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Reducing Sugar Production by Cellulose Immobilized Enzyme from the Oil Palm Empty Fruit Brunch (OPEFB) Treated by Organosolv Pretreatment

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ABSTRACT

Oil Palm Empty Fruit Bunches (OPEFB) is lignocellulosic that consists of 13.20-25.31% lignin, 42.70-65.00% cellulose, and 17.10-33.50% hemicellulose. Cellulose can be used as a material for a new renewable energy source in the term of reducing sugar through a combination of organosolv pretreatment and hydrolysis process using immobilized enzymes. Organosolv pretreatment was used for lignin degradation, by using ethanol as solvent, which are environmentally friendly and easy to recover, with a concentration S/L 10%(w/w), in 160°C for 90-150 min. The following process is to produce crude enzyme from *Aspergillus niger* and *Trichoderma viride*. The resulting crude enzyme cellulase activity of 0.774 U/mL. Then, the crude enzyme is immobilized by Chitosan-GDA. OPEFB hydrolysis process with immobilized cellulase was carried out for 5, 7 and 9 days at 37°C. The best result of lignin degradation reaches out 56.68% lignin removal at 160°C for 150 min, while the hydrolysis of cellulose gives the highest yield, 47.59%, in the 9 days processing time.

***Keywords*:** hydrolysis, Oil Palm Empty Fruit Bunches (OPEFB), organosolv, reducing sugar.

1. INTRODUCTION

Indonesia is the country with the highest level of palm oil production in the world. In 2020, the area of oil palm plantations was 14.586 million hectares [1]. 230 kg of Oil Palm Empty Fruit Bunches (OPEFB) can be produced from 1 ton of oil palm. OPEFB is still allowed to accumulate as organic matter in oil palm plantations today [2].

OPEFB has three main components, including cellulose, lignin, and hemicellulose in 42.70-65.00%, 13.20-25.31% and 17.10-33.50%(w/w), respectively [3]. Cellulose can be converted by enzymatic hydrolysis become simple sugars for further conversion into high-value products such as lactic acid as a biodegradable polymer that can be used to produce conventional plastics and biofuels such as bioethanol and biohydrogen. The presence of lignocellulosic OPEFB is difficult to degrade, and the effective pretreatment is needed to break the lignocellulosic structure.

Several lignocellulosic pretreatment methods have been developed to degrade lignin including acids, bases, hydrogen peroxide, and ozone. According to Mondylaksita et al. [4], acid pretreatment leaves the remaining lignin and cellulose, which will further interfere with the enzymatic hydrolysis process. Alkaline pretreatment resulted in lignin dissolving in the liquid being challenging to recover. Hydrogen peroxide pretreatment is toxic to the environment, and ozone pretreatment requires a large investment. In addition to these 4 methods, there is organosolv pretreatment, which uses organic solvents. Organosolv pretreatment is

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a promising method for degrading lignin. According to Salsabila et al. [5], pretreatment of OPEFB using the organic solvent ethanol produced 98.07% cellulose fractionation, 69.28% lignin, and 98.71% hemicellulose. The organic solvent used is environmentally friendly and more economical because the solvent is easy to recover by distillation or extraction methods. Therefore, organosolv pretreatment using organic solvents, one of which is ethanol, is present to overcome the shortcomings of the previous methods, because ethanol has good solubility of lignin, lower toxicity compared to other alcohol-based solvents, and miscibility with water [4].

Appropriate pretreatment will result in high lignin degradation so that it can increase the activity of enzymatic hydrolysis. Some disadvantages of enzymatic hydrolysis include the uneconomical price of enzymes and the difficulty of separating dissolved enzymes in solvents for reuse [6]. Enzyme immobilization is one way to maintain enzyme activity. Immobilized enzymes can be reused. Chitosan-GDA is a matrix applied to immobilize enzymes and can maintain its activity for up to five cycles [7].

