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Characteristic of oil palm empty fruit bunch pretreated with *Pleurotus floridanus*

Pretreatment biologi tandan kosong kelapa sawit menggunakan Pleurotus floridanus

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Abstrak

Pleurotus floridanus *memiliki kemampuan untuk mendegradasi lignin dengan memproduksi enzim ligninolitik dan lebih memilih untuk mendegradasi lignin daripada karbohidrat (hemiselulosa dan selulosa). Kemampuan unik* P. floridanus *ini dimanfaatkan dalam pretreatment biologi tandan kosong kelapa sawit. Penambahan kation (Cu^{2+}) pada pretreatment biologi menurunkan kandungan lignin dan meningkatkan digestibiliti tandan kosong kelapa sawit. Perlakuan* P. floridanus *mengurangi kandungan lignin dan hemiselulosa dari 23,9% menjadi 10,1% dan dari 20,8% menjadi 16,9%. Perlakuan* P. floridanus *tidak menurunkan kandungan selulosa. Kandungan selulosa tandan kosong kelapa sawit meningkat dari 40,4% menjadi 51,7%. Kristalinitas tandan kosong menurun setelah pretreatment biologi. Kristalinitas yang dinyatakan dalam LOI (LOI, Lateral Order Index) adalah 2,08 untuk tandan kosong tanpa pretreatment biologi dan 1,44 untuk tandan kosong dengan pretreatment biologi. Digestibiliti itandan kosong meningkat dari 17,2% menjadi 60,3%.*

[*Kata kunci: Pretreatment biologi, tandan kosong kelapa sawit, jamur pelapuk putih, lignoselulosa, Pleurotus floridanus*]

Abstract

Pleurotus floridanus have ability on lignin degradation by producing ligninolytic enzyme and prefer to degrade lignin than carbohydrate (hemicellulose and cellulose). Oil palm empty fruit bunches has been pretreated using *P. floridanus*. Addition of cation (Cu^{2+}) on biological pretreatment reduced lignin content and increased digestibility of the empty fruit bunches. *P. floridanus* reduce lignin and hemicellulose content from 23.9% to 10.1% and from 20.8% to 16.9%, respectively. *P. floridanus* did not degrade cellulose. Cellulose content of empty fruit bunches increase from 40.4% to 51.7%. Crystallinity of empty fruit bunches reduced after biological pretreatment. Crystallinity presented as LOI (lateral order index) of un-treated and biological pretreated oil palm empty fruit bunches are 2.08 and 1.44. Digestibility of the empty fruit bunches increased from 17.2% to 60.3% by biological pretreatment.

[*Key words*: biological pretreatment, oil palm empty fruit bunches, *Pleurotus floridanus,* biofuel, white-rot fungi, lignocellulose]

Introduction

Indonesia is the largest producer of crude palm oil (CPO) in the world. Indonesia produced 31 million metric tons of oil palm fruit in 2015 and accumulated 28.65 million metric tons of unused oil palm empty fruit bunches (OPEFB) (Dirjenbun, 2015). OPEFB is containing high lignocellulose and high polysaccharide. OPEFB provides enough potential sources of fermentable sugar for biological conversion and other lignocelluloses base derivate products (Abdulrazik *et al.,* 2017; Piarpuzán *et al.,* 2011). OPEFB has a great potential as feedstock for production of value-added product, such as: xylitol, xylose, glucose, furfural, fuel, pulp, cellulose, microcrystalline cellulose and nano fiber cellulose (Fahma *et al.,* 2010; Piarpuzán *et al.,* 2011; Rahman *et al.,* 2007; Wanrosli *et al.,*2011).

However, OPEFB has low digestibility. The enzymatic digestibility of lignocellulosic materials is limited by a number of factors such as lignin content and its composition, cellulose crystallinity, degree of polymerization, pore volume, acetyl groups bound to hemicellulose, surface area and biomass particle size (Ringkas, 2016; Zhu *et al.,* 2008). The breakdown of the lignin barrier is necessary since the lignin protects the cellulose from an enzyme attack by pretreatment technology, such as biological pretreatment that employs microorganism, such as white-rot fungi.

