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Biodegradation of cocoa shell by phytopatogenic fungi for *Pleurotus ostreatus* production

Biodegradación de cáscara de cacao por hongos fitopatogénicos para la producción de *Pleurotus ostreatus*

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Abstract

Three treatments based on spores *Colletotrichum gloeosporioides* (B1), spores *Rhizopus stolonifer* (B2) and a mixture of spores *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3) were used to increase the degradation of the cocoa shell. Using the treatment (B1) was obtained the highest degradation of the cocoa shell being of 57.18%, and the leachates production was of 84 mL with pH of 4.10. *Pleurotus ostreatus* strain (UE01) was cultivated using a mixture of wheat straw with cocoa shell degraded (without or with the 3 treatments), and the productivity (rate production and biological efficiency) and nutritional composition of the fruiting bodies were determined. *Pleurotus ostreatus* strain (UE01) cultivated on the mixture of wheat straw with cocoa shell degraded with the treatment (B2) showed the highest biological efficiency (124.15%) and the highest production rate (2.71%), while the fruiting bodies obtained in this mixture exhibited the highest protein content being of 27.84%, and lowest carbohydrate content being of 63.83%. The solutions with *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* can be used for the farmers to improve the degradation of cocoa shell and increase the production of *Pleurotus ostreatus* mushrooms with highest protein content.

Keywords: Cáscara de cacao, degradación, *Pleurotus ostreatus*.

Resumen

Tres tratamientos basados en esporas *Colletotrichum gloeosporioides* (B1), esporas *Rhizopus stolonifer* (B2), y una mezcla de esporas *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* (B3) fueron usados para incrementar la degradación de la cáscara de cacao. Usando el tratamiento (B1) se obtuvo la mayor degradación de la cáscara de cacao siendo del 57.18%, y la producción de lixiviados fue de 84 mL con un pH de 4.10. La cepa *Pleurotus ostreatus* (UE01) se cultivó utilizando una mezcla de paja de trigo con cáscara de cacao degradada (sin o con los 3 tratamientos), y se determinó la productividad (tasa de producción y eficiencia biológica) y la composición nutricional de los cuerpos fructíferos. La cepa *Pleurotus ostreatus* (UE01) cultivada en la mezcla de paja de trigo con cáscara de cacao degradada con el tratamiento (B2) mostró la mayor eficiencia biológica (124,15%) y mayor tasa de producción (2.71%), mientras que los cuerpos fructíferos obtenidos en esta mezcla exhibieron el mayor contenido de proteína siendo del 27.84% y el menor contenido de carbohidratos siendo del 63.83%. Las soluciones con *Colletotrichum gloeosporioides* y *Rhizopus stolonifer* pueden ser usados por los agricultores para mejorar la degradación de la cáscara de cacao y aumentar la producción de hongos *Pleurotus ostreatus* con el mayor contenido de proteínas.

Palabras clave: Cocoa shell, degradation, *Pleurotus ostreatus*.

1 Introduction

Cocoa (*Theobroma cacao* L.) is one of the most economically important commodities in Ecuador, with a cultivated area of 434000 hectares (ha) across the country, mainly in the provinces of Guayas and El Oro the most important in the production of this crop (Chavez *et al*., 2015; Manzano *et al*., 2017). Cocoa produced in Ecuador and other South America countries presents highest quality for the production of fine chocolate and other products (Loor *et al*., 2009).

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In Ecuador, the industrial process of cocoa generates about 1632 metric tons of waste per year like cocoa shell (Murillo, 2008). In cocoa producer countries, the processing of cocoa shell is associated with environmental problems (Vriesmann *et al*., 2012). Among them we can cite the foul- smelling appearance, lixiviate generation that filter into the ground and contaminate the soil, due to the high ammonia nitrogen concentrations (Chafla *et al*., 2016).

