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Relationship Between Coffee Husk Caffeine Degradation and Respiration of *Aspergillus* sp. LPBx in Solid-State Fermentation

Débora Brand,[1] Ashok Pandey,[2] Jose A. Rodriguez-Leon,[3] Sevastianos Roussos,[4] Ivo Brand,[1] and Carlos R. Soccol*,[1]

[1]Laboratório de Processos Biotecnológicos, Departamento Engenharia Quimica, Universidade Federal do Paraná (UFPR), CEP 81531-970 Curitiba-PR, Brazil, E-mail: soccol@engquim.ufpr.br; [2]Biotechnology Division, Regional Research Laboratory, CSIR, Trivandrum-695 019, India; [3]Instituto Cubano de Investigacion de los Derivados de la Cana de Azucar (ICIDCA), PO Box 4026, La Habana, Cuba; and [4]Laboratoire de Microbiologie-IRD, Université de Provence, 132288 Marseille, France

Abstract

Studies were carried out in a packed-bed column fermentor using coffee husk as substrate in order to verify a relationship between caffeine degradation and the respiration of *Aspergillus* sp. LPBx. Fermentation conditions were optimized by using factorial design experiments. The kinetic study showed that the caffeine degradation was related to the development of mold and its respiration and also with the consumption of reducing sugars present in coffee husk. From the values obtained experimentally for oxygen uptake rate and CO_2 evolved, we determined a biomass yield of 3.811 g of biomass/g of consumed O_2 and a maintenance coefficient of 0.0031 g of consumed O_2/(g of biomass·h). The maximum caffeine degradation achieved was 90%.

Index Entries: Coffee husk; factorial design; surface response methodology; solid-state fermentation; column fermentor.

Introduction

Brazil is the largest producer of coffee in the world, contributing approx 32% of the world's production. During 2000, its production reached

*Author to whom all correspondence and reprint requests should be addressed.

2,000,000 t. In the preparation of coffee as a drink, only 5.8% of the fruit is used, and the remaining 94.2% is constituted by water and byproducts of the process, which are not well explored and constitute a considerable source of environmental pollution *(1)*. Coffee husk is the most abundant byproduct originated by the "dry processing" of the coffee cherries in Brazil *(2)*. Coffee husk could be beneficially utilized as animal feed provided that antiphysiologic factors such as caffeine and tannins could be partially or entirely removed by biologic detoxification using filamentous fungi. Caffeine is a powerful stimulant and can be harmful to animals owing to its high nitrogen concentration and its diuretic effect. Several studies have been conducted to evaluate coffee husk from an animal nutrition point of view. Low feed intake, protein digestibility, and nitrogen retention are major factors limiting its use *(3,4)*. Consequently, most of the husk remains unutilized or poorly utilized. If the toxic constituents could be partially removed at least, it would open new avenues for its utilization.

Nowadays, it is biotechnology that provides several perspectives for the use of coffee byproducts in a profitable way, allowing final products of high commercial value (enzymes or secondary metabolites) to be obtained. Several attempts have been made to use coffee husk as substrate for the production of edible mushrooms *(5)*, aromatic compounds *(6,7)*, enzymes *(8)*, and gibberellic acid *(9)*.

Strains of *Penicillium* and *Aspergillus* isolated from coffee pulp *(10)*, the byproduct originating from the "wet process" of coffee cherries, were tested in liquid medium in order to verify caffeine degradation. These strains were able to degrade caffeine in a concentration of 20 g/L, by utilizing it as the nitrogen source. Caffeine degradation is mainly a function of pH and saccharose concentration *(11)*.

The use of solid-state fermentation is increasing in the utilization of agroindustrial byproducts *(12–14)*. Solid-state fermentation offers potential advantages in the treatment and value addition of such residues. Microbial growth has been generally estimated using indirect methods such as glucosamine estimation and evolution of CO_2 and O_2 consumption by the microorganism *(15)*.

The objective of the present work was to evaluate the relationship between caffeine degradation present in coffee husk and the respiration of *Aspergillus* sp. LPBx in solid-state fermentation. Solid-state fermentation was employed using a column fermentor that was coupled with a gas chromatography system to evaluate the release of CO_2 and the O_2 production.

Materials and Methods

Microorganism and Media

A strain of *Aspergillus* sp. LPBx was maintained on coffee husk extract agar (CHA) medium, which was prepared by cooking coffee husk in distilled water *(16)*.

