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The effect of adding NaOH and fermentation time on Robusta coffee husk (*Coffea canephora*) bioethanol production with SSF (Simultaneous Sachraffication and Fermentation) method

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Abstract

Robusta coffee husk is one of the largest agricultural wastes and contains lignocellulose as a raw material for bioethanol production. Lignin in Robusta coffee husk can interfere with the enzymatic hydrolysis process, so a delignification process using NaOH solution is needed to remove it. This research aimed to determine the effect of adding NaOH and fermentation time on the bioethanol production from Robusta coffee husks using the SSF (Simultaneous Saccharification and Fermentation) method. Removal of lignin using NaOH with various concentrations of 6, 8, and 10% and fermentation time for 24, 48, 72, 96, and 120 hours. The cellulose, hemicellulose, and lignin contents in the delignification results were tested using the Chesson method. The saccharification and fermentation steps used cellulase enzymes and *Saccharomyces cerevisiae*. Bioethanol levels were analyzed using GCMS. The results showed that the optimal concentration of NaOH to remove lignin was 10% which generated 17.40% lignin content, 20.81% cellulose, and 3.79% hemicellulose. The highest bioethanol content was 100% obtained with a fermentation time of 72 hours. The data showed that adding of NaOH and fermentation time affected bioethanol production.

Keywords: Bioethanol; Robusta Coffee Husk; Lignin; Delignification

1. Introduction

Currently, the dependence on fossil fuels in the world is very high. The world economy also depends on various fossil energy sources [1]. Currently, there is a fossil fuel crisis due to the increasing use of fossil fuels. However, it is not balanced with the supply of natural fossil fuels. Fossil fuels are not renewable, so their quantity is decreasing. Excessive consumption of fossil fuels, especially in urban areas, causes high pollution and global warming [2]. Therefore, renewable and environmentally friendly energy alternatives are needed. All petroleum-based fuels can be replaced with renewable biomass fuels, one of which is bioethanol.

Bioethanol is ethanol produced by fermentation using vegetable raw materials. These raw materials include raw materials for sugar sources, raw materials for starch sources, and raw materials for fiber sources (lignocellulose) [3]. Bioethanol can be used to reduce carbon dioxide emissions, and it is a promising alternative to fossil fuels because it is derived from renewable biomass [4]. One of the natural ingredients used as bioethanol is Robusta coffee husk.

A coffee husk is a husk of the coffee that has been taken from the flesh. Coffee husk is the largest agricultural waste, and is underutilized due to technical and economic factors. Some farmers use coffee husks as an alternative to animal feed in the dry season because animal feed is difficult to obtain. The largest coffee husks are often a problem for farmers. Usually, they cope by burning the coffee husk [5]. Robusta coffee husk contains 6,34% hemicellulose, 15,38% cellulose, 33,79% lignin, 32,38% crude fiber, 1,1% fat, 76,83% carbohydrates, 5,6% ash, and 8,4% water [6].

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The main component of lignocellulose is cellulose which consists of D-glucose monomer units bound by β-1,4-glucosidic bonds [7]. The long cellulose chains are connected by hydrogen bonds and Van der Waals forces. Cellulose forms microfibrils through inter and intra-molecular bonds. There are two types of microfibrils, namely crystal and amorphous [8]. Generally, cellulose fibrils are coated by hemicellulose [9]. Hemicellulose is a heterogeneous polymer of pentoses, hexoses, and sugar acids. Hemicellulose is attached to lignin and forms cellulose with a very complex structure. Lignin is an amorphous heteropolymer consisting of three different phenylpropane units, namely *p-coumaryl*, *coniferyl*, and *sinapyl alcohol*. Lignin is in between cellulose and hemicellulose to bind them together [10]. Cellulose in plants binds to lignin to form lignocellulose so that the cellulose processing process is inhibited. Therefore, a preliminary step is required to degrade lignin from the cellulose structure using a delignification method [11].

Delignification is a process of removing lignin from complex lignocellulosic compounds [12]. The delignification process is essential before hydrolyzing the cellulose material. That is because lignin can inhibit the penetration of acids or enzymes before hydrolysis occurs. Delignification can be conducted biologically, physically, and chemically. Usually, the chemical delignification process is undertaken by hydrolysis of acids, bases, oxidation, organosolv and ionic solutions. Delignification with alkaline hydrolysis can use an alkaline solution, namely NaOH [13].

