A STUDY ON CELLULOSE FROM OIL PALM BIOMASS AS A SOURCE OF BIOETHANOL PRODUCTION

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Oil palm biomass can potentially be an abundant source of cellulose in Indonesia. In this study, we isolated cellulose from oil palm biomass, including fronds, oil palm fibers, and empty fruit bunches. The isolated cellulose was hydrolyzed to produce glucose, and then fermented to ethanol. FT-IR analysis confirmed the presence of cellulose, with absorption peaks at 3563 cm^{-1} assigned to O-H stretching and at 1062 cm^{-1} corresponding to C-O stretching, among other characteristic peaks. The major crystalline cellulose peak was observed at 22.6°. SEM images showed that the cellulose isolated from oil palm biomass presented agglomerated fibers. The amount of glucose from the hydrolysis of cellulose fiber (63.92%) was higher, compared to that of celluloses from oil palm fronds (18.46%) and empty fruit bunches (54.81%). It was found also that oil palm fibers produced higher ethanol content than oil palm fronds and empty fruit bunches.

Keywords: bioethanol, cellulose, oil palm, fronds, fibers, empty fruit bunches

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the most economically important plants in the world due to the edible oil produced from oil palm,[1-2] and many common daily life products.[3-4] Indonesia has been placed as the world's first producer of crude palm oil and palm kernel oils.[5-6] However, the oil palm plantation industry contributes to environmental pollution with huge amounts of agricultural wastes.[7] Most of these wastes originate from oil palm processing. In Indonesia, the production of crude palm oil achieves 6 billion tons per year. The biomass from oil palm mills mainly comprises palm oil mill effluent (POME) (60%), empty fruit bunches (EFB) (23%), mesocarp fiber (MF) (21%), and palm kernel shell (PKS) (5%).[8] The accumulation of such oil palm biomass wastes contributes to environmental problems, such as greenhouse gas emissions, rising temperatures, disturbing weather patterns, and imbalances in the energy sector.[9]

To overcome the disposal issue related to oil palm biomass residues, it has been considered as a promising candidate as a source of renewable energy, with enormous potential in the production of biofuels[10-11] for heat, power, and transportation, as well as for the manufacture of some other value-added materials, such as cellulose-based adsorbents,[12-16] superabsorbents[17-20] *etc*. Oil palm biomass has attracted attention as raw material in the production of bioethanol.[21-23]

Bioethanol is produced through the fermentation process of carbohydrates from plants. Bioethanol is currently being considered as a bio-renewable fuel that can be a potential alternative

to fossil fuels.[24] Bioethanol has a high octane number, being higher than those of gasoline and diesel fuels, leading to lower emission levels, compared to gasoline and diesel fuels, with no CO_2 emission. Moreover, bioethanol has low-cost production, is biodegradable, has low toxicity and does not cause major air pollution. Using bioethanol directly in vehicles or blended with gasoline can potentially reduce greenhouse gas emissions and the consumption of gasoline.[25-26]

With these considerations, in the present study, we investigated the isolation of cellulose from oil palm biomass, namely, oil palm fronds, fibers and empty fruit bunches. Subsequently, the isolated celluloses were hydrolyzed and fermented by using *Saccharomyces cerevisiae* yeast to produce bioethanol.[27-28] Here, we report the comparative production of bioethanol from the mentioned oil palm residues: fronds, fibers, and empty fruit bunches. Such a comparison of oil palm fronds, fibers, and empty fruit bunches can contribute to their more efficient valorization.