Technologies to reduce the time of the degradation of agricultural wastes have been proved like: the use of alkaline subcritical-water treatment and alkaline heat to increase the biodegradability of the agricultural wastes (Fox *et al*., 2003). The organic wastes treated with chemical methods can lose important biological properties because these techniques only have the finality of the reduction of the agricultural wastes volume (Lowor and Ofori, 2018). Studies have presented that the use of phytopatogenic fungi such as: *Colletotrichum* sp. and *Rhizopus* sp. improve the biodegradation of organic wastes such as: mangoes, oranges and banana rachis (Mena-Nevarez *et al*., 2012; Valenzuela-Cobos *et al*., 2020a). These agricultural wastes after using phytopatogenic fungi showed in their chemical composition amino acids such as: leucine, isoleucine, valine and phenylalanine that are related with highest productivity of flowers, fruit and mushrooms (Royse and Sánchez, 2008).

One of the most important use of agricultural wastes: olive cake, tomato tuff, pine needles, wheat straw, banana leaves, leaf of hazelnut, cotton waste, maize stover, palm oil and other wastes is the production of edible fungi like *Pleurotus* spp. (Alananbeh *et al*., 2014; Iossi *et al*., 2018), their cultivation on a variety of agricultural wastes has the advantage of putting these wastes to some use and thus alleviating the incidence of environmental pollution (Lowor and Ofori, 2018). *Pleurotus* spp. are the second genera with the highest production in the word, and can be taken as an important source of proteins, vitamins and minerals. This specie needs different conditions to grow and produce fruiting body; and requires tropical and subtropical climates (Valenzuela-Cobos *et al*., 2017). Ecuatorian native strains of oyster mushroom (*Pleurotus ostreatus*) have been studied for purposes of industrial production using substrates such as: whole plant of bean, stalk part of the banana plant and sugarcane bagasse obtaining low productivity (Pineda *et al*., 2015). For all these reasons, this research pretends to study about the use of cocoa shell as substrate for *Pleurotus* spp. production. The aim of this research was to determine the degradation, the leachate production and pH of leachates of cocoa shell after using the 3 treatments composed by phytopatogenic fungi *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*, and also evaluate the productivity and the nutritional composition of the fruiting bodies obtained in the cultivation of *Pleurotus ostreatus* produced in a mixture of cocoa shell degraded with wheat straw.

2 Materials and methods

2.1 Biological material

In this experiment was used the following phytopathogenic fungi: *Colletotrichum gloeosporioides* (CG03) and *Rhizopus stolonifer* (RS14), and also the edible mushroom strain of *Pleurotus ostreatus* (UE01). The strains were maintained on PDA dishes. Stocks of all strains are deposited at the fungal collection of Research and Development Laboratory of Ecuahidrolizados Industry.

2.2 Obtaining phytopathogenic fungal spores from Colletotrichum gloeosporioides and Rhizopus stolonifer

PDA dishes with the mycelium of phytopathogenic fungi were washed with 100 mL of distilled water, obtaining in the washing the spores of the phytopathogenic fungi. The washing was put in an Erlenmeyer flask until obtain 1 L of distilled water with a concentration of 2.50 $\times 10^6$ spores/mL (propagation solution).

2.3 Preparation of 3 treatments based on the spores of Colletotrichum gloeosporioides and Rhizopus stolonifer

The sugar cane juice was purchased in Virgen de Fatima, Ecuador.

Treatment 1 (B1): 1 L of propagation solution (concentration of 2.50 $\times 10^6$ spores/mL of *Colletotrichum gloeosporioides*) + 1 L of sugar cane juice sterilized.

Treatment 2 (B2): 1 L of propagation solution (concentration of 2.50 $\times 10^6$ spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane juice sterilized.

Treatment 3 (B3): 1 L of propagation solution (concentration of 1.25×10^6 spores/mL of

Colletotrichum gloeosporioides + 1.25×10^6 spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane juice sterilized.

2.4 Test of biodegradation of cocoa shell (without or with the 3 treatments) during 30 days

The cocoa shell was cut in a piece of 0.5 kg (wet basis) and was washed with distilled water. After that, the cocoa shell was inoculated using the 3 treatments composed by phytopatogenic fungi. The inoculated cocoa shell piece was placed in plastic containers (3 kg of capacity) and was incubated at 23°C and 95 to 100% relative humidity (RH) for 30 days. In the experiment was used a piece of 0.5 kg of cocoa shell without treatment. The biodegradation test was evaluated daily for 30 days and the experimentation was realized at the Farm "Sofía" localized in Milagro (Ecuador).

