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AGROINDUSTRIAL WASTE CELLULOSE USING FERMENTED BROTH OF WHITE ROT FUNGI

CELULOSA DE RESIDUOS AGROINDUSTRIALES EMPLEANDO CALDO FERMENTADO DE HONGOS DE PUDRICIÓN BLANCA

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Abstract
The objective of this study was to separate the cellulose contained in the fique spall using different ligninolytic fungi fermented broth. The species *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineous* were adapted to a culture medium containing lignin as a carbon source. The degradation of lignin in the culture medium was present between 12 and 72 h of fermentation for the three species, yielding degradation of 72, 60 and 54% for *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineous* respectively, which coincided with the maximum levels of enzyme activity on Mn-P of 4.59, 2.24 and 4.19 IU/L for *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *P. sanguineous*, respectively. Later, fique spall was subjected to degradation in fermented broth *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *P. sanguineous* at pH 5.0, temperature of 50 °C and 30 min of enzymatic action. The fermented broth from *Pleurotus sp*. CBT1 after 48 h of fermentation showed lignin degradation in the fique spall of 65.2 % higher than other species, decreasing the initial lignin content of 14.1 % to 4.9 % and an increase in cellulose content of 61.8 to 73.1%.
Keywords: lignin, agroindustrial wastes, fique, *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2, *Pycnoporus sanguineous*.

Resumen
El objetivo de esta investigación fue la separación de la celulosa de ripio de fique empleando caldo fermentado de diferentes hongos ligninolíticos. Las especies *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 y *Pycnoporus sanguineous* se adaptaron a un medio de cultivo que contenía lignina como fuente de Carbono. La degradación de lignina en medio de cultivo ocurrió entre 12 y 72 h de fermentación para las tres especies, alcanzando una degradación de 72, 60 y 54% para *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 y *Pycnoporus sanguineous*, respectivamente, lo que coincidió con los niveles máximos de actividad de la enzima en Mn-P de 4.59, 2.24 y 4.19UI / L para *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 y *P. sanguineous*, respectivamente. El ripio de fique se sometió a degradación en caldo fermentado de *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 y *P. sanguineous* a pH 5.0, temperatura de 50 °C y 30 min de acción enzimática. El caldo fermentado a partir de *Pleurotus sp*. CBT1 después de 48h de fermentación mostró una degradación de la lignina en el ripio de fique de 65,2%, disminuyendo el contenido de lignina inicial de 14.1% a 4.9% y un aumento en contenido de celulosa de 61.8 a 73.1%.
Palabras clave: lignina, residuos agroindustriales, fique, *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2, *Pycnoporus sanguineous*.

1 Introduction

The transformation of the raw material in agricultural production processes, forestry and agribusiness present low efficiency which gives rise to a number of misused and discarded wastes. The vegetable biomass is considered a potential industrial valuable material known as lignocellulose; it is mainly composed by cellulose, hemicellulose and lignin molecules (González-Rentería *et al*. 2011; Howard *et al*. 2003).

Around 1550 million tons of agricultural waste is annually generated in the world, while in Colombia around 72 million tons of agricultural biomass residues is annually generated (Dashtban *et al*. 2009; Escalante *et al*. 2011; MADR, 2006). Ramírez-Carmona (2012) conducted the agricultural biomass residues map in Antioquia's province, finding a maximum value of residual biomass of 93.72 ton/ha cultivated for 92 species.

Fique (*Furcraea macrophylla*) is a native plant from South-American Andean region, located within the countries of Colombia, Venezuela and Ecuador (Casierra-Posada and Gómez, 2008; Cauca Government, 2002). In Colombia, fique is cultivated

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for the extraction of a natural fiber called "cabuya", in which extraction process generates juice and bagasse wastes with contents of active industrial potential value ingredients (Ramírez, 2008).

In Antioquia province around 1495 hectares of Fique are grown, whose performance represents 2356 tons per year of cabuya, generating 600 t/year of lignocellulosic agricultural residue called fique spall (MADR, 2010; Cía. de Empaques S. A., Personal Comunication, March 18th 2012).

