Biological Pretreatment of Oil Palm Empty Fruit Bunch by Schizophyllum commune ENN1 without Washing and Nutrient Addition

Authors:

Enis Natasha Noor Arbaain, Ezyana Kamal Bahrin, Mohamad Faizal Ibrahim, Yoshito Ando, Suraini Abd-Aziz

Date Submitted: 2019-09-05

Keywords: fermentable sugar, lignin, Schizophyllum commune, OPEFB, biological pretreatment

Abstract:

Washing and drying are common steps for oil palm empty fruit bunch (OPEFB) preparation prior to pretreatment. However, the mass balance of OPEFB preparation proved a major loss of OPEFB during the washing and drying steps. An indigenous fungus, Schizophyllum commune ENN1 was used for delignification of unwashed OPEFB in biological pretreatment without nutrient addition. S. commune ENN1 achieved a maximum lignin removal of 53.8% after 14 days of biological pretreatment of unwashed OPEFB. S. commune ENN1 was able to grow on unwashed OPEFB during biological pretreatment at 55% of moisture content and 5% of oil residue. The highest amount of reducing sugars obtained from OPEFB pretreated by S. commune ENN1 was 230.4 ± 0.19 mg/g with 54% of hydrolysis yield at 96 h. In comparison, the sugar yield of OPEFB pretreated by Phanerochaete chrysosporium was 101.2 ± 0.04 mg/g. This study showed that S. commune ENN1 was feasible to remove lignin of OPEFB through biological pretreatment for enzymatic saccharification without washing and addition of nutrients.

Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):	LAPSE:2019.0981
Citation (this specific file, latest version):	LAPSE:2019.0981-1
Citation (this specific file, this version):	LAPSE:2019.0981-1v1

DOI of Published Version: https://doi.org/10.3390/pr7070402

processes

MDPI

Communication

Biological Pretreatment of Oil Palm Empty Fruit Bunch by *Schizophyllum commune* ENN1 without Washing and Nutrient Addition

Enis Natasha Noor Arbaain [1], Ezyana Kamal Bahrin [1,2,*], Mohamad Faizal Ibrahim [1,2], Yoshito Ando [3] and Suraini Abd-Aziz [1]

[1] Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang 43400, Malaysia
[2] Institute of Tropical Forestry and Forest Products, Universiti Putra Malaysia, Serdang 43400, Malaysia
[3] Department of Biological Functions and Engineering, Graduate School of Life Science and System Engineering, Kyushu Institute of Technology, 2–4 Hibikino, Wakamatsu, Fukuoka 808-0196, Japan
* Correspondence: ezyana@upm.edu.my

Received: 1 May 2019; Accepted: 5 June 2019; Published: 1 July 2019

Abstract: Washing and drying are common steps for oil palm empty fruit bunch (OPEFB) preparation prior to pretreatment. However, the mass balance of OPEFB preparation proved a major loss of OPEFB during the washing and drying steps. An indigenous fungus, *Schizophyllum commune* ENN1 was used for delignification of unwashed OPEFB in biological pretreatment without nutrient addition. *S. commune* ENN1 achieved a maximum lignin removal of 53.8% after 14 days of biological pretreatment of unwashed OPEFB. *S. commune* ENN1 was able to grow on unwashed OPEFB during biological pretreatment at 55% of moisture content and 5% of oil residue. The highest amount of reducing sugars obtained from OPEFB pretreated by *S. commune* ENN1 was 230.4 ± 0.19 mg/g with 54% of hydrolysis yield at 96 h. In comparison, the sugar yield of OPEFB pretreated by *Phanerochaete chrysosporium* was 101.2 ± 0.04 mg/g. This study showed that *S. commune* ENN1 was feasible to remove lignin of OPEFB through biological pretreatment for enzymatic saccharification without washing and addition of nutrients.

Keywords: biological pretreatment; OPEFB; *Schizophyllum commune*; lignin; fermentable sugar

1. Introduction

The rapid expansion of the Malaysian palm oil industry has significantly increased the total land area of oil palm plantation from 3.4 million hectares in 2000 to 4.7 million hectares in 2009 and reached 5.8 million hectares in 2017 [1]. This scenario has accelerated the accumulation of biomass from palm oil mills and upsurges environmental issues. Each year, about 80 million tonnes of fresh fruit bunch (FFB) is processed in 406 palm oil mills and generating about 18 million tonnes of oil palm empty fruit bunch (OPEFB) [2]. The fruitlets from the FFB is subjected to different processes, including steam sterilisation, stripping, extraction and purification for oil extraction [3]. OPEFB is one of the biomass produced after oil palm fruitlets are stripped from the FFB [4]. Due to the steam sterilisation process, the untreated OPEFB contains approximately 60% of moisture content, which makes it very favourable to be used as direct fermentation feedstock [5].

