Application of Factorial Design for the Optimization of *Pleurotus Afin Ostreatus* Production Using Grass as Substrate

C.O. Tome-Ramos[1], L.A. Bravo-Toledo[2]*, J. Cáceres-Paredes[3], K. Vigo-Ingar[4], S. García-Flores[5], J. Valdivia-Zuta[6], R. E. Solís Farfán[7], J. A. Poma García[8], J. L. Poma García[9].

[1,2]*National University of Callao, Faculty of Environmental Engineering and Natural Resources, Av. Juan Pablo II 306, Bellavista, Callao, Peru; E-mail: labravot@unac.edu.pe*

[3,4,5,6]*Universidad Nacional del Callao, Research Center Science and Technology of Bread-making for Healthy Food, Av. Juan Pablo II 306, Bellavista, Callao, Perú.*

[7]*Universidad Nacional del Callao, Faculty of electrical and electronic engineering, Av. Juan Pablo II 306, Bellavista, Callao, Perú.*

[8]*Universidad Cesar Vallejo, Faculty of Engineering and Architecture, Av. Alfredo Mendiola 6232, Los Olivos 15314, Lima, Perú.*

[9]*Universidad Nacional del Centro del Perú, Architecture Faculty, Huancayo 12006, Perú.*

Abstracts: The organic residue from gardens, which is generally not used efficiently, has been the central focus of this research work. That is why the present work used the factorial methodology in the optimization of the production of *Pleurotus afín ostreatus* using an organic residue, with the primary objective of using this organic residue as a substrate, adjusting it through the balance of the C/N ratio, for the specific cultivation of *Pleurotus*. This is one of the edible mushroom species worldwide, known for its mycelial biomass and bioactive components. The methodology comprised several stages, including propagation of the fungus, generation of seeds using barley grains, inoculation with 5% inoculum in polypropylene bags containing four different types of substrates, with five replicates for each variant. The resulting production was measured, and the nutritional analysis of the fruiting bodies obtained from the substrate with the highest productivity was carried out. The results obtained in the parameters of responses in the production of *Pleurotus afin ostreatus* on an organic residue by the ANOVA method were ($p<0.05$) and the F-values for the models of factor A (type of substrate), factor B (type of crop) and factor AB (type of substrate and crop) were 6.78, 2.99 and 6.10, respectively. The nutritional composition of the fruiting bodies obtained from this substrate was determined. These fruiting bodies exhibited a considerable water content, which reached 75.44%. On the other hand, a low concentration of carbohydrates was observed, with a value of 15.86%, and a minimal amount of fats, barely 0.13%. It is important to note that there was a particularly notable protein content, which reached 8.09% in relation to the wet weight. Finally, it is concluded that the production values obtained in this research work indicate that the best substrate for the growth of the fungus *Pleurotus afín ostreatus* is the organic residue S3, whose biological efficiency is among the highest values recorded (37.849%) in the cultivation of this fungus. In addition, in the optimization by means of the factorial design, it was observed that in order to have a greater productive capacity of *Pleurotus afín ostreatus*, it is necessary to work with a ratio of 40.13C/N and with a type of sampling per day with a mass amount of 77g.

Keywords: Pleurotus Ostreatus, Optimization, Grass, Factorial Design, Mushrooms.

1. INTRODUCTION

Organic waste from lawns in urban environments is not used efficiently and entails an economic cost in its final disposal using many conventional technologie [1]. However, a sustainable alternative for its management is to use it as a substrate for the cultivation of the edible fungus *Pleurotus ostreatus*, thanks to its strong biodegradative actions [1]. It has a fruiting body known as mushrooms, which are occasionally soft-textured, and tend to thrive in soils with a high concentration of organic matter and moisture. In addition, edible mushrooms exhibit outstanding nutritional content, as they contain all the essential amino acids, unsaturated fatty acids, sugars, vitamins, and fiber that make mushrooms an excellent dietary choice [2].

In addition, they have a wide range of compounds that exert beneficial effects against major human diseases [3]. In addition, the mushroom also possesses antitumor properties and contributes to cholesterol reduction [4]. Furthermore, its medicinal qualities influence the regulation of blood pressure and nervous disorders that have

contributed to its consumption [5]. Likewise, the fungus acts as an effective biodegrader and detoxifier, transforming organic waste that is difficult to digest and cannot be consumed into high quality and tasty food for humans and animals [3]. This fact is even more evident in the context of soil, where saprophytic fungi thrive in the environment that they have selected [1].

Many researchers have studied a great variety of organic residues for the cultivation of Pleurotus mushroom, obtaining results of good productivity, such as coffee pulp which turned out to be the best substrate offering a biological efficiency with 1.68 g fresh mushrooms/g dry substrate [6]. On the other hand, tomato stubble obtained a biological efficiency of 92% to 139.8% showing potential as a lignocellulosic source for *Pleurotus spp.* cultivation [7]. In addition, the mixture of wheat straw and eucalyptus, and wheat straw indicated that they are suitable substrates for the cultivation of *Pleurotus* ostreatus [8]. Likewise, the set of substrates based on kenaf combined with straw, followed by the mixture with vine shoots showed better results for *Pleurotus spp*. growth [9] [10]. An adequate growth of aerial mycelia was observed on dehydrated banana leaves with a production rate of 1.5±0.1%, but the best biological efficiency was in the wheat straw substrate, with 129.34±9.1% [11]. However, the studies carried out by [12] compared the yields of wheat straw with different pretreatments (immersion in hot water, steam sterilization and use of fungicide) during the production of *P. ostreatus* fungi*,* obtaining as a result a high yield of 106.93%. Another substrate was also used such as rice husk in weight which reached a higher yield of 350.2 gr for the growth of fungus [13]. In the same way, a higher biological and productive efficiency of *P. ostreatus* was obtained with the rice husk substrate mixed with eucalyptus sawdust [14] and fava bean husks resulted in a biological efficiency of 109% for the production of oyster mushrooms [15].

In most cases, the various substrates used for the cultivation of *Pleurotus* ostreatus do not adequately consider the balance in the C/N ratio. In this particular research, the organic residue from the lawns of the gardens in the university city of the National University of Callao is used as a substrate base. Four different substrates are created, varying the C/N ratio, for the cultivation of a strain of oyster mushroom isolated in the city of Tingo María, Peru, identified as *Pleurotus afín ostreatus*. To achieve this, the seed is developed in barley grains, cultivated for two months in the different substrates, the biological efficiency is measured according to each substrate and the nutritional content of the mushrooms obtained from the substrate that is more efficient in biological terms is analyzed.