Sodium hydroxide (NaOH) is a kind of caustic metallic base of basic oxide Sodium oxide containing water. Researchers have studied sodium hydroxide intensively for several years and showed that NaOH could break the lignin structure bonds. OH^- ions from NaOH will break the bonds of the basic lignin structure, while Na^+ ions will bind to lignin to form sodium phenolate. This phenolic salt is soluble. Dissolved lignin is indicated by a black color in a solution called black liquor. Therefore, NaOH solution is able to separate lignin from cellulose [14].

One method of producing bioethanol is the simultaneous saccharificatian and fermentation (SSF) process or known as the simultaneous saccharification fermentation (SSF) process. The SSF process is a combination of hydrolysis using cellulase enzymes and yeast *Saccharomyces cerevisiae* to ferment sugar into ethanol simultaneously. In fact, the SSF process is almost similar to the separation process between hydrolysis with enzymes and the fermentation process. However, the SSF hydrolysis processes and fermentation are done in one reactor [15]. The advantages of the SSF method are that monosaccharides from polysaccharide conversion do not return to polysaccharides because the monosaccharides are directly fermented into ethanol, speed of hydrolysis, reduce enzyme requirements, increase product yield, and shorter process [16].

The levels of bioethanol produced by saccharification and fermentation are influenced by many factors, including the fermentation time. Fermentation time is required for *Saccharomyces cerevisiae* microorganisms to convert glucose into bioethanol. The role of microorganisms in the fermentation process is relatively time consuming, so optimization of the role of microorganisms is required. Microorganisms undergo several phases, from adaptation to enzyme release and substrate production [11]. The best bioethanol yield can be seen at the highest bioethanol content with the optimum fermentation time so that the fermentation time affects the result of bioethanol concentration [17].

2. Material and methods

2.1. Material and tools

2.1.1. Material

Robusta coffee husk from Jatiarjo Pasuruan coffee plantation, NaOH (Merck), distilled water, H_2SO_4 (Merck), cellulase enzyme (Novoenzym), and *Saccharomyces cerevisiae* (Turbo yeast).

2.1.2. Tools

Blender (Philips), 40 mesh sieve, digital balance (Ohaus), oven (Kirin), pH meter, hotplate stirrer, shaker (DLAB), autoclave (Hirayama), centrifuge (Eppendorf), GCMS and glassware.

2.2. Procedures

2.2.1. Robusta coffee husk preparation

Robusta coffee is dried for 20 days and separated from the husk. The coffee husk was mashed with a blender and sieved with a 40-mesh size.

2.2.2. Delignification step

The sample was put into an erlenmeyer and added NaOH solution with concentrations of 6, 8, and 10% in a ratio of 1: 10. The mixture was allowed to stand for 24 hours, then heated at 160°C and stirred at 100 rpm. The solution was filtered, and the residue was washed with distilled water to a neutral pH. The residue was baked at 105°C for 2 hours.

2.2.3. Lignin, cellulose and hemicellulose analysis steps

Lignin, cellulose, and hemicellulose were analyzed using the Chesson method. One gram of delignified sample was refluxed for 2 hours with 150 mL H_2O at 100°C. The result was filtered and washed. The residue was dried to a constant weight. The sample residue was refluxed with 150 mL of 0.5 M H_2SO_4at 100°C. The result was filtered and dried. The dried sample residue was added with 10 mL of 72% H_2SO_4 (v/v) and kept at room temperature for 4 hours. The mixture was diluted to 0.5 M H_2SO_4 and refluxed at 100°C for 2 hours. The residue was dried and then turned into ash. Calculation using the formula:

$$\text{Hemicellulose (\%)} = \frac{\text{b} - \text{c}}{a}\ x\ 100\%$$

$$\text{Cellulose (\%)} = \frac{c - d}{a}\ x\ 100\%$$

$$\text{Lignin (\%)} = \frac{d - e}{a}\ x\ 100\%$$

Description:

a = Initial dry weight of Robusta coffee husk sample
b = The dry weight of the sample residue refluxed with H_2O
c = Residual weight of the sample after refluxing with H_2SO_4
d = Residual weight of samples after treatment with 72% H_2SO_4
e = Ash from sample residue

