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Biodegradation of Agro-Waste Polysaccharides by *Pleurotus pulmonarius* (Fr.) Quél.

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Abstract

Degradation and solubilisation of plant organic wastes using *P. pulmonarius* is a recycling technology that could be explored and adopted in developing countries. The test substrates; saw dust (SD), sugarcane bagasse (SB) and maize stalk (MS) and in combination (SD+MS, SD+SB, SB+MS, SD+MS+SB) contained polysaccharides that were degraded by *P. pulmonarius*. The hemicelluloses, cellulose and lignin contents of the agro-wastes were significantly ($P<0.05$) degraded by the fungus to varying degrees. The loss of hemicelluloses content of the substrates ranged from 24.98% with MS substrate to 44.02% with SB substrate, cellulose content from 16.13% with SD substrate to 32.79% with SB substrate and lignin content from 8.07 % with SB substrate to 31.25% with MS substrate indicating that the fungus can not only efficiently degrade agricultural wastes and grow at a wide range of substrates but also of importance in biodegradation and bioremediation of a wide range of wastes and pollutants.

Keywords: *Pleurotus pulmonarius, agro-waste, delignification, biodegradation, bioremediation*

Introduction

Pleurotus pulmonarius is a macro fungus known to grow on a wide variety of substrates and habitat (Chang and Miles, 1991). Only the fruiting body of the fungus can be seen whereas the rest remains underground as mycelium on decayed organic matters rich in lignin, cellulose, and other complex carbohydrates (Ananbeh, 2003; Chang and Miles, 1992). The importance of mushrooms in bioremediation and biodegradation has been reported by various scientists (Adenipekun and Fasidi, 2005; Estevez *et al.,* 2005). *Pleurotus species* can efficiently degrade agricultural wastes and grow at a wide range of temperatures (Udayasimha and Vijayalakshmi, 2012). They are among the most potent organisms capable of biodegrading and detoxify a wide range of wastes and pollutants by producing one or more of phenol-targeting redox enzymes, namely the peroxidases and laccases/phenol-oxidases (Ntougias *et al*., 2012; Adebayo *et al*., 2012). Therefore, the huge amounts of lignocellulosic biomass of agro-wastes can be potentially bioconverted into different high value raw materials and products such as bio-ethanol, enriched animal feed, cheap energy sources for cultivation of mushrooms and enzyme production (Anwar *et al*., 2014; Asgher *et al*., 2013; Irshad *et al*., 2013; Ntougias *et al*., 2012). This paper reveals the ability of *P. pulmonarius* to degrade polysaccharides of agro-wastes with desirable properties for biotechnological applications in biodegradation and bioremediation of toxic organic compounds.

Materials And Methods

Source of spawn

Culture of *P. pulmonarius* was obtained from the Federal Institute of Industrial Research, Oshodi, (FIIRO) Lagos, Nigeria and multiplied on sorghum grains (Ali, *et al*. (2007). Sorghum grains were soaked for 24 hours and washed in a running tap water and then spread on a platform to drain excess water. The washed grains (10g) were placed in a medium sized bottle and autoclaved at 121°C and 15 psi for 30 minutes and left overnight to cool. The grains in the bottles were inoculated with 5g culture of *P. pulmonarius,* covered and then shaken for even distribution of the culture on the grain. The inoculated

bottles were placed in an incubator set at a temperature of 25 ± 2°C and observed daily for mycelia growth (white net web) on the grains. The inoculated bottles were removed after 14 days of incubation and stored at 4°C until used.

Substrate preparation

Saw dust (SD), sugarcane bagasse (SB) and maize stalk (MS) obtained from Michael Okpara University of Agriculture Umudike and environ were used as substrates individually and in combination (SD+MS, SD+SB, SB+MS, SD+MS+SB). The method of Adedokun *et al*. (2003) was adopted in the preparation of substrates. The MS substrate was chopped into 5 cm pieces with a knife and each of the substrates was then sterilized separately in tightly covered sack bags and then placed in a drum containing stacks of sticks (30cm). Water was poured into the drum up to the level of the sticks and covered with fresh plantain leaves in order to generate enough heat. The water in the drum containing the substrates was subjected to heating up to 100oC and allowed to steam for 2 hours using industrial gas cooker. The drum and its contents were left overnight to cool. The sterilized substrates were each poured separately into sterile polyethene bags and weighed. Each sterilized substrate (1kg) was placed separately in white sterile transparent buckets perforated with a sterile cork borer (5mm diameter).

Inoculation of substrate

The weighed sterilized substrates in the perforated buckets were each separately inoculated with 5 g of *P. pulmonarius* spawn and then covered properly. The inoculated buckets were watered every two days to maintain high relative humidity. Each treatment was replicated four times and observations made daily for fungal growth.

Treatment

Substrates used for the experiment include;

i. Saw dust (100%) - SD

ii. Maize stalk (100%) - MS

iii. Sugarcane bagasse (100%) - SB

iv. Saw dust (50%) + Maize stalk (50%) - (SD+MS)

v. Saw dust (50%) + Sugarcane bagasse (50%) - (SD+SB)

vi. Sugarcane bagasse (50%) + Maize stalk (50%) - (SB+MS)

vii. Saw dust (33.33%) + Maize stalk (33.33%) + Sugarcane bagasse (33.33%) - (SD+MS+SB).

Data obtained were analyzed with Analysis of Variance (ANOVA) and means separated using LSD at 5% level of probability.