The use of lignocellulosic wastes is subject to the preparation and purification of any of its components. Cellulose obtaining lignocellulosic wastes depends on the degradation of the lignin present in the polymer matrix that covers and protects the cellulose microfibers (Casey, 1990; Colonna, 2010; Sánchez, 2009; Van Dyk and Pletschke, 2012).

The separation of cellulose from other lignocellulosic components is performed by physical and chemical process that seeks to degrade lignin and hemicelluloses that accompany it (Bozzalla, 2010).

A biological alternative to the lignin degradation content in the fique spall is the use of ligninolytic microorganisms. The principal bodies described the ability of degrade and mineralize lignin is a group of Basidiomycetes causing the "*White Rot*" disease on wood (Papinutti *et al*. 2003).

These organisms synthesize enzymes on Laccase, Manganese-Peroxidase and Lignin-Peroxidase to degrade lignin molecule to simple chemical compounds that introduce in their metabolism as a carbon source (Fengel and Wegener, 2003). These enzymes have low specificity catalytic mechanism, so can mineralize a wide range of organic molecules (Lebrun *et al*. 2011). Extracellular enzymes cleave Ether and carbon-carbon linkages that hold type basic lignin monomer by oxidative processes (El Mansouri, 2006; Sánchez, 2009).

The use of fermented broths or crude enzyme extracts from lignin-degrading microorganisms is a simple and effective method for the cellulose separation, compared to the direct use of microorganisms, such as the absence of celluloses enzymes that degrade cellulose to separate, also the difficulties of microorganism growth on a large scale or long incubation processes (Karam and Nicel, 1997).

In this investigation, the cellulose separation of ligniocellulose contained in the fique spall using different ligninolytic "White Rot" fungi fermented broth was performed by enzymatic degradation. The degradation efficiency of lignin by three ligninolytic enzymes of *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineous* was evaluated.

2 Materials and methods

2.1 Microorganisms and growth medium

Fungi *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineous* were used for the fermentation. The microorganisms were provided by Centre for Studies and Research in Biotechnology (CIBIOT) of the Universidad Pontificia Bolivariana of Medellín, Colombia.

In growth medium (MCL) for preservation of microorganisms and fermentations were prepared using lignin of cane bagasse as a Carbon source (10 g/L); NH_4NO_3 (2 g/L); K_2HPO_4 (0.8 g/L); $MgSO_4$ (0.5 g/L); Yeast Extract (2 g/L) and $NaPH_2O_4$ (0.75 g/L).

2.2 Growth kinetics

The growth kinetics of fungi *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineous* were determined by measuring the CO_2 production (mg/L) in a system OXITOP Box 115 WTW. Kinetics were performed in 100 mL of growth medium MCL, inoculum of 1 mL spore suspension for 160 h.

2.3 Delignification in the culture medium

Delignification in the growing medium was performed by the lignin kinetics of fermented broth production. Initially, 1 mL of each suspension was inoculated fungus: *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineous* in 100 mL of growth medium MCL in 250 mL Erlenmeyer flasks. Samples were taken every 24 h for a period of 96 h. The samples were filtered under vacuum using a membrane filter (Schleicher and Schuell, 100) to separate the biomass from the fermented broth. The fermented broth was centrifuged at 4000 rpm (BoecoGermany, U-320) to take the supernatant and filtered again with a filter of 0.45 μm pore size. Subsequently, lignin was quantified.

2.4 Delignification of fique spall

For the enzymatic degradation of lignin contained in the fique spall, 100 mL of fermented broth filtered was added to 1 g of fique spall in 250 mL Erlenmeyer flasks at pH 5.0, temperature of 50 °C and magnetic stirring at a constant speed of 150 rpm for 30 min.

Thereafter, vacuum filtration using a membrane filter (Schleicher and Schuell, 100) to separate the fique spall treated from the fermented broth was performed. The solid residue was dried in an oven (1DIES, VCA115) to consistent weight. Thereafter lignin and Laccase, Manganese-Peroxidase and Lignin-Peroxidase enzyme activities were measured.

Having demonstrated the capacity to degrade lignin in the fique spall, cellulose content was determined after enzymatic treatment with fermented broth of *Pleurotus sp*.