Trichoderma viride and *Aspergillus niger* can produce cellulase enzymes for the enzymatic hydrolysis process. Hydrolysis using *Trichoderma viride* and *Aspergillus niger* fungi have been studied by Juliastuti et al. [8] and this study showed that the using of *Trichoderma viride* and *Aspergillus niger* mix culture, in the ratio of 1:2, can increase reducing sugar levels up to 84.9%.

The combination of pretreatment using organosolv to degrade lignin and the use of *Trichoderma viride* and *Aspergillus niger* to produce immobilized cellulase enzymes by chitosan-GDA is expected to increase the reducing sugar content of OPEFB enzymatic hydrolysis.

2. MATERIALS AND METHODS

2.1 Materials and Experiment Apparatus

The tools used in this research included one set of reflux apparatus, oven (UN 55 53L), digital hot plate stirrer (IKA C-MAG HS), high vacuum autoclave, incubator shaker, spectrophotometer UV-Vis (B-ONE), pH meter, thermometer, a set of Chesson method and Dinitro salicylic acid (DNS) analysis tools such as 3 erlenmeyer 250 mL, 3 pcs filter paper, furnace, 3 crucible cup 20 mL, test tube, and vortex. The organosolv pretreatment equipment is shown in Figure 1.

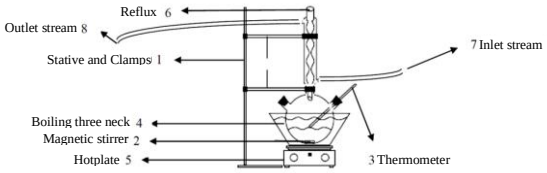

Figure 1. One set of reflux apparatus

The materials used in this study were Oil Palm Empty Fruit Bunches (OPEFB) and some chemicals like ethanol 96%, distilled water, 3,5-Dinitrosalicilyc reagent, NH_4OH 5%, H_2SO_4 20%, fungi (*Aspergillus niger* and *Trichoderma viride*), Potato Dextrose Agar (PDA), yeast extract, glucose, chitosan, glutaricdialdehyde (GDA), citric acid, sodium citrate, sodium acetate, tween-80, NaOH 1.5 M, and mineral salt.

2.2 Pretreatment of Oil Palm Empty Fruit Bunches

The OPEFB which has passed 60 mesh was refluxed in a solvent solution. The solvent solution was made by ethanol and distilled water at the ratio of 1:1 that contained 5% NH_4OH as the catalyst. Reflux was carried out at 160°C for 90, 120, and 150 min with a solid-of-liquid (S/L) ratio of 1:10 by used OPEFB as a solid and solvent solution as a liquid. Then, the reflux solution was extracted with filter paper. The pretreated OPEFB will be used for the subsequent hydrolysis process, while the remaining filtration solution will be subjected to the lignin removal process through precipitation using 20% H_2SO_4 until the solution has a pH of 2 so that lignin and solvent can be separated, allowing the solvent to be reused.

2.3 Production of Crude Enzyme

Production of the crude enzyme was done by inoculated *Trichoderma viride* and *Aspergillus niger* fungi with a ratio of 1:2 in the mineral salt solution that has been sterilized, then added with 5 gr OPEFB as a substrate. The component concentration of the mineral salt solution is presented in Table 1.

Table 1. The mineral salt solution composition

Component	Composition (g/L)
Yeast extract	5
$FeSO_4.7H_2O$	0.7
$(NH_4)_2SO_4$	0.7
$ZnSO_4.7H_2O$	2.5
$CaCl_2.2H_2O$	0.2
KH_2PO_4	1
$MnSO_4.H_2O$	0.8
$MgSO_4.7H_2O$	0.15

The inoculated solution was incubated at 35°C for 8 days. The fermentation medium overgrown with fungi was added to 100 mL citrate buffer (pH 3) contained 0.1% Tween-80. The solution was centrifuged at 4°C and 3500 rpm for 15 min and filtered through filter paper to obtain crude enzyme.