The recalcitrant characteristic of OPEFB is due to the structure of lignin, which contains benzene rings and resists biochemical degradation [6]. Therefore, pretreatment is required to modify and open up the lignocellulosic structure of OPEFB. Biological pretreatment is an environmentally friendly pretreatment using a microorganism or enzyme that offers mild pretreatment, low cost and less energy consumption [7]. *Schizophyllum commune* is one of the white rot fungi that are capable of

degrading lignin and recalcitrant polymer using ligninolytic enzymes mainly known as peroxidases and laccases [8]. The presence of oil residue on OPEFB inhibits fungal growth and consequently hinders lignin degradation. Most of the oily biomass from the agricultural industry must go through the substrate preparation process, which involves washing, drying and grinding steps before any subsequent processes particularly fermentation. Prior to biological pretreatment, the OPEFB has to be washed with tap water and detergent to remove the oil residue and dirt on the surface of the fibres. However, this substrate preparation process is not suitable to be applied as a basis in large-scale pretreatment process due to the cost, labour work and time needed to perform the process [9].

To our knowledge, biological pretreatment by indigenous *S. commune* using unwashed OPEFB as the substrate and without nutrient addition has not been reported in the literature. Moreover, the indigenous fungus is able to grow on an oily pile of OPEFB. Thus, the objective of this study was to evaluate the feasibility of *S. commune* ENN1 for lignin removal of unwashed OPEFB through biological pretreatment for enzymatic saccharification.

2. Materials and Methods

2.1. Oil Palm Empty Fruit Bunch

Fresh shredded OPEFB was obtained from a palm oil mill at Dengkil, Selangor, Malaysia. The untreated OPEFB was stored in an airtight plastic container and kept in a freezer at −40 °C to keep the freshness of the untreated OPEFB and avoid any growth of fungi before biological pretreatment.

2.2. Microorganisms

An indigenous fungus identified as *Schizophyllum commune* ENN1 was obtained from a culture collection of Biorefinery Complex, Universiti Putra Malaysia (UPM), Selangor, Malaysia. The agar (M2 medium) for *S. commune* ENN1 was prepared according to NCIM (2014), and the pH of the agar was adjusted to 6.8 [10]. *Phanerochaete chrysosporium* obtained from International Islamic University Malaysia was used as a positive control in this study. *P. chrysosporium* was grown on the potato dextrose agar (PDA) for mycelia growth. Both fungal strains were cultivated in the control condition at 30 °C for 7 days in an incubator.

2.3. Mass Balance of OPEFB Preparation Process

The OPEFB preparation process was carried out using a total of 1.0 kg of OPEFB on a dry basis as an input to the system. The weight of OPEFB was measured before and after each step throughout the process. A complete washing cycle involved soaking (overnight), washing and rinsing steps. The volume of tap water and discharged wash stream was measured in each washing cycle. Washed OPEFB was oven dried at 60 °C for 12 h, followed by a grinding step using a hammermill with a sieve size of 1 mm (Sima, Kuala Lumpur, Malaysia).

2.4. Biological Pretreatment

Biological pretreatment using *S. commune* ENN1 was carried out using five grams of untreated and unwashed OPEFB with an initial moisture content of 55.2%. The flasks were sterilised at 121 °C for 20 min using autoclave and aseptically inoculated with 5 agar plugs with an average size of 1 cm of 7 days old fungal mycelia that were previously cultured on an agar plate. No additional moistening agent or nutrient was supplied to the OPEFB pretreated with *S. commune* ENN1. Meanwhile, biological pretreatment using *P. chrysosporium* was conducted as a positive control in this experiment according to Hamisan et al. [11], as the fungus was unable to grow on oily biomass. Both biological pretreatments were carried out for 14 days at 30 °C in a 250 mL Erlenmeyer flask with cotton plugs.

