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EVALUATION OF CHEMICAL AND BIOLOGICAL TREATMENTS TO DEGRADE OIL PALM EMPTY FRUIT BUNCHES (*Elaeis guineensis* Jacq.) AND THEIR POTENTIAL USE

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ABSTRACT

The exploitation of oil palm generates lignocellulosic wastes, also known as oil palm empty fruit bunches (EFB), which are difficult to biodegrade and are mainly composed of cellulose, hemicellulose and lignin. The presence of lignin impedes the use of the cellulose from this biomass in industrial processes. This study evaluated the effect of different chemical and biological treatments to remove the lignin present in the EFB from oil palm. The conventional kraft method, the soda-anthraquinone pulping process, microwave-assisted hydrogen peroxide degradation and the peroxy-monophosphoric acid method were the chemical treatments evaluated. Meanwhile, the four biological treatments were tested using different lignocellulolytic native fungi from the genera Xylaria, Psilocybe *and* Agrocybe. *The results showed a greater reduction of the lignin content with an enrichment of cellulose using the kraft method and the soda-anthraquinone pulping process. For the biological treatments, the fungus* Agrocybe *sp. showed a selective delignification, exhibiting a remarkable potential for the utilisation of cellulose present in this type of waste. In contrast, the fungus* Psilocybe *sp. showed high non-selective fibre degradation, which could be used for bioremediation and composting systems.*

Keywords: lignin removal, empty fruit bunch, cellulose, chemical treatment, macrofungi.

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INTRODUCTION

Colombia is the fourth largest producer of palm oil after Indonesia, Malaysia and Thailand and is the primary producer in the Americas. The exploitation of oil palm supplies the raw material for oil, edible fats and soaps, among other industries (Shuit *et al*., 2009). However, during industrial transformation, when fruit removal and oil extraction are performed, a large amount of lignocellulosic wastes that undergo slow degradation is produced. Average oil palm mills are estimated to have the capacity to process 100 000 t yr^{-1} of fruit bunches, producing between 20 000 and 25 000 t of empty fruit bunches (EFB) (20%-25% of the total biomass processed) (Manjarrés *et al*., 2011; Castañeda and Romero, 2012). The planting area reported for Colombia in 2014 was 450 000 ha, resulting in the production of approximately one million tonnes of lignocellulosic wastes (Fedepalma, 2015). At the global level, the production of palm wastes is estimated to be over 190 million tonnes (Awalludin *et al*., 2015).

The vast quantity of EFB generated by year and increased degradation time in the field cause subsequent environmental problems. The slow degradation of these EFB is increased not only for

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its lignocellulosic components but also by the bunches sterilised during the crude oil extraction, which causes the microorganisms and enzymes capable of degrading this biomass to be removed (Prasertsan and Prasertsan, 1996). Nevertheless, this EFB is a potential biomass to produce biofuels and other chemical compounds that might be useful in the fields of energy, industry and agriculture. Therefore, methodologies to accelerate the degradation process, either by selective or general lignin removal of the cell wall components are relevant. For instance, the selective removal of lignin would increase the yield of cellulose, which can be harnessed in areas such as the paper industry, bioethanol production (Shuit *et al.*, 2009), and biohydrogen production (Kothari *et al*., 2010). Also, the reduced lignin content of treated EFB can increase its animal digestibility, which in turn favours its potential use as forage in animal feed (Jung *et al.*, 2012). Meanwhile, with the general removal and recycling of the cell wall components, the wastes could be promoted as soil fertilisers (Kayikcioglu, 2013; Gandahi and Hanafi, 2014).

Among the chemical delignification treatments, the kraft method and the soda-anthraquinone pulping process are widely used in the industrial pulping processes of wood (De Almeida and Gomide, 2013; Pinto *et al*., 2015). These treatments are characterised by the use of high temperatures and the production of pollutants. For this reason, several alternatives for chemical treatments that do not require the use of high temperature have been developed to reduce the pollution rates, for example, the alkaline hydrogen peroxide treatment (Zhao *et al.*, 2009; Sun *et al*., 2000) and the use of peroxy acids (Springer, 1994; 1997).