White-rot fungi are known as the most efficient microorganism in lignin degradation (Wong, 2009). Some species of the white-rot fungi selectively degrade lignin and hemicelluloses more than cellulose and

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leave a cellulose-rich residue. *Pleurotus* spp., a white-rot fungi species, are efficient in lignin degradation and produce ligninolytic enzymes, such as Laccase (Lac), manganese peroxidase (MnP), and versatile peroxidase (VP). Enzymes ligninolityc activities and lignin degradation by white-rot fungi are affected by nutrient content of the substrate and inducer. Coppers are included in the crystal structure of Lac (Glazunova *et al.*, 2015; Polyakov *et al.*, 2017). Addition of Cu^{2+} was reported to improve production of ligninolityc enzymes and was the most efficient inducer for Lac of *P. oestreatus* (Giardina *et al.*, 2000; Tinoco *et al.*, 2011). *Pleurotus* spp. are intensively investigated to be used in pretreatment of lignocellulosic for production of pulp, ruminant feed, bioethanol and biogas (Adamovic *et al.*, 1998; Kinnunen *et al.*, 2017; Nuraini & Trisna, 2017; Taniguchi *et al.*, 2010; Wyman *et al.*, 2017).

Biological pretreatment using white-rot fungi and/or combination with other pretreatment methods has been evaluated for bioethanol, biogas production and other chemical production from lignocellulose biomass (Hamisan *et al.*, 2009; Ma *et al.*, 2010; Salvachúa *et al.*, 2011; Yu & Zhang, 2009; Yu *et al.*, 2009). This results in cellulose that is unprotected and easier to hydrolyze. This study relates to the effects of biological pretreatment of OPEFB using *Pleurotus floridanus* under solid-state fermentation. Dry weight loss, compositional, and structural changes of the OPEFB were discussed.

Material and Method

Oil palm empty fruit bunches and substrate preparation

OPEFB obtained from North Sumatra, Indonesia, and was used as the raw material in this research. The OPEFB was sun-dried and chopped to get a homogenous size of 1–2 cm. The biological pretreatment of the OPEFB using *P. floridanus* was carried out in a series of 300 mL glass bottles. Fifty-five grams of dried OPEFB (51% water content) was placed in a glass bottle and 30 ml of medium (contain 20 ppm of Cu^{2+}) or distillate water for control was added. The bottles were autoclaved at 121°C for one hour.

Biological pretreatment of oil palm empty fruit bunches

P. floridanus cultured on PDA medium in room temperature for at least one week. Fresh culture of *P. floridanus* was used for biological pretreatment. The OPEFB were inoculated with eight pieces ($\varnothing$ 10 mm^2) of mycelia mats that were cut from the plate cultures. Each culture (bottle) was incubated at 30°C for different periods of time, i.e., 0, 7, 14 and 21 days. At the end of the incubation period, the fungal biomass was removed from the substrate as completely as possible, and the solid residue was dried and analyzed for total solid content, hot water soluble (HWS), lignin, cellulose, and hemicellulose. The structural component of the dried sample was analyzed to determine if there were any possible changes. All treatments were carried out in triplicate. The average values for each treatment are presented in the data.

Lignocellulose analysis

The characterization of the raw materials and the pretreated OPEFB was performed according to the Chesson-Datta methods (Isroi *et al.*, 2012). The chemical components of the samples were fractionated step-by-step to various components, as illustrated in Figure 2. The weight loss during every fractionation step gives the weight fraction of the major lignocellulose components: water-soluble, hemicelluloses, cellulose, and lignin. The dry weight was determined after drying the samples at 105±3°C for 24 hours, according to the standard test TAPPI T264 cm-97 (TAPPI Standards, 2007).