2.2.4. Saccharification and fermentation steps

20 grams of the sample with the highest cellulose content and the lowest lignin content was added with 100 mL of distilled water. The solution was heated in an autoclave at 121˚C, cooled to room temperature, and 10 mL of cellulase enzyme added. Then it was tightly closed and shaken at 170 rpm for 24 hours. The solution was added with 4 grams of *Saccharomyces cerevisiae* and stirred until homogeneous. The saccharification process was conducted for 24, 48, 72, 96, and 120 hours. The SSF results were separated between residue and filtrate. The filtrate was distilled at 80˚C, and the obtained ethanol distillate was measured for bioethanol content using GCMS.

3. Results and discussion

3.1. The effect of adding NaOH

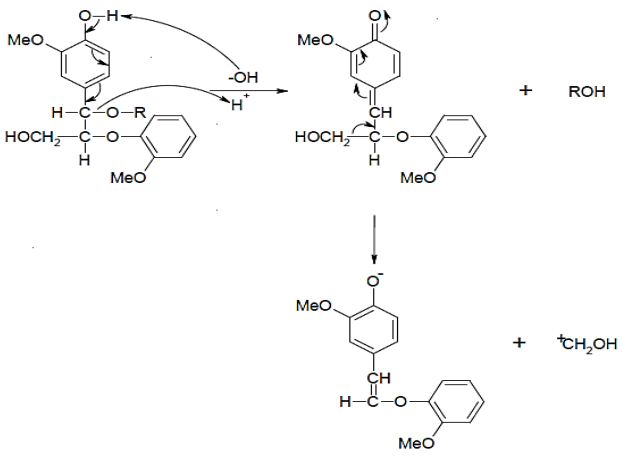


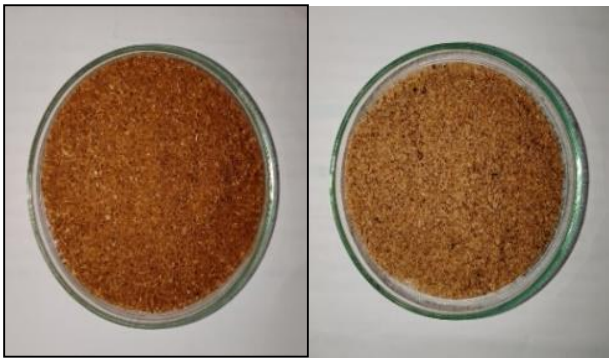
Figure 1 Lignocellulose degradation mechanism

The delignification process aims to destroy the lignocellulosic structure so that the cellulase enzyme easily hydrolyzes cellulose. NaOH was used as a delignifikator because NaOH can destroy the lignin structure in the crystalline and amorphous parts. In addition, NaOH could extract cellulose and hemicellulose in coffee husks [18]. The mechanism of breaking bonds between lignin and cellulose using NaOH is shown in Figure 1.

Lignin degradation was initiated by the offensive of the H atom bound to the phenolic OH group by hydroxide ions (OH) of NaOH. That H atom was acidic because it is bonded to an O atom with high electronegativity. The more electronegativity O atom will attract electrons to the H atom so that the H atom is partially positively charged and easily released into H^+ ions. Ions (OH^-) from NaOH will also break the bonds of the basic structure of lignin, while sodium ions (Na^+) will bind to lignin to form sodium phenolate. This phenolate salt was easily soluble in distilled water. A black color indicated dissolved lignin in a solution called black liquor [11], shown in Figure 2.

Figure 2 Black liquor

Physically, the difference in the color of the biomass before and after the delignification process can be observed, as shown in Figure 3. The coffee skin powder before the delignification stage is light brown. Meanwhile, after the delignification stage, it looks more faded. This shows that lignocellulose is degraded by NaOH solvent.