EXPERIMENTAL

Isolation of cellulose

A mixture of 50 g biomass of palm oil and 3.5% of HNO_3 was stirred and heated at 90 °C for 2 h. The mixture was filtered and washed with distilled water until neutral pH. Then, the residue was added to 250 mL of 2% NaOH and 250 mL of $Na_2S_2O_3$, under continuous stirring and heating at 50 °C for 1 h. The mixture was filtered and washed with distilled water until neutral pH. Subsequently, the residue was added to 335 mL of 1.75% NaOCl under stirring and heated at 70 °C for 30 minutes. The reaction product was filtered and washed with distilled water until neutral pH. Then, 335 mL of 17.5% NaOH was added to the residue and stirred and heated at 80 °C for 30 minutes. The mixture was filtered and the residue was washed with distilled water until neutral pH. Then, it was added to 500 mL of 10% H_2O_2 under stirring at 60 °C for 15 min. The resulting product was filtered and washed with distilled water until neutral pH. Then, it was dried at 60 °C to obtain the isolated cellulose.

Hydrolysis of cellulose

The isolated cellulose was hydrolyzed by the acid method.[29] An amount of 2 g of cellulose was mixed with 32 mL of 30% HCl and heated at 80 °C for 2 h. The resulting product was cooled at room temperature and 10% NaOH was added to it until a pH of 4–4.5 was reached. Then, it was filtered to obtain glucose.

Fermentation of glucose

100 mL of the glucose obtained from the hydrolysis of cellulose was added to 0.1 g of $MgSO_4$; 0.1 g $KMnO_4$; and 0.1 gram $(NH_4)_2SO_4$. Fermentation was carried out using 6 g of *Saccharomyces cerevisiae*. After 6 days of fermentation, the product was filtered and subjected to distillation at 78–80 °C to form bioethanol.

Characterization methods

X-ray diffraction

Cellulose was ground to powder, then the powder sample was placed in the sample container. X-ray diffraction was used to determine the crystallinity of the cellulose samples. An X-ray diffractometer (Bruker), operated with 1.5418 Å CuK radiation, 20 mA current, and 40 kV voltage, was used to examine the samples.

Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy was used to confirm the structure of the cellulose samples by examining its functional groups. The cellulose sample was ground and mixed with KBr in a sample and KBr ratio of 1:100. The spectra were obtained at wavelengths in the range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1}.

Scanning electron microscopy

Scanning electron microscopy (SEM) images of the surface of celluloses extracted from oil palm fibers and oil palm fronds were captured using a Hitachi SEM S3400N. The samples were coated with gold using the sputtering technique prior to scanning.

Gas chromatography with flame ionization detector

In this study, gas chromatography was performed using a Gas Chromatograph 7890B from Agilent Technologies, provided with a Flame Ion Detector with 7697A Headspace Sampler, and an HP DB 624 capillary column with the length of 30 m, internal diameter of 0.25 mm, film thickness of 1.40 μm. Carrier gas was helium at a velocity of 1 mL/min, 50 °C inlet temperature. The oven temperature was maintained at 30 °C for 5 min, and then raised to 130 °C at a rate of 5 °C/min.

Density functional theory

In this study, we used the density functional theory (DFT) method for computational quantum chemical calculations. All DFT calculations were performed using the Orca 5.0.3 program developed by Frank Nesse's research group.[30-31] The DFT calculated data were visualized using the Multiwfn program developed by Tian Lu[32] and Avogadro program 1.2.0[33]. In theoretical analysis, we used the hybrid function and the basis set B3LYP/6-31G* in all calculations.

RESULT AND DISCUSSION

In this work, cellulose was extracted from oil palm fronds, fibers, and empty fruit bunches, hydrolyzed and then fermented by using *Saccharomyces cerevisiae* to produce bioethanol.

Scheme 1 illustrates the procedure followed for the conversion of oil palm biomass into bioethanol.

Characterization of cellulose

The XRD patterns of the celluloses obtained from different oil palm biomass types were recorded and shown in Figure 2. The peaks located at 2θ = 15.7°, 22.6°, and 35.19° are characteristic peaks of the cellulose crystal structure, corresponding to the lattice planes 110, 200, and 004. The major crystalline peak was observed at 22.6°.