Enzymatic hydrolysis

The untreated and pretreated OPEFB were hydrolyzed using a commercial enzyme (Cellulase, 64 FPU/ml and β-glucosidase 58pNPGU/ml, Novozyme Co.). The enzymatic hydrolysis was performed base on a protocol from NERL (Chundawat *et al.*, 2008). A total of 0.15 g of total biomass (dry weight basis) was hydrolyzed with an enzyme dosage of 60 FPU/g substrate of cellulase and 64 pNPGU/g substrate of β-glucosidase in 50mM sodium citrate buffer pH 4.8, and supplemented with 100 µL 2% sodium azide as an antibiotic. The total volume of the hydrolysis mixture was 10 mL. All samples were shaken at 50°C for 72 h using laboratory shaker at 100 rpm and then filtered using a crucible filter. The aliquot obtained from the filtration step was then used for the sugar analysis. The mean and standard deviation were presented. Digestibility of the substrate was calculated using following calculation:

$$Digestibility\ \% = \frac{Glucose\ (g)}{Cellulose\ \ g\ x\ 1.11}\ x\ 100\%$$

FTIR analysis

The structural changes of the OPEFB after the pretreatment were observed based on the changes in the IR spectra. The IR spectra measurements were conducted using the FTIR spectrometer (Impact, 410, Nicolet Instrument Corp., Madison, WI), a resolution of 4 cm-1 in the range of 600 to 4000 cm^{-1} and controlled by Nicolet OMNIC 4.1 (Nicolet Instrument Corp., Madison, WI) (Isroi *et al.*, 2012)and analyzed using eFTIR® (EssentialFTIR, U.S.A.).

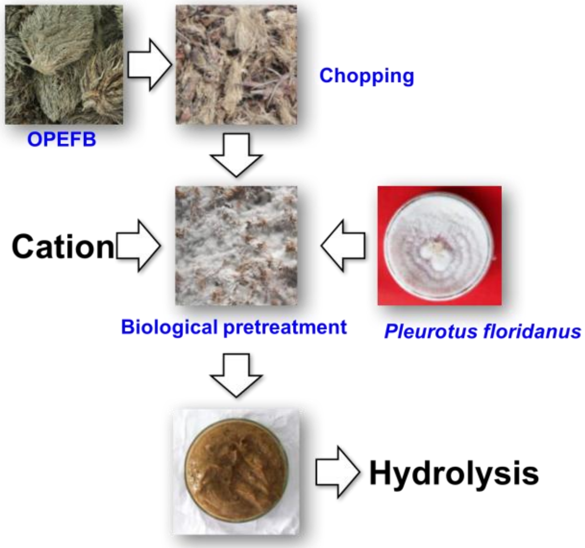

Figure 1. General steps of biological pretreatment of oil palm empty fruit bunches using *Pleurotus floridanus*.

Gambar 1. Langkah-langkah umum pretreatment biologi tandan kosong kelapa sawit menggunakan Pleurotus floridanus.

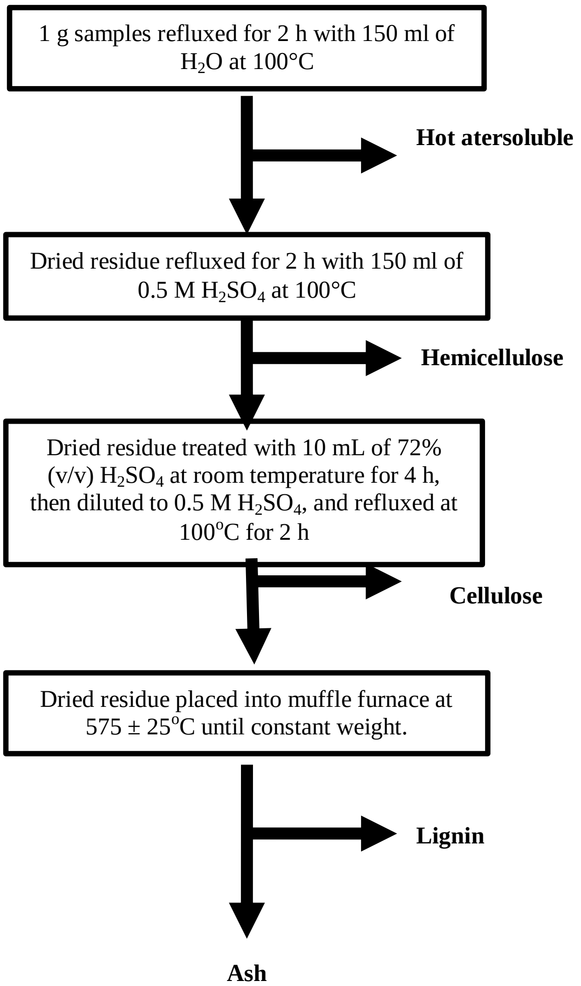

Figure 2. Sequential fractionation of the lignocellulose component, slightly modified from the Chesson Datta methods.