(A) **(B)**

Figure 3 Before delignification (A) and after delignification (B)

The delignification analysis results of robusta coffee husks using the chesson method with variations in NaOH concentrations could be seen in Table 1.

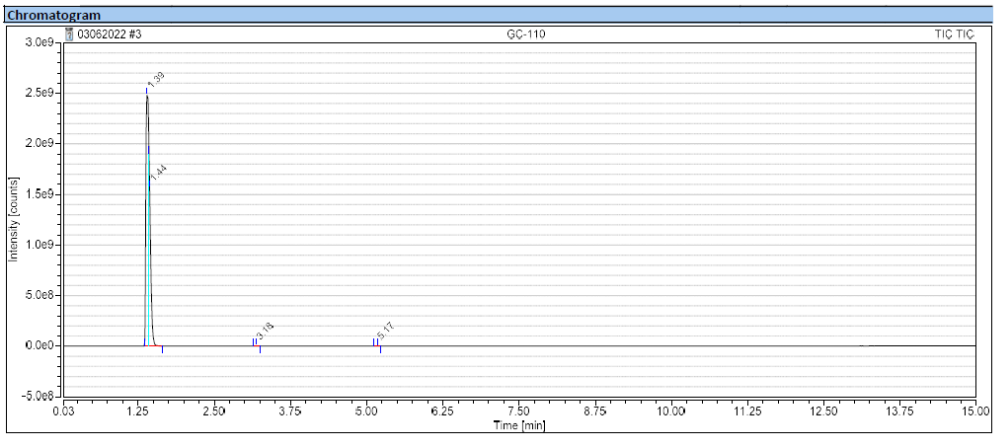
Table 1 Analysis of the lignocellulosic content of robusta coffee husks before and after delignification

Sample condition	Hemicellulose content (%)	Cellulose content (%)	Lignin content (%)
Before delignification	4.97	13.43	37.60
Delignification NaOH 6%	4.52	13.58	30.80
Delignification NaOH 8%	4.20	17.60	23.10
Delignification NaOH 10%	3.79	20.81	17.40

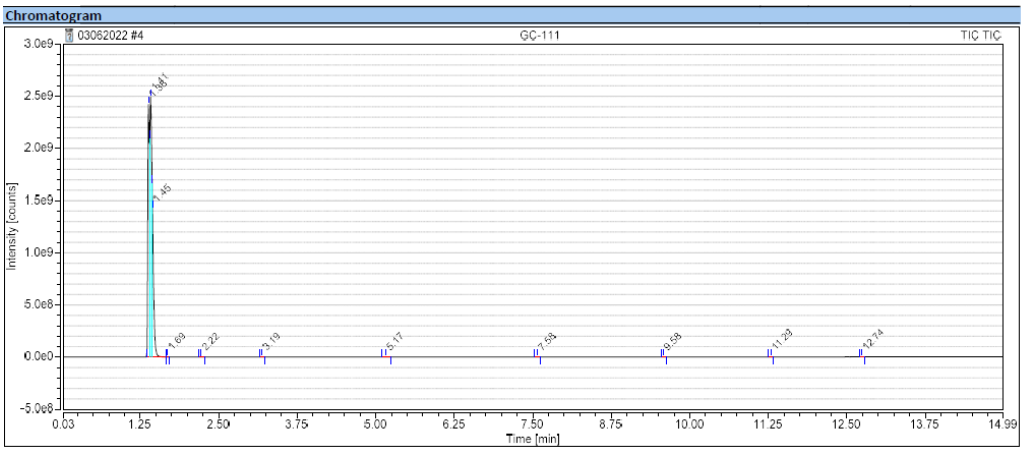
Table 1 showed that the lignin content decreased with increasing NaOH concentration. The lowest lignin content occurred in samples with 10% NaOH delignification, which equals 17.40%. This is because the large concentration of NaOH in the delignification process will add NaOH molecules which destroy the lignin structure so that the lignin content is greatly reduced. The higher of the reduction in lignin content will cause reactive cellulose content for the hydrolysis process [14]. Samples containing a lot of cellulose were found in samples delignified with 10% NaOH concentration, of 20.81%. In addition, the increase in cellulose content was due to some dissolved lignin and hemicellulose in the delignification process so that the cellulose content increased [19]. Meanwhile, the hemicellulose content decreased along with the high concentration of NaOH. The smallest hemicellulose content in the sample with 10% NaOH delignification was 3.79%. It showed that the number of NaOH in the solution system will destroy the bonds that connect hemicellulose with cellulose and lignin, namely hydrogen, ester and ether bonds [20]. Arnata [13], said that sago delignification and 10% NaOH solution were very effective in reducing lignin levels. This is because the high concentration of NaOH, will increase the ability to dissolve lignin and destroy the structure of cellulose, resulting in loose cellulose fibers and high enzyme activity.