2.5 Cellulose content

The Cellulose content in the fique spall was determined by reading High Resolution Liquid Chromatograph HPLC (Shimadzu). The chromatographic separation was performed with a Waters IC-Pack ion exclusion column and refractive index detector Shimadzu, RID-10A model. It was used a mobile phase H_2SO_4 (0.005N) at a pumping flow rate of 0.6 mL/min.

2.6 Lignin content

The lignin content in the fique spall was determined by the Klasson technique (H_2SO_4 al 72%) for the acid insoluble fraction. For determination of the fermented broth, it was filtered using a membrane filter (Schleicher and Schuell, 100). The filtrate was dried in an oven (1DIES, VCA115) at 105 °C to constant weight. The material obtained was subjected to extraction of soluble solvent extractor (Velp Scientifica, SER-148), using ethanol-toluene and distilled water as solvents.

After extracted soluble, acid digestion of the sample was performed following the method Klasson lignin, employing H_2SO_4 (72 %) relative 1:0.1 (H_2SO_4: sample) for a period of 90 min to 30°C. It was then diluted reducing the concentration of sulfuric acid up to 3 % (w/w) by adding distilled water. This solution was placed in an autoclave at 121 °C for 1 h and then filtered using a membrane filter (Schleicher and Schuell, 100). The solid residue was dried in an oven (1DIES, VCA115) to constant weight to obtain the acid insoluble lignin content value.

2.7 Enzyme activity assays

The enzymatic activity of the fungal *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 y *Pycnoporus sanguineous* in growth medium MCL for Laccase, Manganese-Peroxidase and Lignin-Peroxidase was determined.

Quantification of enzyme activity was performed by determining the absorbance in a UV/VIS Spectrophotometer (Shimadzu, UV-1601PC) for three enzymes. Quartz and glass cuvettes of 3 mL as reaction volume were used.

The enzymatic activity of the Laccase enzyme was determined as described Bourbonnais *et al*. (1995). 50 μL sample, 1850 μL de Buffer de Sodium Acetate 100 mM (pH 5.0) and 400 μL reactive Acid 2,2'-azino-bis (3-etilbenzotiazolin-6-sulfonico) (ABTS) 1 mM were taken. The reaction lasted for 1 min. ABTS oxidation was monitored by the increase in absorbance at 420 nm, using absorptivity (s) 36000 M/cm.

The enzymatic activity of the Manganese-Peroxidase enzyme was determined as described Field *et al*. (1992). 1800 μL sample, 600 μL Buffer Sodium Malonate 250 mM (pH 4.5) and 150 μL 2,6-Dimetoxiphenol (DMP) 20 mM were taken. The reaction lasted for 2 min. 150 μL $MnSO_4.H_2O$ was added. The reaction lasted for 2 min. Then, the Mn^{2+} influence on the Mn-P activity was measured for 2 min. Thereafter 300 μL of H_2O_2 was added and absorbance was measured for 3 min. DMP oxidation was followed by the increase in absorbance at 468 nm using absorptivity (s) 18300 M/cm.

The enzymatic activity of the Lignin-Peroxidase enzyme was determined as described Kirk and Tien (1988). 900 μL sample, 1200 μL de Buffer Sodium Tartrate 250 mM (pH 3.0) and 600 μL Veratryl Alcohol (10 mM). The reaction lasted for 1 min. Thereafter 300 μL of H_2O_2 was added and absorbance was measured for 1 min. Veratryl Alcohol oxidation was followed by the increase in absorbance at 310 nm using absorptivity (s) 9300 M/cm.

Enzyme activity was expressed as International Units per volume unit (IU/L).

3 Results and discussion

3.1 Growth kinetics

Pleurotus sp. CBT1, *Pleurotus sp*. CBT2 and *P. sanguineus* showed growth kinetics in MCL medium according to the characteristic pattern of microbial growth kinetics (Madigan *et al*. 2003) and showed a similar ability to grow in this type of environment, reaching population levels corresponding to a production of maximum CO_2 close to 200 mg/L (Figure 1).

