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Utilization of Empty Palm Fruit Bunches as a Carbon Source for Cellulase Production to Reduce Solid Waste from Palm Oil

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Abstract

Cellulase enzymes are widely used in textile, food, detergent, pulp and paper industries and biofuel, so the need for enzymes continues to increase every year. One of many biomass wastes found in Indonesia is empty fruit bunches (EFB) that can be used as a carbon source as a substitute for expensive pure cellulose (CMC) and *Bacillus subtilis* isolates. This study aims to obtain the optimum conditions the production of cellulase enzymes with variations in the pre-treatment of EFB and pH variations of the medium using *Bacillus subtilis*. Pre-treatment was carried out to hydrolysed lignocellulosic biomass was more easily and increased glucose levels which would enter the next production stage. Variations in pre-treatment were carried out by adding acids, bases and organosolv process, as well as variations in pH at 6.5; 7.0 and 7.5, respectively. Enzyme activity was calculated using the Nelson-Somogyi method. When using acid, the enzyme activity is 0.041, while using organosolv, it is obtained 0.057 each at pH 7. The results showed that the highest enzyme activity was obtained at a pH of 7.0 and a temperature of 40 °C on EFB substrate pretreated with a base of 0.204 U/ml. These findings emphasize the potential benefits of using EFB waste as a substrate for cellulase enzyme production, by providing an alternative approach to decrease raw material expenses and mitigate environmental pollution.



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1. Introduction

Enzymes are proteins that act as biocatalysts in chemical or biological reaction [1]. They play a crucial role in various biological processes by speeding up chemical reactions in living organisms. Enzymes are often used in industries related to bioenergy, such as the production of biofuels, to enhance the efficiency of certain biochemical reactions [2].

The use of enzymes in the degradation of polymers has been widely done in various types of industries and other

sectors. One of the enzymes most widely used to break down cellulose is cellulase. Enzyme cellulase is also widely used in the textile, food, detergent, pulp, and paper industries, as well as biofuels. Approximately 20% of the world's enzyme industry sales are made up of cellulase, hemicellulose and pectinase. This value is expected to continue the industry's need for these enzymes increases [3].

Indonesia is one of the countries that leads to use bioenergy as one of environmentally friendly renewable

energies [4]. However, the country still relies on imports from various countries such as China, India, Japan, and parts of Europe to meet 99% of its enzyme demands for local industries. In 2017, enzyme needs in Indonesia are estimated to increase by 2,500 tons with a value of 200 billion imports and are expected to continue to increase each year [5].

Cellulases are inducible enzymes whose expression is mainly stimulated by cellulase [6]. Generally, cellulase is the most abundant biopolymer present in nature [7], as well as in agricultural and may industrial wastes [8], including the waste from the oil palm industry. Unlike the non- sustainable resources such as fossil fuels and minerals, cellulose is the primary product of photosynthesis and the growth of plants and has served as the most abundant renewable resources produced in biosphere [9].

The production of cellulase enzymes requires cellulose as a substrate. Commercially used substrates are pure cellulose such as Avicel and Solka Floc, or organic inducers such as lactose and Carboxy Methyl Cellulose (CMC) [10], but the price of pure cellulose has a cost. Indonesia is the world's largest palm coconut producer with a total area of about 9 million hectares producing about 27,5 million tons of crude palm oil (CPO) per year [11]. One of the supplier regions of palm coconut is Riau Province. In 2017, the area of palm plants was approximately 8,721,148 Ha across Riau Province [12].

By utilizing this abundant resource, namely empty fruit bunches (EFB), Indonesia can potentially address the substrate cost issue for cellulase enzyme production. EFB is the waste produced after the sterilization process of the fresh fruit bunches, which contributes around 24 wt.% of the solid waste. With a cellulose content of 37.53 wt.%, EFB stands out as a lignocellulosic biomass with a substantial amount of cellulose [13]. Generally, EFB is hygroscopic in nature, has high moisture and silica content which renders it unfavourable for energy application. However, the lignocellulosic constituent in EFB, such as high cellulose (24–65 wt%) and low lignin (14–31 wt%) content makes EFB a potential raw material for the CNF [14, 15]. EFB waste is a non-optimally exploited, non-expensive and renewable lignocellulose waste, use of EFB as a substrate to produce cellulase enzymes can be used as an alternative to suppress the cost of raw materials of the substrate as well as reduce environmental pollution due to EFB wastes. The higher the cellulose content of a material the higher its ability to induce the production of cellulase enzymes [16].