3.2. Effect of fermentation time

The ethanol content of robusta coffee husks from the simultaneous saccharification and fermentation (SSF) process with variations in fermentation time was analysed quantitatively using GCMS and the data were shown in Figure 4 and Table 2.



(1)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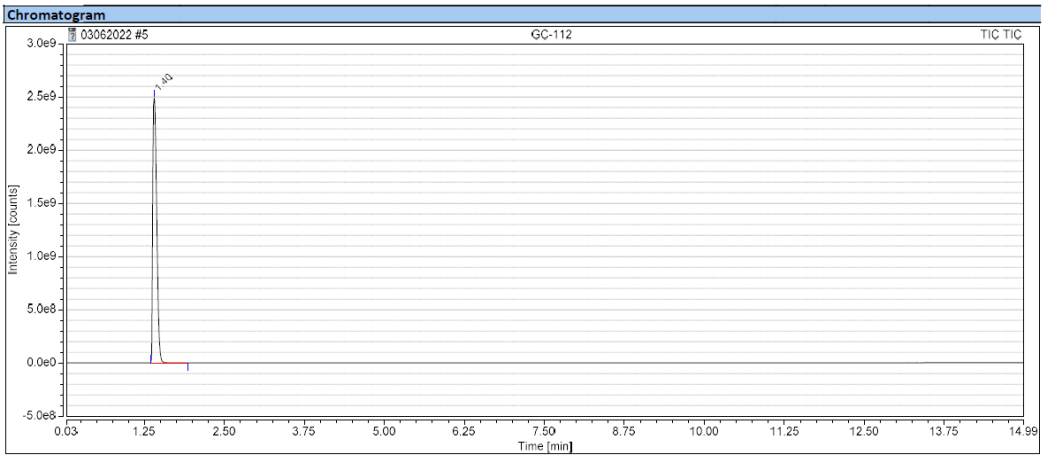
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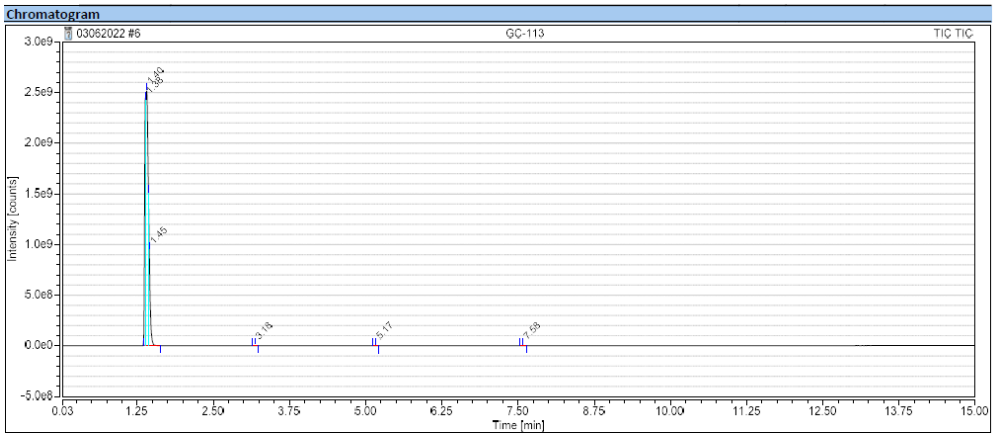
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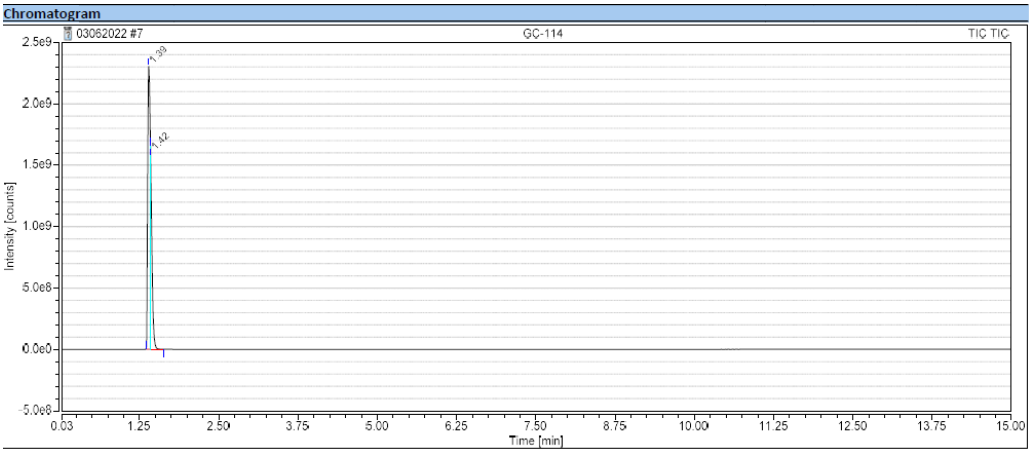
(4)



