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Short communication

Biodegradation kinetics of oil palm empty fruit bunches by white rot fungi

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ABSTRACT

The biodegradation of lignin, cellulose and hemicellulose from oil palm empty fruit bunches by white rot fungi *Phanerochaete chrysosporium* and *Pleurotus ostreatus* was investigated. The results showed higher mass loss and polysaccharide degradation when the biological treatment was carried out with *P. chrysosporium*. Biodegradation curves were modelled by a Weibull kinetic model and the kinetic parameters were obtained for each one of the components. Even though the lignin degradation rates were similar for both fungi, the biodegradation of this component reached 50% with *P. ostreatus*, a higher value than the 41% reached with *P. chrysosporium* after harvesting for four weeks. Higher polysacharides biodegradation rates were observed for *P. chrysosporium* compared to *P. ostreatus*. Consequently, the *P. ostreatus* pretreatment can be considered adequate for the delignification of palm residues without considerably affecting the cellulose fraction, which is important for the production of fermentable sugars.

1. Introduction

Colombia is the world's fifth producer of palm oil and the first in Latin America. Palmiculture is one of the most promising agricultural activities and considered an important source for national development. 4.6 million tons of oil palm fruits were processed in 2012 (Torres et al., 2013) and generated Mt (Million tonnes) of lignocellulosic materials. In 2007, the worldwide annual production of oil palm biomass reached 184.6 Mt (Kelly-Yong et al., 2007).

Lignocellulosic materials are the main source of renewable materials on the surface of the earth. For this reason, their biological degradation has gained interest for investigation, especially for the production of bioenergy and bioproducts (Singh and Chen, 2008). Lignocelluloses are degraded by many microorganisms and, particularly with high efficiencies by white rot fungi. These fungi exhibit an oxidative enzymatic system and a lignolytic extracellular system, and consequently are the most efficient for lignin degradation (Sánchez, 2009). White rot fungi including *Phanerochaete chyrsosporium, Ceriporia lacerata, Cyathus stercoreus, Ceriporiopsis subvermispora, Pycnoporus cinnabarinus* and *Pleurotus ostreatus* have been studied for the degradation of different lignocellulosic biomasses showing high delignification efficiencies (Shi et al., 2008; Kumar et al., 2009). The study of the biodegradation kinetics of the lignocellulosic materials is important considering the fungal treatment breaks the lignin-carbohydrate complex. This improves the availability of cellulose to the hydrolytic enzymes (Taniguchi et al., 2005; Zhang et al., 2007; Shi et al., 2009; Yu et al., 2009), which is considered a requirement for the production of fermentable sugars (Gupta et al., 2011). So far, no studies on the kinetics biodegradation of oil palm residues by white rot fungi have been reported. In the present work, the process of biodegradation of lignin, cellulose and hemicellulose by the fungi *P. chysosporium* and *P. ostreatus* in solid state fermentation was studied.

In the present work, the experimental data for the biodegradation of lignin, cellulose and hemicellulose were described by Weibull kinetic model. It is potentially interesting for the kinetic description of chemical, microbial or enzymatic degradation. One of the advantages of this model is its flexibility when adjusting the experimental data (van Boekel, 2002) using two parameters: the reaction rate constant (α) and the shape factor (β) (Cunha et al., 1998). The distribution has been used with satisfactory results when investigating shelf life (Schmidt and Bouma, 1992; Duyvesteyn et al., 2001), microbial deactivation curves (Peleg, 2000; Unluturk et al., 2010), vitamins and antioxidants degradation (Oms-Oliu et al., 2009; Zheng and Lu, 2011), osmotic dehydration (Corzo and Bracho, 2008), amongst others. It has also been

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evaluated in degradation kinetics of monosaccharides under supercritical conditions (Khajavi et al., 2005). During the literature review, no reports in the use of the Weibull model for biodegradation of lignocellulosic materials were found. In general, a kinetics model is important to study the effect of the culture conditions on the biomass biodegradation.

2. Materials and methods

2.1. Feedstock

The oil palm empty fruit bunches were supplied by the farmhouse Hacienda La Cabaña in Cumaral, Meta, Colombia. The material was washed, dried and chopped (3 cm approx.). The characterization of the material included the determination of cellulose, hemicellulose and lignin using the methodology described by the National Renewable Energy Laboratory NREL (Sluiter et al., 2008). The structural carbohydrate composition was determined based on monomeric sugars content measured after an acid hydrolysis with 72% H_2SO_4 at 30 °C for 60 min and 4% H_2SO_4 at 121 °C for 1 h. This hydrolysis liquid was analyzed by high performance liquid chromatography (HPLC) with refractive index detector, in an AMINEX HPX-87H sugars analysis column (Bio-RAD) operating at 60 °C with 5 mM H_2SO_4 as a mobile-phase (0.6 ml/min). Acid-soluble lignin was determined by UV adsorption and insoluble lignin by gravimetric method.

