were carried out by comparing the pretreatment and non-pretreatment OPEFB. Based on Figs. 4(A) and 4(B), the effect of temperature on the reduction of reducing sugar can be observed. The results show that the highest reducing sugar content is obtained at 50°C.
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(B), optimization at both hydrolysis temperature shows that the highest reducing sugar content is at 40°C. It is due to A. niger included in the mesophyll microbe group with optimum growth at 35-37°C. This is evidenced by an increase in temperature at 45°C which reduces the production of reducing sugar content, this is also certainly correlated with cellulase enzyme production. In the same studies by²⁰ and²⁷, the cellulase enzyme showed high stability at 37°C; however, the activity of β-glucosidase enzyme decreased by approximately 28%, 20%, and 45% after 48, 72 and 96 h incubation at 50°C, respectively. So it can be concluded that temperature optimization is carried out well at 40°C which plays a role in maintaining the stability of enzymes such as endoglucanase and β-glucosidase as variables that are responsible for the hydrolysis process.

Although at this stage we studied the hydrolysis process at various temperatures (as shown in Fig. 4), our focus was on a temperature of 40°C to observe the OPEFB hydrolysis process by A. niger BIOTROP 2173 with a duration of 24, 48 and 72 hours. This is a form of data comparison in optimizing the duration of time required for the hydrolysis process using A. niger. Based on Fig. 4(A), it can be seen that reducing sugar content in non-pretreatment tends to decrease with increasing time. This condition can be related to the optimum performance time and the amount of lignin in the OPEFB non-pretreatment. The optimum time occurs from hydrolysis for 24 hours with the amount of reducing sugar produced is 2.42 mg.mL⁻¹. Although from the graph it can be seen that the hydrolysis for 72 hours produces higher reducing sugars, this time is not the optimum hydrolysis time. Apart from what we mentioned earlier that A. niger requires reducing sugars for growth and other metabolism, it is necessary to think about that A. niger under certain conditions will produce reducing sugars. This condition occurs at 72 hours of hydrolysis. As for the decrease in the amount of reducing sugars at 48 hours of hydrolysis, this can be related to A. niger need for reducing sugars.

3.2.3 pH variations test

Variation in pH has been carried out by correlating the optimization temperature with reducing glucose content as tested using a UV-Vis spectrophotometer. In addition, native enzyme or protein monomer undergoes a conformational change commonly called denaturation caused by pH changes, organic solvents, heat, protein concentration, shaking, or the presence of other proteins or chemical compounds. In some cases, lead to the exposure of “sticky” hydrophobic areas. These areas increase the propensity of the monomer to aggregate or “stick” to each other, causing it to become active. The active monomers or unfolded proteins begin to aggregate forming oligomers that ultimately lead to insoluble fibrils or amorphous aggregates²⁰.

Figure 5 shows how the pH is related to the amount of reducing sugar until it reaches the optimum condition. In general, both OPEFB hydrolysis non-pretreatment (Fig. 5(A)) and OPEFB hydrolysis with pretreatment (Fig. 5(B)) showed the same pattern, namely hydrolysis activity was slow at a more acidic pH (pH 5-6.5 for OPEFB) and pH 5-5.5 for pretreatment OPEFB. Even though the hydrolysis pattern is the same, the amount of reducing sugar and the optimum pH produced is different.

The highest amount of reducing sugar was produced from...
3.2.4 Effect of temperature on cellulase enzyme activity

Furthermore, cellulase enzyme activity was analyzed in each treatment by adding 1.0 mL of sample into a glass tube followed by the insertion of 6.0 mm Whatman paper and the addition of 1 mL of 0.05 M citrate buffer. It was put on a water bath to be heated to 30°C for 30 min and added 3.0 mL DNS solution then analyzed using a UV-Vis spectrophotometer. Figure 6 shows the relationship between hydrolysis temperature and cellulase enzyme activity. As it is understood that temperature will affect the work of enzymes in the production of reducing sugars. Many factors cause enzyme activity to be disrupted, one of which is the high lignin content of OPEFB. This is shown in Fig. 6, where at 24 hours of hydrolysis the enzyme activity shows an irregular pattern. This is influenced by the EFB used, namely the non-pretreatment EFB. When looking at the data shown in Table 1, you will find that non-pretreatment OPEFB contains 26.53% lignin. A very different result is shown in Fig. 6 (B), where the OPEFB used was the 10% NaOH pretreatment OPEFB. The use of OPEFB pretreatment causes a regular pattern of enzyme activity.
This can be observed at the hydrolysis temperature of 40°C, which is the optimum temperature of this work.