2. MATERIEL AND METHODS

The experimental work of the study consisted of a series of processes: first, the expansion of the strain was carried out in a commercial culture medium; then, the seed was created by germinating barley grains. Next, a variety of substrates were formulated and elaborated. These substrates were inoculated with the fungus and the corresponding sowings were carried out in each one. Subsequently, the fruiting bodies of the fungus were harvested, recording the production in each substrate. Finally, an analysis of the nutritional composition of the fruiting bodies obtained from the substrate that showed the best production was carried out. To carry out this experimental process, the procedures detailed in Figure **1** were implemented.

2.1. Obtaining the Strain and Organic Substrate

The seed grain of the fungus strain *Pleurotus afín ostreatus* was obtained from the city of Tingo María-Peru, donated by the Instituto de Investigación de la Amazonia Peruana-IIAP. The organic substrate was obtained according to its C/N balance from a soil analysis laboratory.

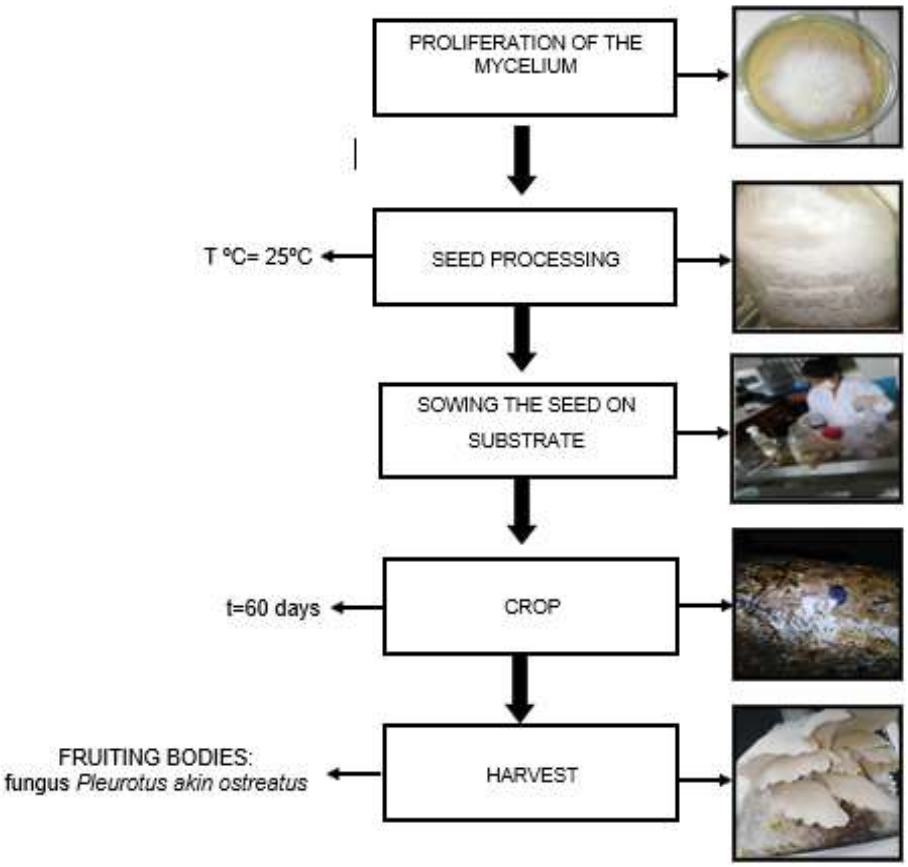


Figure 1: Flow diagram of the *Pleurotus afín ostreatus* application for the optimization of its use by means of a factorial design.

2.2. Mycelial Proliferation

The mycelium of the fungus *Pleurotus afín ostreatus* was seeded in six Petri dishes with potato dextrose agar HIMEDIA [16] The spot inoculation technique was then used. These plates were then incubated at a constant temperature of 25°C for a period of 10 days. During this time, the mycelial growth rate was evaluated and recorded in millimeters per day.

2.3. Seed Processing

A fragment of agar with mycelium of the fungus, with an area of 2 cm^2, was used as inoculum. This fragment was placed on 500 g of barley that had been washed, moistened and sterilized. This mixture was placed in polypropylene bags and/or glass bottles. Then, they were kept at a temperature of 25°C until the grains were completely invaded by the mycelium of the fungus.

2.4. Substrate Preparation

2.4.1. Substrate Formulation

The composition of the substrate and the type of strain influence the performance of *Pleurotus* mushroom cultivation*, which* is why it is of utmost importance to evaluate its physicochemical characteristics, in order to know its potential nutritive nature at the moment of using it as a unique substrate or in mixtures [17]. For this purpose, the substrates were formulated following the balance of the C/N ratio, applying the formula proposed by ITINTEC (Institute of Industrial Technological Research and Technical Standards).

$$\frac{CQ}{NQ} = 30 = \frac{A.X_A.\%C_A + C.X_C\%C_{C/10000}}{A.X_A.\%N_A + C.X_C\%N_{C/10000}}$$

Where: CQ=%C of the mixture, NQ= %N of the mixture, A= % of Sawdust, X_A = Total solids of Sawdust, $\%C_A$ = %C of Sawdust, C= % of turf (%), X_C = Total solids of turf, C_C = %C of turf, N_A =% N of Sawdust and, N_C = %N of turf.

2.4.2. Substrate Characterization

Using the information obtained from the analysis of the percentage of carbon (%C) and nitrogen (%N) of grass and sawdust, provided by the laboratory of soil, plant, water and fertilizer analysis of the Faculty of Agronomy of the Universidad Nacional Agraria La Molina, together with the total solids data provided by the laboratory of Microbiology of the Faculty of Environmental Engineering and Natural Resources of the Universidad Nacional del Callao, the previously mentioned formula was applied. This calculation allowed the determination of the four types of substrates, whose characteristics are presented in Table **1**.

2.4.3. Substrate Preparation

The organic grass debris was subjected to a drying process for one month. Then, these remains were cut into segments of approximately 2 cm in length. On the other hand, both the sawdust and the grass were moistened overnight and then left to air until they released only a drop of water when squeezed. Following the proportions specified in Table **1**. These components were mixed and 1 kg of substrate was taken in its wet state and placed in polypropylene bags. These bags, with the substrate, were subjected to a sterilization process in an autoclave for 30 minutes.