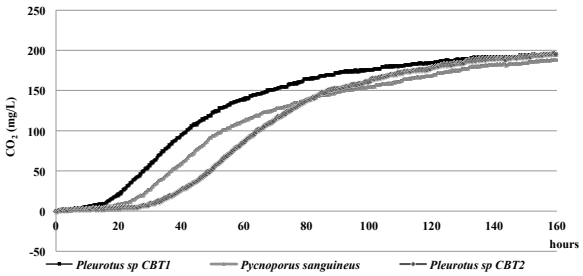

Fig. 1. Growth kinetics in terms of CO_2 (mg/L), of *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *P. sanguineus* in MCL culture medium, for a period of 160 h.

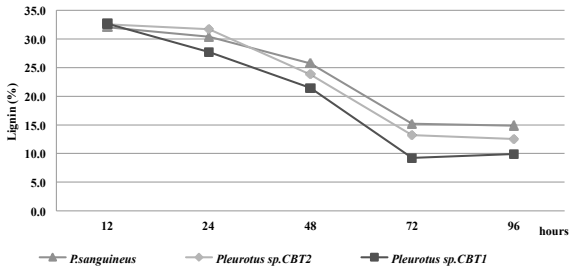

Fig. 2. Lignin content (%) in MCL culture medium for all three microorganisms.

The lag phase for *Pleurotus sp*. CBT1 lasted approximately 15 h, while for *P. sanguineus* and *Pleurotus sp*. CBT2 was 20 and 30 h, respectively. This may mean a greater ability of the organism to synthesize the enzymes needed to catabolize the lignin molecule present in the culture medium and incorporate the carbon to its metabolism, initiating the population increase.

Similarly, *Pleurotus sp*. CBT1 evidenced a rapid increase in its population over the next 40 h. Then, the growth gradually decreases until the end of the assay. It can be inferred that between 24 and 72 h of fermentation, the microorganism is able to get the necessary nutrients for their survival and reproduction. This ability is restricted in the following 72 h due to the limitation of an essential nutrient or the accumulation of any inhibitory waste product.

The behavior of the growth for the microorganisms tested is within the range of values reported by other authors for different fungi known for their ability to degrade lignin (White Rot Fungi) and similar molecules (Chaparro and Rosas, 2006; Osorio and Quintero, 2009; Díaz, 2009; Henao *et al*. 2006).

Studies by Chaparro and Rosas (2006) reported a lag phase around 24 h for *Earliella* sp, *Cookeina* sp, *Xilaria* sp, three ligninolytic species isolated from wood. Cardona *et al*. (2009) obtained lag phases with 10 and 15 h of duration for *Phanerochate sordida* and *Phanerochate chrysosporum*, respectively, in a culture medium composed by wheat bran and glucose-malt extract, respectively. Similarly, *Pleurotus ostreatus* took a period of at least 100 h for adaptation to malt extract medium (Diaz, 2009).

3.2 Delignification of the culture medium

Delignification of the culture medium was determined by lignin content during the fermented broth production kinetics. The initial content of lignin in the culture medium was 33%. Lignin degradation began between 12 and 24 h after the start of fermentation for the three species. This coincides with the end of the lag phase of microorganisms (20 h) on the growth kinetics, reinforcing the hypothesis about the ligninolytic enzymes synthesis by microorganisms during this growth phase (Fig. 2).

72 h after fermentation, all three species obtained the maximum lignin degradation. Lignin content decreased to 15.18% for *P. sanguineus*, 13.2% for *Pleurotus sp*. CBT2 and 9.24% for *Pleurotus sp*. CBT1. The highest lignin degradation was obtained during the exponential growth phase for the three microorganisms. In this phase the ligninolytic enzymes necessary to depolymerize the principal source of carbon in the culture medium was produced in larger quantities.

For the period of the subsequent 72 h, the lignin degradation is reduced, although the microorganisms population growth is still increasing.

Generally, *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineus* showed a lignin degradation efficiency that exceeded 50 %. *Pleurotus sp*. CBT1 was the species with the higher lignin depolymerization level, evidencing an efficiency of 72%. This coincides with a best response of this species in their growth kinetics compared to the other species tested (Fig. 3).