2.5. Analytical Methods

The moisture content analysis of the OPEFB samples was carried out using a digital moisture analyser (A&D, Tokyo, Japan). Lignin, cellulose and hemicellulose content of untreated and biologically pretreated OPEFB were determined using the method by Iwamoto et al. [12]. Determination of oil content in OPEFB was performed with a slight modification from method conducted by Md Yunos et al. [13] on extraction time, which was reduced to 6 h. The determination of water and solvent extractives components was carried out in accordance with the National Renewable Energy Laboratory (NREL) procedure reported by Sluiter et al. [14]. All analyses were carried out in triplicate.

2.6. Enzymatic Saccharification of OPEFB

Enzymatic saccharification of biologically pretreated OPEFB by *S. commune* ENN1 and *P. crysosporium* (positive control) were carried out using enzyme comprising 10 FPU/mL Acremonium cellulase (Meiji Seika, Tokyo, Japan) at substrate loading of 5% (*w*/*v*) in 40 mL of 0.05 M acetate buffer pH 4.8 [15]. The mixtures were incubated at 50 °C in a rotary incubator shaker at 200 rpm for 120 h. Samples were taken from the mixture and centrifuged for 10 min at 10,000 rpm for determination of reducing sugar. The total reducing sugars analysis was determined using the dinitrosalicylic acid (DNS) method [16]. Glucose standard curve (0.2–1.0 g/mL) was used to determine the reducing sugar released. All experiments were performed in triplicate and results were presented as average values. The sugar yield and hydrolysis yield were calculated using Equations (1) and (2), respectively.

$$\text{Sugar yield} = \frac{\text{Sugar from saccharification}}{\text{Sugar from untreated sample}} \times 100, \quad (1)$$

$$\text{Hydrolysis yield} = \frac{\text{Glucose}}{\text{Cellulose}} \times 100. \quad (2)$$

3. Results and Discussion

3.1. OPEFB Preparation Process

Mass balance analysis was carried out to validate the OPEFB preparation process prior to biological pretreatment by common fungi that are unable to grow on the oily substrate. Figure 1 demonstrates the mass balance of the OPEFB preparation process at laboratory scale.

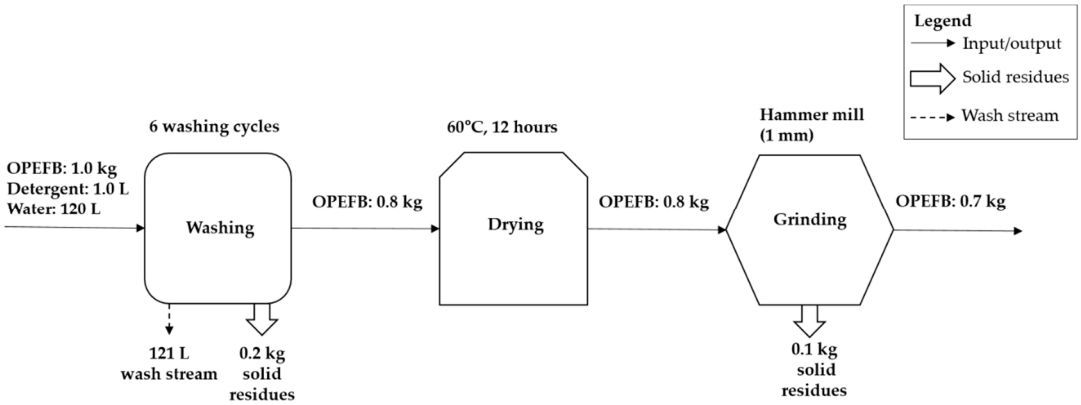

Figure 1. Mass balance diagram of the oil palm empty fruit bunch (OPEFB) preparation process.

A large amount of water was required in order to remove residual oil and foreign particles on the surface of OPEFB. Washing step in the OPEFB preparation process discharged approximately 121 L of wash stream and 0.2 kg of solid residues, which mainly consist of water, detergent and the impurities (residual oil and contaminants) from the surface of OPEFB. The next step involved oven drying, which

reduced the moisture content of OPEFB to less than 10% after the washing step to avoid the growth of fungi on washed OPEFB. The grinding step using a hammermill reduced the size of OPEFB to 1 mm and discharged approximately 0.1 kg of solid residues. From the mass balance, the highest mass loss in the OPEFB preparation process was derived from the washing step (0.2 kg). In a single input of 1.0 kg of untreated OPEFB, around 70% of OPEFB was recovered by the end of the process. This suggests that a total of 30% of the OPEFB sample was lost after the OPEFB preparation process. The mass loss after each operation in the process is an important aspect in the determination of the efficiency of the process [17]. Moreover, 120 L of water per kg of OPEFB could be saved by omitting the washing step prior to pretreatment. The substrate preparation process requires a large proportion of water in scale-up pretreatment. This forced the industries to discover a new approach to skip the substrate preparation process [18]. Biological pretreatment by *S. commune* ENN1 enabled it to grow on untreated OPEFB and omit the substrate preparation process. This served as an added value compared to the existing procedure in the pretreatment of OPEFB.