Another alternative for lignin degradation with an environmental-friendly approach includes biological treatments that use organisms or enzymes. Fungi are essential for this type of process because they modify lignin using ligninolytic enzymes such as laccases and peroxidases; brown rot fungi are also essential in some non-enzymatic systems, such as the Fenton method (Cullen and Kersten, 2004; Martínez *et al*., 2005; Arantes *et al*., 2012). Whereas white rot basidiomycetes are recognised as having the best characteristics to degrade plant material – completely altering the lignocellulosic structure – some of the ascomycetes, known as soft rot fungi, are capable of modifying the lignin without dramatically altering the structure. This enables the subsequent use of the cellulose and other fibres (Kuhad *et al*., 1997; Hatakka and Hammel, 2010; Liers *et al*., 2011). Notably, this activity could be selective or non-selective, that is, it could induce the exclusive degradation of lignin or the simultaneous degradation of all components of the plant cell wall, including cellulose, hemicellulose and lignin (Arantes *et al*., 2012).

This study evaluates and compares the effect of four chemical and biological treatments on the oil palm EFB to determine the effectiveness and selectivity of each in the degradation of these agro-industrial wastes.

MATERIALS AND METHODS

Plant Material

EFB was obtained immediately after oil extraction from the oil palm extraction processing plant Unipalma S.A., which is located in Cumaral, Meta, Colombia (4° 13′ 33″ north latitude, 73° 14′ 50″ west longitude). The material was dried at 70°C for four days, milled and sifted with a 10 mm and 1 mm sieve, and sterilised at 121°C for 20 min before being used for the biological treatments. The 1 mm sieved samples were used for all cases except when the treatments involved the direct growth of fungi (solid-state fermentation). Each of these treatments was performed in triplicate, and the percent yield was determined by calculating the ratio between the mass recovered at the end of the treatment and the starting mass.

Chemical Treatments

Kraft pulping. The sample was subjected to kraft pulping according to Oudia *et al*. (2007). A total of 10 g of palm rachis (1 mm) was weighed and placed in a high-pressure reactor. Thereafter, 70 ml (for a 7:1 proportion) of pulping liquor was added. The pulping liquor was an aqueous solution of NaOH (0.70 M) and Na_2S (0.15 M). The mixture was heated at 160°C for 2 hr. The resulting solid was thoroughly filtered and washed with hot distilled water until a colourless supernatant was observed. The treated rachis was dried at 50°C for 24 hr.

Soda-anthraquinone pulping process. An adaptation of the treatment proposed by Nandkumar (2009) was performed. A total of 10 g of palm rachis (1 mm) was placed in a high-pressure reactor, and 50 ml (for a 5:1 proportion) of the pulping liquor was added (4.20 M NaOH, 0.1% anthraquinone). This mixture was subjected to 160°C for 2 hr. The resulting solid was thoroughly filtered and washed with hot distilled water until a colourless supernatant was observed. The treated rachis was dried at 50°C for 24 hr.

Microwave-assisted hydrogen peroxide treatment. An adaptation of the treatment proposed by Infante *et al*. (2007) was performed. A total of 10 g of palm rachis (1 mm) was weighed and placed in a 250 ml beaker with 100 ml of 30% hydrogen peroxide (H_2O_2). Once the sample was completely wetted, the partially covered beaker was placed inside a

microwave (LG) with an output power of 900 W and a frequency of 2450 MHz. The sample was subjected to three irradiation cycles of 10 s with 30 s intervals between each cycle. Subsequently, 100 ml of 1% NaOH was added, and the mixture was allowed to stand for 30 min. Excessive foaming was avoided using a cold-water bath to cool the mixture. After 30 min, 250 ml of distilled H_2O was added. The resulting samples were thoroughly filtered and washed with hot distilled water until a colourless supernatant was observed. The treated rachis was dried at 50°C for 24 hr.

Peroxy-monophosphoric acid treatment. The treatment method proposed by Springer (1997) was followed. A total of 10 g of palm rachis (1 mm) was immersed in 0.1 M sulphuric acid (H_2SO_4) for 30 min and then thoroughly washed with hot distilled water. The sample was filtered and dried at 50°C for 24 hr. A solution of 3.8% peroxy-monophosphoric acid (H_3PO_5) was prepared by hydrolysing 32.38 g of potassium peroxy-diphosphate ($K_4P_2O_8$) in 260 ml of a 3.80 M nitric acid (HNO_3) solution. The hydrolysis was performed at 50°C for 30 min. The residue obtained during the treatment with H_2SO_4 was placed in a beaker in which the peroxy-monophosphoric acid solution was added, maintaining a ratio of 25:1 with respect to the initial amount of rachis. The mixture was left standing at room temperature for 40 hr. Subsequently, washes with 10 volumes of 100 ml of 1% NaOH were performed at 60°C, followed by hot water washes. The sample obtained was dried at 50°C for 24 hr.