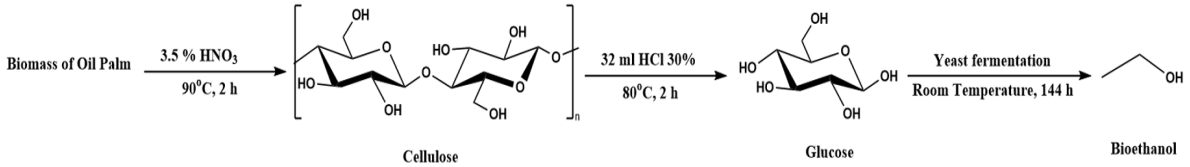

Figure 1: Schematic procedure for conversion of oil palm biomass into bioethanol

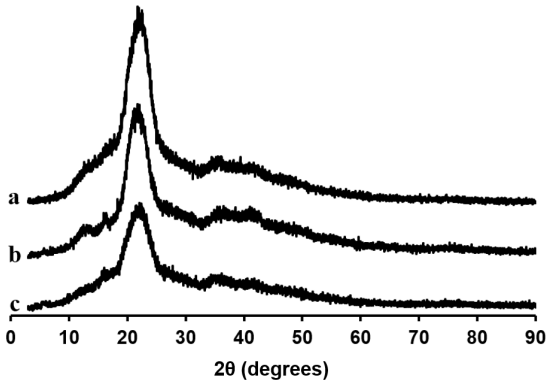

Figure 2: XRD patterns of celluloses obtained from (a) oil palm fronds, (b) oil palm fibers, and (c) oil palm empty fruit bunches

The DFT computational method was carried out at the B3LYP/6-31G* level in order to predict the theoretical normal vibrations of cellulose as a cellubiose unit. The theoretical IR spectrum shown in Figure 3 reveals that the cellulose molecule has absorption peaks at the wavenumbers of 3563 cm^{-1}, assigned to O-H stretching, at 3042 cm^{1} corresponding to C-H stretching, at 1062 cm^{-1} corresponding to C-O stretching, and at 1352 cm^{-1} assigned to O-H bending.

Figure 4 presents the actual spectra recorded for the obtained celluloses. The sharp transmittance peak around 1364 cm^{-1} represents the bending of OH groups, which is similar to the calculated peak for O-H bending. The peak at 1025 cm^{-1} corresponds to C-O stretching, being similar to the calculated value for C-O stretching. The peak at 3358 cm^{-1} represents the stretching vibration of the OH group in the broad band in the 3600-3100 cm^{-1} region. The absence of a shoulder peak at 1630 cm^{-1} indicates that there are no acetyl and uronic ester groups of the hemicelluloses or the ester linkage of lignin. Similarly, the absence of peaks at 1411 and 1630 cm^{-1} indicates the absence of C=C of the aromatic ring of lignin. Thus, the absence of peaks at 1411, 1630, and 1227 cm^{-1} indicates the absence of hemicelluloses and lignin. The FTIR spectra of the isolated celluloses shown in Figure 4 display the characteristic absorption patterns corresponding to the specific functional groups of α-cellulose, which are in good agreement with the calculated vibrations determined by DFT calculations.

SEM micrographs were recorded to observe the surface morphology of the cellulose fibers. As can be seen in the SEM images in Figure 5, the cellulose fibers from each type of biomass are agglutinated, with a rigid appearance.