2.5. Inoculation, Sowing and Harvesting

The fungal seed inoculum was added at a ratio of 5% to the wet weight of the substrate. This was equivalent to 50 g of seed per bag containing 1 kg of substrate. This process was carried out on a disinfected surface and under the constant presence of a lighted Bunsen burner flame to maintain sterile conditions. The seed was mixed in the first 4 cm depth of the substrate. After this stage, all the bags that were inoculated were placed in a dark place for 15 days, maintaining the environmental conditions of a greenhouse. Subsequently, the bags were exposed to light for approximately 45 days, continuing regular irrigation of the crop until harvest time. The fruiting bodies were collected using a previously disinfected scalpel. After collection, the fruiting bodies were weighed with a high precision balance.

2.6. Factorial experimental design

Table 1: Factors and their levels for the response surface method-composite central design

Substrates	Turf (%)	Sawdust (%)	C/N
S_1	100	-	20,37
S_2	61	39	30
S_3	39	61	40,13
S_4	-	100	94,12

The focus of the study is on the harvesting of *Pleurotus akin ostreatus* mushroom grown on an organic substrate. For a more detailed understanding of the independent factors intended to be adjusted, an optimal number of experiments was calculated. This was done through the response surface method, used to optimize the process. The details of these factors and the assignment of experiments are presented in Table **2**. On the other hand, it is known that the Response Surface Central Composite Design (CSD) is the most commonly used design [18]. Considered as a factorial or fractional factorial design with central points, extended with a group of axial points (also called star points) that allow estimating the curvature.

The factorial design provides the possibility of comparing how the manipulated factors affect various response parameters, allowing the identification of statistically significant differences. For the analysis and interpretation of the data, analysis of variance (ANOVA) was employed according to [19]. The regression model used is presented below through the following equation (1).

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{12} X_2^2 + \beta_{23} X_3^2 \quad (1)$$

Table 2: Factors and levels used by levels

Factor	Name	Units	Type	Minimum	Maximum		
A	Type of substrate	C/N Ratio	category	S1	S4	levels:	4
B	harvest	Harvest/day	category	Harvest 1	Harvest 3	levels:	3

Where: Y is a response, X1 and X2 are manipulated factors and β0, β1, β12 are unknown parameters.

To validate the model, an acceptability analysis was carried out depending on the F-value and p-value, in addition to evaluating the model fit considering R^2, adjusted R^2 and predictive R^2 (Table **5**).

The present investigation employed a central composite design derived from a full factorial design, and 60 experiments were conducted as part of the analysis of variance (ANOVA). This methodology allows the evaluation of the effects of the 4 factors. The independent factors and their respective levels used in the design are detailed in Table **2**. The intervals selected for these factors were adjusted to the closest possible values.

2.7. Optimization By Desirability Model To Optimize

Within the methodology used, the multiple response approach is integrated, in which the desirability model is implemented. The purpose is to achieve an objective function that jointly encompasses all transformed responses. This is achieved by combining the desirable ranges for each response using Equation (2):

$$D = (d_1 \times d_2 \times d_3 \times d_4 \ldots \times d_n)^{1/n} = (\prod_i^n d_i)^{1/n} \quad (2)$$

Where, D, di and n are the desirability function, each individual response and the total number of responses, respectively. For simultaneous optimization, high and low values are searched for each response. If any of the responses is outside its desired range, the overall desirability is set to zero.

3. RESULTS

3.1. Characterization Of Organic Substrates

In the process of obtaining samples of *Pleurotus afín ostreatus* harvests from substrates S1, S2, S3 and S4, initial conditions are established. Three different levels of harvesting per day were carried out. The total yield of the harvests was as follows: 91.6g for substrate S1, 121.2g for substrate S2, 105.6g for substrate S3 and 23.20g for substrate S4. However, the analyses carried out with the factorial model in relation to the harvests of *Pleurotus afín ostreatus* revealed the following factors: Factor 1 (A, which corresponds to the type of substrate as a function of the C/N ratio); and factor 2 (B, which reflects the harvest measured in days), as detailed in Table **3**. It is observed that substrate S1 registered the greatest amount of mass in the harvest of the fruiting bodies of *Pleurotus afín ostreatus*, reaching a total of 113g on the second day. In contrast, the lowest value was 0g in the harvest of the fungus cultivated on substrate S4.

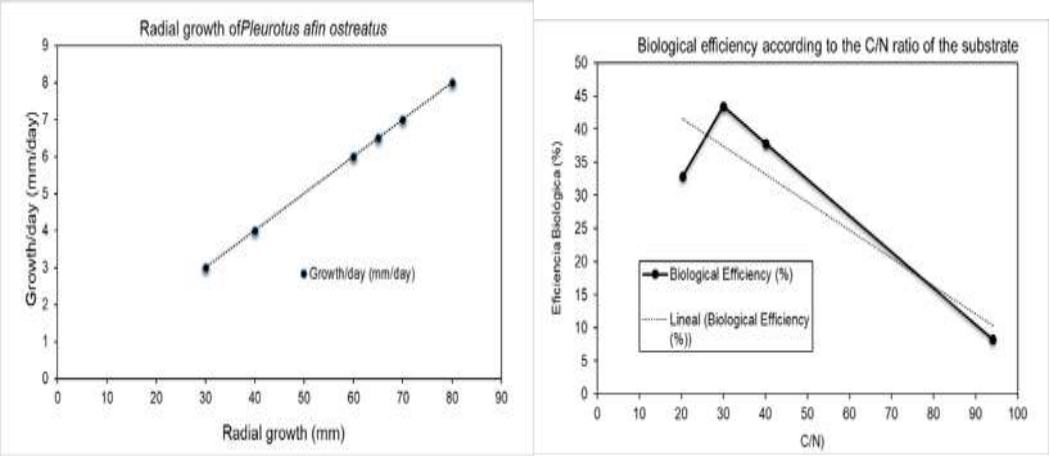
Table 3: Experimental setup for the model