(5)

Figure 4 Chromatogram of ethanol GCMS based on the length of fermentation time (1) 24 hours, (2) 48 hours, (3) 72 hours. (4) 96 hours, (5) 120 hours

Table 2 The ethanol content of robusta coffee rind variations in fermentation time

Fermentation time (Hour)	Retention time (mm)	Area (pA*) × 10^6	Ethanol content (%)
24	1.44	54.23	28.94
48	1.42	82.80	42.49
72	1.40	18.06	100,00
96	1.41	89.81	48.82
120	1.43	43.50	30.41

The results showed that the fermentation time from 24 hours to 72 hours would produce high levels of bioethanol. However, at 96 hours to 120 hours of fermentation, there was a decrease in bioethanol levels. It showed that the optimal fermentation time was 72 hours with 100% bioethanol content. Bioethanol levels at the fermentation time of 96 hours decreased when the fermentation time of 120 hours decreased. It was because substrate conversio to the product by microorganism *Saccharomyces cerevisiae* has been exhausted. The decrease in bioethanol content was due to ethanol turning into organic acids such as acetic acid or esters [21]. Acetic acid can be produced from C_2H_5OH compounds (bioethanol) or fruits containing these compounds by biological oxidation processes using microorganisms. Bioethanol was oxidized to acetaldehyde and water. Then, the hydrated acetaldehyde was oxidized to acetic acid and water [22]. According to Novia [14], the fermentation time also affected the number of CO_2 gas produced. The longer fermentation time would produce higher CO_2 gas. This increase in gas production decreased in the pH value. This condition distruotes *Saccharomyces cerevisiae's* growth, producing less bioethanol. The lowest level of bioethanol was found in the 24-hour fermentation, which was 28.94%. This is because *Saccharomyces cerevisiae* was less than optimal. It was still adapting to the environment and utilizing glucose to grow and reproduce [23].

Febriana [24] investigated the bioethanol content of coffee husk by fermentation for 72 hours to obtain 1.46%, and Harsono [25] explained that the optimal bioethanol fermentation time of coffee husk was 48 hours with 60.2% bioethanol content. The delignification process in robusta coffee husks affected the bioethanol content of robusta coffee husks. The optimal time for fermentatio was 72 hours, and the bioethanol content was 100%.

4. Conclusion

Based on the research results above, it can be concluded that the optimal concentration of NaOH to remove lignin with 10% NaOH delignification generated lignin content, hemicellulose content decreased, and cellulose content in robusta

coffee husk increased. The variation of the fermentation time, which is 24-72 hours, shows an increase in ethanol conteny but decrease after passing through 72 hours.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no potential conflicts of interest, financial interest, or personal relationships concerning this article's research, authorship, and publication.