Biological Treatments

Fungal strains. Two of the strains used were fungi from the genera *Agrocybe* and *Psilocybe*, which were isolated from Unipalma S.A. plantation (4° 13′ 33″ north latitude, 73° 14′ 50″ west longitude), and DNA was extracted for molecular identification using the internal transcribed spacer region (ITS) from rDNA (ITS1-5′-TCCGTAGGTGAACCTGCGG-3′ and ITS4-5′-TCCTCCGCTTATTGATATGC-3′) (*Table 1*). The other was a strain from the genus *Xylaria* isolated from a high Andean forest ecosystem in the region of San Antonio del Tequendama (Cundinamarca) (4° 36′ 24″ north latitude, 74° 18′ 21″ west longitude) (Castaño *et al*., 2015). The strains were maintained at 4°C in wheat bran agar media [50 g litre^{-1} wheat bran, 17 g litre^{-1} agar, 1.4 g litre^{-1} $(NH_4)_2SO_4$; 2 g litre^{-1} KH_2PO_4; 0.4 g litre^{-1} $CaCl_2.2H_2O$; 0.3 g litre^{-1} $MgSO_4.7H_2O$; 5 mg litre^{-1} $FeSO_4.7H_2O$; 1.18 mg litre^{-1} $MnSO_4.H_2O$; 1.4 mg litre^{-1} $ZnSO_4.7H_2O$, and 2.6 mg litre^{-1} $CoCl_2.6H_2O$] and were preserved in slant tubes with a layer of mineral oil in the culture collection of the Agrobiotechnology Laboratory at the Universidad Nacional de Colombia. To obtain the fresh inocula needed for the testing treatments, fungi were inoculated in plates with the same media and incubated at 28°C for seven days.

Treatment with crude extract of the fungus* Xylaria *sp. A total of 10 g of rachis palm (1 mm) was weighed and placed in a 250 ml Erlenmeyer flask, to which 100 ml of crude extract of the fungus *Xylaria* sp. culture was added. The crude extract was obtained by culturing for six days under submerged fermentation, as described by Castaño *et al*. (2015). The initial pH of the crude extract was 5.0, and the laccase activity was 17416 U.litre^{-1}, determined as described by Castaño *et al.* (2015). The Erlenmeyer flask was incubated at 40°C for 48 hr with orbital shaking at 150 rpm. This temperature was chosen considering the optimal temperature and thermal stability for this enzyme as discussed by Castaño *et al.* (2015). The 48 hr of treatment was established analysing the enzyme stability at 40°C for different periods of time. After 48 hr of incubation at 40°C, the enzyme retained around 5% of its initial activity, which was deemed as an activity level low enough as to not extend the incubation any longer while still

TABLE 1. TAXONOMIC CHARACTERISATION OF ISOLATED STRAINS

Strain	Genus	Closest relative sequence	% Coverage	% Similarity	Access number	E value
UPB1	*Agrocybe* sp.	*Agrocybe praecox* isolate AFTOL-ID 728 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence	97	83	AY818348.1	2, 00E-172
UPB6	*Psilocybe* sp.	*Psilocybe coprophila* strain CBS 417.82 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	100	98	HM035073.1	0.0

obtaining as much degradation as possible (data not shown). The samples obtained were thoroughly filtered and washed using distilled water until a colourless supernatant was observed. The treated rachis was dried at 50°C for 24 hr. A control incubating the rachis with uninoculated liquid medium was included.

