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# Design of a Bioreactor for the Bioconversion of Lignocellulosic Residues into Protein for Human Feeding

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***Abstract:*** *World food security has become seriously compromised and in danger of an irreversible crisis; as a result, there are a billion people on the planet in a state of undernutrition.The designed reactor was loaded with perforated cylindrical biocells of 10 cm in diameter, filled with 4 kg of bean harvest residue (Phaseolus vulgaris) as substrate, which had previously had its humidity adjusted to 70.68%, and had been pasteurized at 103.4 kPa and 121°C. 10%Pleurotus ostreatus myceliumceba-po-gliie-010106 was used as the inoculum. The bioreactor operated at a temperature of 20°C and a relative humidity of 85%.It was established with 95% confidence that both the biocell distribution inside the bioreactor and the packing density exert significant influence over the bioconversion (BC) and the specific biomass productivity (SBP); it was determined that the best biocell distribution is the square one, and that the best packing density is 21.7kg of substrate dry matter (SDM)/m3. Under these conditions, a BC of 101.47 g of fungal drymatter (FDM)/kg of SDM, and a specific biomass productivity (SBP) of 5.07 g of FDM/kg of SDM per day was obtained. The designed bioreactor represents a technological contribution to scientific research of the biotechnology of dry fermentation, and has wide usefulness to small local production.*
***Keywords:*** *residues, biotechnology, food*

## I. Introduction

During the first half of this century, as the world population reaches 9 billion, the world demand for food, livestock feed and fibers will almost double. At the same time, crops can also be used to produce bioenergy and for other industrial uses [1].

From 2007 to 2009, the food price crisis followed by the financial crisis and the world economic recession brought about an unprecedented increase in the number of people experiencing hunger and undernutrition, exceeding the record figure of 1 billion in 2009[2].

Ecuador, as well as the rest of the developing world, does not escape the worldwide problem of food insecurity and climate change. Intending to minimize the negative impact of these issues, the Constitution of the Republic of Ecador, Art. 281, states that “food sovereignty constitutes a strategic objective and an obligation on the part of the State to guarantee that persons, communities, peoples and nationalities reach self-sufficiency in healthy and culturally appropriate food in a permanent way”[3]. The Organic Code of Production, Commerce and Investments, for the transformation of the production matrix, in its Article 5, states that the State will incentivize productive investment through the encouragement of the “improvement in the productivity of the actors in the popular and caring economy, and of the micro, small, and medium-sized businesses, in order to participate in the domestic market and, eventually, to reach economies of scale and production quality levels which will allow them to offer their products abroad” [4].

Throughout the world, businesses and governments’ strategies to confront climate change, energy, agricultural, technological and materials production are increasingly converging around the same concept: biomass. Biomass comprises more than 230 billion tons of living matter produced by the earth every year, such as trees, shrubs, grasses, algae, grains, microbes and others [5]. To date, humans have used only a quarter, about 24% of this earthly resource to satisfy their basic necessities and industrial production, which leaves 76% of the total existing biomass on the planet unutilized [5].

Ecuador bases its economy on agriculture and oil. It is considered the top banana exporter in the world. Among its main crops are cocoa, bananas, coffee, African oil palm, sugar cane, corn, rice, soybeans, potatoes, beans, and others [6]. For these crops, residues represent on average about 80 or 90% of the harvest, which, if not adequately handled, would become a source of environmental contamination [7, 8].

One of the important avenues in the fight against hunger is the use of lignocellulosic residues for the production of non-conventional protein, such as that of unicellular microorganisms [9-17].

The consumption of protein of microbial origin is very ancient and known on all continents. This comes, in the early 20th century, a biotechnological option for the use of agro-industrial crop residues [13, 18-23]. The most used microorganisms for these purposes belong to the families of microalgae, bacteria, yeasts and fungi. The consumption of protein from bacteria is limited, due to the problems of toxicity; protein from yeasts has a high content of ribonucleic acid (RNA), which limits its consumption by humans. Protein from microalgae has a certain toxicity in levels approaching 100 g daily in tests on humans[24].

The fungi kingdom is an attractive alternative due to its low production cost and natural nutritional advantages over other microorganisms [25]: good protein content (20 to 30% dry basis); it has all the essential aminoacids, high content of vitamin B and is low in fat. The most used filamentous fungi for this purpose belong to the genera: *Agaricus, Pleurotus, Lentinula, Auricularia, Volvariella y Flammulina*[26]. One of the most cultivated genera worldwide is*Pleurotus* spp. There are several scientific studies about the solid-state fermentation (SSF)by fungus *Pleurotusspp* of agro-industrial lignocellulosic residues. The following species excel at producing edible protein: *P. ostreatus, P. eryngii, P.sajor-caju, P. pulmonarius, P. sapidus, P. cornucopiae, P. djamor, P. citrinopileatus* [27].