2.4 Immobilized Enzyme with Chitosan-GDA

In the immobilized enzyme process, 30 mL of crude enzyme that has been produced was dissolved in 60 mL chitosan solution. Chitosan solution was made by 2.7 g chitosan dissolved in 60 mL of 2% acetic acid solution that has been stored for 24 hours under room temperature. Then, the crude enzyme and chitosan solution was dripped into 1.5 M NaOH solution using a syringe to form the solution into beads. The beads were rinsed with distilled water before being soaked it in 1% GDA solution for 10 min. After that, the beads were rinsed again with distilled water before can be stored at 4°C.

2.5 Enzymatic Hydrolysis of Cellulose and Hemicellulose

In the enzymatic hydrolysis process, 1 g of treated OPEFB was dissolved in 0.1 M citrate buffer (pH 3) until the volume reach out 100 mL. Then, it was added by 18.6 U/g cellulose into the flask. Cellulose weight is calculated using Equation 1:

$$\text{Cellulose weight (g)} = \frac{18.6\ \text{U}}{\text{Enzyme activity (U/g)}} \quad (1)$$

The hydrolysis process was carried out for 5, 7 and 9 days in an incubator shaker with a shaker speed of 125 rpm and a temperature of 37°C.

2.6 Data Analysis

Lignin, cellulose and hemicellulose content was analyzed by the chesson method, while the enzyme activity test and reducing sugar content was analyzed used the DNS method.

3. RESULTS AND DISCUSSION

3.1 Pretreatment of Oil Palm Empty Fruit Bunch

Initial OPEFB characterization before organosolv pretreatment is needed to compare cellulose, hemicellulose and lignin levels with those after pretreatment. The results of the initial characterization of OPEFB are presented in Table 2.

Table 2. Result of OPEFB characterization before organosolv pretreatment

Component	This Study	Rahmasita et al. [3]
Cellulase	38.91%	42.70-65.00%
Hemisellulose	13.75%	17.10-33.50%
Lignin	24.83%	13.20-25.31%
Extractives and ash	22.51%	27.00%

Based on the data in Table 2, the levels of cellulose, hemicellulose, extractives and ash obtained were lower than in previous study while the lignin content is still in the same range as the previous study. The difference in composition can be caused by several factors,

namely different varieties of oil palm and the lignocellulosic composition of OPEFB. The diverse of the lignocellulosic composition of OPEFB can be affected by harvesting techniques, storage areas, land conditions, drying processes, milling and other processing processes.

The initial OPEFB that has been characterized then pretreated using the organosolv technique with ethanol as the solvent. In ethanol (C_2H_5OH), there is a hydroxyl functional group (OH^-) which will break the ester bonds of lignin and minor hydrolysis of hemicellulose glycosidic bonds so that the aromatic compounds and polysaccharides in lignocellulose in OPEFB will break [9]. This is shown by the differences in the results of OPEFB characterization before and after organosolv pretreatment in Figure 2.

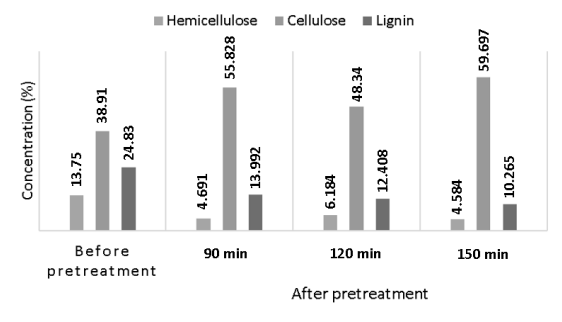

Figure 2. The content of hemicellulose, cellulose and lignin before and after organosolv pretreatment

Figure 2 shows an increase in cellulose content and a decrease in lignin and hemicellulose levels as the pretreatment time increases. Most hemicelluloses are soluble in solvents because hemicelluloses consist of short and amorphous polymer chains. Meanwhile, adding the NH_4OH catalyst can accelerate the delignification and breaking of lignin intra-molecular bonds due to the increased concentration of hydroxyl ions in ethanol. The breakdown of lignin compounds causes lignin to dissolve in the solvent [10]. In addition, theoretically, the cellulose content after pretreatment will increase over time due to the increasing amount of dissolved lignin and hemicellulose. However, at 120 min, the cellulose content decreased because cellulose ß and cellulose γ could be dissolved in the NH_4OH base catalyst.