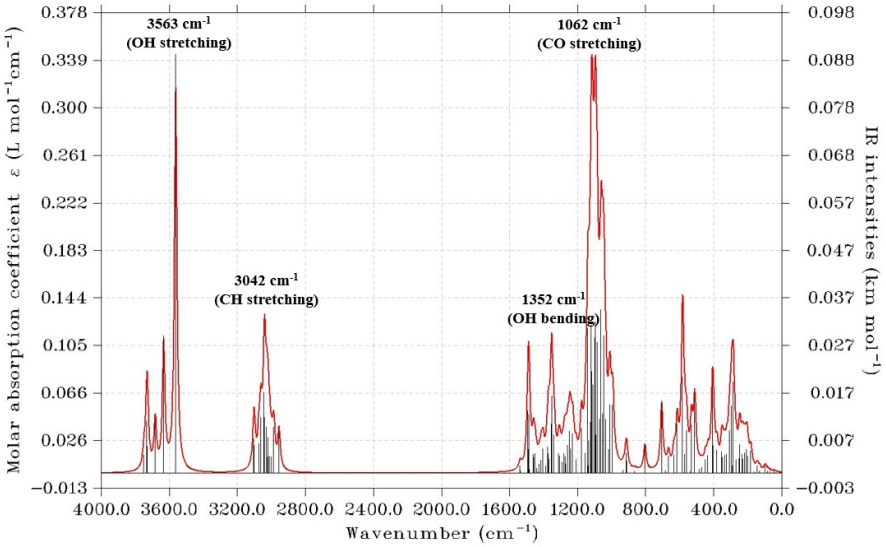

Figure 3: IR spectrum of cellulose obtained by the DFT method at B3LYP/6-31G*

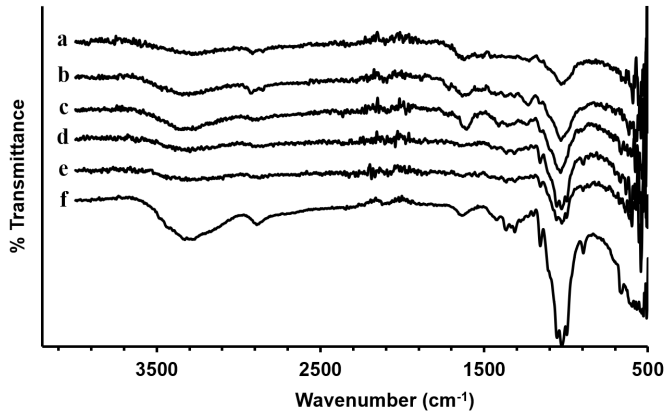

Figure 4: FTIR spectra of (a) oil palm empty fruit bunches, (b) oil palm fibers, (c) oil palm fronds, (d) cellulose from oil palm empty fruit bunches, (e) cellulose from oil palm fibers, and (f) cellulose from oil palm fronds

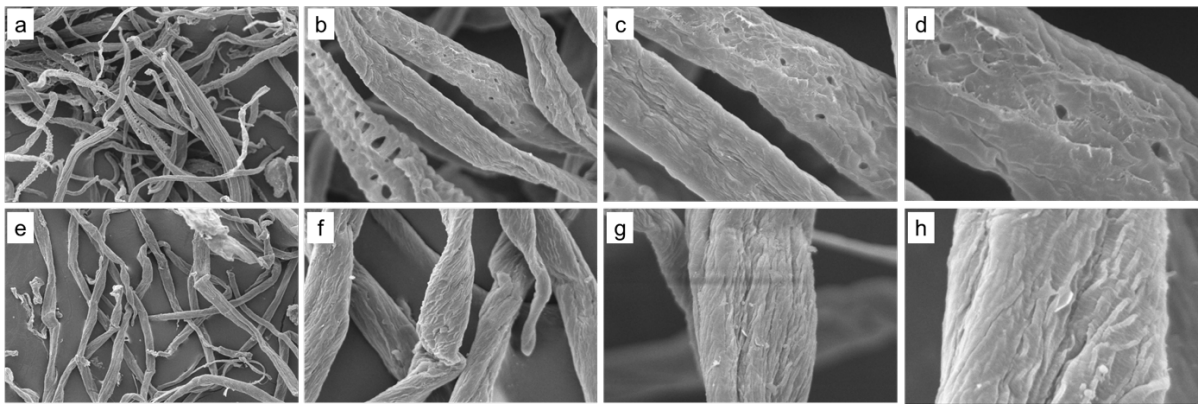

Figure 5: Scanning electron microphotographs of celluloses from oil palm fibers at (a) 1K×, (b) 5K×, (c) 10K×, (d) 20K×; from oil palm fronds at (e) 1K×, (f) 5K×, (g) 10K×, and (h) 20K×

Hydrolysis of cellulose to glucose

Before hydrolysis, pretreatment of lignocelluloses from fronds, fibers, and empty fruit bunches of oil palm was carried out by the acid method to reduce crystallinity, so that cellulose compounds were more easily to isolate.[34] After isolation, the cellulose derived from oil palm fronds, fibers, and empty fruit bunches was hydrolyzed with 30% HCl.