Regarding the most used raw materials, in the majority of studies, they are agro-industrial residues [28-34]: wheat, rice, barley, and sorghum chaff, sawdust, sugar cane bagasse, coffee hulls, and, recently, pastures, such as elephant grass.

In Ecuador, the solid-state fermentation by *Pleurotus*of the following residues: cocoa, corn, quinua, African oil palm (rachis), wheat, barley, oats, vetches, páramo grasses, and flower (proteas) residues, among others, has been studied. The reported biological efficiency varies depending on the type of residue being studied, and ranges from 19 to 96.67% [34-38]. The majority of the studies used strain CP-184 of *Pleurotus ostreatus var. Florida*, from the culture collection of the Center of Industrial Biotechnology (Centro de Biotecnología Industrial, CEBI) at theUniversity of the Orient in Cuba. The use of strains imported from Mexico has also been reported [34]. For the first time in Ecuador, exploratory studies have been carried out of solid-state fermentation by *Pleurotus ostreatus* of flower (proteas) residues using native strain R24 of *Pleurotus*from the collection of the Ecuadorian Center of Mushroom Research (Centro Ecuatoriano de Investigación de Setas, CEIS). They report a 19% bioconversion with a protein content of 23.74% (DB) [39]. It is observed that the bioconversion is low. More in-depth studies of this variable need to be performed, particularly of the native strains as a function of the selected residue type.

The production of edible mushrooms arose historically as a craft with a high empirical component in rural setting and in a small scale. Even today, a good part of the production retains these features, especially the one destined for local consumption [40]. From the literature that was consulted about the production technologies of this Basidiomycota, it is apparent that it is fragmented, sparse, and, in some aspects, scarce.

The literature contains reports of different types of fermenters for the SSF process. In general, these devices perform static or dynamic fermentation [41]. Examples are the tray, static bed, tunnel, rotary disc, agitation tank, and continuous screw fermenters [41]. The literature reports five types of fermenters for the production of *Pleurotus* bySSF: mound-type fixed-bed, beds in trays or shelving, in bottles, in plastic bags and in perforated columns [20, 42, 43]. The mound process is the oldest and the least costly. The bed-in-shelving type of fermenter is much more efficient as far as the available space is concerned, given that it best uses the vertical dimension. Fermentation in bottles is very efficient regarding asepsis automation and control, but its startup and operational costs are high [44]. The culture of *Pleurotus* in bags and in filled-in perforated columns has emerged as an economical technology with good production results, occupying less space per unit of volume in the bioreactor chamber. The culture of fungi in different containers with specific features is often protected by patents [45-47].

The design of these processes requires the knowledge of certain factors associated with the growth needs of the microorganism: the quantity and type of nutrients, the influence of the water activity, temperature and ph, to name the ones reported the most [12, 31, 41, 48-52]. Problems such as the limited growth of the biomass, the overheating of the medium and low yields vis-à-vis the raw materials consumption and product synthesis, are some of the consequences deriving from designs based on a limited knowledge of the system. These deficiencies endanger the economic feasibility of the process, even when using inexpensive raw materials, as is the case with most of agro-industrial residues.

The scientific *problem* identified is centered around the design and efficient operation of the solid-state fermentation bioreactor by fungus *Pleurotus ostreatus* of lignocellulosic residues, such that it allows for the maximum conversion of the residue into protein-rich fungal biomass.

The *hypothesis* that is put forth is that if a type of bioreactor is designed with an adequate biocell distribution and packing density, a viable production process can be developed that is useful for small-scale local production.

The general *objective* that serves to evaluate the hypothesis of this paper is to design an experimental solid-state fermentation bioreactor using *Pleurotus ostreatus* with lignocellulosic residues by means of scaling and engineering techniques, allowing the speedy introduction of the technology for practical production, and, through the technology's simplicity, its implementation in small-scale local production.

## II. Materials And Methods

***Design of the experimental bioreactor***

One of the most traditional ways to produce protein from mushrooms is to place the inoculated substrate inside a container with the surrounding air suitable perforations to exchange oxygen and carbon dioxide, and to allow the expansion of the fruitbody that grows from the solid substrate. If such a container is placed in an environment where the key parameters for growth, such as air temperature, the solids' temperature, and relative humidity can be kept under control, then the fungi's growth will be optimal. Following this idea, the bioreactor has been conceived for the fungi's growth as a fermentation chamber where various perforated tubes are placed, containing the necessary raw material for the protein's production.

