

Decomposition of Sugarcane Bagasse by Edible Mushrooms Estimated by Microbial Respiration

João Manoel da Silva^{1*}, Micheline Thais dos Santos¹, Jéssica Raimundo da Rocha², Tania Marta Carvalho dos Santos¹, Yamina Coentro Montaldo¹ and Raíza Rocha Oliveira Teixeira¹

¹Laboratório de Microbiologia Agrícola, Universidade Federal de Alagoas, Brazil.

²Departamento de Química e Biotecnologia, Universidade Federal de Alagoas, Brazil.

*Corresponding author email id: jm.agro@hotmail.com

Abstract – The aim of this study was to investigate sugarcane bagasse decomposition through the technique of microbial respiration measurement. The following treatments were used: T1 (autoclaved soil + sugarcane bagasse), T2 (autoclaved soil + 2% sugarcane bagasse + *Pleurotus ostreatus*), T3 (autoclaved soil + 2% Sugar cane + *Lentinula edodes*), T4 (sugarcane bagasse + *P. ostreatus*) and T5 (sugarcane bagasse + *L. edodes*). Vigorous mycelial growth was observed on all substrates. The treatment T2 (soil autoclaved + 2% of sugarcane bagasse + *P. ostreatus*), presented the highest respiratory rates differing significantly from all others in all the collections, the values varied from 3.74 to 7.37 with an average of 5.5. The T3 treatments (soil autoclaved + 2% sugarcane bagasse + *L. edodes*), and T4 (sugar cane bagasse + *P. ostreatus*) did not differ significantly between them, but were higher than the control with Averages of 3.30 and 3.50 respectively. The other treatments did not differ significantly from the control.

Keywords – Agroindustrial Residue, CO₂ Evolution, *Lentinula Edodes*, *Pleurotus Ostreatus*.

I. INTRODUCTION

Edible mushrooms are high-quality protein sources, which can be produced with greater biological efficiency than animal protein and therefore may be of great importance in developing countries for the enrichment of diet populations with protein deficiencies [1].

Mushrooms of the genus *Pleurotus ostreatus* (shimeji) represent a low-cost food, which contains high protein, essential amino acids, high proportion of unsaturated fatty acids, various vitamins and minerals, and low levels of fats, nucleic acids and calories. *Lentinula edodes* (shiitake) is an edible mushroom of global importance due to good economic returns, possibility of being cultivated in small areas and requiring low initial investment. Besides the ability to convert lignocellulosic materials into food of appreciated taste and texture, it also has medicinal and nutritional properties beneficial to human health, which puts it in front of other cultivated mushrooms.

The cultivation of edible mushrooms has become, increasingly, an important practice in modern society. This is due to the biotechnological process of bioconversion of residues by the action of these fungi, for the production of foods of high nutritional value from agroindustrial residues, making possible the more efficient use of the materials, besides reducing the volume of residues or accelerating the decomposition process [2]. In addition to the important bioconversion role of the residue in food, the residual substrate resulting from the cultivation of edible

mushrooms can still be used as animal fodder, soil conditioner or natural fertilizer or as feed, closing the cycle of raw material utilization [3].

Microbial soil respiration represents the oldest and most widely used parameter for quantifying metabolic activity in soils [4], and vegetable and agro-industrial waste has therefore been applied to soils, especially those with a tropical climate, characterized by rapid microbial decomposition of their organic constituents [5].

The release of carbon dioxide or soil respiration is directly related to the decomposition of organic matter. The rate of decomposition of the organic material and the consequent release of CO₂ is mainly determined by the intrinsic characteristics of the organic matter itself, such as: C/N ratio; Carbohydrate contents, lignin; Degree of aggregation of soil particles and soil type, which model the structure of the microbial community.

However, a large amount of agroindustrial residues are deposited daily in the soils, being these sources of secondary energies for several decomposing organisms, among them the mushrooms, that besides participating in the cycling of nutrients in the soil through the decomposition of organic matter, are able to provide biological quality to the soil. Thus, agroindustrial wastes are highly applicable to the production of edible mushrooms, thus reusing waste and environmental conservation through the recycling of this organic material.

The objective of this study was to evaluate the decomposition of sugarcane bagasse by *P. ostreatus* and *L. edodes*, estimated by microbial respiration as a function of two substrates.

II. MATERIALS AND METHODS

A. Description of the Experimental Area

The experiments were conducted at the Center of Agricultural Sciences of the Federal University of Alagoas in the city of Rio Largo-AL located at 9° and 29'45" south latitude, 35° and 49'54" longitude and 165 m altitude. The classification of Köppen includes the climatic type As', is tropical humid coastal, with sun in the months of September to May, from spring to summer, with temperatures varying from 19 °C to 32 °C, with rain and thunderstorms from June to August, from autumn to winter, with temperatures varying around 15 °C to 26 °C. Relative humidity is 79.2% and rainfall is 1.410 mm/year.