Run	Factor 1	Factor 2	mass
	A: Type of substrate	B: Harvest	(g)
	C/N relation	harvest/day	g
1	S4	harvest 2	0
2	S3	harvest 1	95
3	S2	harvest 1	45
4	S3	harvest 1	75
5	S3	harvest 1	55
6	S4	harvest 3	20
7	S4	harvest 1	0
8	S3	harvest 2	16
9	S2	harvest 2	85
10	S4	harvest 1	0
11	S4	harvest 1	0
12	S1	harvest 3	10
13	S1	harvest 2	80
14	S1	harvest 2	113
15	S1	harvest 3	5
16	S4	harvest 1	0
17	S4	harvest 3	27
18	S4	harvest 3	27
19	S2	harvest 2	0
20	S4	harvest 3	18
21	S2	harvest 2	105
22	S1	harvest 1	42
23	S4	harvest 2	0
24	S3	harvest 2	18
25	S3	harvest 1	85
26	S4	harvest 2	0
27	S1	harvest 1	12
28	S2	harvest 2	45
29	S1	harvest 1	24
30	S3	harvest 1	75
31	S3	harvest 2	13
32	S2	harvest 1	43
33	S1	harvest 2	27
34	S1	harvest 3	43
35	S1	harvest 2	30
36	S2	harvest 3	30
37	S4	harvest 1	0
38	S3	harvest 3	12
39	S3	harvest 3	14
40	S1	harvest 2	5
41	S2	harvest 1	67
42	S4	harvest 2	0
43	S1	harvest 3	10
44	S4	harvest 3	24
45	S1	harvest 1	20
46	S2	harvest 1	38
47	S2	harvest 3	53
48	S1	harvest 3	25
49	S3	harvest 3	13
50	S3	harvest 3	0
51	S3	harvest 3	17
52	S2	harvest 3	0
53	S2	harvest 3	15
54	S2	harvest 1	43
55	S2	harvest 2	0
56	S3	harvest 2	15
57	S2	harvest 3	37
58	S3	harvest 2	25

59	S1	harvest 1	12
60	S4	harvest 2	0

3.2. Relationship Between Growth and Biological Efficiency

A linear correlation was observed between daily growth of *Pleurotus afín ostreatus* and its radial growth. In addition, the highest daily growth of 8 mm/day reached the highest growth value in a Petri dish with potato dextrose agar with a value of 80 mm, while the daily growth of 3 mm/day reached the lowest growth value in a Petri dish with a value of 30 mm (Figure **2a**). On the other hand, the biological efficiency was obtained according to the C/N ratio of the biological residue whose results are represented in (Figure **2b**) where the correlation of the biological efficiency and the carbon/nitrogen ratio (C/N) of the substrate in the production of *Pleurotus afín ostreatus* can be appreciated. In addition, it was observed that substrates S2 and S3 presented a higher carbon nitrogen ratio 40.13 C/N and 30.00 C/N respectively, with a biological efficiency of 43.441% and 37.849% respectively.



(2a) (2b)

Figure 2: Graph of the relationship between radial growth and growth/day (2a) and graph of the biological efficiency according to the C/N ratio of the biological waste (2b).

3.3. Analysis Of The Amount Of Substrate Mass

The effects of the studied factors such as the type of substrate and the harvest of *Pleurotus afin ostreatus* mushroom in relation to the amount of mass were determined. Figure **3** shows the semi-normal probability that allows to analyze in a first instance the parameters with greater effects, where the color coding provides details on the type of substrate and the mushroom harvest per day, obtaining as a result an interaction of both with a positive effect on the amount of substrate mass used.

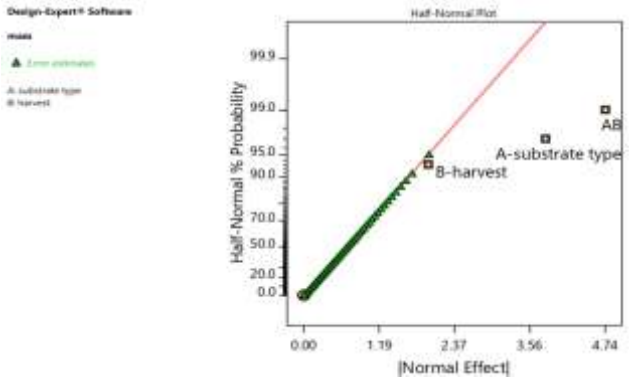


Figure 3: Normal probability plot for the model

3.4. ANOVA Analysis Of Factorial Designs

The significant main and interaction effects of the factors influencing the response parameters in the production of *Pleurotus afin ostreatus* on an organic residue by the ANOVA method are shown in Table **4**. For this, the significance of each parameter of the model was evaluated through the F-value test and the p-value values for each variable, including linear interactions, interactions and quadratic interactions. Table **4** presents the statistical values for each factor. The p-value was below 0.05, which means that the coefficients of the model are statistically significant. In addition, the quadratic models developed for each response were found to be significant. F-values of 6.78, 2.99 and 6.10 were obtained for the models of factor A (substrate type), factor B (crop type) and factor AB (substrate type and crop), respectively. On the other hand, the accuracy of the statistical models developed to predict the responses was confirmed by small probability values ($p<0.05$).

Table 4: ANOVA analysis for the quadratic model of response surface %DQO removed

Source	Sum of Squares	df	Mean Square	F-value	p-value
Mass (g) =28.46+2.01 A[1]+11.93 A[2]+6.73 A[3]+8.08 B[1]+0.38 B[2]-16.62 A[1]B[1]-1.28 A[2]B[1]+33.72 A[3]B[1]+20.08 A[1]B[2]+6.22 A[2]B[2]-18.18 A[3]B[2].					
Model	28840.13	11	2621.83	5.72	< 0.0001
A-substrate type	9328.27	3	3109.42	6.78	0.0007
B-harvest	2743.43	2	1371.72	2.99	0.0596
AB	16768.43	6	2794.74	6.1	< 0.0001
Pure Error	22002.8	48	458.39		
Cor Total	50842.93	59			

3.5. Statistical Model Analysis

Significant factors are shown so a statistical model has been developed where the equation was calculated in terms of study factors as independent variables and the variable response to the mass in grams of the substrate. However, for the determination of the repeatability of the equation of the generated model, an R^2 was calculated with a value of 0.5672, an adjusted R^2 with a value of 0.4681, a value that is in reasonable agreement with the predictive R^2 of 0.3238. In addition, the Adeq Precision indicator measured the mass ratio, giving a ratio of 8.0419 as shown in Table **5**. Furthermore, the accuracy of the regression model is supported by the existence of an adequate correspondence between the empirical response values and the expected values, i.e., it has a positive relationship indicating a consistent agreement between the actual data and the model predictions (Figure **4**).