Figure 1 presents a simplified diagram of the bioreactor. Its design has been conceived such that it will allow fermentation to take place with the following characteristics: adiabatic operation, aerobic, semi-continuous flow. The fermentation system is made up of the following components:

- A fermentation chamber of the irregular-hexahedron type, with a hermetic front door for loading and unloading.Inside this chamber can be found the perforated cylindrical biocells, which contain the substrate inoculated with the fungus's mycelium.
- An air humidification system for the bioreactor's air inflow, supplied with a humidification tower, blower, mist spray and heating system.

The operating parameters are: relative humidity, and the temperatures associated with the solid phase and the gas phase.The operating parameters are controlled via automatic proportional–integral–derivative (PID) controllers, both for the temperatures and the relative humidity.

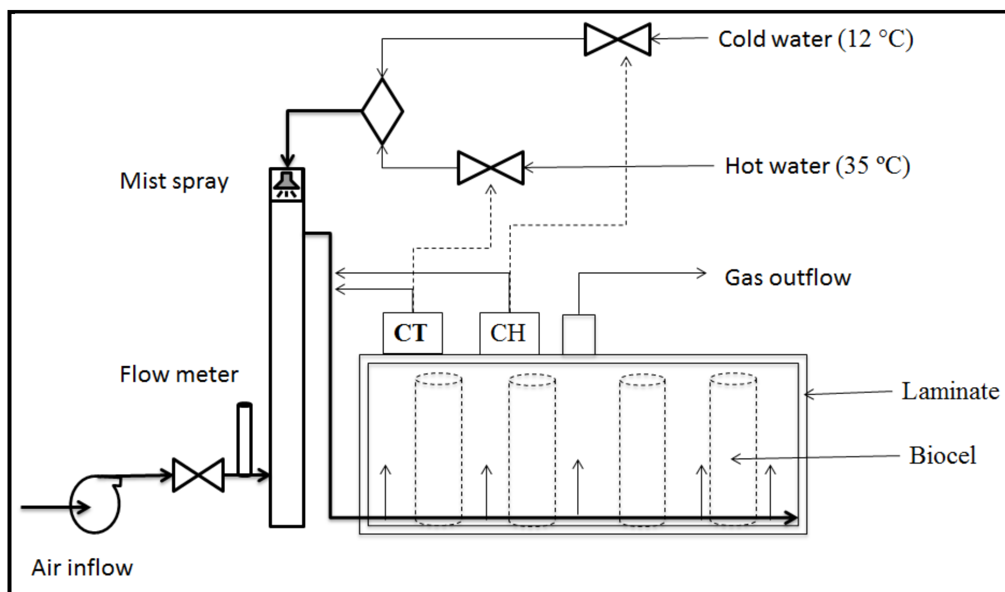


**Figure 1.** Simplified diagram of the experimental bioreactor for Pleurotus Ostreatus

***Construction of the experimental bioreactor***

The experimental bioreactor has the shape of an irregular hexahedron of 1.2x0.6x0.6 m of length, width and height, respectively. The bioreactor occupies a surface of 0,72 $m^2$ and an internal volume of 0.430 $m^3$. Built from a water-proof melamine laminate 1.5 cm thick, it has a sliding side door for loading and unloading. Figure 2 shows the experimental reactor built at the Ecuadorian Center for Biotechnology and the Environment (Centro Ecuatoriano de Biotecnología y Ambiente, CEBA).

The fermentation biocell was built from a polyvinyl-carbonate cylindrical tube with an internal diameter of 0.1016 m and a height of 0.6 m. 74 perforations with a diameter of 0.0127 m each were made to the biocell, distributed in a square pattern with a spacing of 0.0508 m.

The air humidification system wich feeds the biorreactor is made up of a centrifugal blower and a humidification column supplied with a micro spray-homogenizer.

The experimental bioreactor is supplied with an automatic temperature, humidity and air flow controller to allow for:

- Temperature, air flow, and humidity control at the bioreactor's intake.
- Control of the operating parameters inside the bioreactor.
- The measuring of the parameters and the taking of samples inside the bioreactor.
- The quantitative and qualitative evaluation of the final product.
- The study of the yield of the various inputs used in the process.
- The continuous quality control of the production operation.

As was stated before, the objective of this study is to develop processes for small-scale local production, whether individual and cooperative, in order to drive the popular and caring economy from the micro-enterprise sector, which is a priority of the present Ecuadorian government, captured in its National Plan for the Development of Good Living [53].