EFB have a very complex structure of the lignocellulose material where cellulose, hemicellulose and lignin are

bind together. Cellulose and hemicelluloses cannot be hydrolysed by the enzymes cellulase and hemicellulose unless lignin. Lignin is the most difficult component to degrade due to its complex and heterogeneous structure. Therefore, a preliminary treatment is needed to degrade lignin. This pre-treatment aims to break down the protective lignin, change the structure of the lignocellulose, and make cellulose and hemicellulose easier to hydrolyse. The crystalline part of cellulose will shrink and become decreased in crystallinity. Early treatment is an important stage in the process of the biomass conversion of lignocellulose, which aims to eliminate lignin, reduce cellulose crystallinity, and increase the porosity of the material, thus facilitating the hydrolysis process and the fermentation of sugars.

A variety of early treatment methods and techniques have been tried on different biomasses. The results vary for each method or type of lignocellulose biomass. Each initial treatment method also has its own advantages and disadvantages. Initial chemical treatments aim to increase cellulose biodegradation by removing lignin and/or hemicellulose. The method also aims to reduce the level of polymerization and crystallinity of cellulose components. Some of the initial treatment methods commonly used are acid treatment, base treatment, and organosolv treatment [17].

This study aims to determine the influence of pre-treatment variations of raw materials on the activity of enzymes producing cellulase. The findings of this study contribute to the development of more efficient and sustainable biofuel production processes by identifying the most effective pre-treatment method for enhancing cellulase activity.

2. Materials and Methods

The materials used in this study are palm cane, H_2SO_4 1.5%, NaOH 0.5 M, ethanol 70%, *Bacillus subtilis*, H_2SO_4 0,045 N, CMC 1%, NaCl, Medium Luria Bertani (LB), Phosphate buffer solution, Nelson-Somogyi reagent, aquadest. Some of the chemicals used were purchased from Aldrich products. The equipment used for this experiment was Erlenmeyer, oven, autoclave, Petridis cup, analytical weights, incubator, centrifuge, cuvette, hotplate stirrer, pH meter, and UV-Vis spectrophotometer.

Table 1. Composition of solutions A and B for a particular pH.

pH	A solution (ml)	B solution (ml)
6.5	68.5	31.5
7.0	39.0	61.0
7.5	16.0	84.0

Pre-treatment is divided into several ways such as acid treatment using 1.5% H₂SO₄, basic treatment using NaOH 0.5 M, and organosolv treatment using 70% ethanol with H₂SO₄ 0.045 N. The maintenance medium used is LB with a composition of 10 gr peptone, 5 gr yeast extract (nitrogen source) and 5 gr NaCl. LB added with CMC substrate 1% (carbon sources). The media is dissolved with aquadest according to the size of the desired production volume. In this study production was carried out in a volume of 45 ml pH buffer of 7,0 and added 0.45 gr CMC (CMC 1% in 45 ml of solution). Then the medium is sterilized in an autoclave at 121 °C for 15 minutes.

A total of 1-2 doses of pure *Bacillus subtilis* isolates are inoculated into LB Agar Media then incubated for 24 hours at 37 °C. This reimagined isolate is used as a cultural inventory for this research. A total of 1-2 inoculum doses are inserted into liquid LB media as much as 10% of the production media. The total production media is 50 ml and a starter media of 5 ml is used. This starter medium is made by dissolving 0.125 g of LB powder into 5 ml of aquadest. Incubation is carried out for 6 hrs at 37 °C and stirred at 150 rpm. After 6 hours, 5 ml of the starter media that has already contained the inoculum is inserted into 45 ml of standard production media, the total production media is 50 ml. The media is incubated for 24 hours at 37 °C and agitated at 150 rpm.

The method used is the same as the enzyme production method above, only modified on the substrate, i.e., used EFB as a replacement for CMC, as much as 10%. The initial pH is adjusted to 6.5, 7.0 and 7.5, at 40 °C. The media is sterilized in an autoclave at 121 °C for 15 minutes. A phosphate buffer solution with a specific pH can be made with the amount of solutions A and B as shown in Table 1.

After 24 hours, the raw extract of the enzyme is inserted into the reaction tube and centrifugated in a cold state at 4 °C and stirred at 4000 rpm for 15 minutes. The raw enzymes produced are then analysed for their enzyme activity.