Studies with *Pleurotus sp*. have shown greater ability to degrade aromatic compounds like lignin, compared to microorganisms known for their ligninolytic ability. Sahoo and Gupta (2005) obtained aromatic dyes degradation levels higher to 80%, 72 hours after fermentation, while *Trametes versicolor* and *Phanerochaete chrysosporium*, known ligninolytic fungi, only reached around 60% values. Meanwhile, MacDonald (2012) obtained a modification of lignin of 30% using *Phanerochaete chrysosporium*, while that Antai and Crawford (1981)

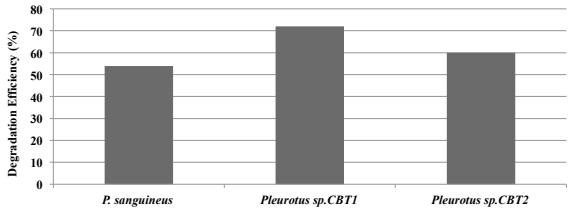

Fig. 3. Maximum Lignin degradation efficiency (%) in culture medium for *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *P. sanguineus*.

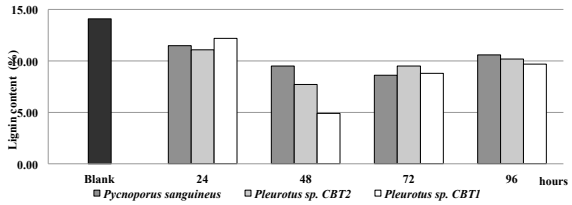

Fig. 4. Lignin content (%) in fique spall before and after enzymatic treatment with fermented broth for all three species.

obtained a lignin loss of 31.9% using different strains of *Streptomyces* sp. in herbaceous plants.

Lignin degradation by *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *Pycnoporus sanguineus* reflects their ability to synthesize extracellular ligninolytic enzymes, which interact with the lignin polymer through oxidation reactions resulting in the rupture of the bonds that hold the monolignols into the molecule (Bertrand, *et al*. 2013; Dashtban *et al*. 2009; Feng *et al*. 2011;*et al*. 2012; Lebrun *et al*. 2011; Howard *et al*. 2003; Papinutti *et al*. 2005; Pinto *et al*. 2012).

The depolymerization of lignin molecule by ligninolytic fungi or bacteria begins outside of the microorganism. There, peroxidase enzymes (Lignin-Peroxidase and Manganese-Peroxidase) and phenol oxidase enzymes (Laccase) break ether bonds (α-O-4, β-O-4, 4-O-5) and Carbon-Carbon bonds, generating lower molecular weight compounds, which can be dilignoles and/or monolignols (Dashtban *et al*. 2009; Feng *et al*. 2011; Fackler *et al*. 2006; Gassara *et al*. 2012; Lebrun *et al*. 2011; Howard *et al*. 2003; Papinutti *et al*. 2005; Pinto *et al*. 2012).

Once finished the extracellular enzymatic depolymerisation, the residual lignin oligomers are mineralized into the microorganism through a metabolic pathway that leads to cellular respiration processes (Sylvia *et al*. 2005)

3.3 Delignification of the fique spall

The initial content of lignin in the fique spall was 14.1% (control). The fermented broth of *Pleurotus sp*. CBT1 and *Pleurotus sp*. CBT2 and *P. sanguineus* showed ability to degrade lignin contained in the treated material. This behavior was obtained under conditions of pH 5.0, temperature 50 °C temperature, and fique spall concentration of 1% and 30 min of enzymatic action time.

In Figure 4, the behavior of the lignin content in the fique spall (in terms of percentage of lignin) after the enzymatic treatment with the fermented broth of the three microorganisms for a period of 96 h is shown.

24 h after fermentation, the fermented broths of all three organisms presented a ligninolytic activity on the fique spall. Lignin degradation for this period was about 12% for the three species.

48 h after fermentation, the fermented broth begins to show differences in the degradative capacity of the three species. For this period, the lignin content in the fique spall is less than 10%, reaching levels of 4.9 and 7.7% in the case of *Pleurotus sp*. CBT1 and *Pleurotus sp*. CBT2, respectively. These values are the greater levels of lignin reduction in the fique spall for these two species. *P. sanguineus* reached a lower content of lignin in the fique spall (8.6%) with a 72 h fermented broth.