2.5 Degradation test of cocoa shell

The cocoa shell (without or with 3 treatments) was weighed daily using a digital capacity scale, the duration of the test was 30 days. The degradation was determined based on the initial and final weight of the rachis and was presented as a percentage of the initial weight.

2.6 Leachate production and pH of leachates

The cocoa shell (without or with 3 treatments) was put in a box strainer, and then it was placed over a plastic container where the leachate was collected. The volume and pH of leachates produced using a graduated glass test tube and a pH meter respectively on 30^{th} day.

2.7 Test of phytotoxicity of cocoa shell degraded (without or with the 3 treatments)

The phytotoxicity test was determined according to the percentage of viable seeds (germination index) and only with the substrate with the highest biodegradation. The seeds of lettuce and turnip were tested in 5 mL of water-soluble extracts of compost "C1" (from 10 g of fresh sample in 50 mL of distilled water), and also the seeds of lettuce and turnip were tested only in 5 mL of distilled water "C2", using conditions of darkness at 25 ºC for 72 h (Fels *et al*., 2014). To determine the germination index (GI) was necessary the number of germinated seeds (tests 72 h), and growth of roots (tests 72 h), see Eq. (1)

$$GI\% = \frac{NGext \times LRext}{NGwater \times LRwater} \times 100 \qquad (1)$$

where: NGext, NGwater= number of seeds germinated in water-soluble extracts and distilled water, respectively; and LRext, LRwater= the length of rootlets in soluble extracts and distilled water, respectively.

2.8 Substrates preparation for Pleurotus ostreatus production

Mushrooms were cultivated in a mixture of wheat straw 50% and cocoa shell degraded 50% (without or with the 3 treatments): D= Mixture of wheat straw and cocoa shell degraded without treatment. D1= Mixture of wheat straw and cocoa shell degraded with the treatment B1. D2= Mixture of wheat straw and cocoa shell degraded with the treatment B2. D3= Mixture of wheat straw and cocoa shell degraded with the treatment B3. The mixture was hydrated reaching 80%. After that, the substrate was placed (0.5 kg wet weight) in plastic bags and sterilized for 2 h at 15 psi (121°C). Subsequently, the bags were cooled down and then inoculated with 10% (w/w) of wheat grain (colonized with *Pleurotus ostreatus* strain) and were incubated in a dark room at temperature of 28±2°C (Valenzuela-Cobos *et al*., 2019a).

2.9 Induction to form fruiting bodies

As soon as the mycelium of the strain had completely colonized the substrates, the bags with substrate were transferred to the fructification room with favorable conditions: relative humidity was maintained between 85 and 90%, temperature of 18±1°C, air recirculation and period of illumination of 12 h (Valenzuela-Cobos *et al*., 2019a).

2.10 Productivity parameters of the fruiting bodies

The productivity of the fruiting bodies was evaluated based on the biological efficiency (BE; fresh weight of harvested mushrooms / substrate dry weight x 100), yield (Y; fresh weight of harvested mushrooms / substrate fresh weight x 100) and production rate, daily average biological efficiency (PR; ratio of BE

/ total number of production days starting from inoculation) (Royse, 1989; Salmones *et al*., 1997)

2.11 *Nutritional composition of the fruiting bodies*

Fruiting bodies were dried at 60°C for 24 h and then were milled to perform proximal analysis using standard methods. Moisture, ash, crude fiber and crude fat were determined according to the Association of Official Analytical Chemists methods (AOAC, 1997). Total nitrogen were evaluated with the micro-kjeldahl method, crude protein was calculated from total nitrogen content by employing the converting factor 4.38, total carbohydrates were calculated by the formula: 100 - (% protein + % fat + % ash contents), and energy value was estimated according to the equation: energy = 4 x (% protein + % carbohydrate) + 9 x (% fat) (Manzi *et al*., 2004; Valencia del Toro *et al*., 2018). The energy value of mushrooms was estimated based on the content of crude protein (Nx4.38), fat and carbohydrate using specific modified factors 3.75, 8.37 and 4.2 kcal g^{-1} of each component, respectively (Lau, 1982).