Enzyme cellulase activity is expressed in international units U/ml. The activity of the cellulase enzyme is calculated based on the data of the relative glucose level as 1 mg of glucose produced by 1 ml of the gross cellulase enzymatic filter. One unit of enzyme activity is defined as the amount of 1 µmol of glucose produced from the hydrolysis of the medium by 1 ml of raw cellulase extract during the incubation period. Equation 1 used to calculate the size of one unit of enzyme activity [18]:

$$\text{Enzyme activity } \left(\frac{U}{ml} \right) = \frac{C}{BM \text{ Glucose} \times t} \times \frac{H}{E} \quad (1)$$

3. Results and Discussions

EFB before pre-treatment process is performed are characterized to know the composition of cellulose, hemicellulose and lignin contained in them. The result is presented in Figure 1.

The percentage of each chemical component of EFB is 36% cellulose, 25% hemicellulose, 18% lignin, ash and other about 21%. The results correspond in the composition range of components in biomass generally namely cellulose (35-50%), hemicellulose (20%-35%), lignin (15-20%), ash and other (15-20%) [19].

The chemical composition of EFB after acid pre-treatment using 1.5% H₂SO₄ produced 39% cellulose, 21% hemicellulose, 15% lignin, and 25% ash & other. This suggests that pre-treatment with acid solvents was able to break the bond between cellulose, hemicellulose and lignin. The addition of acid will increase the level of decomposed lignin which means decreasing the lignin contained in EFB. In addition, acid will decrease the pH, is one of the things that affect the solubility of lignin, low pH will make phenolate hydroxyl clusters protonated, condensed, and settled in polar solvents [20].

The chemical composition of EFB after pre- treatment using NaOH 0.5M produced 52% cellulose, 8% hemicellulose, 17.1% lignin, and 22.9% ash & other. This suggests that initial treatment with a base solvent was also able to break the bond between cellulose, hemicellulose and lignin in EFB. The addition of alkaline bases of NaOH will facilitate the breakdown of lignin bonds. NaOH particles will enter the sample and break the structure of lignin so that lignin is more easily soluble resulting in a decrease in lignin levels [21]. The decrease in the percentage of lignin is also affected by the pH. Lignin will dissolve at a high pH in black liquor because the hydroxyl phenolate group lignin is in an ionized state forming its salts and is polar. The treatment will break down lignin into smaller particles [20].

The chemical composition of EFB after pre- treatment using 70% ethanol produce 48% cellulose, 12% hemicelluloses, 9% lignin, and 31% ash & other. This suggests that pre-treatment with ethanol solvents was able to break the bond between cellulose, hemicellulose and lignin in EFB. The longer the reaction time, the greater the cellulose composition. However, compared with the preliminary study conducted by Salapa et al [22]

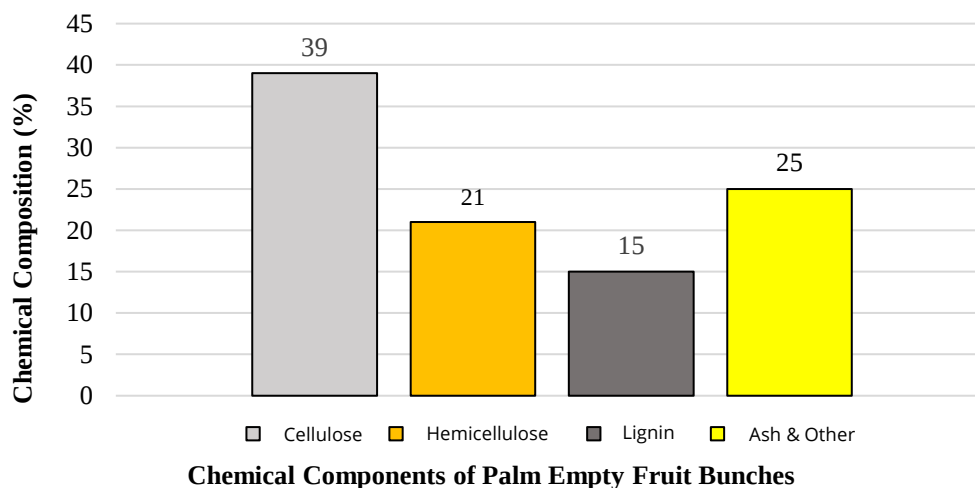


Figure 1. Chemical composition of empty fruit bunch.