4 Conclusions

Based on these results, we can conclude the optimization of hydrolysis to the next step for the saccharification process to produce bioethanol can be using A. niger to produce high glucose levels at 40°C with a pH value of 6-7. We also report that high reducing sugar levels are found in non-delignification of OPEFB. Meanwhile, the addition of A. niger can cause a decrease in glucose levels due to the lignin content in lignocellulose can inhibit the hydrolysis process. Lignin has hydrophobic properties so that it interferes with the hydrolysis process to react with cellulose and by binding to enzymes and acting as inhibitors.

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Conflict of Interest

All authors declare that there is no conflict of interest in this paper.

References

1) Asghar, A.; Rasool, M.S.; Younas, T.; Basit, M.; Haq, A. Food, water and energy nexus: a cockpit for implementing the sustainable future. in Food-Energy-Water Nexus Resilience and Sustainable Development. Springer, pp. 291-304 (2020).
2) Purnomo, H.; Okarda, B.; Dermawan, A.; Ilham, Q.P.; Pacheco, P.; Nurfatiani, F.; Suhendang, E. Reconciling oil palm economic development and environmental conservation in Indonesia: A value chain dynamic approach. For. Policy Econ. 111, 102089 (2020).
3) Matsuo, Y.; Yanagisawa, A.; Yamashita, Y. A global energy outlook to 2035 with strategic considerations for Asia and Middle East energy supply and demand interdependencies. Energy Strateg. Rev. 2, 79-91 (2013).
4) Wik, M.; Pingali, P.; Broca, S. Global agricultural performance: Past trends and future prospects. (2008).
5) Oh, T.H.; Pang, S.Y.; Chua, S.C. Energy policy and alternative energy in Malaysia: issues and challenges for sustainable growth. Renew. Sustain. Energy Rev. 14, 1241-1252 (2010).
6) Hoon, M.; Tan, X. Depletion of fossil fuels and anthropogenic climate change—A review. Energy Policy 52, 797-809 (2013).
7) Lee, S.; Speight, J.G.; Loyalka, S.K. Handbook of Alternative Fuel Technologies. CRC Press (2015).
8) Suharyati; Pambudi, S.H.; Wiswojo, J.L.; Pratiwi, N.I. Indonesia Energy Outlook 2019 (Abdurrahman, S.; Pertivami, M.; Wahjanto, eds.). Secretary General of the National Energy Council, Jakarta (2019).
9) Hosseini, S.E.; Wahid, M.A. Feasibility study of biogas production and utilization as a source of renewable energy in Malaysia. Renew. Sustain. Energy Rev. 19, 454-462 (2013).
10) Nieves, D.C.; Karim, K.; Horváth, I.S. Improvement of biogas production from oil palm empty fruit bunches (OPEFB). Ind. Crops Prod. 34, 1097-1101 (2011).
11) Steg, L. Promoting household energy conservation. Energy Policy 36, 4449-4453 (2008).
12) Maulidiyah; Nurdin, M.; Fatma, F.; Natsir, M.; Wiswojo, D. Characterization of methyl ester compound of biodiesel from industrial liquid waste of crude palm oil processing. Anal. Chem. Res. 12, 1-9 (2017).
13) Balat, M.; Balat, H. Recent trends in global production and utilization of bio-ethanol fuel. Appl. Energy 86, 2273-2282 (2009).
14) Demirbas, A. Progress and recent trends in biofuels. Prog. Energy Combust. Sci. 33, 1-18 (2007).
15) Hall, D.O.; Scrase, J.I. Will biomass be the environmentally friendly fuel of the future? Biomass Bioenergy 15, 357-367 (1998).