2.12 *Statistical analysis*

In all analyzes a completely randomized design, and the results were studied using one-way analysis of variance (ANOVA) to determine the significance of individual differences at $p<0.05$ level, the degradation, the leachates production and pH of leachates of the cocoa shell (without or with 3 treatments) after of the biodegradation test, the productivity parameters and nutritional composition of the fruiting bodies when statistical differences were found, the Duncan Test with $\alpha = 0.05$ was applied. The analyses were carried out using statistical software (Statgraphic ver. 16).

3 Results and discussion

3.1 *Characteristics of cocoa shell after of the degradation test*

The characteristics of cocoa shell after using the different treatments for 30 days (Table 1).

The cocoa shell piece using the treatment (B1) composed by spores of the mixture of *Colletotrichum gloeosporioides* presented the highest degradation being of 57.18%, while using the treatment (B2) of spores of *Rhizopus stolonifer* showed the lowest degradation being of 45.96%. On the other hand, the cocoa shell using the treatment (B3) composed by the mixture of spores of *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* presented degradation of 42.01%, while the cocoa shell without treatment (control) showed biodegradation of 25.41%. Mena-Nevarez *et al*. (2012) presented biodegradation since 28 to 33% on mangoes using different mixtures of spores of Colletotrichum sp. and Rhizopus sp., whereas (Valenzuela-Cobos *et al*., 2020a) presented biodegradation on banana rachis using spores of *Colletotrichum gloeosporioides* being of 28.35%, by using spores of *Rhizopus stolonifer* showing degradation of 35.58% and using spores of *Colletotrichum gloeosporioides* + *Rhizopus stolonifer* presenting degradation of 42.91%. The treatment (B1) can used in farms for the highest degradation of the cocoa shell, whereas the cocoa shell without treatment (only natural degradation) showed the lowest degradation.

Table 1. Characteristics of cocoa shell after using the different treatments.

Treatments	Degradation (%)	Leachate production (mL)	pH of leachates
Control	25.41 ± 0.82^{d}	15.00 ± 0.28^{d}	3.54 ± 0.15^{c}
B1	57.18 ± 0.31^{a}	84.00 ± 0.25^{a}	4.10 ± 0.12^{a}
B2	45.96 ± 1.52^{b}	64.00 ± 0.07^{b}	3.85 ± 0.84^{b}
B3	42.01 ± 0.03^{c}	58.00 ± 0.59^{c}	3.96 ± 0.03^{b}

*Treatment 1 (B1): 1 L of propagation solution (concentration of 2.50×10^{6} spores/mL of *Colletotrichum gloeosporioides*) + 1 L of sugar cane sterilized, Treatment 2 (B2): 1 L of propagation solution (concentration of 2.50×10^{6} spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane sterilized, Treatment 3 (B3): 1 L of propagation solution (concentration of 1.25×10^{6} spores/mL of *Colletotrichum gloeosporioides* + 1.25×10^{6} spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane sterilized.

*The degradation of the cocoa shell was calculated according to the following equation: %Degradation = 100-(%Final weight of the cocoa shell*100/% Initial weight of the cocoa shell).

*Different letters in each column indicated significant difference among the characteristics of the cocoa shell degraded at level $p<0.05$, according to Duncan's test, n = 3.

The cocoa shell degraded with the treatment (B1) showed the highest leachates production being of 84 mL on the 30th day of the experimentation, whereas the lowest leachates production (15 mL) was presented by the cocoa shell without treatment (control). In addition, the pH of the leachates on the 30th day was since 3.54 (from cocoa shell without treatment) and 4.10 (from cocoa shell degraded with the treatment B1). The rise of the pH can be explained by the production of ammonia from the degradation of amines which can release bases already existing in the organic waste (Ouatmane *et al*., 2000), or can correspond to the degradation of organic acids and to the release of exchangeable bases (Fels *et al*., 2014). The leachates of cocoa shell can be used like fungicide in different kind of plants.