References

[1] S. A. Jambo, R. Abdulla, H. Marbawi, and J. A. Gansau, "Response Surface Optimization Of Bioethanol Production From Third Generation Feedstock - Eucheuma Cottonii," *Renew. Energy*, vol. 132, pp. 1–10, 2019, doi: 10.1016/j.renene.2018.07.133.

[2] S. Singh, S. T. P. Bharadwaja, P. Kumar, V. S. Moholkar, and A. Goyal, "Mechanistic investigation in ultrasound-assisted (alkaline) delignification of Parthenium hysterophorus biomass," *Ind. Eng. Chem. Res.*, vol. 53, no. 37, pp. 14241–14252, 2014.

[3] N. D. Siswati, P. S. Dara, and R. A. Wardana, "Fermentasi buah sukun menjadi bioetanol," *J. Tek. Kim.*, vol. 11, no. 2, pp. 56–59, 2017.

[4] R. F. Azzahra and Meilianti, "Produksi Bioetanol Berbahan Dasar Limbah Kulit Kopi Sebagai Bahan Bakar Alternatif," *J. Kinet.*, vol. 12, no. 02, pp. 58–63, 2021.

[5] Murni, F. Arifan, and Z. Abidin, "Optimasi Proses Bioetanol dari Kulit Kopi dengan Menggunakan Proses Hidrolisis Vibrous Bed Bioreaktor," *Traksi*, vol. 15, no. 1, pp. 1–9, 2015.

[6] D. lndra Wardhana, E. Ruriani, and A. Nafi, "Karakteristik Kulit Kopi Robusta Hasil Samping Pengolahan Metode Kering Dari Perkebunan Kopi Rakyat Di Jawa Timur," *Agritrop*, vol. 17, no. 2, pp. 214–223, 2019.

[7] B. Sharma, C. Larroche, and C. G. Dussap, "Comprehensive Assessment of 2G Bioethanol Production," *Bioresour. Technol.*, vol. 313, pp. 1–39, 2020, doi: 10.1016/j.biortech.2020.123630.

[8] H. Chen, "Chemical Composition and Structure of Natural Lignocellulose," in *Biotechnologi of lignocellulose*, Dordrecht: Springer, 2014, pp. 25–71. [Online]. Available: https://link.springer.com/chapter/10.1007/978-94-007-6898-7_2

[9] H. V. Lee, S. B. A. Hamid, and S.K.Zain, "Conversion of Lignocellulosic Biomass to Nanocellulose: Structure and Chemical Process," *Sci World J*, vol. 2014, pp. 1–20, 2014.

[10] H. Bamdad, K. Hawboldt, and S. MacQuarrie, "A Review on Common Adsorbents for Acid Gases Removal: Focus on Biochar," *Renew. Sustain. Energy Rev.*, vol. 81, no. 2, pp. 1705–1720, 2018.

[11] V. A. P. Putra and I. G. M. Sanjaya, "Pengaruh Waktu Sakarfikasi Dan Fermentasi Pada Produksi Bioetanol Dari Rumput Alang - Alang (Imperata cylindrica) Menggunakan Metode SSF (Simultaneous Saccharicatin and Fermentation)," *UNESA J. Chem.*, vol. 9, no. 2, 2020.

[12] I. B. W. Gunam, Y. Setiyo, N. S. Antara, I. M. . Wijaya, I. W. Arnata, and I. W. W. P. Putra, "Enhanced delignification of corn straw with alkaline pretreatment at mild temperature," *Rasayan J. Chem.*, vol. 13, no. 2, pp. 1022–1029, 2020, doi: 10.31788/RJC.2020.1325573.

[13] I. W. Arnata, S. Suprihatin, F. Fahma, N. Richana, and T. Candra Sunarti, "Cellulose Production from Sago Frond with Alkaline Delignification and Bleaching on Various Types of Bleach Agents," *Orient. J. Chem.*, vol. 35, no. Special Issue 1, pp. 08–19, 2019, doi: 10.13005/ojc/35specialissue102.