Fungal growth on EFB. Solid-state fermentation (SSF) was performed with each of the three strains of fungi. A total of 12 g of palm rachis (10 mm) was weighed and placed in a 250 ml glass flask with 30 mL of a salt solution composed of: 1.4 g litre^{-1} $(NH_4)_2SO_4$, 2 g litre^{-1} KH_2PO_4, 0.4 g litre^{-1} $CaCl_2.2H_2O$, 0.3 g litre^{-1} $MgSO_4.7H_2O$, 5 mg litre^{-1} $FeSO_4.7H_2O$, 1.18 mg litre^{-1} $MnSO_4.H_2O$, 1.4 mg litre^{-1} $ZnSO_4.7H_2O$, 2.6 mg litre^{-1} $CoCl_2.2H_2O$ and 1.5 mg litre^{-1} $CuSO_4.5H_2O$. The material was sterilised at 121°C for 15 min, and the substrate was inoculated using three mycelia disks of 5 mm diameter; the mycelia had been previously grown in wheat bran agar for one week. The experiment was carried out in a Sanyo MLR-351H phytotron, under dark conditions, at 28°C and at a relative humidity of 90% for three months to ensure the total fungal colonisation of the substrate and also because a significantly higher degradation of cellulose, hemicellulose and lignin was found in the substrate at the third month of incubation (data not shown), after which the material was dried at 50°C for 24 hr; the residual biomass of each treatment was then weighed. The fibres were washed with distilled water to remove parts of the mycelium. Finally, the treated rachis was dried at 50°C for 24 hr before being milled and sifted with a 1 mm sieve for fibre analysis. A control consisting of incubating the rachis with an uninoculated salt solution was included.

Electron Microscopy

Variation in the structural stability of the palm fibres that had been subjected to biological treatments was determined by scanning electron microscopy (SEM), according to the methodology reported by Xian-Ling *et al*. (2012). The experiments were conducted at the Life Science Microscopy Facility (LFMF) at Purdue University, USA.

Fibre Determination

The determination of lignin, cellulose and hemicellulose content was performed using standard methods (Van Soest *et al*., 1991) implemented in the Laboratory of Ruminant Nutrition, Faculty of Veterinary Medicine, National University of Colombia, Bogotá.

Statistical Analysis

Statistical analysis was performed using the software SAS version 9.2; an analysis of variance (ANOVA) was performed, and a comparison of means was conducted using the Tukey test with an alpha value of 0.05.

RESULTS AND DISCUSSION

Efficiency of Chemical and Biological Treatments

The effect of the chemical and biological treatments performed on the empty palm rachises was calculated based on the yield obtained after the treatment, where a percentage close to 100% implies a minimal loss of biomass. The results showed that in the case of the chemical treatments, the yield of the kraft pulping and the soda-anthraquinone pulping processes were less than 48% and 32%, respectively, representing a high loss of the starting material during the processes (*Table 2*). These results agree with other studies using the same treatments for the delignification of other lignocellulosic substrates such as aspen, poplar, pine, and beech, wherein the pulp yield was around 50% or even lower (Buzala *et al*., 2017). Different researchers have shown that the content of alkali charge, temperature, and treatment time are negatively correlated with yield while positively correlated with delignification (Anupam *et al*., 2018; Leh *et al*., 2008; Oldroyd and Wadley, 1997). In addition, important interactions have been described between temperature and treatment time (Leh *et al*., 2008), as well as temperature and alkali charge (Oldroyd and Wadley, 1997) in the delignification process of different lignocellulosic substrates, including EFB. These interactions and

TABLE 2. PERCENTAGE OF YIELD FOR THE CHEMICAL AND BIOLOGICAL TREATMENTS TESTED

Chemical treatments	% Yield	Biological treatments	% Yield
Kraft pulping	46.8-48.0^a	Crude extract *Xylaria* sp.	95.8-97.0^a
Soda-anthraquinone pulping	30.6-32.0^b	*Xylaria* sp.	76.29-78.31^b
Hydrogen peroxide	74.1-75.9^c	*Agrocybe* sp.	66.39-72.19^c
Peroxy-monophosphoric acid	74.0-74.3^c	*Psilocybe* sp.	39.01-44.07^d

Note: Percent yield is calculated according to the equation presented in the Materials and Methods section. Means with the same letter do not show a statistically significant difference according to the Tukey test ($\alpha = 0.05$).

complex effects on delignification and yield suggest that complex optimisation designs intended to optimise both variables should be applied. For instance, Wan Omar and Amin (2016) carried out the optimisation of oil palm frond pre-treatment by ozonolysis using a multi-response analysis with a desirability function, wherein lignin degradation and total reducing sugars recovery were used as optimisation targets.