Gambar 2. Fraksinasi sekuensial dari komponen lignoselulosa, modifikasi dari metode Chesson-Datta.

Statistical analysis

Statistical calculations were performed with SPSS software (Statistical Product Service Solutions, Chicago, IL, USA). All data presented as averaged value. Linear correlations between degradation of the lignocelluloses component were examined by Duncan Multiple Range Test (DMRT). Subsequently, an analysis of variance (ANOVA) was applied to determine if the data series presented statistical significant difference.

Results and Discussion

Effect of biological pretreatment on lignocellulose component of the oil palm empty fruit bunches

The initial content of OPEFB is presented in Table 1. The biological pretreatment of the lignocellulosic materials degrades the solid components into less complex structures, water-soluble materials, and gaseous products. It is generally observed that biological pretreatment resulted in the reduction of the oven dry weight (ODW) of OPEFB (Figure 3). There are no significant different in reduction of ODW between control and Cu addition, but significant different found in the reduction of HWS, hemicellulose and lignin content. Biological pretreatment reduced all component of OPEFB except cellulose. Generally white-rot fungi has all enzyme machinery to degrade all lignocellulose components including *Pleurotus* spp (Cohen *et al.,* 2002; Kuforiji & Fasidi, 2009; Pedraza-Zapata *et al*., 2017; Wong, 2009). Most biological pretreatment using white-rot fungi degraded all cellulose component in various amount (Hongbo Yu *et al.,* 2009; Zhang *et al.,* 2017). *P. floridanus* used in this research has unique ability to selectively degrade lignin and hemicellulose than cellulose. Biological pretreatment by *P. floridanus* reduced lignin and hemicellulose content from 23.9% to 10.1% and from 20.8% to 16.9%, respectively. *P. floridanus* did not degrade cellulose. Cellulose content of empty fruit bunches increase from 40.4% to 51.7% after biological pretreatment.

The fact that the addition of cation (Cu^{2+}) accelerates the degradation of lignin and hemicellulose in the lignocellulosic materials by fungi has also been observed in others works (Tinoco *et al*., 2011; Tychanowicz *et al.,* 2006). The addition of cation can induce and control the ligninolytic enzymes production, resulting in the improvement of the lignin degradation. The cation can affect the ligninolytic enzymes activities and lignin degradation.

Structural *changes and crystallinity of the oil palm empty fruit bunches*

Biological pretreatment altered the physical characteristics of the OPEFB, by turning its color from dark brown to a lighter color, and it became more brittle and easier to grind. The color change may be used as an initial indication of the lignin reduction or removal.

Table 1. Initial lignocelluloses content of the oil palm empty fruit bunches.

Tabel 1. Kandungan lingoselulosa awal tandan kosong kelapa sawit.

Components *komponen*	Contents (%) *Kandungan* (%)
Lignin/*lignin*	35.82±0.0232
Cellulose/*selulosa*	40.37±0.0012
Hemicellulose/hemiselulosa	20.05±0.0004
Hot water soluble/*komponen larut air panas*	14.47±0.0004
Ash/*abu*	1.219±0.0056

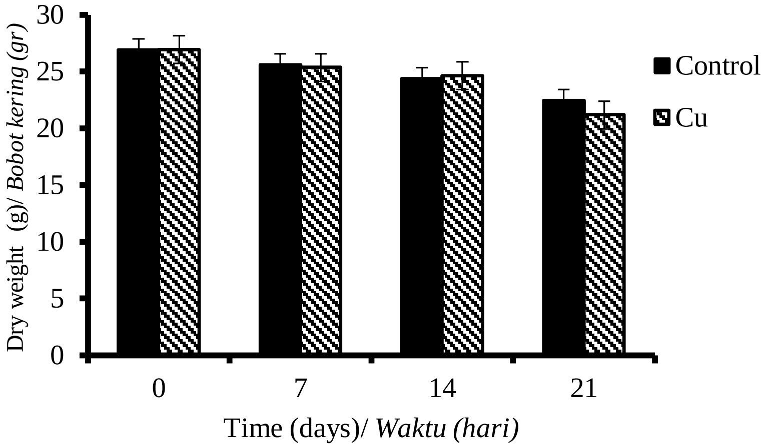

Figure 3. Decrease of the dry weight (ODW) of the OPEFB during the pretreatment using *P. floridanus*: Control (without cation) and Cu (addition of Cu^{2+}).