**Figure2.**Experimental biorreactor built at the CEBA for the SSF by Pleurotus

***Operation of the experimental bioreactor:*** The requirement is to determine the best geometric distribution of the biocells and the packing density inside the bioreactor through the study of the biomass's productivity, such that the use of the physical space inside the bioreactor can be maximized.
***Strain and inoculum:*** the inoculum that was used was the mycelium of strain ceba-gliie-po-010106 of fungus *Pleurotus ostreatus*of Ecuadorian origin, from the CEBA's pure culture bank.
***Culture medium*:** the culture medium that was used was the one recommended by Pineda (2014), based on residues from the bean of genus *Phaseolus vulgaris*, harvested in Ecuador, originating from the Afro-Ecuadorian community "La Concepción", Carchi province. The residue was enriched with 0.13% DB ammonium sulfate, 0.036% DB magnesium sulphate, and 25% DB depleted substrate, with a moisture content of 70.7% [54].
***Experimental unit*:** The experimental unit was a cylindrical biocell with a diameter of 10.16 cm, made of PVC, with a capacity of 4 kg of wet substrate.
***Factors studied*:** two levels were selected for the packing density (PD), 21.7 and 32.6 kgSDM/$m^3$.For the biocell distribution (BCD) inside the biorreactor, square, triangular and staggered-rows layouts were used. The biocells were placed in such a way that each one occupied between 0.06 and 0.09 $m^2$. This would guarantee living space for the development of the fungus's fruitbody. This parameter was not known up until now.
***Outcome variables*:** the outcome variables were the bioconversion (BC) and the specific biomass productivity (SBP).
***Operating parameters*:** the operating parameters were the medium's humidity, set to 70.68%, the relative humidity, set to 85%, the room temperature, obtained from a growth kinetics study [55], set to 20°C, and the fermentation time, set to 20 days.
***Experimental design and statistical treatment*:** the experimental analysis and design was done using the statistical package *STATGRAPHICS Centurión XV*, version 15.2.05. Although there is a quantitative factor, a multi-factor categorical design was selected. The experiment was carried out with three replicates, for a total of 18 experimental runs. The experimental design will estimate the effects of the numerical factor, packingdensity, and those of the categorical factor, biocell distribution inside the bioreactor.
***Experimental procedure*:** the bean residues were ground to a size of 9 mm. The nutrient salts were dissolved in distilled water and were mixed with the solids to reach a 70.68% humidity. The substrate was pasteuried following the technique described by Stames (2003) in a 200-liter capacity steel tank, operating at 100°C and 101.3 kPa for two hours[44]. The cold substrate was manually mixed with the inoculum in a proportion of 10%.

The biocells were filled with four kg of inoculated substrate, and the bioreactor was loaded with the biocells. The bioreactor operated for 20 days. The biomass was then separated using a stainless-steel knife.

## III. Results And Discussion

Table 1 presents the results matrix obtained for variables PD and SBP for each treatment carried out.Figure 3 shows the new bioreactor in operation.

**Tabla 1.**Experimental results matrix for packing density and biocell distribution

| Nº | PD kg SDM/m$^3$ | BCD | BC g FDM/kg SDM | PEB g MSH/(kg SDM.día) |
|---|---|---|---|---|
| 1 | 32.6 | Triangular | 55.71 | 2.79 |
| 2 | 21.7 | Triangular | 89.53 | 4.48 |
| 3 | 21.7 | Triangular | 90.72 | 4.54 |
| 4 | 32.6 | Staggered | 50.93 | 2.55 |
| 5 | 21.7 | Square | 99.32 | 4.97 |
| 6 | 21.7 | Square | 100.27 | 5.01 |
| 7 | 21.7 | Staggered | 83.56 | 4.18 |
| 8 | 32.6 | Triangular | 54.12 | 2.71 |
| 9 | 32.6 | Triangular | 53.32 | 2.67 |
| 10 | 21.7 | Square | 101.47 | 5.07 |
| 11 | 21.7 | Triangular | 95.50 | 4.77 |
| 12 | 32.6 | Square | 60.48 | 3.02 |
| 13 | 32.6 | Staggered | 51.73 | 2.59 |
| 14 | 32.6 | Staggered | 47.75 | 2.39 |
| 15 | 21.7 | Staggered | 71.62 | 3.58 |
| 16 | 21.7 | Staggered | 79.98 | 4.00 |
| 17 | 32.6 | Square | 55.71 | 2.79 |
| 18 | 32.6 | Square | 57.30 | 2.86 |

FDM: fungal dry matter, SDM: substrate dry matter

**Figura 3.**New bioreactor for the conversion of lignocellulosic residues into a protein-rich biomass

***Analysis of variance for the bioconversion***

Tabla 2,present the analysis of variance for the bioconversion variable. It is observed that both the packing density and the biocell distribution inside the reactor exert a significant effect, in the range studied for these factors, with a 95% confidence level.