In contrast to what happened in the kraft and anthraquinone pulping, the samples treated with hydrogen peroxide and peroxymonophosphoric acid presented a high yield that was close to 74%, which means that no significant loss of mass had occurred. It is generally expected that these methods result in a higher pulp yield because their mechanism of delignification is based on the capacity of the peroxy acids and H_2O_2 of hydroxylating the aromatic rings in the lignin molecule (Kadla and Chang, 2001). Consequently, the cellulose and hemicellulose polymers are less susceptible to be degraded during these pulping strategies. Other authors have also reported high pulp yields using different substrates such as aspen wood or rice straw (Springer, 1997; Verma *et al*., 2011).

For the biological methods, when the crude extract of *Xylaria* sp. was used, the yield detected was approximately 97%, in contrast to the percentage of efficiency observed in treatments based on the direct fungal growth on the palm fibres. In the latter case, the treatments resulted in a radical reduction of the initial mass after 90 days of SSF. Notably, the yield of the treatment with *Psilocybe* sp. was less than 45%. Other authors have evaluated the total weight loss after a fungal treatment such as Singh *et al*. (2013), who used *Trametes versicolor* to degrade oil palm trunk chips, achieving 8.45% of weight loss after 30 days of incubation. Also, Piñeros-Castro and Velásquez-Lozano (2014) reported that *Pleorutus ostreatus* and *Pleorotus chrysosporium* were able to cause 43% and 33% of weight loss in oil palm EFB after four weeks of incubation, respectively. The weight loss is influenced by the degradation rate, which in turn depends on the type of fungal rot (white, brown, or soft), and the specific strains. In that sense, high variability is expected in weight loss when using a soft rot (*Xylaria* sp.), white rot (*Agrocybe* sp.), and an uncategorised wood degrader (*Psilocybe* sp.).

Minimal loss of mass with respect to the initial mass is important and desirable if the intention is to utilise the cellulose from the treated waste, but this outcome must be complemented with efficient lignin removal. For this reason, the percentage of lignin, cellulose and hemicellulose was determined before and after each treatment to allow the observation of the change in the rachis composition. Additionally, the quantitative loss of each component was calculated to determine whether the delignification of each treatment was general or selective.

Effect of Chemical and Biological Treatments on the Rachis Fibres Composition

The determination of the waste composition after the application of each chemical treatment demonstrated that all of the methods lead to a reduction in the lignin and hemicellulose concentration in the rachis, increasing the cellulose concentration. These results suggest that there is a minimal effect on this component during these treatments (*Figure 1*). The chemical treatments are classified into two groups according to their lignin content reduction. The first group corresponds to

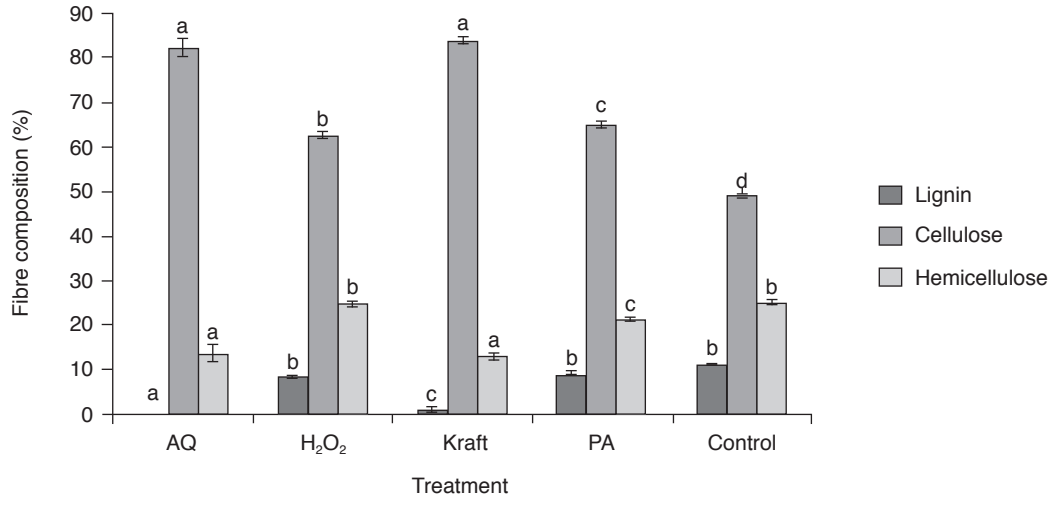

Figure 1. Composition of samples before and after the application of the chemical treatments. A significantly higher reduction was observed in lignin content with respect to the control for the kraft pulp and anthraquinone (AQ) pulping methods compared to the processes using hydrogen peroxide (H_2O_2) and peroxy-monophosphoric acid (PA). Means with the same letter show no statistically significant difference according to the Tukey test ($\alpha = 0.05$).