Table 5: Fit indicators of the factorial design of the experiment

Statistician	Quantity
R^2	0.5672
Adjusted R^2	0.4681
Predicted R^2	0.3238
Adeq Precision	8.0419
Std. Dev.	21.41
Mean	28.47
C.V. % C.V. % C.V. % C.V.	75.21

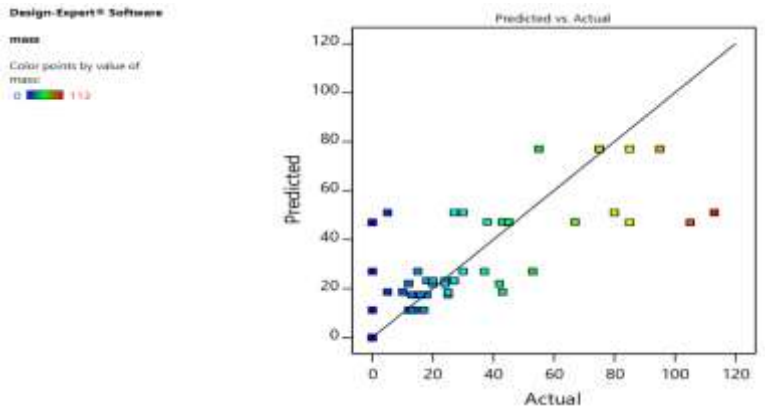


Figure 4: Graphs of the evaluation between predicted and experimental values

3.6. Effect Of Mass Quantity

Figure **5** shows the interaction graph, which shows the statistically significant impacts of the type of substrate in relation to the mass obtained. It is observable that substrate S3 reveals an increase in the production of *Pleurotus afín ostreatus compared* to substrates S1, S2 and S4. This difference is represented and analyzed in a three-dimensional graphical representation presented in Figure **6**.

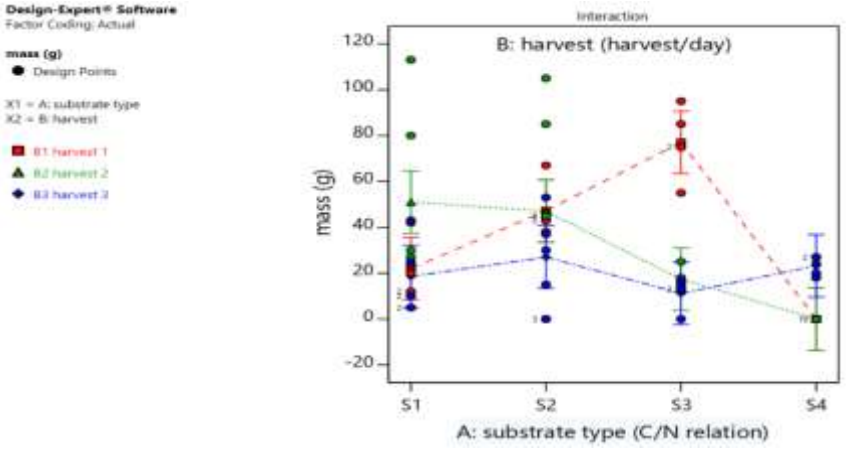


Figure 5: Plots of the substrate type in iteration of the amount of mass(g)

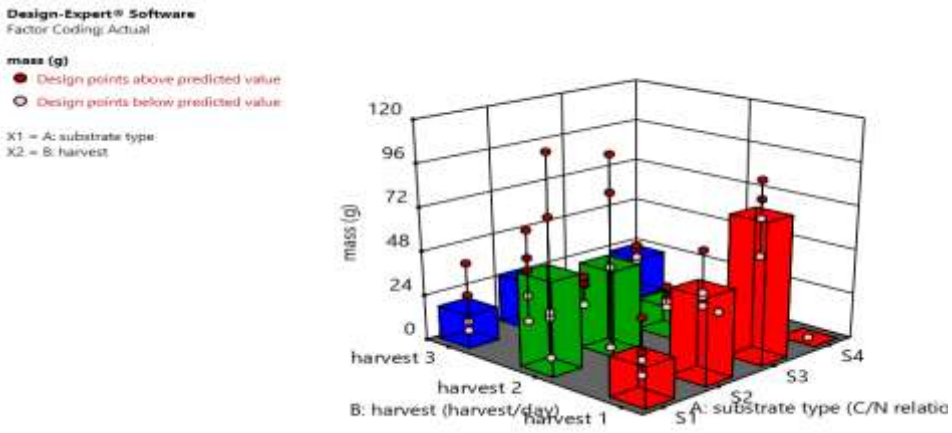


Figure 6: 3D plots of the iteration harvesting of the amount of mass(g)

3.7. Model Validation

To verify the validity of the proposed model, a normality analysis was performed. This analysis made it possible to visualize how the values are distributed along a normal line. As can be seen in Figure **7**, the results follow a distribution similar to that of a normal line. In addition, plot 8 of the residuals as a function of the fitted values for the mass quantity data is presented. In this plot, the distributions of the results do not show a clear trend, which means that the variance of the observations does not increase as the magnitude of the observation increases. Therefore, in this case, it does not appear that the error or background noise of the experiment is significantly low

3.8. Optimization And Comparison With Standards

Response surface methodology was applied to optimize results related to substrate type and harvest stage of *Pleurotus afin ostreatus* mushroom. Figure **8** presents three graphs illustrating how to select the appropriate substrate type treatment most suitable for the growth of *Pleurotus* species. The results indicate that the optimal substrate type optimization is achieved with S3, with one sampling type per day with a mass amount of 77g.

3.9. Analysis Of The Nutritional Composition Of *Pleurotus Afín Ostreatus*

From the optimization of the data on the production of fruiting bodies according to the type of substrate, the optimum biological efficiency was obtained with substrate S3, which has a C/N ratio of 40.13 for the cultivation of *Pleurotus afin ostreatus*. In addition, with regard to the nutritional composition of the fruiting bodies obtained from this substrate, as presented in Table **6**, a high-water content, a low concentration of carbohydrates and fats, and especially a notable content of 8.09% protein on a wet weight basis, stand out.