The delignification process also needs to be supported by heating. Heating to 160°C will make the solvent consisting of ethanol and water evaporate. The evaporating solvent will put pressure on the substrate, thereby helping break down lignocellulose in OPEFB [11]. The success parameter of the fractionation with organosolv pretreatment was measured by the highest percentage of lignin degradation. The rate of lignin degradation is presented in Figure 3.

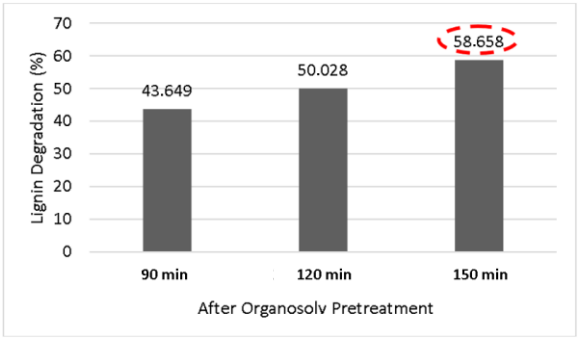

Figure 3. Percentage of lignin removal after the organosolv pretreatment

Figure 3 shows the increase in the percentage of lignin removal before and after pretreatment. As the pretreatment time increases, the longer contact time between the substrate and the lignin solvent which results in an increasing percentage of lignin degradation. The largest lignin degradation was 58.658% obtained under operating conditions of 150 min pretreatment time, S/L ratio of 1:10 and temperature of 160°C. The success of the lignin degradation process is supported by the mechanical pretreatment of OPEFB, which aims to increase the surface area of OPEFB to make it easier to break down the cellulose structure into glucose. After conducting organosolv pretreatment for 150 min, there was a color change in the solvent from clear to brown due to the presence of lignin dissolved in the solvent.

The remaining solvent after organosolv pretreatment is presented in Figure 4.

(a) (b)

Figure 4. Visualization of solvent: (a) before pretreatment; (b) after pretreatment

Lignin in the solvent can be separated by lignin precipitation and can also be separated by distillation or extraction [12]. With this separation, the remaining ethanol can be reused for the organosolv process, while lignin can be utilized as a high-value product.

3.2 Production of Crude Enzyme Cellulase from *Aspergillus niger* and *Trichoderma viride*

The enzymes used for the hydrolysis process in this study were crude enzymes derived from the *Aspergillus niger* and *Trichoderma viride* fungi with OPEFB as substrate. One of the advantages of crude enzyme in the hydrolysis process is its higher enzyme activity compared to ordinary enzymes. The crude enzyme activity of *Aspergillus niger* and *Trichoderma viride* obtained was 0.774 U/mL. The comparition results of the enzyme activity using multicultural and monoculture fungi in the production of cellulase enzymes are presented in Table 3.

Table 3 shows the comparison of the enzyme activity which produced in this study with the other references. Based on the table, *Aspergillus niger* monoculture produced higher cellulase enzyme activity than *Trichoderma viride* monoculture. Meanwhile, the multicultural of the two types of mold increased enzyme activity. This is because *Aspergillus niger* produces high β-glucosidase, but low exoglucanase and endoglucanase content, while *Trichoderma viride* can produce high exoglucanase and endoglucanase, but low β-glucosidase content [13]. Therefore, the *Aspergillus niger* and *Trichoderma viride* multiculture can improve the performance of enzymes to degrade cellulose, so that the hydrolysis process runs optimally.

3.3 Crude Enzyme Cellulase Immobilized on Chitosan-GDA

Crude enzyme cellulase, whose enzyme activity was measured then immobilized on chitosan-GDA beads. One of the goals of immobilizing the enzyme is using chitosan-GDA beads so the enzyme can be reused.