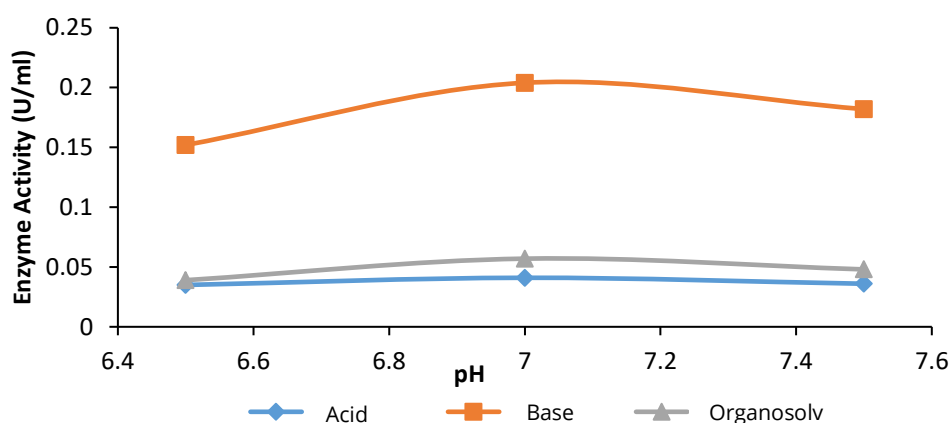


Figure 2. Results of cellulase enzyme activity with pre-treatment using acids, bases and organosolv.

with wheat saliva organosolv using 50% ethanol, the results showed that the content of wheat silk cellulose increased from 31.6% to 89% and approximately 72% was converted into glucose. This suggests that pre-treatment with organosolv is both in degrading cellulose and converting cellulose into glucose but less good in extracting hemicellulose.

Figure 2 shows a comparison of cellulase activity produced using EFB substrate using acid, base, and organosolv showed that the highest cellulase activity was produced on EFB substrates with pre-treatment using base solution of 0.204 U/ml at pH of 7.0 and 40 °C at the 24th hour. The enzyme cellulase activity produced on EFB substrate with pre-treatment of acid and organosolv at pH 7.0 was 0.041 U/ml and 0.057 U/ml. This suggests that changes in pH can cause changes in the load on the enzyme molecules, which can affect the activity of enzymes, both by structural changes and by changes in load [23].

4. Conclusions

This study focused on the pre-treatment EFB as a substrate for cellulase enzyme production. The results showed that acid, base, and organosolv pre-treatments effectively broke down the bond between cellulose, hemicellulose, and lignin in EFB. Acid pre-treatment increased cellulose content, while base pre-treatment facilitated lignin breakdown. The highest cellulase activity was observed in EFB substrates pre-treated with a base solution at pH 7.0 and 40 °C. These findings highlight the potential of utilizing EFB waste as a substrate for cellulase enzyme production, offering an alternative to reduce raw material costs and minimize environmental pollution. Further research and optimization of pre-treatment methods can contribute to the development of efficient and sustainable biofuel production processes.

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preparation, N.N.N.; writing—review and editing, Z.H.; visualization, S.Z.A.; supervision, S.Z.A.; project administration, M.M.; funding acquisition, S.Z.A. All authors have read and agreed to the published version of the manuscript.