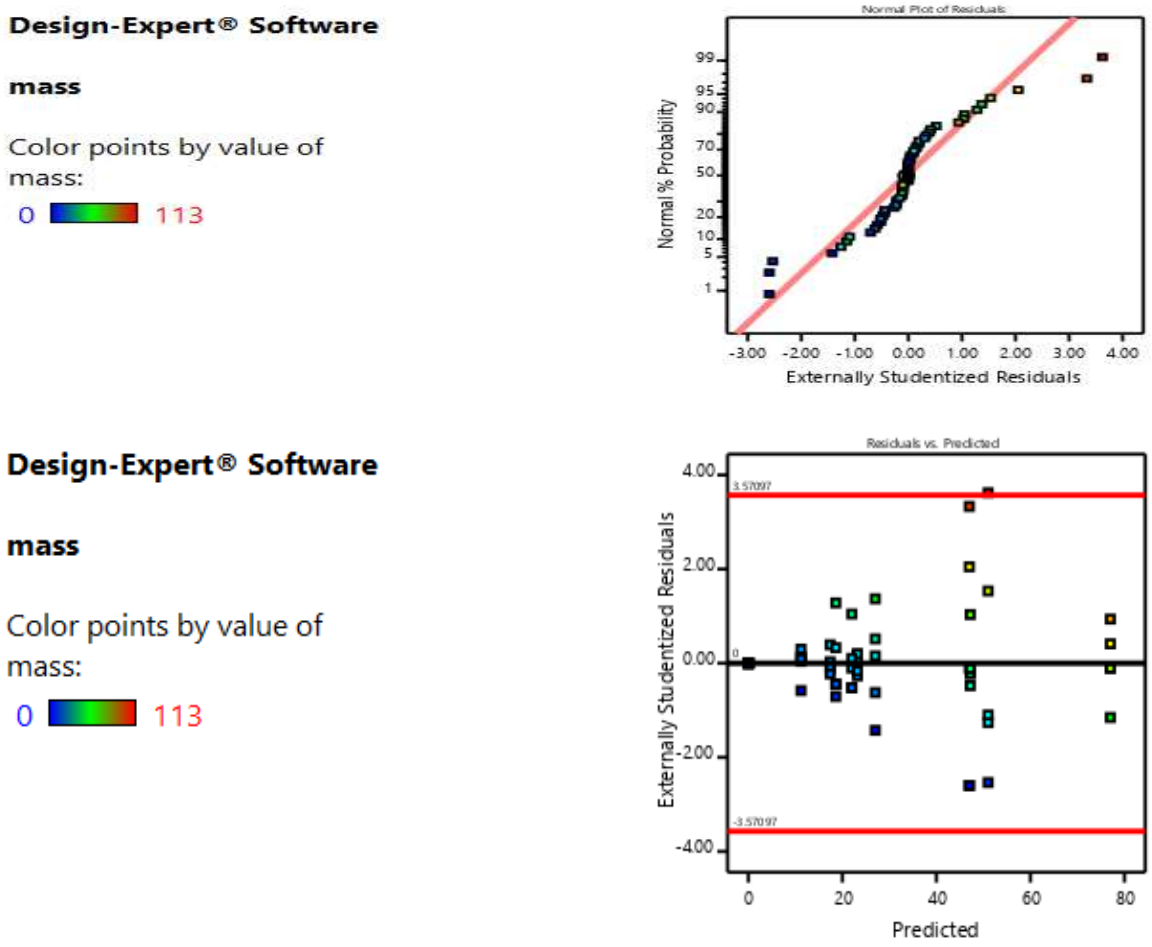


Figure 7: Normality plot and plots of homogeneity of variance of the residuals of the different parameters of treatment

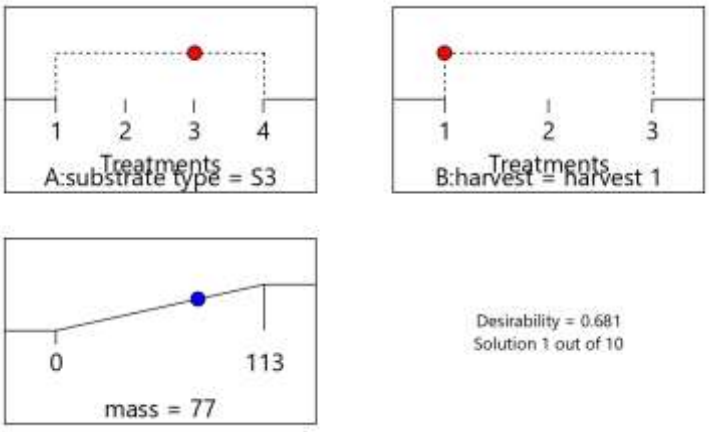


Figure 8: Optimization of substrate type and harvesting of *Pleurotus afin ostreatus* mushroom.

Table 6: Nutritional composition of fruiting bodies of *Pleurotus afín ostreatus*

Analysis	Percentage
Humidity	75,44±0,25
Protein	8,09±0,01
Crude Fat	0,13±0,00
Gross Fiber	3,34±0,20
Ash	0,48±0,00
Total Carbohydrates	15,86

4. DISCUSSION

The study conducted by [20] determined that the formula for the cultivation of *Pleurotus ostreatus* under controlled conditions depends on the type of substrate. For this reason, three organic substrates were used: Bamboo spp. leaves, Traveler's palm leaves and Livistona palm leaves. In addition, an agro-industrial residue, coconut tow, was used. The results of this study were evaluated by analysis of variance (ANOVA). Significant differences were found at 95% significance level. The findings indicated a notable impact on biological efficiency and productivity rate, with respective values of 11.9% and 17%. On the other hand, the least effective treatment was the second formulation, which registered an efficiency and rate of 3% and 4.2%, respectively. Likewise, [21] reported that the biological efficiency of S2 of C/N equal to 30 was 43.44%, S3 of C/N equal to 40 was 44%, S1 of C/N equal to 20 was 32.83%, S4 of C/N equal to 94.12 was 8.315% according to ANOVA analysis of variance for α=0.05.Concliyoi that the best substrate for *Pleurotus afin ostreatus* production was residual turf with 39% sawdust.