Pleurotus sp. CBT1 fermented broth provided further lignin degradation in the fique spall than those of *Pleurotus sp*. CBT2 and *P. sanguineus*. Lignin content of fique spall fell to 4.9% with the fermented broth of *Pleurotus sp*. CBT1. This value represent a reduction of the lignin content on fique spall of 20 and 26 % higher than *Pleurotus sp*. CBT2 and *P. sanguineus*, respectively.

Varnaite and Raudoniene (2005) conducted tests using the full microorganism, reporting a decrease in lignin content in rye straw (16.2% initial) to 8.4 and 9.4% using *Galactomyces geotrichum* and *Myrothecium verrucaria* respectively.

In Figure 5 the behavior of lignin degradation efficiency on fique spall by all three organisms, in terms of percent degradation is shown.

Maximum levels of lignin degradation on fique spall for the fermented broths of *Pleurotus sp*. CBT1 and *Pleurotus sp*. CBT2 was reached at 48 h of fermentation. These levels were of 65 and 45% of lignin degradation, respectively. The highest efficiency for *P. sanguineus* was presented with the fermented broth of 72 h (39%).

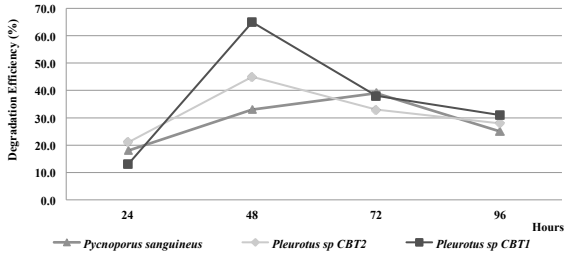

Fig. 5. Lignin degradation efficiency on fique spall (%) by all three microorganisms.

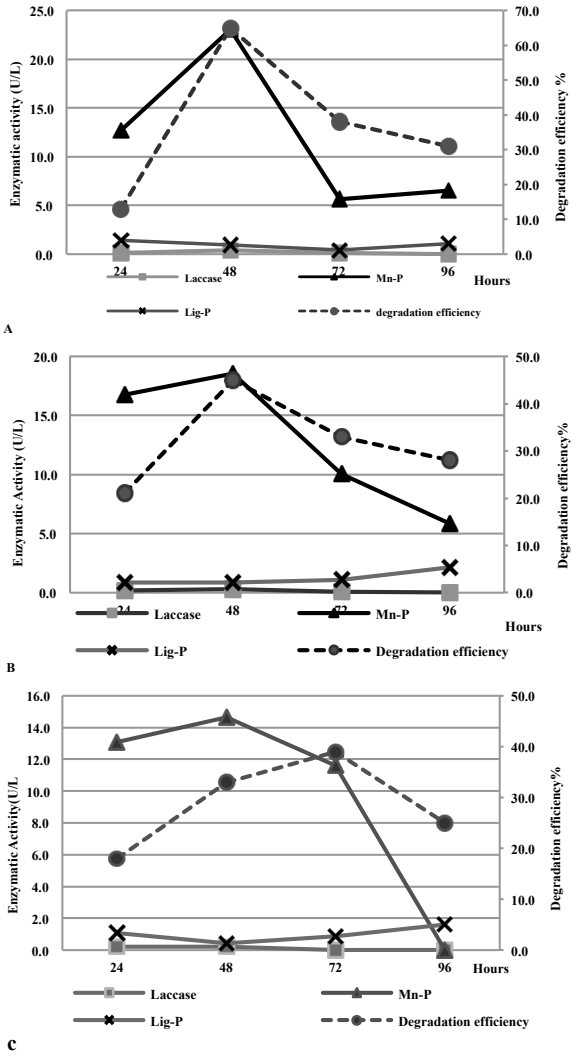

Fig. 6. Enzymatic Activity (IU/L) of Laccase, Manganese-Peroxidase and Lignin-Peroxidase in MCL culture medium and lignin degradation efficiency in fique spall (%) for *Pleurotus sp*. CBT1 (A), *Pleurotus sp*. CBT2 (B) and *P. sanguineus* (C).