3.2. Composition of Pretreated OPEFB

The composition of untreated and biologically pretreated OPEFB by *S. commune* ENN1 and *P. chrysosporium* is shown in Table 1. OPEFB pretreated by *S. commune* ENN1 showed higher lignin removal of 53.8% compared to *P. chrysosporium*, with only 38.6% of lignin removal. Increment in hemicellulose (10.9%) and cellulose (14.6%) contents indicated that hemicellulose and cellulose content of the pretreated OPEFB were exposed due to the modification of lignocellulosic structure in OPEFB after pretreating by *S. commune* ENN1. This exhibited that *S. commune* ENN1 was able to modify and degrade the lignin structure of OPEFB through biological pretreatment without washing and addition of nutrients. The amount of solvent extractive in biologically pretreated OPEFB by *P. chrysosporium* was decreased to 3.7% due to the washing and drying steps prior to the pretreatment. The washed OPEFB contained a low amount of solvent extractive such as terpenoids, waxes, fatty acids and phenolic substances [19]. *P. chrysosporium* was unable to grow on unwashed OPEFB and needed the addition of nutrient throughout the biological pretreatment (unpublished data). The results proved that *S. commune* ENNI is a good candidate to pretreat OPEFB for the enzymatic saccharification process without the initial washing step to remove excess oil residues.

Table 1. Comparison of the composition of untreated and biologically pretreated OPEFB by *Schizophyllum commune* ENN1 and *Phanerochaete chrysosporium* after 14 days of incubation.

OPEFB Samples	Composition [1] (%)					
	Lignin	Hemicellulose	Cellulose	Extractives		Ash
				Water	Solvent	
Untreated OPEFB	21.0 ± 0.1 [c]	39.0 ± 0.4 [b]	32.8 ± 0.5 [d]	2.3 ± 0.4 [a]	5.9 ± 0.4 [b]	1.16 ± 0.2 [b]
Schizophyllum commune ENN1 (this study)	9.7 ± 1.0 [b]	43.8 ± 4.6 [b]	38.4 ± 4.4 [c]	15. 3 ± 1.1 [c]	5.7 ± 0.4 [b]	2.57 ± 0.4 [c]
Phanerochaete chrysosporium [2]	12.9 ± 2.5 [b]	37.0 ± 0.5 [b]	44.2 ± 0.0 [b]	7.5 ± 0.2 [b]	3.7 ± 0.1 [b]	1.45 ± 0.3 [b]

[1] indicates the values reported to represent average values ± standard deviation of triplicate samples. [2] indicates the positive control of the experiment [a,b,c] indicate significant differences between OPEFB samples at $p < 0.05$ level.

Physical changes on the surface of untreated and pretreated OPEFB were examined using a scanning electron microscope (SEM), as shown in Figure 2. The surface of untreated OPEFB was rough where the craters were filled with spiky round shaped silica bodies on the surface of the fibre, as stated by Abdul et al. [20] Meanwhile, the surface of OPEFB pretreated with *S. commune* ENN1 shows dense growth with a network of filamentous hyphae on the surface of the strand compared to OPEFB pretreated with *P. chrysosporium*. *S. commune* has an irregularly branched format, with filamentous hyphae in typical conidial chains and well-defined rod clusters [21]. The fungal hyphae moved toward the open pores inside the crater and penetrated inside the OPEFB strands.