The following treatments were made: Sterilized treatments were autoclaved for 1 hour at 121 °C in two

cycles. Each substrate was moistened with 70% water retention capacity, and 100 cm³ of the sample was packed in a 1000mL volume hermetically sealed vessel, where a small, wide-mouthed vessel containing 10mL of NaOH (2N) was also placed as shown in Fig. 1.



Fig. 1. Experimental unit for microbial respiration mediation containing a small wide mouth container containing 10mL of NaOH (2N).

B. Testing Treatments

The following treatments were used: T1 (autoclaved soil + sugarcane bagasse), T2 (autoclaved soil + 2% sugarcane bagasse + *P. ostreatus*), T3 (autoclaved soil + 2% sugarcane bagasse + *L. edodes*), T4 (sugarcane bagasse + *P. ostreatus*) and T5 (sugarcane bagasse + *L. edodes*).

The edible mushrooms used in the experiments belong to the collection of microorganisms of the Laboratory of Agricultural Microbiology - Federal University of Alagoas, conserved from the primary matrix. Previously, the strains cultured in rice were inoculated in potato dextrose agar (PDA) culture medium at room temperature ($\pm 28^\circ\text{C}$) for 15 days. After this time, the above-mentioned substrates were packed into the sterile containers as described and the mushrooms were inoculated. Five discs containing vigorous mycelia were inoculated onto their respective substrates and the containers were sealed.

At approximately 7 day intervals, the containers were opened and the stock solutions were removed, adding 2.5mL BaCl₂ and three to five drops of the acid / base phenolphthalein 0.1% indicator. After titration the beakers were replaced with a new NaOH solution. The amount of CO₂ released was considered after titration with 0.1N HCl. The calculation of respiration was done using the titration method with CO₂ capture by NaOH by the following formula:

$$\text{meq C-CO}_2 \cdot 100 \cdot \text{cm}^3 = (\text{BR} - \text{AM}) \times \text{M} \times \text{F} \times \frac{\text{V}_1}{\text{V}_2},$$

wherein;

BR = white titration

AM = sample titration

M = HCl molarity

F = correction factor of HCl

V₁ = volume of NaOH used to capture CO₂ (mL)

V₂ = vapor of the NaOH used in the titration (mL)

C. Experimental Design and Statistical Analysis

The experimental design was completely randomized, with 5 treatments and 5 replications, totaling 25 plots in a

factorial scheme, with time, mushroom species and substrate factors. Data were submitted to analysis of variance ($t = p \leq 0.05$) and the means were compared by Student's t test (Bonferonni) ($p \leq 0.05$). The statistical software SISVAR 5.3 was used [6].

III. RESULTS

By means of the analysis of variance (ANOVA) by the Bonferonni T test, was possible to detect that there is no dependence between the species of edible mushrooms studied, where they did not present statistical difference ($t = p \leq 0.05$). However, there was difference in the evaluation of the studied substrates (Table 1).

Table 1. CO₂ release in function of substrate and mushrooms strains.

Variables	meq CO ₂ m ³ *
Substrates	
Autoclaved soil and sugarcane bagasse	3.23 a
Sugarcane bagasse	3.72 b
Mushroom species	
<i>P. ostreatus</i>	3.35 a
<i>L. edodes</i>	3.40 a
Without mushroom	3.74 a

*Averages followed by the same letter do not differ statistically by Bonferonni test ($t = p \leq 0.05$).

Thus, it was possible to observe that the studied variables, individually, do not present statistically significant effects, being therefore dependent on each other, mainly as a function of the collection time, being this one of continuous growth gradient (Fig 2), where the regression analysis was able to obtain significance for this last variable ($R^2 = 99.03\%$).

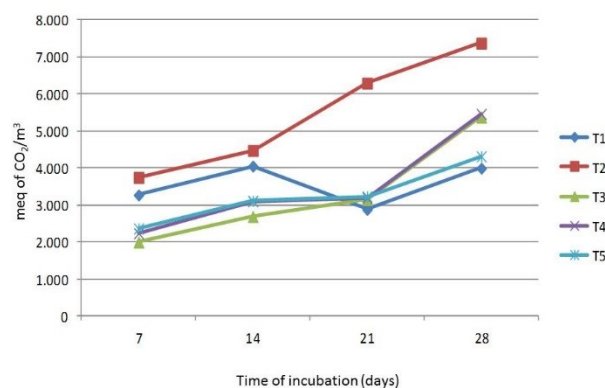


Fig. 2. Evolution of C-CO₂ in function of the times of incubation. T1 (autoclaved soil + sugarcane bagasse), T2 (autoclaved soil + 2% sugarcane bagasse + *P. ostreatus*), T3 (autoclaved soil + 2% sugarcane bagasse + *L. edodes*), T4 (sugarcane bagasse + *P. ostreatus*) and T5 (sugarcane bagasse + *L. edodes*). ($R^2 = 99.03\%$).