Gambar 3. Penurunan berat kering oven (ODW, oven dry weight*) dari TKKS (Tandan Kosong Kelapa Sawit) selama pretreatment menggunakan* P. floridanus*: Kontrol (tanpa penambahan kation) dan Cu (penambahan* Cu^{2+}*)*

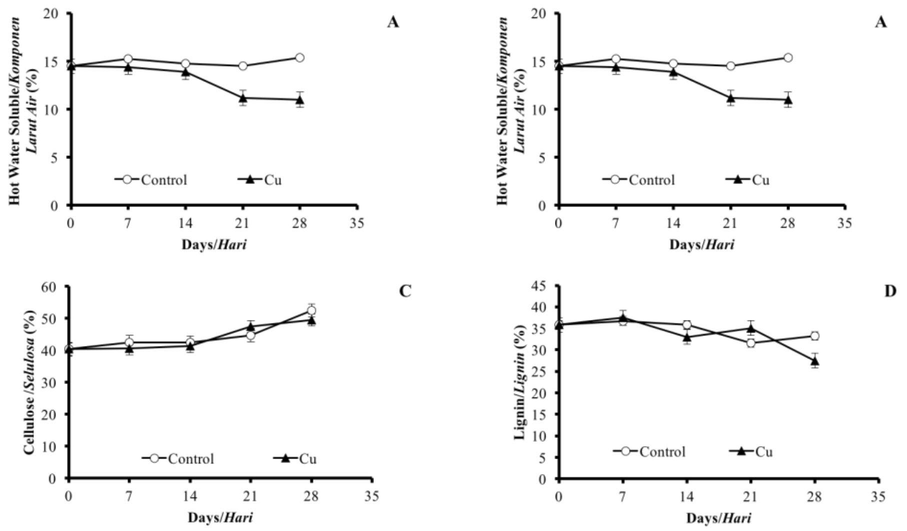

Figure 4. Changes in the OPEFB components of (**a**) hot water soluble (HWS), (**b**) hemicellulose, (**c**) cellulose, and (**d**) lignin during the biological pretreatment using *P. floridanus*: Control (without cation) and Cu (addition of Cu^{2+}).

Gambar 4. Perubahan komponen tandan kosong kelapa sawit (a) komponen larut air panas (HWS, hot water soluble*), (b) hemiselulosa, (c) selulosa, dan (d) lignin selama pretreatment biologi menggunakan* P. floridanus*: kontrol (tanpa penambahan kation) dan Cu (penambahan Cu^{2+}).*