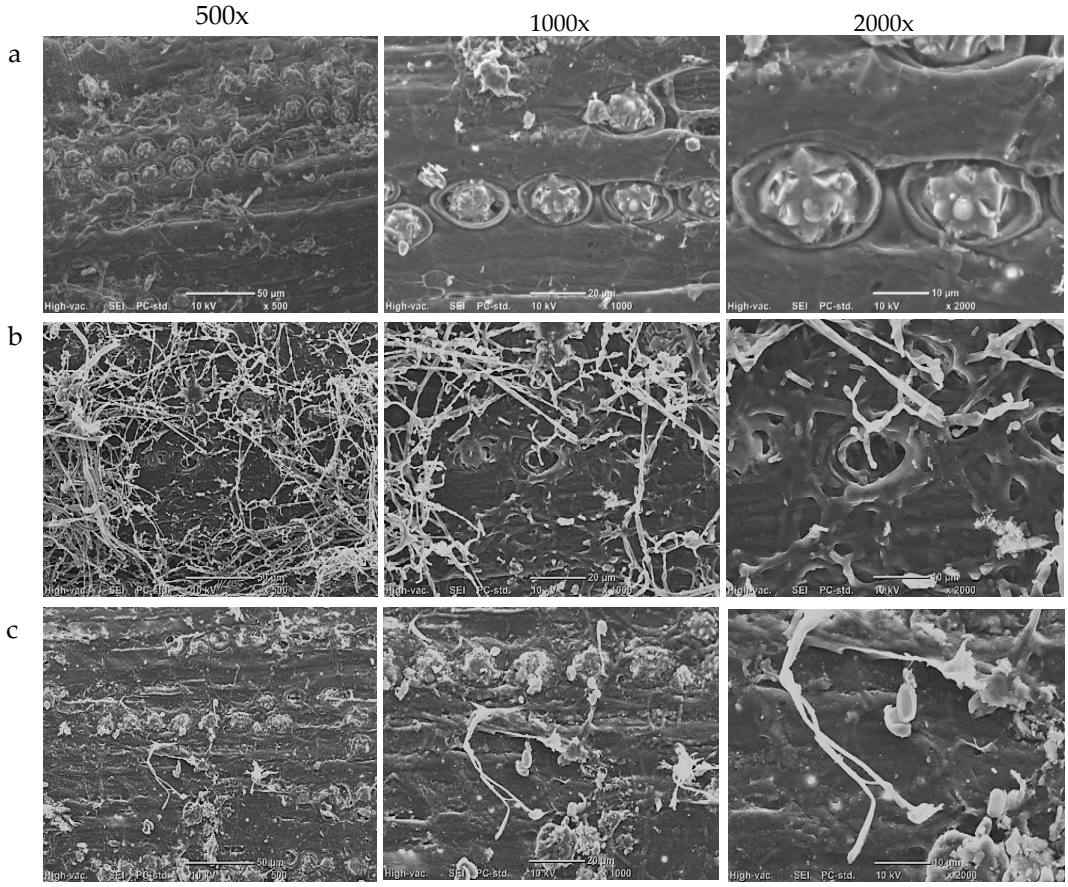

Figure 2. SEM images on the surface of OPEFB. (**a**) Untreated, (**b**) biological pretreatment with *Schizophyllum commune* ENN1, (**c**) biological pretreatment with *Phanerochaete chrysosporium* at magnification 500×, 1000× and 2000×.

3.3. Moisture and Oil Residue Contents of Pretreated OPEFB

Moisture content is one of the key elements in fungal growth during the biological pretreatment process. The initial moisture content of untreated OPEFB was recorded at 55.2%, which is relatively high due to the steam sterilisation process at the palm oil mill. The moisture content of pretreated OPEFB is able to be maintained throughout the biological pretreatment without a substrate preparation process. Therefore, biological pretreatment by *S. commune* ENN1 can omit the addition of nutrient or moistening agent. From day 7 to 14 of incubation time, *S. commune* ENN1 was able to grow at moisture content less than 50% and still able to delignify the OPEFB (Figure 3a). By contrast, *Daedalea flavida* demonstrated minimal growth at the low moisture content (45%) and thus reduced delignification of cotton stalks [22].