Table 1
Experimental Design for Optimization of Physical Parameters on Coffee Husk Caffeine Degradation by *Aspergillus* sp. LPBx in Column Fermentor

Parameter	Low level	Central point	High level
Factorial design 1			
Moisture (%)	55	60	65
Inoculum rate (spores/substrate)	10^5	10^6	10^7
Aeration (mL/min)	20	40	60
Factorial design 2			
Moisture (%)	50	55	60
Aeration (mL/min)	10	20	30

Coffee Husk

Coffee husk was obtained from Café Damasco, Curitiba. It was dried in an air oven at 55°C for 48 h, milled manually, and sieved to obtain fractions between 0.8- and 2.0-mm particle size.

Preparation of Inoculum

Spores of *Aspergillus* growing on CHA medium in 250-mL Erlenmeyer flasks (10 d old) were harvested by homogenization with distilled water (50 mL with five drops of Tween-80) and glass beads. The spores were counted in a Neubauer cell.

Solid-State Fermentation

Solid-state fermentation was carried out in glass columns as described by Raimbault and Alazard *(17)*. Each column had the capacity for 40 g of the substrate (dry wt basis). Substrate preparation was similar as described previously *(16)*. Preinoculated substrate was packed in the columns. Humidified air passed through the columns at a desired flow rate. Solid-state fermentation was carried out at 28°C for a desired length of time. Experiments were conducted in order to find the best conditions of inoculum size, initial moisture content of the substrate, and aeration flow in caffeine degradation (Table 1). All the experiments were conducted with two replicates.

Kinetic Studies of Coffee Husk Caffeine Degradation

Experiments were conducted at the optimized conditions of pH 4.5, aeration rate of 30 mL/min, inoculum rate of 1.75×10^7 spores/g of substrate, and initial moisture content of 55%. The experiment was run with two replicates. Every 15 h, samples were collected for evaluation of caffeine degradation as well as pH, reducing sugars, and protein with a total fermentation time of 105 h.

Estimation of Growth

In the kinetic study, the respiratory metabolism of the microorganism was evaluated by determining the O_2 consumption and the production of CO_2. This was utilized to estimate the biomass biosynthesis by the fungal culture. The exit gases from the fermentor were passed through silica gel columns to dry and then analyzed by gas chromatography to determine the oxygen uptake rate (OUR), the CO_2 evolved, and the respiration quotient (RQ) during the process following the mathematical model developed by Rodríguez-León *(15)*.

Analytical Methods

Caffeine assays were carried out following the methodology described by Instituto Adolpho Lutz *(18)* and proteins by the Stutzer method *(19)*. Total and reducing sugars were analyzed by the Somogyi method *(20)*.

Results and Discussion

Factorial Design 1

The fractionated factorial design 2^{3-1} consisted of aeration, moisture, and inoculumn size as variables, which were distributed in two levels with one central point. The response measured was caffeine degradation and the results were submitted to Pareto analysis (Fig. 1). The factors that affected caffeine degradation in the column fermentor at the 5% level were the initial moisture content and aeration rate. The inoculum size was not a significant factor in caffeine degradation present in coffee husk.

Factorial Design 2

Since the variables aeration and initial moisture content were significant factors at the 5% level in the first experiment, it was necessary to implement another factorial design with the aim of reaching the best conditions on the degradation of caffeine. A complete factorial design 3^{2-0} was employed in which the experimental variables were distributed in three levels, and the analysis was based on surface response. From the statistical analysis of the data obtained for caffeine degradation in the column fermentor, it was concluded that moisture and aeration rate were critical factors in the fermentation.

The surface response obtained in this experiment is represented in Fig. 2. The maximal caffeine degradation (88%) occurred with an aeration flow of 30 mL/min and initial moisture content of the substrate of 55%. In Fig. 2 the region where caffeine was metabolized in greater quantity can be observed. It comprised 54–60% of the initial moisture content. The final moisture content (data not shown) increased proportionally to the initial moisture content employed for each experiment, probably because of microbial respiration. With rates lower than 50%, the fungal culture was not able to metabolize caffeine efficiently, which could be the