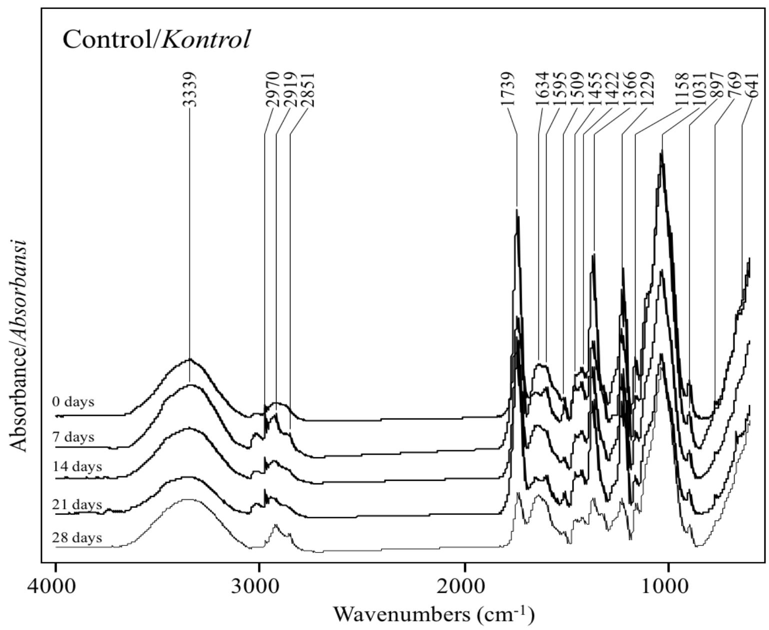

Figure 5. FTIR spectra of the biologically pretreated OPEFB without the cation addition for 0, 7, 14 and 21 days.

Gambar 5. Spektra FTIR dari TKKS yang sudah dipretreatment biologi tanpa penambahan kation selama 0, 7, 14 dan 21 hari.

The structural changes of the materials were analyzed using the FTIR, which reflects the changes in the functional groups of the OPEFB. The peaks of the IR Spectrum at certain wavelengths could be lower, higher, and/or shifted, which indicates the alteration of certain functional groups associated with that wavelength. The intensities of the C=O stretch in the un-conjugated ketone, carbonyl, and ester groups at wavenumbers 1739–1738 cm^{-1}, mainly from the polysaccharides, were significantly reduced after the pretreatment with the cation addition. In this peak, there may be linkages between the lignin and the carbohydrate (Takahashi & Koshijima, 1988). The degradation of the hemicellulose and the lignin as well as the break linkages between the carbohydrate and the lignin by the fungi may contribute to the reduction of this peak.

The crystallinity of cellulose could be predicted using the intensities ratio of certain bands at the IR spectra, which was A1418/A895 known as the Lateral Order Index (LOI) (Balogun *et al.,* 2016; Liquid, 2010). The LOI value of the biologically pretreated OPEFB is shown in Figure 7. The crystallinity of the cellulose decreased during the pretreatment. Meanwhile, the decreasing rate for the OPEFB pretreated with the Cu^{2+} addition was higher than for those without the cations addition. As indicated by the FTIR analysis of the cellulose IR band, although there was no significant degradation of the cellulose, the structure of the cellulose could be changed, such as its crystallinity.

Digestibility

The digestibility compares the sugar produced from the hydrolysis of the pretreated OPEFB with that of the untreated one. Its reveals that the digestibility of all the pretreated OPEFB increases as the time of the incubation increased (Figure 8). Un-pretreated OPEFB has very low digestibility as reported by other researcher (Hamzah *et al*, 2011). The digestibility of the control OPEFB was 17.2±4.8 (0-day incubation) to 22.0±0.1% (28-day incubation). The maximum digestibility of the pretreated OPEFB with the Cu^{2+} was 60.3±5.1% at 28-day incubation. The highest digestibility for the pretreated OPEFB increased 95%, compared to the untreated OPEFB. This result is affirmation of others references, that biological pretreatment could improve the digestibility of the lignocellulosic materials (Ma *et al.*, 2010; Yu & Zhang, 2009).

The enzymatic digestibility of lignocellulosic materials is limited by a number of factors such as lignin content, cellulose crystallinity, hemi-cellulose, degree of polymerization, pore volume, acetyl groups bound to surface area and biomass particle size (Alvira, *et al.*, 2010). In this study, increasing of the OPEFB digestibility has significant correlation with reduction in lignin and hemicellulose content, and increasing the cellulose content.