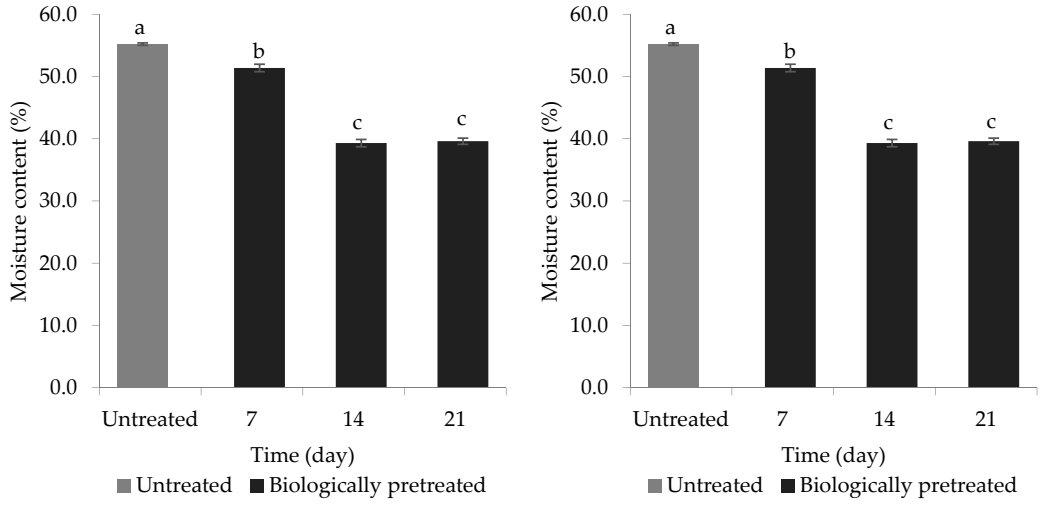

Figure 3. (**a**) Moisture content of untreated and biologically pretreated OPEFB by *Schizophyllum commune* ENN1; (**b**) oil residue of untreated and biologically pretreated OPEFB by *Schizophyllum commune* ENN1. Error bars represent the standard deviation of triplicate samples. Different letters on error bar indicate significant differences between time (day) at $p < 0.05$ level.

After 7 days of biological pretreatment with *S. commune* ENN1, the oil residue decreased by 26.2%. The amount of residual oil significantly reduced to 0.8%, which is 85.3% oil residue loss after 14 days (Figure 3b). The reduction of oil residue probably due to the lipolytic enzyme's activity that hydrolysed the thin oil layer on the surface of press-shredded OPEFB. This is in agreement with a study that stated *Schizophyllum commune* is the type of basidiomycete able to produce lipolytic enzymes [23].

3.4. Enzymatic Saccharification

Table 2 shows the total reducing sugar and hydrolysis yield of OPEFB before and after biological pretreatment. The highest amount of sugar yield obtained from biological pretreatment using *S. commune* ENN1 was 230.4 ± 0.19 mg/g, with 54% of hydrolysis yield at 96 h. The amount of sugar yield obtained is 1.8-fold from untreated OPEFB with 128.2 ± 0.00 mg/g and higher than the sugar yield of OPEFB pretreated with *P. chrysosporium* (101.2 ± 0.04 mg/g). A similar finding by Hermiati et al. [24] also exhibited that the reducing sugar yield of oil palm frond (OPF) pretreated with *Trametes versicolor* was higher than OPF pretreated with *P. chrysosporium*.

Table 2. Enzymatic saccharification of untreated and biologically pretreated OPEFB.

Time (hour)	Untreated		Biological Pretreatment			
			S. commune ENN1		*P. chrysosporium* (control) [2]	
	Sugar Yield [1] (mg/g)	Hydrolysis Yield (%)	Sugar Yield [1] (mg/g)	Hydrolysis Yield (%)	Sugar Yield [1] (mg/g)	Hydrolysis Yield (%)
24	96.4 ± 0.05 [b]	26.5	151.9 ± 0.16 [a]	35.6	78.6 ± 0.08 [c]	15.8
48	106.8 ± 0.08 [b]	29.3	187.1 ± 0.04 [a]	43.9	45.0 ± 0.13 [c]	9.4
72	123.8 ± 0.06 [b]	34.0	200.4 ± 0.00 [a]	47.0	58.7 ± 0.02 [c]	11.8
96	128.2 ± 0.00 [b]	35.2	230.4 ± 0.19 [a]	54.0	101.2 ±0.04 [c]	20.3
120	147.2 ± 0.04 [b]	40.4	224.2 ± 0.05 [a]	52.5	102.4 ± 0.06 [c]	20.6

[1] indicates the values reported to represent average values ± standard deviation of triplicate samples. [2] indicates the positive control of the experiment. [a,b,c] indicate significant differences between OPEFB samples at $p < 0.05$ level.