[14] N. Novia, Khairunnas, and G. T. Purbojoyi, "Pengaruh Konsentrasi Natrium Hidroksida Saat Pretreatment Dan Waktu Fermentasi Terhadap Kadar Bioetanol Dari Daun Nanas," in *Jurnal Teknik Kimia*, 2015, vol. 21, no. 3, pp. 14–24.

[15] S. Mandari, E. Yenie, and S. R. Muria, "Pembuatan Bioetanol dari Kulit Nanas (ananas comosus l.) Menggunakan Enzim Selulase dan Yeast Saccharomyces Cerevisiae dengan Proses Simultaneous Sacharificatian and Fermentation (SSF)," *Dr. Diss. Riau Univ.*, pp. 28–30, 2014.

[16] D. Anggriani, U. Kulsum, and N. Nurjanah, "Journal of Chemical Process Engineering Pengaruh Konsentrasi Enzim Silanase Dan Saccharomyces Cerevisiae," *J. Chem. Process Eng.*, vol. 5, no. 2655, pp. 45–49, 2020.

[17] L. Nuryanti, S. R. Muria, and Chairul, "Pembuatan Bioetanol dari Limbah Padat Sagu menggunakan Enzim Selulase dan Yeast Saccharomyces Cerevisiae dengan Proses Simultaneous Sacharificatian and Fermentation (SSF) Dengan Variasi Konsentrasi Substrat dan Volume Inokulum," 2014.

[18] M. D. Lestari, J. Kimia, F. Matematika, P. Alam, and U. N. Semarang, "Ekstraksi Selulosa dari Limbah Pengolahan Agar Menggunakan Larutan NaOH sebagai Prekursor Bioetanol," *Indones. J. Chem. Sci.*, vol. 7, no. 3, pp. 236–241, 2018.

[19] E. O. Sari *et al.*, "Pengaruh Konsentrasi NaOH Terhadap Kadar Selulosa Pada Proses Delignifikasi dari Serat Kapuk sebagai Bahan Baku Biodegradable Plastic Berbasis Selulosa Asetat," in *Seminar Nasional AVoER XII*, 2020, pp. 305–308.

[20] S. Winarsih, "Pengaruh Konsentrasi NaOH dan Lam Pemaparan Microwave Terhadap Kandungan Selulosa, Hemiselulosa dan Lignin Tongkol Jagung," in *Seminar Nasional dan Gelar Produk*, 2016, pp. 285–290.

[21] D. L. Pramita, E. Yenie, and S. R. Muria, "Pembuatan Bioetanol dari Kulit Nenas Menggunakan Enzim Selulase dan Yeast Saccharomyces Cerevisiae dengan Proses Simultaneous Sacharifacation and Fermentation (SSF) terhadap Variasi Konsentrasi Inokulum dan Waktu Ferment," 2014.

[22] Z. F. Khaira, E. Yenie, and S. R. Muria, "Pembuatan Bioetanol Dari Limbah Tongkol Jagung Menggunakan Proses Simultaneous Saccharification And Fermentation (SSF) Dengan Variasi Konsentrasi Enzim Dan Waktu Fermentasi," 2015.

[23] D. R. Saputra, A. Ridlo, and I. Widowati, "Kajian Rumput Laut Sargassum duplicatum J. G. Agardh sebagai Penghasil Bioetanol dengan Proses Hidrolisis Asam dan Fermentasi," *J. Mar. Res.*, vol. 1, no. 2, pp. 145–151, 2012.

[24] R. V. Febrina, R. S. Nasution, and F. Arfi, "Pengaruh Variasi Massa Ragi Saccharomyces cerevisiae dan Waktu Fermentasi Terhadap Kadar Bioetanol Berbahan Dasar Limbah Kulit Kopi Arabika (Coffea arabica L.)," *Amina*, vol. 2, no. 1, pp. 19–25, 2020, [Online]. Available: https://doi.org/10.22373/amina.v2i1.498

[25] S. S. Harsono, Salahuddin, M. Fauzi, G. S. Purwono, D. Soemarno, and Kissinger, "Second Generation Bioethanol from Arabica Coffee Waste Processing at Smallholder Plantation in Ijen Plateau Region of East Java," *Procedia Chem.*, vol. 14, pp. 408–413, 2015, doi: 10.1016/j.proche.2015.03.055.