Hydrolysis using these acidic compounds is intended to produce fermentable glucose.[35]

As the main component of lignocelluloses, cellulose is a biopolymer consisting of many glucose units linked by β-1,4-glycosidic bonds. Breakage of the β-1,4-glycosidic bonds by acids leads to the hydrolysis of the cellulose polymer, producing oligosaccharides or glucose molecules.[36] In this study, the hydrolysis processes of cellulose to glucose were carried out using the acid method and the obtained glucose yields are shown in Table 1. After analysis, it was found that the quantity of glucose obtained from the hydrolysis of cellulose fibers was higher than that obtained from oil palm fronds and empty fruit bunches.

Fermentation of glucose into bioethanol

The hydrolyzed glucose was fermented to bioethanol using the *Saccharomyces cerevisiae* yeast at room temperature, under anaerobic conditions for 6 days.

During the fermentation process, one molecule of glucose will produce two molecules of ethanol (C_2H_5OH) and two molecules of carbon dioxide (CO_2). Fermentation is influenced by many factors, for instance, the type of substrate, bacterial strains, operating conditions, and others.[37] The results of the fermentation process in this study were analyzed using a Gas Chromatograph-Flame Ionization Detector (GC-FID), and the ethanol content is shown in Figure 6. The higher the glucose level, the higher the ethanol yield produced.[38] Wardani *et al*. studied bioethanol production from oil palm trunk biomass.

Table 1
Glucose yields from hydrolysis processes of oil palm biomass samples

No	Sample	Glucose levels (%)
1	Oil palm fronds	18.46
2	Empty fruit bunches	54.81
3	Oil palm fibers	63.92

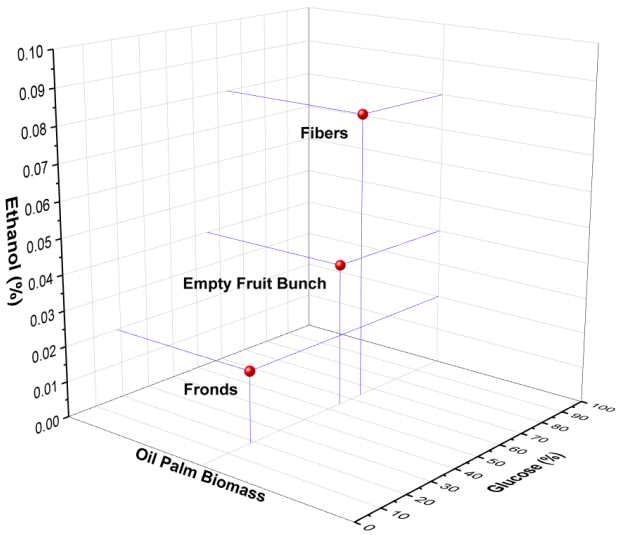

Figure 6: Ethanol production from glucose fermentation for various oil palm biomass samples

They performed simultaneous saccharification and cofermentation (SSCF), using *Saccharomyces cerevisiae* NCYC 479 and *Pichia stipitis* NCYC 1541, which yielded ethanol amounts up to the maximum of 1.89%.[39] Based on our study results, the bioethanol yield using *Saccharomyces cerevisiae* was only about 0.08% from oil palm fibers, 0.04% from empty fruit bunches and 0.02% from oil palm fronds. This significant difference in the results may be explained by different reaction conditions used, as opposed to the simultaneous saccharification and cofermentation in the cited study. However, in our work, it was also noticed that the ethanol yields varied as a function of the fermentation substrate used, namely, higher concentrations of ethanol were obtained from oil palm fibers, compared to

those from oil palm empty fruit bunches and fronds.