2.2. Strains, culture preparation and solid substrate

Two fungi from the white rot were used: the *Phanerochaete chrysosporium* CECT 2798 and the *P. ostreatus* CECT 20311 from the Spanish Type Culture Collection. The strains were kept using potato dextrose agar (PDA) as medium, and were cultivated for six days at 30 °C to prepare the inoculants. For *P. chrysosporium*, spores were suspended in sterile distilled water (1.4×10^8 spores/mL) and 2 mL were added to each experimental unit. For *P. ostreatus*, 4 cm^2 of the agar surface were scraped for each experimental unit.

Polyetylene bags were used as experimental units. 10 g of lignocellulosic material were added (dry basis), and humidity was adjusted to 67% (w/w) using saline supplement (Kirk medium) (Kirk et al., 1986). Subsequently, the units were sterilized for 20 min at 121 °C and the inoculant was added after cool down. The experimental units were incubated at 30 °C for four weeks.

2.3. Biomass biodegradation

The progress of the oil palm empty fruit bunches pretreated with *P. ostreatus* and *P. chrysosporium* was monitored for 4 weeks. Each week, three random samples were removed and washed. Weight loss was calculated as the percentage of total solids lost after each week. Lignin content and polysaccharide composition (cellulose and hemicellulose) of biopretreated palm biomass were determined as described in section 2.2.1. The weight loss and the kinetic parameters of the Weibull model (α and β) were set as response variables.

2.4. Methods for Weibull model parameters estimation

Weibull model kinetic parameters were obtained by adjusting experimental data of lignin, cellulose and hemicellulose degraded fraction. Such procedure was carried out by minimizing the square of the experimental error using the Levenberg–Marquardt algorithm (Bates and Watts, 2008) available in the SciDavis software v.0.2.4. (http://scidavis.sourceforge.net/).

2.5. Statistical analysis

All experiments and measurements were made by triplicates and the mean values were used for calculations and data analysis. The ANOVA ($p = 0.05$) and the means difference tests (Tukey test) were carried out using Statgraphics 5.0 (Statistical Graphics Corp USA).

3. Results and discussion

3.1. Weight loss percentage

The weight loss percentage of the palm residues caused by the biological treatment is showed in Table 1. During first week, the weight loss was similar between both fungi.

However, a higher weight loss was observed for bunches treated with the fungus *P. chrysosporium* during the following two weeks. The results are similar to those obtained by Camarero et al. (1994), who reported that *P. chrysosporium* causes higher weight losses when compared to *Pleurotus eryngii* in the biodegradation of wheat residues. This can be attributed to the simultaneous attack to lignin and polysaccharides by *P. chrysosporium*, whereas *P. eryngii* preferentially degrades lignin and xylans while slightly affecting the cellulose. The observed values are within the ranges reported for white rot fungi degradation of eucalyptus and grasses (Akin et al., 1995) as well as rubberwood (Pandey and Nagveni, 2007).

3.2. Biodegradation of palm residues

Experimental data obtained for the degradation of the different components present in the palm residues is presented in Fig. 1. Both *P. ostreatus* and *P. chrysosporium* fungi degraded the lignin to a similar extent, but also degraded the polysaccharides at the evaluated conditions. The fungus *P. ostreatus* can be considered as selective for delignification, since the degradation of cellulose starts only after the third week of treatment. In contrast, *P. chrysosporium* simultaneously removes lignin and structural carbohydrates, homogeneously degrading the material as has been reported for rubber in previous studies (Pandey and Nagveni, 2007). Other authors have also reported the results of the biodegradation of rice (Oriza sativa L.) and corn residues (Zea maize L.) by *P. chrysosporium*, which degraded cellulose and hemicellulose indiscriminately (Karunanandaa and Varga, 1996).

Table 2 summarizes the characterization results for the palm residues after the third week of treatment. Data reported in other scientific studies using the same fungi is also presented for comparison. The wide range of variation observed for the biodegradation of lignin reflects differences in the complexity of this molecule depending on the feedstock (Agosin et al., 1985). With regard to the degradation of polysaccharides, the behavior observed for the palm residues has a similar tendency to that reported for wheat straw (Salvachúa et al., 2011), where higher degradation was observed for *P. chrysosporium*.

It has been reported that the lignin degradation by *P. chrysosporium* reached 70% for olive residues (Tomati et al., 1995),

Table 1
Weight loss percentage observed during the biological treatment (wt %).

Week	*P. chrysosporium*	*P. ostreatus*
0	0.00 ± 0.00	0.00 ± 0.00
1	3.24 ± 0.03	3.33 ± 0.03
2	23.24 ± 0.61	6.63 ± 0.27
3	27.48 ± 3.08	14.63 ± 0.57
4	33.43 ± 2.24	42.69 ± 2.37