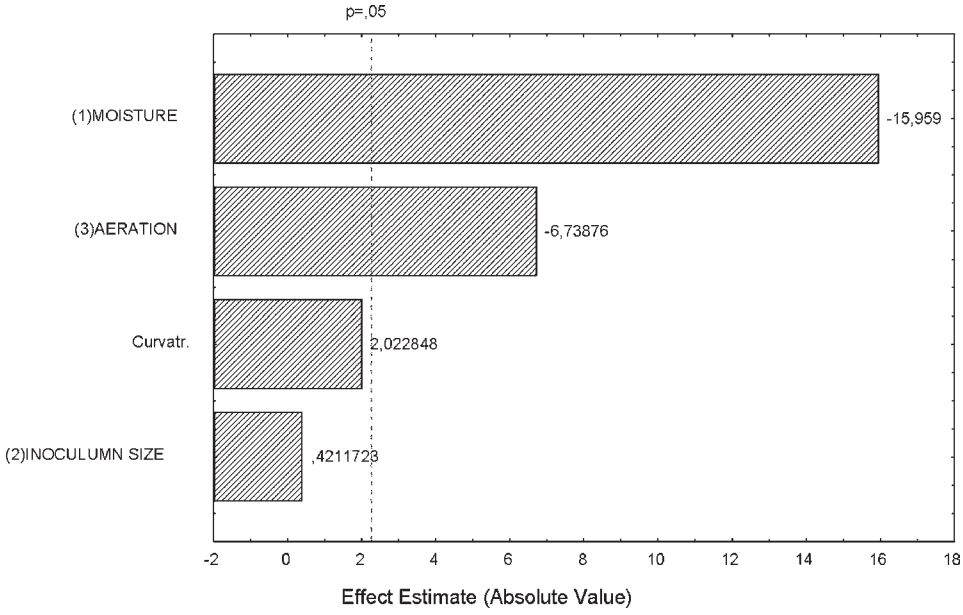

Fig. 1. Pareto chart of effects for 2^{3-1} experimental design for coffee husk caffeine degradation by *Aspergillus* sp. LPBx in solid-state fermentation.

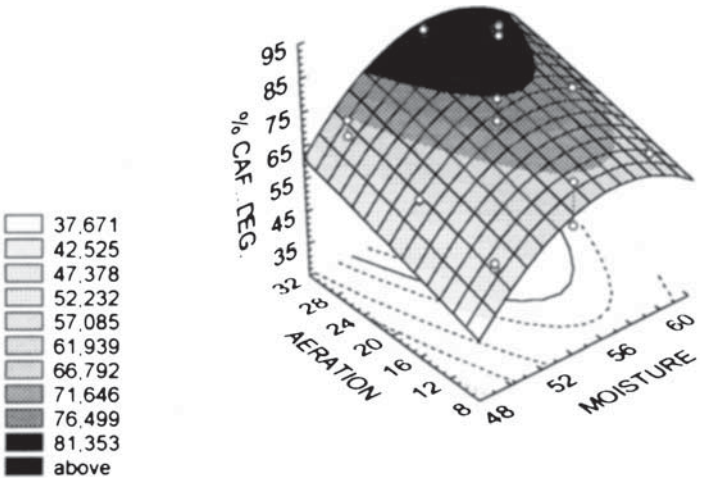

Fig. 2. Response surface of coffee husk caffeine degradation as function of aeration rate and initial moisture content.

result of the nutrients and enzyme transfer being affected owing to lack of sufficient moisture in the substrate to solubilize them. Increase in moisture content results in decreased porosity and low oxygen diffusion, leading to a superficial mycelium formation, a reduction in gas volume and its exchanges, and still the risk of bacterial contamination.

A low aeration rate (10 mL/min) seemed to be inefficient for the fungal culture to degrade the caffeine. Probably, it was not enough to reduce uniformly the temperature of the substrate that was increased as a function of the metabolic activity of the culture. Aeration rates higher than 30 mL/min could slightly enhance the degradation, but observing the fitted surface obtained, it could be noticed that this parameter was in a limiting zone and was best achieved between 30–40 mL/min. Aeration rates higher than 40 mL/min showed that an atmosphere rich in O_2 negatively influenced degradation. Higher rates of aeration also facilitate sporulation, which is not desirable in the process.