Studies on the biological efficiency of *Pleurotus pulmonarius* strains on an organic substrate such as, [22] obtained an average biological efficiency of 55.73% (fermented straw) and 71.25% (unfermented straw), with a production rate of 0.64% (fermented straw) and 0.92% (unfermented straw), with an average production period of 66 days and 58 days, respectively. Prior to planting, the pH of the fermented straw was 9.4 with 74% moisture, and the control straw had a pH of 8.5 and 73% moisture. In addition, they concluded that the initial C: N ratio in the control straw was 76% (dry basis) and in the fermented straw it gradually decreased to 47% at day 7 and [23] showed that the C/N content fluctuated between 50.12 and 56.55 % and 0.38-1.29 % respectively. They concluded that there is a direct relationship between nitrogen content with biological efficiency and protein in the fungus. However [24] determined the effect of the substrate on the production of *Pleurotus* genus strains and obtained as a result that the treatment with 100% grass obtained high values of EB with 38.2 % and 48.8 %, TP with 0.5 % and 0.6 % and R with 14.7 and 18.8 %, for strains ECS-123 and ECS-1123 respectively. They concluded that 0.5 % nitrogen concentration and C/N of 96.76 is enough to obtain a good production of *Pleurotus spp*.

The present research obtained significant effects among the factors that influence the production of Pleurotus afín ostreatus on an organic residue by the ANOVA method. Where p-value values below 0.05 were obtained, which means that the coefficients of the model are statistically significant. In the same way, the studies carried out by [25] optimized the productive capacity of the oyster mushroom (Pleurotus ostreatus), by means of a Simplex Design with Centroid for a total of 10 treatments. They used organic residues such as eucalyptus shavings, pine shavings and corn stover and applied the analysis of variance (ANOVA), finding a significant difference ($p<0.05$) among the treatments. They obtained as a result that pine chips increase the productive capacity and eucalyptus chips decrease it. Finally, they obtained a corresponding model for each dependent variable, with a determination coefficient ($R^2 >0.98$). On the other hand [23] evaluated cassava bagasse (YB) and wheat straw (WS) as substrate for cultivation of *Pleurotus ostreatus.* They obtained as a result a ratio of 75:25 for (WS/YB) with an average biological efficiency of 115% and 143%, respectively. In addition, they found a significant difference ($p<0.05$) between strains and substrate.

On the other hand, studies by [26] evaluated the effect of chemical and biological supplements of oat straw to increase the production of the fungus *Pleurotus ostreatus* by means of an analysis of variance and Tukey's test. As a result, sorghum paste obtained a significant difference ($p<0.05$) in yield and biological efficiency, with 31.6%. [27] corroborated the parameters such as biological efficiency (EB), productivity rate (TP), size, fat, fiber, total carbohydrates, energy value and total proteins. They obtained a size of 5 and 10cm with a high EB for *Pleurotus* pulmonarius RN2 (75.65%) and with a high protein content for the fungus *Pleurotus djamor RN82* grown on corn stover (43.07%). Concluding that there is a significant difference ($p<0.05$) for biological efficiency, protein and fiber by effect of each substrate.

The results of this study showed that the percentage of protein for the fruiting bodies of *Pleurotus afín ostreatus according to* the type of substrate (organic residue) was 8.09 % on a wet weight basis, in addition to 75.44% moisture, 0.13% crude fat, 3.34% crude fiber, 0.48% ash and 15.86% total carbohydrates. Similar studies have shown that [26] which obtained a nutritional composition of the *Pleurotus ostreatus* mushroom cultivated in coffee pulp. They obtained as results 28.6 % in crude protein content and 29.7 %, in dry sample, with a high water content (86 %) and a low content in carbohydrates (< 8 %) and fats (< 1 %) in fresh sample. They concluded that *Pleurotus ostreatus* mushroom can improve people's health, nutrition and control diseases.

In turn, [21] where it was determined that the adequate substrate for the production of *Pleurotus afín ostreatus* was an organic residue (garden pruning) which allowed a rapid invasion, with a high protein content (32.94%), which was formulated with 61% of residual grass mixed with 39% of sawdust for a C/N balance equal to 30. In relation to the nutritional composition of the fruiting bodies of *Pleurotus afin ostreatus* obtained from the culture on the substrate of C/N equal to 30, it is distinguished the low content of total carbohydrates and fat and, a considerable protein content of 32.940% in dry basis. In addition, [8] used four treatments with poplar chips, eucalyptus chips, a mixture of wheat straw and eucalyptus, and wheat straw as a control. The results of the research indicated that certain substrates, particularly wheat straw and wheat straw with eucalyptus, were suitable for Pleurotus ostreatus production. The researchers concluded that this species exhibited a high protein content in all substrates investigated. However, they noted that the carbon/nitrogen ratio decreased after the harvesting process.

CONCLUSION

In conclusion, the production results obtained in this study indicate that the optimum substrate for the growth of the fungus *Pleurotus afín ostreatus* is the organic residue S3, whose biological efficiency of this substrate is one of the highest recorded (37.849%) in the cultivation of this fungus. In addition, in the optimization by means of the factorial design it was observed that in order to have a greater productive capacity of *Pleurotus afín ostreatus,* it is advisable to work with a ratio of 40.13C/N and with a type of sampling per day with a mass amount of 77g.

REFERENCES

[1] Daccò C, Girometta C, Asemoloye MD, Carpani G, Picco AM, Tosi S. Key fungal degradation patterns, enzymes and their applications for the removal of aliphatic hydrocarbons in polluted soils: A review. Int Biodeterior Biodegradation 2020;147:104866. https://doi.org/10.1016/j.ibiod.2019.104866

[2] Heleno SA, Barros L, Sousa MJ, Martins A, Ferreira ICFR. Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. Food Chem. 2010;119(4):1443–50. https://doi.org/10.1016/j.foodchem.2009.09.025

[3] Molina AMG, Cardona NV, Alzate SR, D'León JGS, Holguín ES. Evaluación de algunos residuos orgánicos como sustrato para el cultivo de hongos comestibles. Rev Lasallista Investig. 2005;2(2):15–20.

[4] Yoshioka Y, Emori M, Ikekawa T, Fukuoka F. Isolation, purification, and structure of components from acidic polysaccharides of Pleurotus ostreatus (Fr.) Quél. Carbohydr Res. 1975;43(2):305–20. https://doi.org/10.1016/S0008-6215(00)83495-3

[5] Beelman RB, Royse DJ, Chikthimmah N. Bioactive components in button mushroom Agaricus bisporus (J. Lge) Imbach (Agaricomycetideae) of nutritional, medicinal, and biological importance. Int J Med Mushrooms. 2003;5(4). https://doi.org/10.1615/InterJMedicMush.v5.i4.10

[6] García-Oduardo N, BermúdezSavón RC, Serrano-Alberni M. Formulaciones de sustratos en la producción de setas comestibles Pleurotus. Tecnol química. 2011;31(3):272–82.