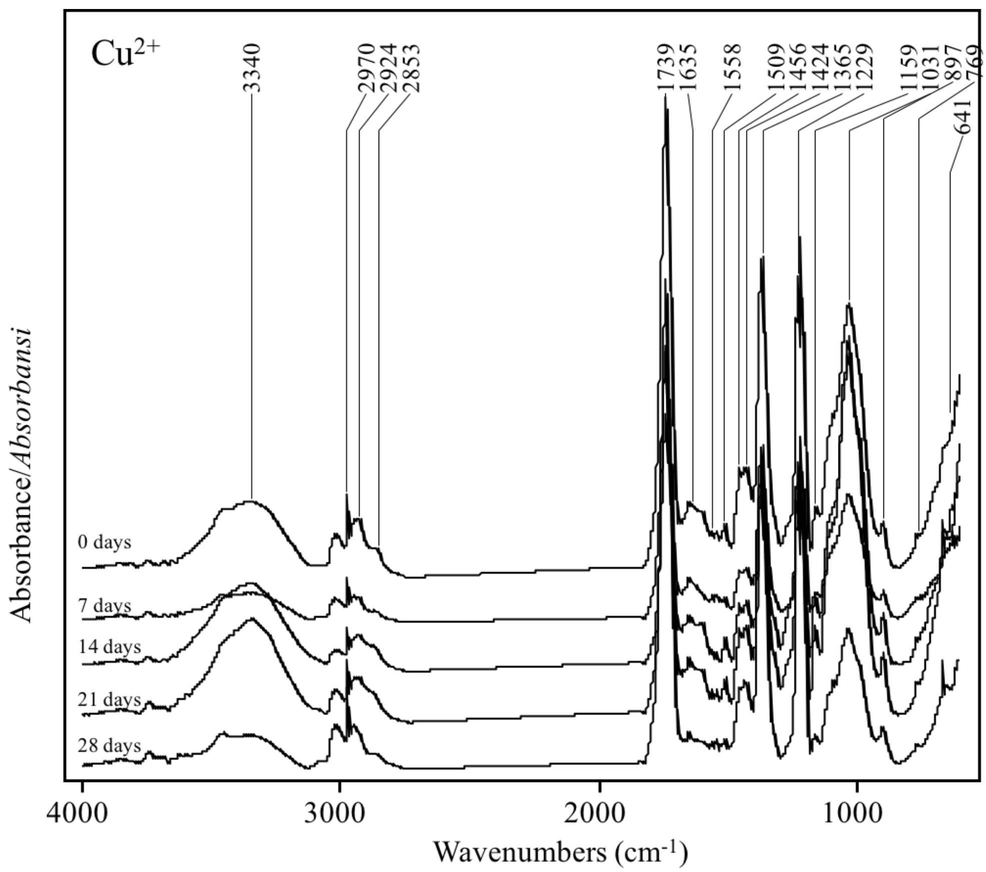

Figure 6. FTIR spectra of the biologically pretreated OPEFB with the Cu^{2+} addition for 0, 7, 14 and 21 days.

Gambar 6. Spektra FTIR dari TKKS yang sudah dipretreatment biologi dengan penambahan Cu^{2+} selama 0, 7, 14 dan 21 hari.

Table 2. Assignment of the FTIR-Absorption Bands (cm^{-1}) to various components of the oil palm empty fruit bunches according to existing literature (Isroi et al., 2012).

Tabel 2. Penetapan pita absorbs FTIR (cm^{-1}) ke dalam berbagai komponen tandan kosong kelapa sawit berdasarkan literature (Isroi et al., 2012).

Wavenumber (cm^{-1})	Assignments/*Penetapan*	Source/*Sumber*
670	C-O out-of-plane bending mode	Cellulose
715	Rocking vibration CH_2 in Cellulose $I_β$	Cellulose
858–853	C-H out-of-plane deformation in position 2,5,6	G-Lignin
897	Anomeric C-groups C(1)-H deformation, ring valence vibration	Polysaccharides
996–985	C-O valence vibration	
1035–1030	Aromatic C-H in-plane deformation, G>S; plus C-O deformation in primary alcohols; plus C=O stretch (unconj.)	Lignin
1162–1125	C-O-C asymmetric valence vibration	Polysaccharides
1230–1221	C-C plus C-O plus C=O stretch; G condensed > G etherified	Polysaccharides
1227–1251	C=O stretch, OH i.p. bending	
1270–1260	G-ring plus C=O stretch	G-Lignin
1315	O-H blending of alcohol groups	Carbohydrate
1375	C-H deformation vibration	Cellulose
1470–1455	CH_2 of pyran ring symmetric scissoring; OH plane deformation vibration	
1430–1416	Aromatic skeletal vibrations with C-H in plane deformation CH_2 scissoring	Lignin
1460	C-H in pyran ring symmetric scissoring; OH plane deformation vibration	Cellulose
1515–1505	Aromatic skeletal vibrations; G > S	Lignin
1605–1593	Aromatic skeletal vibrations plus C=O stretch; S>G; G condensed > G etherified	Lignin
1675–1655	C O stretch in conjugated p-substituted aryl ketones	Lignin
1738–1709	CO stretch unconjugated (xylan)	Polysaccharides
2940–2850	Asymmetric CH_2 valence vibration	
2980–2835	CH_2, CH_2OH in Cellulose from C6	Cellulose
2981–2933	Symmetric CH_2 valence vibration	
3338	Hydrogen bonded O-H valence vibration; O(3)H...O(3) intermolecular in cellulose	Cellulose