From this finding, the reducing sugar yield is strongly dependent on the extent of delignification from the lignocellulosic materials. Reducing the lignin content of the lignocellulosic biomass exposes the ordered crystalline structure of cellulose and facilitates cellulase accessing the substrate during enzymatic saccharification [25]. In the meantime, the sugar yield obtained from this study is higher compared to washed OPEFB pretreated using *Aspergillus niger* EB4, where the amount of sugar yield obtained is 16.2 mg/g after 144 h of saccharification time [26].

4. Conclusions

The indigenous fungus of *S. commune* ENN1 was capable of modifying and degrading the lignin structure of OPEFB in biological pretreatment. The results showed high lignin removal at 53.8%, followed by the increment of cellulose by 14.6% after 14 days of biological pretreatment using *S. commune* ENN1. The highest sugar yield 230.4 ± 0.19 mg/g with 54% of hydrolysis yield was obtained after enzymatic saccharification using the biological pretreated OPEFB at 96 h. *S. commune* ENN1 was able to remove the lignin of OPEFB through biological pretreatment without washing and nutrient addition.

Author Contributions: E.N.N.A., E.K.B., M.F.I., Y.A. and S.A.-A. conceived and designed the experiments; E.N.N.A. performed the experiments and analysed the data; all of the authors contributed to writing the article.

Funding: This research was funded by Putra Grant IPM, Universiti Putra Malaysia (UPM) (Project grant number: 9484600).

Acknowledgments: The authors would like to thank MyBrain 15 scholarship from the Ministry of Education Malaysia, UPM Graduate Research Fellowship (GRF) and Kyushu Institute of Technology, Japan for their financial and technical support for this research.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Malaysian Palm Oil Board. Overview of the Malaysian oil palm industry 2017. *Malays. Palm Oil Board MPOB* **2018**, *1*, 1–6.
2. Begum, S.; Kumaran, P.; Jayakumar, M. Use of oil palm waste as a renewable energy source and its impact on reduction of air pollution in context of Malaysia. *IOP Conf. Ser. Earth Env. Sci.* **2013**, *16*, 012026. [CrossRef]
3. Law, K.-N.; Daud, W.R.W.; Ghazali, A. Morphological and chemical nature of fiber strands of oil palm empty-fruit-bunch (OPEFB). *BioResources* **2007**, *2*, 351–362.
4. Simarani, K.; Hassan, M.A.; Abd-Aziz, S.; Wakisaka, M.; Shirai, Y. Effect of palm oil mill sterilization process on the physicochemical characteristics and enzymatic hydrolysis of empty fruit bunch. *J. Am. Med. Assoc.* **2009**, *1*, 57–66. [CrossRef]
5. Abdullah, N.; Sulaiman, F. The oil palm wastes in Malaysia. In *Biomass Now—Sustainable Growth and Use*; Intech Open: London, UK, 2013; pp. 75–100. ISBN 978-953-51-1105-4.
6. Mathew, G.M.; Sukumaran, R.K.; Singhania, R.R.; Pandey, A. Progress in research on fungal cellulases for lignocellulose degradation. *J. Sci. Ind. Res. (India)* **2008**, *67*, 898–907.
7. Ishola, M.; Millati, R.; Syamsiah, S.; Cahyanto, M.; Niklasson, C.; Taherzadeh, M. Structural changes of oil palm empty fruit bunch (OPEFB) after fungal and phosphoric acid pretreatment. *Molecules* **2012**, *17*, 14995–15012.
8. Asgher, M.; Wahab, A.; Bilal, M.; Nasir Iqbal, H.M. Lignocellulose degradation and production of lignin modifying enzymes by *Schizophyllum commune* IBL-06 in solid-state fermentation. *Biocatal. Agric. Biotechnol.* **2016**, *6*, 195–201. [CrossRef]
9. Afzanizam, N.; Nazri, M.; Jaafar, M.; Tung, C.; Jo-han, N. A review of palm oil biomass as a feedstock for syngas fuel technology. *J. Teknol. Sci. Eng.* **2015**, *5*, 13–18.
10. NCIM. National collection of industrial micro-organisms. *NCIM Cat. Strains* **2014**, *4*, 1–14.
11. Hamisan, A.F.; Abd-Aziz, S.; Kamaruddin, K.; Shah, U.K.M.; Shahab, N.; Hassan, M.A. Delignification of oil palm empty fruit bunch using chemical and microbial pretreatment methods. *Int. J. Agric. Res.* **2009**, *4*, 1–7. [CrossRef]