[7] Sánchez A, Esqueda M, Gaitán-Hernández R, Córdova A, Coronado ML. Uso potencial del rastrojo de tomate como sustrato para el cultivo de Pleurotus spp. Rev Mex Micol. 2008;28(SPE):17–24.

[8] Varnero MT, Quiroz MS, Álvarez CH. Utilización de residuos forestales lignocelulósicos para producción del hongo ostra (Pleurotus ostreatus). Inf tecnológica 2010;21(2):13–20. http://dx.doi.org/10.4067/S0718-07642010000200003

[9] Pardo Giménez A, Perona Zamora MA, Pardo Núñez J. Utilización de fibra de kenaf (Hibiscus cannabinus L.) en la elaboración de sustratos específicos para cultivo de Pleurotus ostreatus (Jacq. ex Fr.) Kummer. Rev Iberoam Micol. 2008;25(1):57–61. https://doi.org/10.1016/S1130-1406(08)70015-9

[10] Romero O, Huerta M, Damián MA, Macías A, Tapia AM, Parraguirre JFC, et al. Evaluación de la capacidad productiva de Pleurotus ostreatus con el uso de hoja de plátano (Musa paradisiaca L., cv. Roatan) deshidratada, en relación con otros sustratos agrícolas. Agron Costarric. 2010;34(1):53–63. https://doi.org/10.15517/rac.v34i1.6699

[11] Fernández A, Romero J. Evolución de las bacterias ácido lácticas durante la elaboración del tocosh fresco, aislamiento y concentración por liofilización 2020.

[12] Jaramillo S, Albertó E. Heat treatment of wheat straw by immersion in hot water decreases mushroom yield in Pleurotus ostreatus. Rev Iberoam Micol. 2013;30(2):125–9. https://doi.org/10.1016/j.riam.2012.11.004

[13] Baysal E, Peker H, Yalinkiliç MK, Temiz A. Cultivation of oyster mushroom on waste paper with some added supplementary materials. Bioresour Technol. 2003;89(1):95–7. https://doi.org/10.1016/S0960-8524(03)00028-2

[14] Costa AFP, Steffen GPK, Steffen RB, Portela VO, Santana NA, dos Santos Richards NSP, et al. The use of rice husk in the substrate composition increases Pleurotus ostreatus mushroom production and quality. Sci Hortic (Amsterdam). 2023;321:112372. https://doi.org/10.1016/j.scienta.2023.112372

[15] Ivarsson E, Grudén M, Södergren J, Hultberg M. Use of faba bean (Vicia faba L.) hulls as substrate for Pleurotus ostreatus – Potential for combined mushroom and feed production. J Clean Prod. 2021;313:127969. https://doi.org/10.1016/j.jclepro.2021.127969

[16] Suárez C, Holguín MS. Evaluación de medios de cultivo sintéticos y cereales para la producción de semilla de setas comestibles. Rev Colomb Ciencias Hortícolas. 2011;5(1):130–40. https://doi.org/10.17584/rcch.2011v5i1.1259

[17] Ruilova M, Hernandez A. Diseño de sustratos de diferente composición físico-química utilizando el programa de mezclas de residuos agrícolas para el cultivo del hongo Pleurotus Ostreatus. Rev Investig Talent. 2016;3(2):30–5.

[18] Parra LVB. Optimización multi-objetivo en el problema de metodología de superficie multi-respuesta. Trab fin máster, CIMAT, México, Guanajuato 2007.

[19] Khan, T. I., Jam, F. A.., Anwar, F., Sheikh, R. A., & Kaur, S. (2012). Neuroticism and job outcomes: Mediating effects of perceived organizational politics. African Journal of Business Management, 6(7), 2508-2515.

[20] Guzmán TM, Pérez O, Valdés O. Biodegradación de residuos sólidos urbanos utilizando cultivos microbianos y biofiltro estático de Eisenia foetida. Tecnol Química. 2020;40(1):81–92.

[21] Sánchez CA. Evaluación de la productividad del hongo comestible Pleurotus ostreatus sobre un residuo agroindustrial del Departamento del Valle del Cauca y residuos de poda de la Universidad Autónoma de Occidente 2013.

[22] Tome CO. Fructificación del hongo comestible Pleurotus afín ostreatus sobre un sustrato según su balance de relación c/na base del césped residual de jardines 2022.

[23] Gaitán R, Salmones D, Pérez R, Mata G. Evaluación de la eficiencia biológica de cepas de Pleurotus pulmonarius en paja de cebada fermentada. Rev Mex Micol. 2009;30:63–71.

[24] Cueva MBR, Monzón AH. Evaluación de residuos agrícolas para la producción del hongo Pleurotus ostreatus. ICIDCA Sobre los Deriv la caña azúcar. 2014;48(1):54–9.

[25] ., S., Hanifati, S. N. ., ., D., & ., J. (2023). The Influence of Instructional Models and Innovation Ability on Learning Outcomes of Alternative Energy Courses. International Journal of Membrane Science and Technology, 10(2), 542-549. https://doi.org/10.15379/ijmst.v10i2.1264

[26] Roblero DO, Aguilar L, Sánchez JE. Efecto de la variación del sustrato en la productividad de dos cepas de Pleurotus spp. Sci fungorum.

2021;52. https://doi.org/10.33885/sf.2021.52.1377

[27] García JA, BarrazaG, Miano AC. Sustrato óptimo entre viruta de Pino, viruta de Eucalipto y coronta de Maíz para maximizar la capacidad productiva de Hongo Ostra. LACCEI Inc 2020. http://dx.doi.org/10.18687/LACCEI2020.1.1.137

[28] Aceves ACM, Flores RA, Sánchez MAO, Ayala AB. Productos químicos y biológicos como suplementos que incrementan la producción del hongo ostra Pleurotus ostreatus. Interciencia. 2015;40(8):542–8. http://dx.doi.org/10.4067/S0718-07642013000100009

[29] Nieto JI, Cuzcano ÁD, Reyes WA. Estudio preliminar de la composición nutricional del hongo Pleurotus ostreatus cultivado en pulpa de café. Rev la Soc Química del Perú. 2019;85(4):422–31. http://dx.doi.org/10.37761/rsqp.v85i4.256

DOI: https://doi.org/10.15379/ijmst.v10i3.1905