**Table 2.**Analysis of variance for the bioconversion (BC)

| Source | Sums of squares | DF | Mean squares | F-Ratio | P-Value |
|---|---|---|---|---|---|
| Main effects | | | | | |
| A:PD | 5902.41 | 1 | 5902.41 | 584.86 | 0.0000 |
| B:BCD | 668.924 | 2 | 334.462 | 33.14 | 0.0000 |
| Interactions | | | | | |
| AB | 157.94 | 2 | 78.97 | 7.83 | 0.0067 |
| Residual var. | 121.104 | 12 | 10.092 | | |
| Total (corrected) | 6850.38 | 17 | | | |

Table3presents a multiple range test for bioconversion with respect to packing density. A significant difference is observed, with the one with the highest mean, a packing density of 21.7 kg SDM/m$^3$, being the best one. With a bioconversion of 90,29 g FDM/kg SDM, this result makes sense from the standpoint that the *Pleurotus*could have a living space, as is the case with plants[56].

**Tabla 3.**Multiple range tests for BC by packing density (95.0%)

| Packing density | Cases | Mean LS | Sigma LS | Homogeneous groups |
|---|---|---|---|---|
| 32.6 | 9 | 54.0789 | 1.05893 | X |
| 21.7 | 9 | 90.2956 | 1.05893 | X |
| | | | | |
| **Contrast** | **Sig.** | **Difference** | **+/- Limits** | |
| 21.7 – 32.6 | * | 36.2167 | 3.2629 | |

* indicates a significant difference

Table4 presents the multiple range test for bioconversion with respect to biocell distribution. A significant difference is observed among the three types of distribution.The best one, the one with the highest mean, is the square distribution, followed by the triangular distribution. This result cannot be explained with the avilable information, and must be investigated further in the future.

**Tabla 4.**Multiple range tests for BCbydistribution (95.0%)

| Distribution | Cases | Mean LS | Sigma LS | Homogeneous groups |
|---|---|---|---|---|
| Staggered | 6 | 64.2783 | 1.29692 | X |
| Triangular | 6 | 73.17 | 1.29692 | X |
| Square | 6 | 79.1133 | 1.29692 | X |
| | | | | |
| **Contrast** | **Sig.** | **Difference** | **+/- Limits** | |
| Square - Staggered | * | 14.835 | 3.99622 | |
| Square - Triangular | * | 5.94333 | 3.99622 | |
| Staggered - Triangular | * | -8.89167 | 3.99622 | |

* indicates a significant difference

***Analysis of variance for the biomass productivity (SBP)***

The results coincide with those obtained for the bioconversion variable. Both the packing density and the biocell distribution exert a significant effect with a 95.0% confidence level. The multiple range test for productivity with 95% confidence, both for packing density and for biocell distribution, reveals that the best packing density is 21.7 kg SDM/$m^3$, that and the best distribution is the square one, achieving a productivity of 4,51 g FDM/(kg SDM.day).

## IV. Conclusions

A new type of heterogeneous bioreactor was designed, built, and put into operation for the bioconversion of bean residues into a protein-rich biomass by the native strain of *Pleurotus ostreatus* isolated from the Ecuadorian fungal biodiversity. This new bioreactor exceeds the productivity reported in the literature for the production of mushrooms.Furthermore, it was determined that when the bioreactor is operated with a packing density of 21.7 kg SDM/m3 and a square distribution of biocells, the highest bioconversion is obtained, equal to 101.47 g FDM/kg SDM, and a productivityof 5.07 g FDM/(kg SDM.day) is reached.The new bioreactor design represents a novelty in the category of utility model for the technological development of solid-state fermentation (SSF) of lignocellulosic residues, and their conversion into protein-rich fungal biomass. The device may be used for scientific research and for small-scale local production. The industrial harnessing of the native fungal strain of *Pleurotus ostreatus* (ceba-gliie-po) isolated from Ecuadorian biodiversity constitutes a contribution to Ecuador's biotechnological development.The bioconversion process of lignocellulosic residues in fungal protein for human consumption, based on the operation of the new SSF bioreactor, represents an efficient alternative in the fight against food insecurity, and contributes to the change of the country's production matrix.

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