Results

The effects of *P. pulmonarius* growth on substrate polysaccharide composition (Table 1) showed significant (P<0>P. *pulmonarius* attack ranged from 24.98% with MS substrate to 44.02% with SB substrate, cellulose content from 16.13% with SD substrate to 32.79% with SB substrate and lignin content from 8.07 % with SB substrate to 31.25% with MS substrate. In general, the highest average polysaccharide degrading potential of the fungus was recorded with SB substrate (28.29%), followed by MS substrate (20.54%) and SD+MS substrate (19.19%) indicating that the decomposition of the polysaccharides and loss were highest in SB substrate followed by MS substrate which were made readily available for use in the development and growth of *P. pulmonarius* fruit bodies. Saw dust (SD), sugarcane bagasse (SB) and maize stalk (MS) obtained from Michael Okpara University of Agriculture Umudike and environ were used as substrates individually and in combination (SD+MS, SD+SB, SB+MS, SD+MS+SB). The water in the drum containing the substrates was subjected to heating up to 100oC and allowed to steam for 2 hours using industrial gas cooker. The drum and its contents were left overnight to cool. The sterilized substrates.

Discussion

The agro-wastes contained polysaccharides that were degraded by extracellular enzymes of *P. pulmonarius* to provide energy for the oyster mushroom that possess bioactive compounds (Gunde-Cimerman, 1999). The decrease or loss in the values of hemicelluloses, cellulose and lignin contents of the agro-wastes indicated the delignification and polysaccharide degrading potentials of the fungus (Akinfemi *et al.,* 2009). Fungi have been reported to produce extracellular lignin modifying enzymes, in which the best characterized enzymes were laccase, lignin peroxidase and manganese peroxidases (Isikhuemhen and Nerude, 1999). Akinfemi *et al.* (2010) and Sivaprakasam and Kandasawmy (1981) reported that hemicelluloses and cellulose present in the substrates are reduced when *Pleurotus ostreatus* was used during biodegradation of agricultural waste and the emergence of mushrooms from the substrates. Degradation of polysaccharide contents of the agro-wastes/substrates by *P. pulmonarius* during delignification process appears to have increased the digestibility of the spent/used substrates for livestock

Table 1: Polysaccharide composition of substrates before and after cultivation of Pleurotus pulmonarius

Polysaccharide content of substrate (mg/100g) and loss (%)												
Substrate	***Hemicelluloses***			***Cellulose***			***Lignin***			***Mean***		
	A	***B***	***%Loss***	***A***	***B***	***%Loss***	***A***	***B***	***%Loss***	***A***	***B***	***%Loss***
SD	*19.48*	*26.30*	*25.93*	*39.25*	*46.80*	*16.13*	*28.45*	*35.44*	*19.72*	*29.06*	*35.44*	*18.00*
SB	*16.52*	*29.51*	*44.02*	*30.75*	*45.75*	*32.79*	*24.28*	*26.41*	*8.07*	*23.85*	*26.41*	*28.29*
MS	*19.37*	*25.82*	*24.98*	*34.33*	*49.46*	*30.59*	*21.76*	*31.65*	*31.25*	*25.15*	*31.65*	*20.54*
SD+SB	*18.00*	*27.91*	*35.51*	*35.00*	*46.28*	*24.37*	*26.37*	*30.93*	*14.74*	*26.46*	*30.93*	*14.45*
SD+MS	*19.43*	*26.06*	*25.44*	*36.79*	*48.13*	*23.56*	*25.11*	*33.55*	*25.16*	*27.11*	*33.55*	*19.19*
SB+MS	*17.95*	*27.67*	*35.13*	*32.54*	*47.61*	*31.65*	*23.02*	*29.03*	*20.70*	*24.50*	*29.09*	*15.78*
SD+SB+MS	*18.46*	*27.21*	*32.16*	*34.78*	*47.34*	*26.53*	*24.83*	*31.17*	*20.34*	*26.00*	*31.17*	*16.59*
LSD (0.05)	*1.87*	*1.02*	*4.65*	*3.60*	*2.43*	*5.12*	*2.56*	*3.45*	*1.26*	*1.86*	*2.70*	*2.54*

Values are means of four replicates in two separate experiments, A = substrate after cultivation, substrate before.

feed. Some species of *Pleurotus* have been reported to possess the ability to upgrade cattle feed by colonizing different types of crop/vegetable wastes thereby increasing their digestibility through delignification (Rajarathnam *et al.,* 1997; Salmones *et al.,* 2005). *Pleurotus spp.* have been used in the degradation of organo pollutants and bioconversion of agro-wastes due to the presence of non-specific oxygenases, as well as being explored in bioremediation efforts including biodegradation of xenobiotic compounds (Rajarathnam *et al.,* 1997), purification of air, water and soil, clean-up of contaminated soils and in the treatment of industrial effluents (Reid *et al.,* 2002). Studies have shown that *Pleurotus spp.* are able to degrade a variety of polycyclic aromatic hydrocarbons (Sack and Gunther, 1993) indicating their importance in biodegradation as reported by several scientists (Adenipekun and Fasidi, 2005; Estevez *et al.,* 2005) on various types of agro-wastes such as spent beer grain (Wang *et al.,* 2001), elephant grass, sugarcane baggase wastes and coffee husk (Obodai *et al.,* 2003; 2014) which have been evaluated as alternative substrates for mushroom cultivation (Nwokoye *et al*., 2010). The fact that mushrooms can be cultivated on materials that would otherwise be considered as waste makes it a valuable venture in self-sustaining and empowerment of communities in future (Taurachand, 2004). Growing of *P. pulmonarius* as edible mushroom using agricultural wastes could increase food production and reduce the hazards and pollution problems associated with crop residues in developing countries. In Nigeria, large volumes of unused lignocellulosic by-products are readily available and their degradation and solubilisation using *P. pulmonarius* is therefore a recycling technology that could be explored and adopted in biodegradation and bioremediation of toxic wastes.

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