16) Maulidiyah, M.; Natsir, M.; Fitrianingsih, F.; Arham, Z.; Wiswojo, D.; Nurdin, M. Lignin degradation of oil palm empty fruit bunches using TiO2 photocatalyst as anti-fungal of Fusarium oxysporum. Orient. J. Chem. 33, 3101-3106 (2017).
17) Maulidiyah, M.; Mardhans, F.T.; Natsir, M.; Wiswojo, D.; Nurdin, M. Lignin black liquor degradation on oil palm empty fruit bunches using FeO3 TiO2 and its activity as antibacterial. In Journal of Physics: Conference Series; IOP Publishing; Vol. 1242, p. 12017 (2019).
18) Natsir, M.; Maulidiyah, M.; Ansharullah, A.; Arham, Z.; Wiswojo, D.; Nurdin, M. Natural biopesticide preparation as antimicrobial material based on lignin photo-degradation using mineral ilmenite (FeO3 TiO2). Int. Res. J. Pharm. 9, 170-174 (2018).
19) Fahma, F.; Iwamoto, S.; Hori, N.; Iwata, T.; Takenura, A. Isolation, preparation, and characterization of nanofibers from oil palm empty-fruit-bunch (OPEFB). Cel- lulose 17, 977-985 (2010).
20) Yunus, R.; Salleh, S.F.; Abdullah, N.; Biak, D.R.A. Ef-
fect of ultrasonic pre-treatment on low temperature acid hydrolysis of oil palm empty fruit bunch. Bioresour. Technol. 101, 9792-9796 (2010).
21) Dahnum, D.; Tasum, S.O.; Triawahyuni, E.; Nurdin, M.; Abimanyu, H. Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. Energy Procedia 68, 107-116 (2015).
22) Triawahyuni, E.; Hariyanti, S.; Dahnum, D.; Nurdin, M.; Abimanyu, H. Optimization of saccharification and fermentation process in bioethanol production from oil palm fronds. Procedia Chem. 16, 141-148 (2015).
23) Muryanto, M.; Alvin; Nurdin, M.; Hanifah, U.; Sudiyani, Y. Extraction of glutathione from EFB fermentation waste using methanol with sonication process. in AIP Conference Proceedings. AIP Publishing, Vol. 1904, p. 20011 (2017).
24) Sari, A.A.; Kurniawan, H.H.; Nurdin, M.; Abimanyu, H. Decolorization of black liquor wastewater generated from bioethanol process by using oil palm empty fruit bunches. Energy Procedia 68, 254-262 (2015).
25) Arutoni, O.; Sari, A.A.; Berkah, A.; Nurdin, M.; Fitriady, M.A.; Parmawati, Y.; Rinaldi, N.; Yuniarto, A.; Hadibarata, T. Advanced degradation of lignin from palm oil mill effluent (POME) by a combination of photocatalytic-fenton treatment and TiO₂ nanoparticle as the catalyst. Water Air Soil Pollut. 231, 266 (2020).
26) Ahmad, A.; Baung, A.; Bhat, A.H. Renewable and sustainable bioenergy production from microalgal co-cultivation with palm oil mill effluent (POME): A review. Renew. Sustain. Energy Rev. 65, 214-234 (2016).
27) Kresnowati, M.; Mardawati, E.; Setiadi, T. Production of xylitol from oil palm empty fruits bunch: A case study on biofinery concept. Mod. Appl. Sci. 9, 206-213 (2015).
28) Zheng, Y.; Zhao, J.; Xu, F.; Li, Y. Pretreatment of lignocellulosic biomass for enhanced biogas production. Prog. energy Combust. Sci. 42, 35-53 (2014).
29) Gaurav, N.; Sivasankari, S.; Kiran, G.S.; Ninawe, A.; Selvin, J. Utilization of bioresources for sustainable biofuels: A review. Renew. Sustain. Energy Rev. 73, 205-214 (2017).
30) Gupta, A.; Verma, J.P. Sustainable bio-ethanol production from agro-residues: A review. Renew. Sustain. Energy Rev. 41, 550-567 (2015).
31) Li, X.; Zhou, J.; Ouyang, S.; Ouyang, J.; Yong, Q. Fumaric acid production from alkali-pretreated corn cob by fed-batch simultaneous saccharification and fermentation combined with separated hydrolysis and fermentation at high solids loading. Appl. Biochem. Biotechnol. 181, 573-583 (2017).