CONCLUSION

Cellulose has been successfully isolated from different oil palm biomass, such as fiber, fronds and empty fruit bunches, as confirmed by the IR absorption peaks located at 3563 cm^{-1} assigned to O-H stretching, and at 1062 cm^{-1} corresponding to C-O stretching. The cellulose was then hydrolyzed using 30% HCl to produce glucose. The highest glucose percentage (63.92%) was produced from oil palm fibers. The results showed that the greater the glucose percentage is in the cellulose fiber, the higher the yield of the ethanol produced.

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REFERENCES

[1] B. M. Siddique, A. Ahmad, M. H. Ibrahim, S. Hena, M. Rafatullah *et al*., *Grasas Y. Aceiteis*, **6**, 423 (2010)
[2] K. O. Purnama, D. Setyaningsih, E. Hambali and D. Taniwiryono, *Int. J. Oil Palm*, **3**, 40 (2020), https://doi.org/10.35876/ijop.v3i2.47
[3] Jusman, Syamsuddin and S. Handayani, *J. Phys. Conf. Series*, **1763**, 1 (2021)
[4] P. Hariyadi, *IOP Conf. Series Earth Environ. Sci*., **418**, 1 (2020)
[5] Junaedi, *Int. J. Soc. Sci*., **2**, 1779 (2022), https://doi.org/10.53625/ijss.v2i4.4137
[6] D. A. F. Zuhdi, M. F. Abdullah, M. S. W. Suliswanto and S. T. Wahyudi, *Jurnal Ekonomi Pembangunan*, **19**, 111 (2021), https://doi.org/10.29259/jep.v19i1.13193
[7] V. I. Otti, H. I. Ifeanyichuwu, F. C. Nwaorum and F. U. Ogbuagu, *Civil Environ. Res*., **6**, 121 (2014), https://core.ac.uk/download/pdf/234677783.pdf
[8] E. Hambali and M. Rivai, *IOP Conf. Series Earth Environ. Sci*., **65**, 1 (2017)
[9] H. A. Umar, S. A. Sulaiman, M. A. M. Said, A. Gungor, M. Shahbaz *et al*., *Biomass Bioenerg*., **151**, 106179 (2021), https://doi.org/10.1016/j.biombioe.2021.106179
[10] T. M. I. Mahlia, N. Ismail, N. Hossain, A. S. Silitonga and A. H. Shamsuddin, *Environ. Sci. Pollut. Res*., **26**, 14849 (2019), https://doi.org/10.1007/S11356-019-04563-X
[11] R. Sindhu, P. Binod, A. Pandey, S. Ankaram, Y. Duan *et al*., in "Current Developments in Biotechnology and Bioengineering", edited by A. Pandey, S. Negi and C. R. Soccol, Elsevier, 2019, pp. 79-92
[12] A. Alipour, S. Zarinabadi, A. Azimi and M. Mirzaei, *Iran. J. Energ. Environ*., **13**, 258 (2022), https://doi.org/10.5829/IJEE.2022.13.03.06
[13] N. Nairat, O. Hamed, A. Berisha, S, Jodeh, M. Algarra *et al*., *BMC Chem*., **16**, 1 (2022), https://doi.org/10.1186/S13065-022-00837-7
[14] S. N. H. Azmi, W. M. A. Lawati, U. H. A. A. Hoqani, E. A. Aufi, K. A. Hatmi *et al*., *Pharmaceuticals*, **15**, 1 (2022)
[15] R. Si, J. Pu, H. Luo, C. Wu and G. Duan, *Polymers*, **14**, 1 (2022), https://doi.org/10.3390/polym14245479
[16] I. Elsayed, G. T. Schueneman, E. M. El-Giar and E. B. Hasan, *Gels*, **9**, 1 (2023)