However, in this study, after the hydrolysis process, there was a small amount of OPEFB residue attached to the beads so that they had the same color as OPEFB. That makes it difficult to separate the OPEFB from the beads. A comparison of the visualization of the beads before and after hydrolysis is present in Figure 5.

Table 3. Result of comparison of enzyme activity using multiculture and monoculture of fungi

Fungi	Enzyme Activity (U/mL)	Substrate	Ref.
Trichoderma viride and *Aspergillus niger*	0.774	OPEFB	This study
Aspergillus niger	0.172	Bagase	Idiawati et al. [14]
Aspergillus niger	0.123	Rice straw	Parwita et al. [15]
Trichoderma viride	0.00535	CMC	Kurniawan et al. [16]

(a) (b)

Figure 5. Visualization of beads: (a) before hydrolysis; (b) after hydrolysis

The beads condition, as shown in Fig. 5(b), can further reduce the enzyme performance if the beads will be reused because the presence of OPEFB residue attached to the beads will limit the contact between the new OPEFB substrate and the enzymes in the beads.

3.4 Performance of Immobilized Enzyme Hydrolysis by Chitosan-GDA Effect of Hydrolysis Time on Concentration of Reducing Sugar

The cellulase enzyme beads immobilized by chitosan-GDA used for the hydrolysis process of organosolv pretreatment results at optimum conditions. The concentration of reducing sugars resulting from hydrolysis is presented in Figure 6.

Figure 6 shows that the concentration of the reducing sugars increases with the longer of the hydrolyzation process. It indicates that each cellulase enzyme variable can hydrolyze cellulose and hemicellulose substrates in OPEFB into reducing sugars. This is supported by an increase in the activity of cellulase enzyme production from *Trichoderma viride* and *Aspergillus niger* as more active sites of the cellulase enzyme work to cut carbon chains in cellulose structure into simple structures in the form of glucose. So that, the glucose levels produced are higher with the longer hydrolyzation process. In this study, the hydrolysis process using enzymes immobilized by chitosanGDA had the highest concentration of reducing sugar on day 9 of 4.370 g/L with 1 gram of OPEFB substrate in 100 mL of solution or equivalent to 10 g/L of OPEFB substrate. The comparison results of the reducing sugar concentrations from this study with other studies is presented in Table 4.

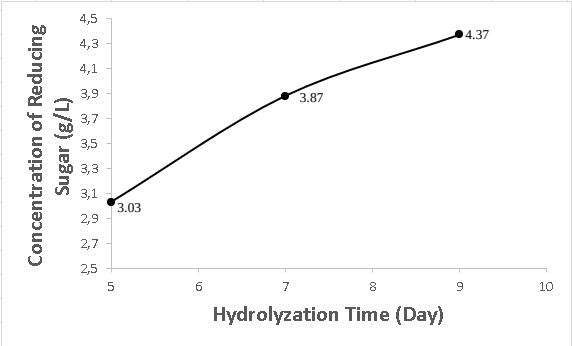

Figure 6. The condition of the reducing sugar results from the hydrolysis process.

Table 4. Comparison of the result reducing sugar concentrations with other studies using Hydrolysis Sample at Day-5

Concentration Reducing Sugar (g/L)	Enzyme Type	Substrate	Ref
3.875	Immobilized crude enzyme	OPEFB	This study
5.537	Free enzyme without immobilization	Sugarcane bagase	Yuwono, et al. [17]

Based on Table 4, the five days hydrolyzation results lower concentration of the reducing sugar compared to the result obtained by Yuwono, et.al. [17]. The different in results can be caused by the different enzymes used. A previous study conducted by Yuwono, et.al. [17] used free enzyme without immobilization, which makes the active sites of the enzymes could work to hydrolyze cellulose and hemicellulose substrates. In contrast with this study, immobilization of enzymes with chitosan-GDA could reduce enzyme activity due to partitioning, obstruction of the mass diffusion process, and the presence of steric barriers [15]. In addition, cross-linking can result in changes to the active site of the enzyme. So that, the resulting enzyme activity after immobilization is not too high as the shape of the active center of the enzyme and no longer suitable for the substrate. Thus, it causes the enzyme to lose its activity. According to Rulianah et al. [18], one way to increase enzyme activity is by adding more substrate to its optimum condition.