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References

1. Fogler, H. S. (2006). *Elements of Chemical Reaction Engineering Fourth Edition*, Pearson Education, Inc, United States of America
2. Helwani, Z., Amraini, S. Z., Asmura, J., Siregar, T. N., Triwahyuni, V. E., Abd, A. A. (2023). Palm Frond Waste as a Carbon Source in the Synthesis of CaO/Biochar Catalysts for the Biodiesel Production Process, *Heca Journal of Applied Sciences*, Vol. 1, No. 1, 8–13
3. Howard, R. L., Abotsi, E., Van Rensburg, E. L. J., Howard, S. (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production, *African Journal of Biotechnology*, Vol. 2, No. 12, 602–619
4. Idroes, G. M., Syahnur, S., Abd Majid, M. S., Idroes, R., Kusumo, F., Hardi, I. (2023). Unveiling the Carbon Footprint: Biomass vs. Geothermal Energy in Indonesia, *Ekonomikalia Journal of Economics*, Vol. 1, No. 1, 10–18
5. Kementrian Riset dan Teknologi. (2017). *Siaran Pers Kemenristekdikti*, Gresik
6. Sukumaran, R. K., Singhanian, R. R., Pandey, A. (2005). Microbial cellulases-production, applications and challenges
7. Zhang, Y.-H. P., Lynd, L. R. (2006). Biosynthesis of radiolabeled cellobioses by the *Clostridium thermocellum* cellobiose and cellobiosyl phosphorylases for measurement of intracellular sugars, *Applied Microbiology and Biotechnology*, Vol. 70, 123–129
8. Hii, K.-L., Yeap, S.-P., Mashitah, M. D. (2012). Cellulase production from palm oil mill effluent in Malaysia: Economical and technical perspectives, *Engineering in Life Sciences*, Vol. 12, No. 1, 7–28. doi:10.1002/elsc.201000228
9. Zhang, Y. P., Lynd, L. R. (2004). Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems, *Biotechnology and Bioengineering*, Vol. 88, No. 7, 797–824
10. Umikalsom, M. S., Ariff, A. B., Zulkifli, H. S., Tong, C. C., Hassan, M. A., Karim, M. I. A. (1997). The treatment of oil palm empty fruit bunch fibre for subsequent use as substrate for cellulase production by *Chaetomium globosum* Kunze, *Bioresource Technology*, Vol. 62, Nos. 1–2, 1–9. doi:10.1016/S0960-8524(97)00132-6
11. GAPKI. (2013). *Diplomasi Kelapa Sawit*
12. Badan Pusat Statistik. (2017). *Pedoman Pendataan Survei Perkebunan Tahun 2017*, Badan Pusat Statistik, Jakarta Pusat
13. Ngadi, N., Lani, N. S. (2014). Extraction and characterization of cellulose from empty fruit bunch (EFB) fiber, *Jurnal Teknologi*, Vol. 68, No. 5, 35–39
14. Chang, S. H. (2014). An overview of empty fruit bunch from oil palm as feedstock for bio-oil production, *Biomass and Bioenergy*, Vol. 62, 174–181. doi:10.1016/j.biombioe.2014.01.002
15. Grande, P. M., Weidener, D., Dietrich, S., Dama, M., Bellof, M., Maas, R., Pauly, M., Leitner, W., Klose, H., Domínguez de María, P. (2019). OrganoCat Fractionation of Empty Fruit Bunches from Palm Trees into Lignin, Sugars, and Cellulose-Enriched Pulp, *ACS Omega*, Vol. 4, No. 11, 14451–14457. doi:10.1021/acsomega.9b01371
16. Ramadhan, R. F., Montesqrit, M., Marlida, Y. (2020). PRODUKSI ENZIM SELULASE TERMOSTABIL DARI BAKTERI NG2 MENGGUNAKAN BERBAGAI SUMBER SELULOSA ASAL LIMBAH PERTANIAN DAN PERKEBUNAN (Production of Thermostable Cellulase Enzyme by NG2 Bacteria Using Various Cellulose Sources from the Agriculture Waste), *Jurnal Ilmu Dan Teknologi Peternakan*, Vol. 8, No. 2, 64–72. doi:10.20956/jitp.v8i2.8171
17. Hidayat, M. R. (2013). Teknologi pretreatment bahan lignoselulosa dalam proses produksi bioetanol, *Biopropal Industri*, Vol. 4, No. 1, 33–48
18. Kombong, H. (2004). Evaluasi daya hidrolitik enzim glukamilase dari filtrat kultur *Aspergillus niger*, *Jurnal Ilmu Dasar*, Vol. 5, No. 1, 16–20
19. Mood, S. H., Golfeshan, A. H., Tabatabaei, M., Jouzani, G. S., Najafi, G. H., Gholami, M., Ardjmand, M. (2013). Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment, *Renewable and Sustainable Energy Reviews*, Vol. 27, 77–93
20. Arianie, L., Idawati, N. (2011). Penentuan lignin dan kadar glukosa dalam hidrolisis organosolv dan hidrolisis asam, *Jurnal Berkala Ilmiah Sains Dan Terapan Kimia*, Vol. 5, No. 2, 140–150
21. Lutfi, M., Hendrawan, Y. (2014). Analisis pengaruh waktu pretreatment dan konsentrasi naoh terhadap kandungan selulosa, lignin dan hemiselulosa eceng gondok pada proses pretreatment pembuatan bioetanol, *Jurnal Keteknik Pertanian Tropis Dan Biosistem*, Vol. 2, No. 2
22. Sidiras, D. K., Salapa, I. S. (2015). Organosolv pretreatment as a major step of lignocellulosic biomass refining
23. Dali, S., Arfah, R., Karim, A., Patong, A. R. (2013). Eksplorasi enzim amilase dari mikroba yang diisolasi dari sumber air panas di Sulawesi Selatan dan aplikasinya dalam produksi maltodekstrin, *Laporan Penelitian BOPTN, Universitas Hasanuddin, Makassar*