3.2 Phytotoxicity testing

The germination index was determined with the cocoa shell degraded with treatment B1 that presented the highest degradation in comparison with the others treatments. The germination index of the turnip species using water-soluble extracts of compost "C1" was of 21%, while using 5 mL of distilled water "C2" was of 15%. On the other hand, the germination index of the lettuce species using water-soluble extracts of compost "C1" was of 12%, whereas using 5 mL of distilled water "C2" was of 6%. Similar results have been reported, Fels *et al*. (2014) presented germination index value for turnip species ranged from 16 and 58% using two different kind of compost during the thermophilic phase, while Valenzuela-Cobos *et al*. (2020a) showed the GI value for lettuce species using water-soluble extracts of compost (banana rachis degraded) was of 10%. The highest value of germination index was obtained by the turnip species using the cocoa shell degraded with the treatment B1.

3.3 Productivity of the fruiting bodies

Table 2 presents the productivity parameters of the *Pleurotus ostreatus* (UE01) grown in a mixture of wheat straw with cocoa shell degraded (without or with the 3 treatments).

The strain of *Pleurotus ostreatus* (UE01) cultivated in the mixture D2 presented the highest biological efficiency being of 124.15%, whereas produced in the mixture D1 showed the lowest biological efficiency being of 87.43%. Mandeel *et al*. (2005) cultivated one strain of *Pleurotus ostreatus* on four different substrates (paper, cardboard, fiber, sawdust) presenting biological efficiencies between 59.60 and 117.50%, while (Valenzuela-Cobos *et al*., 2019b) reported biological efficiencies since 73.10 to 116.19% for one strain of *Pleurotus ostreatus* cultivated in two mixtures of different proportions of peat moss and wheat straw.

Pleurotus ostreatus (UE01) cultivated in the mixture D2 presented the highest production rate being of 2.71%, whereas produced in the mixture D1 showed the lowest production rate being of 2.10%. Valenzuela-Cobos *et al*. (2020b) showed production rates between since 1.79 to 1.94% for one strain of *Pleurotus ostreatus* cultivated on two different substrates (wheat straw and mixture of oak sawdust with wheat straw). *Pleurotus ostreatus* strain (UE01) produced in the mixture D2 showed the highest productivity parameters in relation with the same strain using the others mixtures. The use of different substrates is directly related with the productivity of the edible mushroom.

Table 2. Productivity parameters of the strain of *Pleurotus ostreatus* cultivated on mixtures of agricultural wastes.

Strains	Substrates	Total cultivation time (days)	Biological efficiency (%)	Production rate (%)
UE01	D	40.30 ± 1.02^{c}	95.84 ± 2.08^{c}	2.37 ± 0.04^{b}
	D1	41.60 ± 1.43^{c}	87.43 ± 0.85^{d}	2.10 ± 0.24^{d}
	D2	45.80 ± 0.94^{b}	124.15 ± 1.54^{a}	2.71 ± 0.09^{a}
	D3	50.40 ± 0.29^{a}	110.37 ± 2.84^{b}	2.19 ± 0.16^{c}

*All values are means ± standard deviation of ten replicates. Different letters in each column indicated significant difference among the productivity parameters of *Pleurotus ostreatus* strain cultivated in a mixture of wheat straw with cocoa shell degraded (without or with the 3 treatments at level $p<0.05$, according to Duncan's test, n = 10.

*D= mixture of wheat straw and cocoa shell degraded without treatment, D1= mixture of wheat straw and cocoa shell degraded with the treatment B1, D2= mixture of wheat straw and cocoa shell degraded with the treatment B2, D3= mixture of wheat straw and cocoa shell degraded with the treatment B3.

*Treatment 1 (B1): 1 L of propagation solution (concentration of 2.50×10^{6} spores/mL of *Colletotrichum gloeosporioides*) + 1 L of sugar cane sterilized, Treatment 2 (B2): 1 L of propagation solution (concentration of 2.50×10^{6} spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane sterilized, Treatment 3 (B3): 1 L of propagation solution (concentration of 1.25×10^{6} spores/mL of *Colletotrichum gloeosporioides* + 1.25×10^{6} spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane sterilized.

Table 3. Chemical composition of the mushrooms of the strain of *Pleurotus ostreatus* cultivated on mixtures of agricultural wastes.