those methods using hydrogen peroxide and peroxy-monophosphoric acid, which are characterised by a discrete effect that reduces the lignin content by 1.2 and 1.3 times, respectively, with respect to the control. The second group corresponds to a larger delignification effect as seen in the kraft pulping and the soda-anthraquinone pulping processes, in which the lignin concentration is reduced by 10 times, reaching final concentrations of approximately 1% lignin. With these two methods, a greater reduction in hemicellulose occurs, which might be associated with the characteristic thermal degradation suffered by sugars (Whistler and Daniel, 1985), as these methods are conducted at a temperature of 160°C. In contrast, the enrichment of cellulose could be attributed to the structure of the polymer, which is complex and organised, with protofibres that are associated with cellulose fibres and microfibres (Lynd *et al*., 2002). This renders cellulose more recalcitrant than hemicellulose, a polymer with a less compact structure.

As for the biological treatments, two different approaches were evaluated. The first was the use of enzymatic crude extracts with high ligninolytic activity, as was the case for *Xylaria* sp. and its laccase activity (Castaño *et al*., 2015.). The second was the direct growth of fungi on the lignocellulosic wastes (Brijwani *et al*., 2010; Piñeros and Velásquez, 2014) to avoid an extra process in the pre-treatment of lignocellulose. In these cases, the determination of the waste composition after each treatment showed that *Agrocybe* sp. was the fungus that achieved the highest decrease in lignin concentration (1.8 times) compared to the other biological treatments, which presented similar values to those found in the untreated rachises (*Figure 2*). Notably, in the treatments involving the direct growth of *Xylaria* sp. and *Agrocybe* sp., an increase in the cellulose concentration was observed.

Even though information about the composition may show variations in the proportion of the compounds tested, indicating which of them is decreased or increased with respect to the control, a determination of the percentage of each component lost is needed to better understand the effect of each treatment, whether chemical or biological, on the hemicellulose, cellulose and lignin fibres. These results are shown in *Table 3*.

As shown in *Table 3*, and consistent with observations from the composition analysis, the

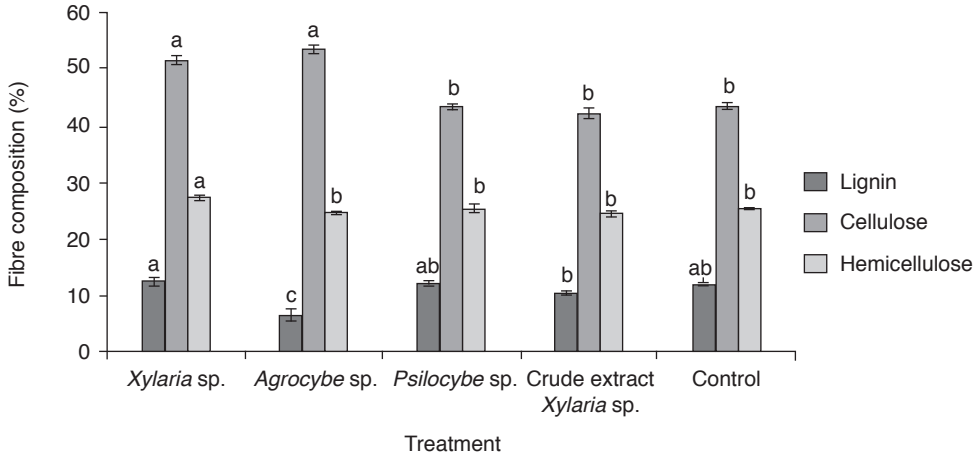

Figure 2. Composition (%) of samples before and after application of biological treatments. Means with the same letter show no statistically significant difference according to the Tukey test (α = 0.05). Controls for the crude extract extract treatment and the incubation treatments were not statistically different from each other. Therefore, they are presented in the figure as one control for better clarity.