32) Saini, J.K.; Saini, R.; Tewari, L. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: Concepts and recent developments, 3 Biotech 5, 337-353 (2015).
33) Rahman, S.H.A.; Choudhury, J.P.; Ahmad, A.L.; Kamarruddin, A.H. Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose. Bioreour. Technol. 98, 554-559 (2007).
34) Mardawati, E.; Purwadi, R.; Setiadi, T. Evaluation of the enzymatic hydrolysis process of oil. ARPN J. Eng. Appl. Sci. 12, 5286-5292 (2017).
35) Duangwang, S.; Sangwichien, C. Optimization studies on acid hydrolysis of pretreated oil palm empty fruit bunch for production of xylose by application of Response Surface Methodology. Adv. Mater. Res. 699, 77-82 (2013).
36) Noratiqah, K.; Madihah, M.S.; Aisyah, B.S.; Eva, M.S.; Suraini, A.A.; Kamalrulzaman, K. Statistical optimization of enzymatic degradation process for oil palm empty fruit bunch (OPEFB)/in rotary drum bioreactor using crude cellulase produced from Aspergillus niger EFBI. Biochem. Eng. J. 75, 8-20 (2013).
37) Muliyangtyas, A.; Sediawan, W.B. Effect of combined pretreatment of lignocellulose and the kinetics of its subsequent bioconversion by Aspergillus niger. Biocatal. Agric. Biotechnol. 21, 101292 (2019).
38) Lynd, L.R.; Laser, M.S.; Bransby, D.; Dale, B.E.; Davison, B.; Hamilton, R.; Himmel, M.; Keller, M.; McMillan, J.D.; Sheehan, J. How biotech can transform biofuels. Nat. Biotechnol. 26, 169-172 (2008).
39) Abdel-Rahman, M.A.; Tashiro, Y.; Sonomoto, K. Lactic acid production from lignocellulose-derived sugars using lactic acid bacteria: overview and limits. J. Biotechnol. 156, 286-301 (2011).
40) Rosales-Calderon, O.; Tranjao, H.L.; Duff, S.J.B. Stability of commercial gluconase and β-glucosidase preparations under hydrolysis conditions. PeerJ 2, e402 (2014).
41) dos Santos, T.C.; Filho, G.A.; Oliveira, A.C.; Rocha, T.J.O.; Machado, F.P.P.; Bonomo, R.C.F.; Mota, K.I.A.; Franco, M. Application of response surface methodology for producing cellulolytic enzymes by solid state fermentation from the purple mombin (Spondias purpurea L.) residue. Food Sci. Biotechnol 22, 1-7 (2013).
42) dos Santos, T.C.; dos Santos Reis, N.; Silva, T.P.; Machado, F. de P.P.; Bonomo, R.C.F.; Franco, M. Prickly palm cactus husk as a raw material for production of ligninolytic enzymes by Aspergillus niger var. EFBI. Food Sci. Biotechnol. 25, 205-211 (2016).
43) Carvalho, T.; Cavalcanti, I.S.; Bonomo, R.C.F.; Santana, N.B.; Franco, M. Optimization of production of cellulolytic enzymes by Aspergillus niger using residue of mango a substrate. Ci. Rural 41, 2210-2216 (2011).
44) dos Santos, T.C.; dos Santos Reis, N.; Silva, T.P.; Bonomo, R.C.F.; Aguiar-Oliveira, E.; de Oliveira, J.R.; Franco, M. Production, optimisation and partial characteri-
sation of enzymes from filamentous fungi using dried forage cactus pear as substrate. Waste and Biomass Valorization 9, 571-579 (2018).

45) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. Lab. Anal. Proced. 1617, 1-16 (2008).

46) Nelson, N. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153, 375-380 (1944).

47) Farinas, C.S.; Loyo, M.M.; Junior, A.B.; Tardioli, P.W.; Neto, V.B.; Couri, S. Finding stable cellulase and xylanase: Evaluation of the synergistic effect of pH and temperature. N. Biotechnol. 27, 810-815 (2010).