12. Iwamoto, S.; Abe, K.; Yano, H. The effect of hemicelluloses on wood pulp nanofibrillation and nanofiber network characteristics. *Biomacromolecules* **2008**, *9*, 1022–1026. [CrossRef] [PubMed]
13. Yunos, N.S.H.M.; Baharuddin, A.S.; Yunos, K.F.M.; Hafid, H.S.; Busu, Z.; Mokhtar, M.N.; Sulaiman, A.; Som, A.M. The physicochemical characteristics of residual oil and fibers from oil palm empty fruit bunches. *BioResources* **2015**, *10*, 14–29.
14. Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of extractives in biomass. *Natl. Renew. Energy Lab.* **2008**, *1*, 1–9.
15. Xu, F.; Sun, J.X.; Geng, Z.C.; Liu, C.F.; Ren, J.L.; Sun, R.C.; Fowler, P.; Baird, M.S. Comparative study of water-soluble and alkali-soluble hemicelluloses from perennial ryegrass leaves (Lolium peree). *Carbohydr. Polym.* **2007**, *67*, 56–65. [CrossRef]
16. Miller, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* **1959**, *31*, 426–428. [CrossRef]
17. Akanksha, K.; Sukumaran, R.K.; Pandey, A.; Rao, S.S.; Binod, P. Material balance studies for the conversion of sorghum stover to bioethanol. *Biomass Bioenergy* **2016**, *85*, 48–52. [CrossRef]
18. Zahari, M.A.K.M.; Ariffin, H.; Mokhtar, M.N.; Salihon, J.; Shirai, Y.; Hassan, M.A. Case study for a palm biomass biorefinery utilizing renewable non-food sugars from oil palm frond for the production of poly(3-hydroxybutyrate) bioplastic. *J. Clean. Prod.* **2015**, *87*, 284–290. [CrossRef]
19. Tan, J.P.; Jahim, J.M.; Harun, S.; Wu, T.Y.; Mumtaz, T. Utilization of oil palm fronds as a sustainable carbon source in biorefineries. *Int. J. Hydrog. Energy* **2016**, *41*, 4896–4906. [CrossRef]
20. Abdul, P.M.; Jahim, J.M.; Harun, S.; Markom, M.; Lutpi, N.A.; Hassan, O.; Balan, V.; Dale, B.E.; Mohd Nor, M.T. Effects of changes in chemical and structural characteristic of ammonia fibre expansion (AFEX) pretreated oil palm empty fruit bunch fibre on enzymatic saccharification and fermentability for biohydrogen. *Bioresour. Technol.* **2016**, *211*, 200–208. [CrossRef]
21. Kumar, N.S.; Min, K. Phenolic compounds biosorption onto Schizophyllum commune fungus: FTIR analysis, kinetics and adsorption isotherms modeling. *Chem. Eng. J.* **2011**, *168*, 562–571. [CrossRef]
22. Meehnian, H.; Jana, A.K.; Jana, M.M. Effect of particle size, moisture content, and supplements on selective pretreatment of cotton stalks by Daedalea flavida and enzymatic saccharification. *3 Biotech.* **2016**, *6*, 1–13. [CrossRef] [PubMed]
23. Kam, Y.C.; Hii, S.L.; Sim, C.Y.Y.; Ong, L.G.A. *Schizophyllum commune* lipase production on pretreated sugarcane bagasse and its effectiveness. *Int. J. Polym. Sci.* **2016**, *2016*, 6. [CrossRef]
24. Hermiati, E.; Anita, S.H.; Risanto, L.; Styarini, D.; Sudiyani, Y.; Hanafi, A.; Abimanyu, H. Biological pretreatment of oil palm frond fiber using white-rot fungi for enzymatic saccharification. *Makara Seri. Teknol.* **2013**, *17*, 39–43.
25. Saha, B.C.; Qureshi, N.; Kennedy, G.J.; Cotta, M.A. Biological pretreatment of corn stover with white-rot fungus for improved enzymatic hydrolysis. *Int. Biodeterior. Biodegrad.* **2016**, *109*, 29–35. [CrossRef]
26. Baharuddin, A.S.; Asma, N.; Razak, A.; Aini, N.; Rahman, A. Bioconversion of oil palm empty fruit bunch by *Aspergillus niger* EB4 under solid-state fermentation. *Pertanika J. Trop. Agric. Sci.* **2009**, *32*, 143–151.