TABLE 3. PERCENTAGE OF FIBRE LOSS FOR EACH APPLIED TREATMENT

Treatment	% Lost hemicellulose	%Lost cellulose	% Lost lignin
Chemical			
Kraft pulping	75.57 ± 0.37^{b}	18.84 ± 1.21^{c}	94.43 ±0.08^{a}
Soda-anthraquinone pulping	82.96 ± 0.70^{a}	47.64 ± 0.97^{b}	99.86 ± 0.19^{a}
Hydrogen peroxide	26.83 ± 1.86^{f}	4.10 ± 0.31^{f}	40.09 ± 1.89^{c}
Peroxy-monophosphoric acid	37.45 ± 0.52^{d}	1.76 ± 0.28^{f}	37.83 ± 1.57^{c}
Biological			
Agrocybe sp.	32.08±0.79^{e}	14.88±2.0^{d}	61.44±6.4^{b}
Psilocybe sp.	58.13±1.12^{c}	58.58±0.37^{a}	57.60±1.34^{b}
Xylaria sp.	16.34±1.18^{g}	8.80±1.61^{e}	19.08±4.78^{d}
Crude extract *Xylaria* sp.	4.64 ± 1.87^{h}	16.75 ± 1.19^{cd}	7.22 ± 1.55^{e}

Note: Means with the same letter are not statistically significantly different according to the Tukey test (α = 0.05).

kraft method and the soda-anthraquinone pulping process were the chemical treatments with the highest delignification effect on the starting material, almost completely removing the lignin (> 94%). In the same way, a high loss of hemicellulose was presented in these treatments, exceeding 75% in both cases. Notably, despite the enrichment of cellulose observed in the waste (*Figure 1*), an important degradation of cellulose was observed, especially through the soda-anthraquinone process, where almost 50% of this material was lost. Although carbohydrates tend to be resistant to basic hydrolysis, high temperatures can result in the thermal degradation of sugars (Whistler and Daniel, 1985), thereby explaining this observation. From this analysis, these treatments can be considered to be suitable if the objective is to maximally degrade the lignin waste for the subsequent use of cellulose. This is particularly interesting in fields such as the paper industry, wherein the strength of the product is associated with high cellulose content (Ververis *et al*., 2004). However, in many cases, the harnessing of cellulose may be achieved just by reducing other components such as lignin and hemicellulose. Indeed, the removal of hemicellulose can cause a decrease in the mechanical strength that prevents access to cellulose due to the role of the cross-linking features of the polymeric chains of hemicellulose, which generate stronger associations between cellulose microfibres. This mechanical strength decrease also allows the remaining cellulose fibres to swell (Zhao *et al.,* 2009; Sun *et al.*, 2000), which in the end contributes to a higher subsequent hydrolysis potential. Considering the above, the treatments using hydrogen peroxide and peroxymonophosphoric acid constitute two important alternatives for the selective delignification of oil palm EFB, which additionally present higher yield and omit the use of aggressive conditions of pressure and temperature.

In general, the chemical treatments are characterised by the production of adverse environmental effects during the process, especially in the kraft method (Pokhrel and Virara Ghavan, 2004). Therefore, the strategy of using biological treatments that generate less pollution is an important option to explore. In this case, the most outstanding alternatives are those involving the direct growth of the fungus over the use of the crude extract from *Xylaria* sp. given the higher reduction achieved in the hemicellulose, cellulose and lignin content in the direct growth strategies. The development of the organism directly on the waste supposes that the fungus must modify the lignin to access the cellulose, which is used as the carbon source (Tuomela *et al.*, 2000), thus, explaining the effectiveness of the direct growth methods. Additionally, enzyme secretion is maintained throughout the incubation period, which contributes to improve the substrate degradation, as reported by Manjarrés *et al.* (2011). In contrast, when using the crude extract of the fungus, a significant loss of enzyme activity occurs beyond the second day of incubation (data not shown), restricting the enzyme effect to a short period and limiting the degradation of the vegetal material. Notably, the direct growth strategies generate a non-selective loss of lignin that can be attributed to the presence of glycosyl hydrolases (*i.e.*, endoglucanases, mannanases, xylanases, pectinases, *etc*.) that degrade cellulose and other cell wall components. In this regard, for instance, the fungus *Xylaria* sp. has been described as a soft rot fungus, characterised by a moderate degradation of lignin and cellulose (Schwarze, 2007; Hatakka and Hammel, 2010; Liers *et al.*, 2011), which may explain the low rate of lignin loss. In contrast, *Psilocybe* sp. degrades the three evaluated components in similar proportions, explaining the lack of selectivity to one of the fibres of interest. This unspecific activity has also been reported for other white rot fungi from the order Agaricales, including *Pholiota conissans*, *Mycena* sp. and *Lycoperdon* sp. (Barrasa *et al*., 2014). Based on these results, the *Psilocybe* sp. fungi could be used in bioremediation or biofertilisation processes in which further degradation of the cell wall components is required (Gandahi and Hanafi, 2014). On the other hand, the effect achieved by the fungus *Agrocybe* sp. could be considered to be selective, as it achieves a high loss of lignin (61.44%) and a decrease of only around 15% of cellulose, coinciding with the results reported for other basidiomycete species (Nerud *et al*., 1991; Blanchette, 1995; Piñeros and Velásquez, 2014).