Strains	Substrates	%Moisture	%Fat	%Crude Protein	%Ash	% Carbohydrate	Energy value (kcal/100g dm)
UE01	D	91.75±1.52^{a}	1.75±0.38^{a}	23.05±0.85^{c}	8.95±0.18^{a}	66.25±2.05^{b}	372.95±2.08^{c}
	D1	90.86±2.03^{a}	1.10±0.03^{d}	26.93±1.01^{b}	7.41±0.64^{b}	64.56±3.04^{c}	375.86±1.98^{b}
	D2	84.52±0.48^{c}	1.63±0.19^{b}	27.84±0.97^{a}	6.70±0.09^{c}	63.83±2.97^{d}	381.35±4.41^{a}
	D3	87.19±0.89^{b}	1.24±0.28^{c}	20.49±1.08^{c}	5.24±0.17^{d}	73.03±2.87^{a}	385.24±4.09^{a}

*All values are means ± standard deviation of ten replicates. Different letters in each column indicated significant difference among the chemical composition of the mushrooms cultivated in a mixture of wheat straw with cocoa shell degraded (without or with the 3 treatments at level $p<0.05$, according to Duncan's test, n = 10.

*D= mixture of wheat straw and cocoa shell degraded without treatment, D1= mixture of wheat straw and cocoa shell degraded with the treatment B1, D2= mixture of wheat straw and cocoa shell degraded with the treatment B2, D3= mixture of wheat straw and cocoa shell degraded with the treatment B3.

*Treatment 1 (B1): 1 L of propagation solution (concentration of 2.50 $\times 10^{6}$ spores/mL of *Colletotrichum gloeosporioides*) + 1 L of sugar cane sterilized, Treatment 2 (B2): 1 L of propagation solution (concentration of 2.50 $\times 10^{6}$ spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane sterilized, Treatment 3 (B3): 1 L of propagation solution (concentration of 1.25×10^{6} spores/mL of *Colletotrichum gloeosporioides* + 1.25×10^{6} spores/mL of *Rhizopus stolonifer*) + 1 L of sugar cane sterilized.

3.4 Nutritional composition of the fruiting bodies

Table 3 presents the nutritional composition of mushrooms of *Pleurotus ostreatus* (UE01) grown in a mixture of wheat straw with cocoa shell degraded (without or with the 3 treatments).

Fruiting bodies of the strain of *Pleurotus ostreatus* cultivated in the mixtures D and D1 presented the highest value of moisture content since 90.85 to 91.75%, while the lowest value of moisture was presented for the mushrooms of the strain of *Pleurotus ostreatus* produced in a mixture D2 being of 84.52%. The fruiting bodies of the strain of *Pleurotus ostreatus* cultivated in a mixture D presented the highest value of fat content being of 1.75%, whereas the mushrooms bodies of the strain of *Pleurotus ostreatus* produced in a mixture D1 showed the lowest fat content being of 1.10%. The moisture and fat content of the fruiting bodies is influenced by the composition of the substrates used in the production of the mushroom (Valencia del Toro *et al*., 2018; Valenzuela-Cobos *et al*., 2020b) .

Fruiting bodies of the strain of *Pleurotus ostreatus* cultivated in a mixture D2 presented the highest value of crude protein being of 27.84%, whereas the mushrooms bodies of the strain of *Pleurotus ostreatus* produced in a mixtures D and D3 showed the lowest value of crude protein since 20.48 to 23.05%. In addition, the mushrooms of the strain of *Pleurotus ostreatus* cultivated in a mixture D2 presented the lowest carbohydrate content being of 63.83%, whereas the fruiting bodies of the strain of *Pleurotus ostreatus* cultivated in a mixture D3 presented the highest carbohydrate content being of 73.03%. Valenzuela-Cobos *et al*. (2019a) indicated that the nutritional composition of the fruiting bodies is influenced by the substrate used in the cultivation of the mushrooms and the strain. The mushrooms of the strain (UE01) using the mixture D2 exhibited the better nutritional parameters: highest crude protein and lowest carbohydrate content.

Conclusions

The cocoa shell after using the solution of *Colletotrichum gloeosporioides* spores (B1) showed the highest degradation in comparison with the others solution with phytopathogenic fungi spores.

Pleurotus ostreatus strain cultivated on the mixture of wheat straw and cocoa shell degraded with the solution of *Rhizopus stolonifer* spores (B2) presented the highest productivity parameters and also the better nutritional composition such as: highest crude protein and lowest carbohydrate content.

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