In general, it was detected that the substrate inoculated with *P. ostreatus* strain gave the highest levels of C-CO₂ release. Since the collection time was able to make a

difference in the increase in CO₂ release rate, it is also elucidated in this study that this release of CO₂ is highly correlated with the substrate, which makes these species, especially *P. Ostreatus*, are perfect candidates for use in the reuse of agro-industrial waste in the production of human food proteins.

IV. DISCUSSION

Measurement of respiration is a way of estimating the activity of soil microorganisms and an indication of the rate of decomposition of organic matter or of materials added to it. Several factors are active in respiration, presence of microbial growth inhibitory substances, and the chemical composition of the substrate have been considered responsible for the reduction in microbial activity [7].

Therefore, the use of substrates rich in lignin and cellulose as the sugarcane bagasse favors the release of C-CO₂, since the edible mushrooms studied here are recognized as microorganisms with high enzymatic capacity for degradation of these compounds. These processes are essential in their development and the knowledge of their efficiency of degradation of lignocellulosic compounds are able to provide a way to reuse agroindustrial residues that would be discarded and thus be used as substrates to increase the production of edible mushrooms in regions of sugar and alcohol production.

CO₂ loss occurs as a consequence of fungus metabolism, consumption of components of polysaccharide components and lignin. The treatments with the highest rates of mineralization occur more intensely and their nutrients are released available quickly.

The increase in the microbial respiration rate of the *L. edodes* and *P. ostreatus* strains from this study in the last collection can be considered due to the already high metabolic activity, causing the lignin and cellulose molecules to be degraded almost completely, so the mushrooms start to use other food sources with less complex molecules and of greater ease of enzymatic breakdown and degradation, causing their release of C-CO₂ to be more intense with the passage of time. In addition, it was also noted the high respiratory activity of the T2 treatment, which was composed of the *P. ostreatus* strain, so it is possible to affirm that this species has higher growth efficiency and metabolic activity in the presence of not only sugarcane bagasse but also of the soil, since these micro-organisms are found naturally in this habitat, with a simulation of their natural development environment.

Basal respiration is an indicator of the organic carbon available not only to heterotrophic microorganisms. The higher the amount of CO₂ released per unit weight of soil, the greater the amount of substrate assimilable for the development of microbial biomass [8]. Thus, the greater mycelial mass conferred to *P. ostreatus* strain due to its greater capacity of assimilation of the substrate, providing greater CO₂ release.

In addition, these fungi are capable of producing extracellular proteins produced for health instruments for

testing alkaline pretreated lignocellulosic substrates. Relevant proteins span multiple families of glycoside hydrolases, including endoglucanases GH5 and GH45, GH3-β-glucosidases and GH10-xylanases [9].

In addition, there are no studies that relate the degradation of substrates through the decomposition performed by edible mushrooms, estimated by basal respiration, and microbial respiration of the species. A lignocellulosic capacity of edible mushrooms is highly related to specific related genes as well as the evolutionary pattern of the species [10]. In addition, other factors such as carbon source, temperature, humidity and other mechanisms strongly influence fungal biomass [11].

V. CONCLUSIONS

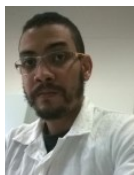
The incubation of substrates with the addition of *P. ostreatus* strain were able to produce a larger volume of mycelial mass and to provide higher rates of CO₂ release, indicating that this edible mushroom species is more efficient than *L. edodes* due to the mineralization of Sugar cane bagasse.

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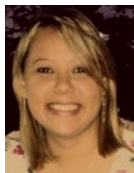
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AUTHORS' PROFILES



João M. da Silva

Agronomist, MSc. In Agriculture and Biodiversity, Acting in the line of research, management and recovery of degraded environments, with emphasis on soil microbiology and phytopathology.



Micheline T. dos Santos

Zootechnist, Master in Animal Production, with emphasis on quality and microbiological characterization of foods.



Jéssica R. da Rocha

Bachelor in Chemistry, MSc in Chemistry and Biotechnology, with emphasis on organic chemistry and metabolomic characterization of fungi.



Tania M. C. dos Santos

PhD in Applied Microbiology, with emphasis on prospecting of functional microorganisms with application to agriculture, as well as endophytic microorganisms, rhizospheric microorganisms, edible mushrooms and food microbiology.



Yamina Coentro Montaldo - Dra. Biotecnologia Agropecuária, acting in the research line prospecting of soil microorganisms with applicability in agriculture.



Raíza Rocha Oliveira Teixeira

Agronomic Engineer, MSc. In Plant Protection. Researcher in the areas of microbiology and entomology.