[17] P. Susmanto, A. R. Putri and M. Z. Nugraha, *J. Ecol. Eng*., **24**, 98 (2023), https://doi.org/10.12911/22998993/162786
[18] M. Manuel and A. Jennifer, *J. Chem. Rev*., **5**, 182 (2023)
[19] J. Diaz-Ramirez, L. Urbina, A. Eceiza, A. Rategi and N. Gabilondo, *Int. J. Biol. Macromol*., **191**, 1212 (2021)
[20] A. Sorze, F. Valentini, A. Dorigato and A. Pegoretti, *Molecules*, **28**, 1 (2023)
[21] H. Li and S. Li, *ACS Sustain. Chem. Eng*., **10**, 9820 (2022)
[22] M. E. Hage, B. N. Rajha, Z. Maache-Rezzoug, M. Koubaa and N. Louka, *Energies*, **15**, 1 (2022)
[23] P. Dhungama, B. Prajapati, S. Maharjan and J. Joshi, *Int. J. Appl. Sci. Biotechnol*., **10**, 1 (2022)
[24] M. Takano and K. Hoshino, *Int. J. Modern Phys.: Conf. Series*, 6, 715 (2012)
[25] M. Balat, H. Balat and C. Oz, *Progress Energ. Combust. Sci*., **34**, 551 (2008), http://dx.doi.org/10.1016/j.pecs.2007.11.001
[26] K. Collins, in *EIA Energy Outlook, Modeling, and Data Conference*, March 28, 2007, Washington, DC, https://www.eia.gov/outlooks/archive/aeo07/conf/pdf/collins.pdf
[27] L. S. Hong, D. Ibrahim and I. C. Omar, *Int. J. Biochem. Biotechnol*., **1**, 7 (2012), https://www.internationalscholarsjournals.com/articles/oil-palm-frond-for-the-production-of-bioethanol.pdf
[28] A. Ahmad, S. R. Muria and M. Tuljannah, *J. Phys.: Conf. Series*, **1295**, 1 (2019)
[29] J. K. W. Chang, X. Duret, V. Berberi, H. Z. Niaki and J. M. Lavoie, *Frontiers Chem*., **6**, 117 (2018), https://doi.org/10.3389/fchem.2018.00117
[30] F. Neese, *Wiley Interdiscip. Rev.: Comput. Mol. Sci*., **2**, 73 (2012), https://doi.org/10.1002/wcms.1606
[31] F. Neese, F. Wennmohs, U. Becker and C. Riplinger, *J. Chem. Phys*., **152**, 224108 (2020), https://doi.org/10.1063/5.0004608
[32] T. Lu and F. Chen, *J. Comput. Chem*., **33**, 580 (2012), https://doi.org/10.1002/jcc.22885
[33] M. D. Hanwell, D. E. Curtis, D. C. Lonie, T. Vandermeersch, E. Zurek *et al*., *J. Cheminform*., **4**, 17 (2012), https://doi.org/10.1186/1758-2946-4-17

[34] C. S. Goh, K. T. Lee and S. Bhatia, *Bioresour. Technol.*, **101**, 7362 (2010), https://doi.org/10.1016/j.biortech.2010.04.048
[35] C. N. G. Hamelinck, *Biomass Bioenerg.*, **28**, 384 (2005), https://doi.org/10.1016/j.biombioe.2004.09.002
[36] Y. B. Huang and Y. Fu, *Green Chem.*, **15**, 1095 (2013), https://doi.org/10.1039/C3GC40136G
[37] N. Adela, A. B. Nasrin, S. K. Loh and Y. M. Choo, *J. Appl. Environ. Biol. Sci.*, **4**, 234 (2014), https://www.textroad.com
[38] Y. H. Chang, K. S. Chang, C. Y. Chen, C. L. Hsu, T. C. Chang *et al.*, *Fermentation*, **4**, 45 (2018), https://doi.org/10.3390/fermentation4020045
[39] A. K. Wardani, A. Sutrisno, T. N. Faida, R. D. Yustina and U. Murdiyatmo, *Int. J. Microbiol.*, 2021, ID 2509443 (2021), https://doi.org/10.1155/2021/2509443