In the case of biological treatments, the effect on the rachis structure was evaluated using SEM. In agreement with the percentage loss for each component discussed above, the SEM images showed that each of the tested fungi degraded the cell wall in a different manner depending on the strain, either selectively (*Agrocybe* sp.), indiscriminately (*Psilocybe* sp.) or with only a slight modification, as observed with *Xylaria* sp. (*Figure 3*).

To sum up, the comparison of chemical and biological treatments used for the delignification of oil palm waste revealed that obtaining selective lignin degradation is possible, especially through chemical treatments and also by using the *Agrocybe* sp. fungus. Thus, this organism shows a great potential to be applied in pre-treatments of lignocellulosic oil palm wastes, constituting a good environmental-friendly delignification alternative. Additionally, the treatment with the fungus *Psilocybe* sp. could be useful not only in bioremediation, given the high degradation rates, but also in biofertilisers production, considering that mineralisation can accelerate the availability of nutrients and organic matter (Huang *et al.*, 2008).

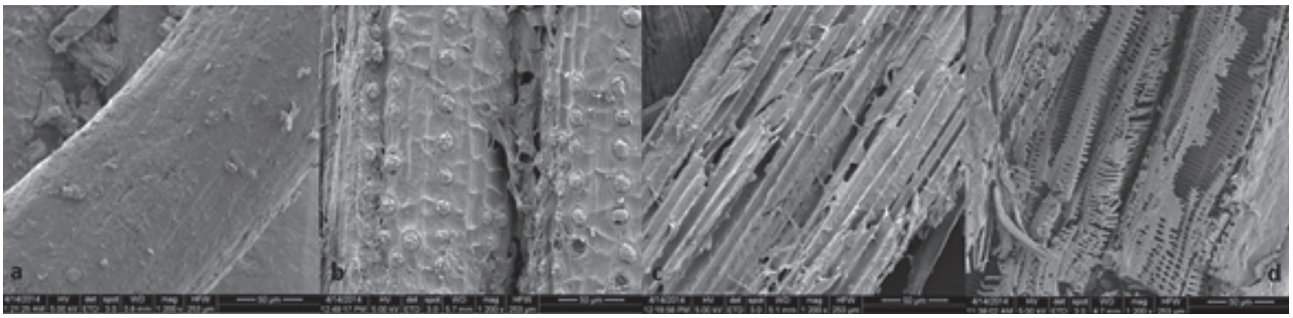

Figure 3. Images obtained by scanning electron microscopy (SEM) of palm fibres biologically treated for three months: a. Control, b. Xylaria *sp., c.* Agrocybe *sp. and d.* Psilocybe *sp.*

CONCLUSION

Chemical treatments that are widely used in the industry to remove lignin such as the kraft method and the soda-anthraquinone pulping process, showed the greatest reduction in lignin content from the oil palm EFB. This reduction was accompanied by an enrichment of cellulose in the waste, which potentiates the oil palm wastes for use in various processes of industrial interest.

In the analyses of the biological treatments, that with the fungi *Agrocybe* sp. is noteworthy. This fungus presented the greatest selectivity during the degradation process. As a result, this strain could be used in the pre-treatment processes, especially in cases in which there is a need to reduce the lignin content. The *Psilocybe* sp. fungal strain achieved a reduction superior to 50% of the biomass during the 90 days of evaluation, which makes this strain useful for optimising the composting process. In general, the use of the direct growth of fungi on this substrate could be highly interesting in bioremediation processes, along with the use of other degrading organisms. However, more studies on this topic are required.

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