These results are similar to those obtained in studies in which used fermented broth of ligninolytic fungi. Husaini *et al*. (2011) achieved lignin degradation efficiency up to 25% in 60 days by solid state fermentation using *Acacia magnium* wood with crude extracts of *Coriolus versicolor* and *Pycnoporus coccineus*. Meanwhile, Yu *et al*. (2006) reached 80% of decolourization by degradation of aromatic components from textile Red Dye K-2BP, using the fermented broth of 72 h of *Phanerochaete chrysosporum*.

Figure 6 shows the enzymatic activity in terms of International Units (IU/L) of Laccase, Manganese-Peroxidase and Lignin-Peroxidase in the fermented broths of *Pleurotus sp*. CBT1, *Pleurotus sp*. CBT2 and *P. sanguineus* and their lignin degradation efficiency on fique spall.

The lignin degradation efficiency on fique spall is directly related to the activity of the ligninolytic enzymes present in the fermented broth for all three organisms, particularly with Mn-peroxidase. The decrease of enzymatic activity of Mn-P coincides with a lower response in the lignin degradation on the fique spall for fermented broths after 48 h for *Pleurotus sp*. CBT1 and *Pleurotus sp*. CBT2 and after 72 h for *P. sanguineus*.

In 2009, Pant *et al*. reported similar results for *Aspergillus* sp. in the degradation of aromatic components of textile dyes using its fermented broth. These authors obtained a decolourisation percentage close to 30% when was presented with a high enzymatic activity of Mn-P (4.5 IU / L).

The maximum enzymatic activity of Mn-P in the fermented broth of *Pleurotus sp*. CBT1 and *Pleurotus sp*. CBT2 was submitted after 48 h of fermentation, coinciding with the maximum lignin degradation efficiency. In contrast, the higher efficiency of degradation for *P. sanguineus* fermented broth appears to 72 h, period at which the enzymatic activity of Mn-P starts decreasing.

The enzymatic activity of Laccase and Lignin-Peroxidase was lower than 0.5 and 2.0 IU/L, respectively for the three microorganisms throughout the test, keeping in line with evidenced in previous assays.

Having demonstrated the ability to degrade lignin, the cellulose content of fique spall was determined after enzymatic treatment with fermented broth of *Pleurotus sp*. CBT1, as the micro-organism showed higher lignin degradation (65%).

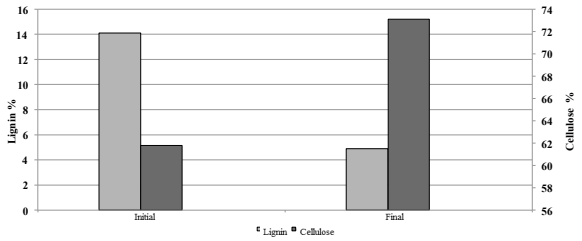

Fig. 7. Lignin and Cellulose content in fique spall before and after enzymatic treatment with fermented broth of *Pleurotus sp*.CBT1.

After enzymatic treatment with the fermented broth, the cellulose content in the fique spall increased to 73.1% (Figure 7).

The increase in the proportion of the cellulose confirms the selective lignin degradation in fique spall by action of ligninolytic enzymes present in the fermented broth. It can be inferred that the lignin presence and the cellulose absence in the culture medium during the production of fermented broth redirected the metabolism of the microorganisms to the synthesis of ligninolytic enzymes in detriment of celluloses, so that the cellulose content is not affected during treatment.

Conclusions

The fermented broth of *Pleurotus sp*. CBT1 after 48 h of fermentation had higher lignin degradation in the fique spall than those ones of *Pleurotus sp*. CBT2 and *P. sanguineus*, decreasing the initial lignin content of 14.1% to 4.9%. This representing an increase in the cellulose content in the spall of 61.8 % to 73.1 % after treatment.

The lignin biodegradation allows increasing the cellulosic fraction in agricultural wastes. Therefore, this process is useful to the cellulose exploitation as a raw material or the natural cellulose based materials development.

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