Respiratory Metabolism Analysis

The kinetics of solid-state fermentation was determined by measuring the OUR, the CO_2 evolved, and the RQ during the process. A balance was made for the estimation of the OUR and the CO_2 evolved in terms of volumetric flow (L/h), considering an initial weight of 40 g of dry matter. The exhausted airflow (F_{out}) was 0.045 L/(h·g) of initial dried weight and the inlet airflow (F_{in}) to the fermentor. The following equations were considered:

$$V_{O_2out} = (\%O_{2out}/100)F_{out}$$

$$V_{CO_2out} = (\%CO_{2out}/100)F_{out}$$

$$V_{N_2out} = [(100 - \%O_{2out} - \%CO_{2out})/100]F_{out}$$

From the balance of O_2 and N_2 we obtained:

$$V_{O_2uptake} = (20.9/100)F_{in} - (\%O_{2S}/100)F_{out}$$

$$V_{N_2in} = V_{N_2out}$$

Relating the several equations considered, the following relationship for the inlet and the outlet airflow was obtained:

$$F_{in} = [(100 - \%O_2 - \%CO_2)F_{out}]/79.1$$

For the estimation of OUR and CO_2 evolved in mass flow units (mmol/h), it was considered that the air was an ideal gas at the respective volumetric flows ($V_{O_2uptake}$ and V_{CO_2out}) and the proper corrections for temperature conditions considering a temperature value of 28°C.

Figure 3 shows the evolution of the OUR and CO_2 evolved during solid-state fermentation of coffee husk by *Aspergillus* sp. LPBx. The process showed characteristics of an aerobic system, until approx 80 h of fermentation, with a medium respirometric coefficient (RQ) of 1.05 for this time interval. After 80 h of fermentation, the RQ started to increase owing to a considerable raise in CO_2 production in relation to oxygen consumption. This increase could be related to an improper airflow distribution owing to canalization of air after complete development of mycelium.

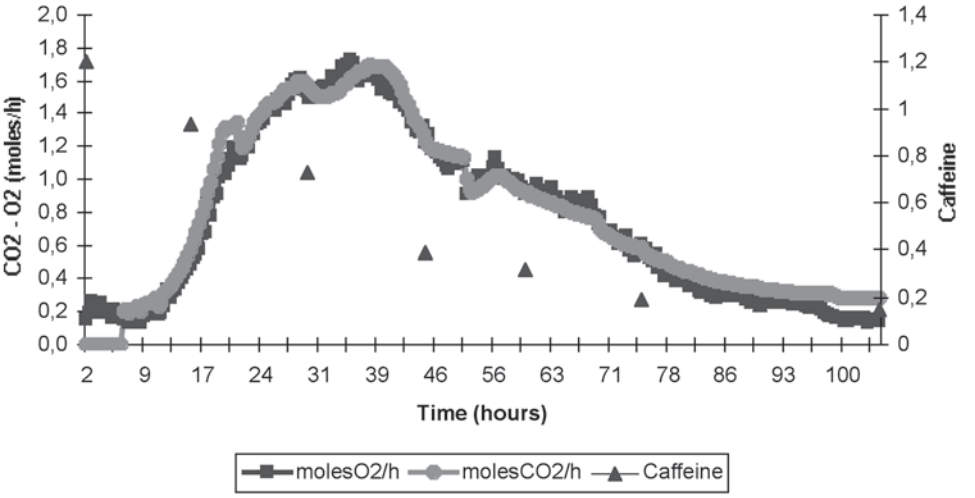

Fig. 3. Kinetic pattern of the OUR and CO_2 evolved during coffee husk caffeine degradation by *Aspergillus* sp. LPBx.

Based on the results of the OUR and CO_2 evolved, the bioprocess parameters were estimated. Considering the balance of OUR, the following equation was obtained:

$$X_n = \left(Y_{xo}\Delta t \left\{ \frac{1}{2}\left[\left(\frac{dO_2}{dt} \right) t = 0 + \left(\frac{dO_2}{dt} \right) t = n \right] + \sum_{t=1}^{t=n-1} \left(\frac{dO_2}{dt} \right) t = 1 \right\} + \left(1 - \frac{a}{2} \right) X_o - a \sum_{i=1}^{i=n-1} X_i \right) / \left(1 + \frac{a}{2} \right)$$

in which $a = m\ (Y_{x/o})\Delta t$.