3.5 Effect of Hydrolysis Time on Yield and Gain Reducing Sugar

The yield and gain of the reducing sugar resulting from the enzymatic hydrolysis process with immobilized enzymes are presented in Table 5.

Table 5. The yield and gain of the reducing sugar results from enzymatic hydrolysis process

Hydrolysis Sample	Yield	Gain Reducing Sugar (mg/g)
Day 5	42.42%	424.23
Day 7	48.23%	482.26
Day 9	47.59%	475.88

Table 5 shows an increase in the yield and gain of the reducing sugars over time. The gain of the reducing sugar and the yield on Day 7 was the highest and then decreased on Day 9. It indicates that the maximum hydrolyzation time to produce high yield is 7 days. Hydrolyzation that goes too long can cause degradation of the reducing sugar, resulting in lower yield [19]. In addition, lower yield on Day 9 can also be caused by the formation of mold that uses the reduced sugar obtained as a source of energy or nutrients for its growth and decreasing enzyme performance due to the large number of products that have been formed.

4. CONCLUSION

The optimum conditions for organosolv pretreatment were obtained at a temperature of 160°C, an S/L ratio of 1:10, and a pretreatment time of 150 min. Under these conditions, lignin was degraded by 58.658%. The hydrolyzation process was carried out enzymatically using crude enzyme obtained from *Aspergillus niger* and *Trichoderma viride* with enzyme activity of 0.774 U/mL. Hydrolyzation using OPEFB from organosolv pretreatment at optimum conditions and immobilized crude enzyme in chitosan-GDA was able to produce the reducing sugar on Day 5, 7, and 9, respectively, with a concentration of 3.03; 3.875; and 4.370 g/L. By the reducing sugar concentration, the higest yield of hydrolysis process is 47.59%. The optimum time for the enzymatic hydrolysis process using immobilized crude enzyme cellulase is 9 days.

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REFERENCES

[1] Badan Pusat Statistik, Statistik Kelapa Sawit Indonesia, 2020.

[2] R. Siswanto, M. I. Arsyad, R. A. Wicaksono, Studi Karakteristik

Terhadap Uji Impact Dan Bending Komposit Tandan Kosong Kelapa Sawit-Polyester Dengan Perlakuan Alkali Dan Hidrogen Peroksida, *J. Teknol. Rekayasa Tek. Mesin*, vol. 2, no. 2, pp. 129–134, 2021.

[3] M. E. Rahmasita, M. Farid, H. Ardhyananta, Analisa Morfologi Serat Tandan Kosong Kelapa Sawit Sebagai Bahan Penguat Komposit Absorpsi Suara, *J. Tek. ITS*, vol. 6, no. 2, pp. A584–A588, 2017.

[4] K. Mondylaksita, J. A. Ferreira, R. Millati, W. Budhijanto, C. Niklasson, M. J. Taherzadeh, Recovery of high purity lignin and digestible cellulose from oil palm empty fruit bunch using low acid-catalyzed organosolv pretreatment, *Agronomy*, vol. 10, no. 5, pp. 674, 2020.

[5] S. R. Salsabila, A. S. Hasanah, E. M. Widyanti, L. Elizabeth, Studi Literatur Pengaruh Kondisi Operasi Fraksionasi TKKS dengan Proses Organosolv Terhadap Kadar Selulosa dan Lignin, *Pros. 12th Ind. Res. Work. Natl. Semin.*, pp. 770–778, 2021.

[6] L. L. Sitompul, I. N. F. Putri, Produksi Gula Reduksi dari Sabut Kelapa dengan Menggunakan Kombinasi Enzim Selulase dari *A. Niger* dan *T. Reesei* Terimobilisasi pada Chitosan Magnetic Microparticle, Bachelor Thesis, Institut Teknologi Sepuluh Nopember, Indonesia, 2017.