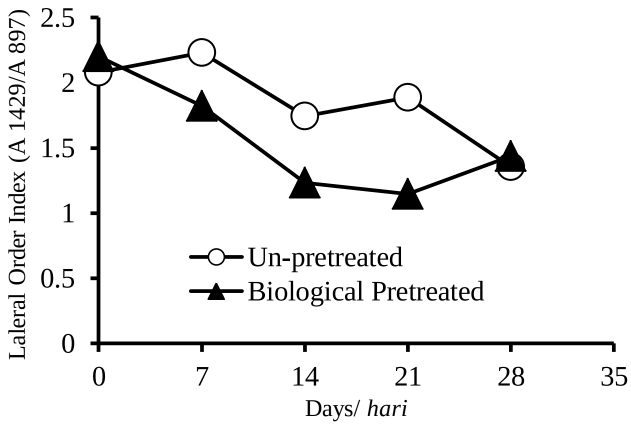

Figure 7. Lateral Order Index (A 1429/A 897) of the un-pretreated and biological pretreated OPEFB using *P. floridanus*.

Gambar 7. Lateral Order Index (A 1429/A 897) dari TKKS yang tidak dipretreatment dan dipretreatment menggunakan P. floridanus.

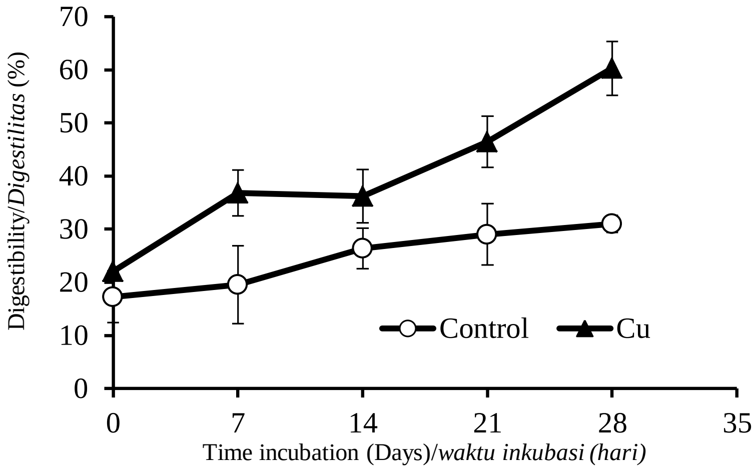

Figure 8. Hydrolysis yield of the OPEFB samples biologically pretreated using *P. floridanus* without the cation addition (control) and with Cu^{2+} addition.

Gambar 8. Hasil hidrolisis contoh TKKS yang sudah dipretretment menggunakan P. floridanus *tanpa penambahan kation (kontrol) dan penambahan Cu^{2+}.*

Conclusion

The *P. floridanus* used in the biological pretreatment of the OPEFB selectively degrades the lignin, hemicelluloses, and HWS, but not the cellulose. There is no correlation between the cellulose degradation and the dry weight loss, which implies that the fungi used in this work does not degrade the cellulose. The analysis of the FTIR spectra reveals significant changes in the OPEFB in its functional group in various regions, mainly the lignin and hemicellulose. Although there was no significant degradation of the cellulose, structural changes in the cellulose were observed using the FTIR spectra and could imply a reduction in the crystallinity. The degradation of the lignin and the hemicellulose may contribute to the improvement of the OPEFB digestibility.

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