The procedure to estimate biomass content in a certain time (X_n) consisted of making a trial-and-error estimation, assuming values for the biomass yield based on oxygen consumption ($Y_{x/o}$) and maintenance coefficient (m) using FERSOL software *(15)*. Biomass values were analytically determined at 0, 15, 30, 45, 60, 75, 90, and 105 h of fermentation. By successive approach, the software allowed determination of the equation coefficients. From the values obtained experimentally for OUR and CO_2 evolved, the system determined a biomass yield ($Y_{x/o}$) of 3.811 g of biomass/g of consumed O_2 and a maintenance coefficient (m) of 0.0031 g of consumed O_2/(g of biomass·h). The value of $Y_{x/o}$ considered to solve the OUR balance seemed to be relatively high while the m value seemed to be low. This could be explained by the microorganism's characteristics. During the exponential growth phase, the energy employed by the fungi was not utilized for the synthesis of metabolites partially associated, which meant that the enzymatic pattern seemed to be more constitutive and not inductive, considering that the adaptation phase practically did not exist as demonstrated by the kinetics pattern (Fig. 3).

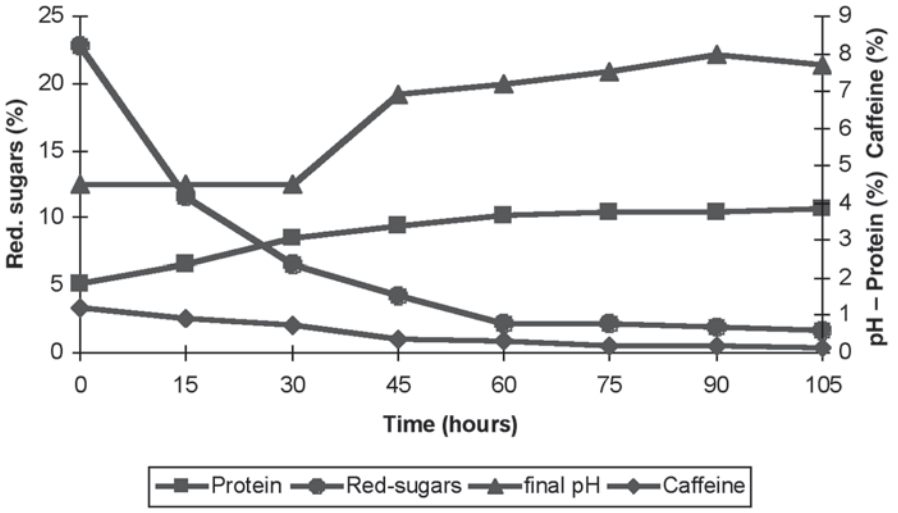

Fig. 4. pH influence, reducing sugar consumption, and protein synthesis in caffeine degradation during solid-state fermentation of coffee husk by *Aspergillus* sp. LPBx.

Caffeine Degradation

For better evaluation of caffeine degradation kinetics, the evolution of important factors such as reducing sugars, protein synthesis, and pH was followed. Figure 4 presents the relationship among pH, reducing sugar consumption, protein synthesis, and caffeine degradation. From 30 h of fermentation, the pH increased drastically from 4.5 to 6.9. This resulted in a significant increase in caffeine degradation—67% in 45 h. After 75 h, the pH was about 7.5 and the degradation yield was 84%. At the end of the aerobic phase of fermentation (in approx 90 h of fermentation), no further changes in pH were noticed and the degradation started to stabilize. At the end of the fermentation, 90% caffeine degradation was reached and the pH dropped from 8.0 to 7.7. The sugar consumption appeared to be intimately related to the reduction in caffeine as well as the protein synthesis by the mold. The reduction in caffeine could also be related to the utilization of caffeine as the nitrogen source by the mold, as previously described *(10)*. In 30 h of fermentation, when higher CO_2 production was obtained, the sugar content lowered drastically from 22.7 to 6.5%. After this phase, the degradation of caffeine increased as well as the protein synthesis until approx 60 h of fermentation, when only 2.3% of reducing sugars remained in the substrate. In 75 h of fermentation, when the aerobic phase finished, the protein synthesis started to stabilize along with the metabolism of caffeine and practically all the reducing sugar was consumed. At the end of fermentation, a considerable increase in the protein content of coffee husk was obtained (the crude husk contained 5.2% protein, which at the end was 10.6%).

Conclusion

The results proved the feasibility of using the strain *Aspergillus* sp. LPBx in solid-state fermentation using a column fermentor for coffee husk caffeine degradation. The use of factorial design experiments and surface response methodology helped in selecting the suitable bioprocess parameters. There was no need to pretreat the coffee husk for its effective utilization as substrate by the fungal culture nor was there any need to supplement the substrate with any nutrients.

Acknowledgments

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