[7] N. F. Putri, M. R. G. Dwinanda, A. Widjaja, A. Hamzah, H. F. Sangian, Mass Transfer and Reaction in Hydrolysis of Coconut Husk Using Immobilized Enzyme on Chitosan Magnetic Nanoparticle, *IOP Conf. Ser.: Mater. Sci. Eng.*, vol. 1053, pp. 012046, 2021.

[8] S. R. Juliastuti, N. Hendrianie, K. R. Sabar, S. Anggita, Glucose production from oil palm empty fruit bunch (OPEFB) using microwave and fungal treatment method, *IOP Conf. Ser.: Earth Environ. Sci.*, vol. 963, pp. 012053, 2022.

[9] A. Nuryahya, D. A. P. Kartika, Pra Rancangan Pabrik Asam Levulinat dari Tandan Kosong Kelapa Sawit dengan Kapasitas Produksi 25.000 Ton/Tahun, Bachelor Thesis, Institut Teknologi Kalimantan, Indonesia 2021.

[10] H. Heradewi, Isolasi Lignin dari Lindi Hitam Proses Pemasakan Organosolv Serat Tandan Kosong Kelapa Sawit (TKKS), Bachelor Thesis, Institut Pertanian Bogor, Indonesia, 2007.

[11] P. Schulze, A. Seidel-Morgenstern, H. Lorenz, M. Leschinsky, G. Unkelbach, Advanced process for precipitation of lignin from ethanol organosolv spent liquors, *Bioresour. Technol.*, vol. 199, pp. 128–134, 2016.

[12] H. A. Darojati, R. Purwadi, C. B. Rasrendra, Proses Fraksionasi Biomassa dari Tandan Kosong Kelapa Sawit melalui Metode Organosolv Etanol dengan Penambahan Katalis, *J. Selulosa,* vol. 10, no. 2, pp. 73–80, 2020.

[13] Elwin, Optimasi Produksi Enzim Selulase Oleh *Aspergillus niger* dan *Trichoderma viride* untuk Hidrolisis Selulosa Eceng Gondok (*Eichornia crassipes*), Master Thesis, Universitas Brawijaya, Indonesia, 2016.

[14] N. Idiawati, E. M. Harfinda, L. Arianie, Produksi Enzim Selulase oleh Aspergillus niger pada Ampas Sagu, *J. Natur Indones.*, vol. 16, no. 1, pp. 1–9, 2014.

[15] G. E. K. Parwita, D. R. Tardan, Produksi Gula Reduksi Dengan Hidrolisis Sabut Kelapa Hasil Pretretament Alkali Menggunakan Crude Enzim Terimobilisasi, Bachelor

Thesis, Institut Teknologi Sepuluh Nopember, Indonesia, 2016.

[16] F. Kurniawan, T. T. Nugroho, A. Dahliaty, Isolasi dan Pemekatan Enzim Selulase *Trichoderma sp* LBKURCC28 Menggunakan Metode Penggaraman $(NH_4)_2SO_4$ 80% Serta Penentuan Aktivitas dan Aktivitas Spesifik Enzim, *JOM FMIPA*, vol. 1, no. 2, pp. 1–6, 2014.

[17] T. Yuwono, E. Rolanda, A. Widjaja, S. Soeprijanto, Fermentasi hidrolisat enzimatik bagasse tebu menjadi hidrogen, *J. Teknik POMITS*, vol. 1, no. 1, pp. 1–5, 2012.

[18] S. Rulianah, Z. Irfin, M. Mufid, P. Prayitno, Produksi *Crude* Selulase dari Bahan Baku Ampas Tebu Menggunakan Kapang *Phanerochaete chrysosporium*, *J. Tek. Kim. dan Lingkung.*, vol. 1, no. 1, pp. 17–27, 2017.

[19] D. De Idral, M. Salim, E. Mardiah, Proses Hidrolisis Asam Dan Menggunakan *Saccharomyces cerevisiae*, *J. Kim. Unand*, vol. 1, no. 1, pp. 34–39, 2012.