Chapter 36

MICROBIAL DEGRADATION OF CAFFEINE AND TANNINS FROM COFFEE HUSK

D. Brand[1], A. Pandey[1a], S. Roussos[2], I. Brand[1], C. R. Soccol[1]

[1]Laboratorio de Processos Biotecnologicos, Departamento Engenharia Quimica, Universidade Federal do Parana (UFPR), 81531-970 Curitiba-PR, Brazil; [2]Laboratoire de Microbiologie, IRD, IFR-BAIM, Université de Provence, 13288 Marseille, France; [a]currently at Biotechnology Division, Regional Research Laboratory, Trivandrum-695 01, India

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1. Introduction

Brazil contributes approximately 25% of the world's coffee production. During 1998, it's production reached two million tons coffee beans. Parana State is one of the most important States for coffee cultivation in the country. Its production was 280,000 tons (representing about 14% of total Brazilian production) during 1998. Only 6% of the fresh grain is utilized for the production of the beverage, the remaining 94% is constituted by water and sub-products of the process (Zuluaga, 1989). In Brazil, coffee cherries are generally sun dried and subsequently the outer layers of husk covering the green coffee are removed by a hulling machine as and when needed. This residue poses a serious environmental concern due to its disposal in rivers, lakes located near the coffee processing regions. In view of its richness in proteins, fibres, carbohydrates and minerals, it has been suggested that it could be used as animal feed and organic fertilizer with suitable bio-treatments. It could also be used as substrate for the production of biogas, enzymes edible mushrooms, etc. (Gaime-Perraud, 1996; Fan *et al.*, 1999a, b, c 2000a, b; Pandey and Soccol, 2000).

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The use of the coffee husk as animal feed has some controversies. The presence of substances that have been termed as anti-physiological factors such as caffeine, polyphenols and a high potassium content could cause problems with the intake of husk (Cabezas *et al.*, 1978). Caffeine has some limitations due to its high nitrogen content and its diuretic and physical stimulant effects causing a decrease in urinary retention and loss of nitrogen. Polyphenols interfere with protein digestibility, thiamine utilization and cause reduced iron intake. The high content of potassium may cause ions unbalance at the tissues. (Velez *et al.*, 1985)

In order to make effective use of the huge amounts of coffee husk, thus, it seems important to remove (or reduce to a reasonably low level) the anti-physiological factors present in it. Use of solid state fermentation (SSF) could be a good alternative to aggregate value in its utilization as animal feed, since there are some micro-organisms that are capable to use caffeine as sole nitrogen source and polyphenols as carbon source (Roussos, 1989.) SSF has great potential to utilize agro-industrial residues for value-addition (Soccol, 1996; Pandey, 1991, 1992, 1994; Pandey and Soccol, 2000; Pandey *et al.* 1999a,b, 2000). The filamentous fungi are the micro-organisms best adapted to SSF processes due to their hyphal mode of growth and their good tolerance for low water activity (aw) (Pandey, 1992, 1994; Pandey *et al.*, 2000). High osmotic pressure conditions make fungi efficient and competitive in natural micro-flora for bioconversion of solid substrates (Raimbault, 1997)

There are a few reports, which describe removal or degradation of toxic substances from crops or crop-residues using filamentous fungi. For example, Wang *et al.* (1969) detoxified the cyanogenic compounds of cassava using filamentous fungi. Some authors have shown that fungi increased the digestibility and protein content of foods (Daubresse *et al.*, 1987; Soccol, 1996; Stertz *et al.*, 1999) and produced anti-carcinogenic substances (Wang *et al.*, 1969). The mould Phanerochaete chrysosporium is known to degrade lignin (Mudgett and Paradis, 1985). Attempts were made to degrade caffeine and tannins present in coffee husk using filamentous fungi belonging to three genera (Brand, 1999; Brand *et al.*, 1999; 2000)

2. Chemical composition of the husk

The chemical composition of the coffee husk has not been studied so extensively as that of coffee pulp, although both appear to have several similarities in their compositions. Table 1 shows a comparative profile of the components present in the coffee pulp and coffee husk. Vasco (1989) described the chemical composition of the pulp. It was interesting to note a difference between the values obtained for protein (total N) determined by Kjeldahl and for True Protein determined by Stutzer method, possibly due to the nitrogen content present in caffeine and other nitrogenous compounds present in the husk. The high content of tannins could be probably because the coffee grains are sun dried which favours the production of these compounds in the coffee husk. The contents of different nutrients of the coffee husk and pulp are reasonably good in comparison to other agricultural products or agro-industrial residues such as oats, rice meal, rice bran and wheat bran, all of each are increasingly being used in the diet of man (Christensen, 1981). However, the contents of caffeine and tannins make coffee husk and pulp different from all of these.

Table 1. Chemical composition of coffee husk and coffee pulp*

Components	Coffee pulp	Coffee husk
Moisture	6.93	11.98
Lipids	2.50	1.50
Fibers	21.00	31.86
Ash	8.3	6`.03
Carbohydrates	59.10	26.5
Protein (N*6,25)	8.25	6.8
True Protein (Stuzer)	-	4.8
Caffeine	0.75	1.2
Tannins	3.70	9.3

*Source-Brand, 1999

3. Selection of micro-organisms

The micro-organisms were selected by radial growth velocity and biomass produced in a coffee husk extract agar medium containing coffee husk extract (Brand, 1999). Eleven strains of *Rhizopus* sp., two strains of *Phanerochaete* and one strain of *Aspergillus* sp. were screened. The radial growth was realized by the inoculation of the micro-organisms in the centre of a Petri dish. Mycelial growth was measured every two hours for *Rhizopus* strains and every 12 hours for the strains of *Phanerochaete* and *Aspergillus* sp. Biomass was measured by the dissolution of the agar and separation of mycelia on filter paper (Table 2) (Brand, 1999; Brand *et al.*, 1999, 2000).

Table 2. Growth of strains on coffee husk extract agar medium*

Strain	Radial Growth velocity ($MM.H^{-1}$)	Biomass ($mg.plate^{-1}$)
R. oryzae LPB 68	2.19 ± 1.12	10,20 ± 0,56
R. oryzae LPB 95	2.05 ± 0.87	8,70 ± 1,20
R. delemar LPB 12	2.13 ± 2.12	10,80 ± 6,00
R. circicans LPB 75	2.09 ± 0.83	9.20 ± 0.70
R. arrhizus LPB 79	2.03 ± 0.34	12.10 ± 2.20
R. arrhizus LPB 25	1.94 ± 0.76	6.6 ± 0.40
R.oryzae LPB 27	1.88 ± 1.15	7.90 ± 4.30
Rhizopus sp. LPB 975	1.78 ± 1.32	2.80 ± 0.20
R. oligosporus LPB 67	1.78 ± 0.87	3.60 ± 0.20
R. formosa LPB 22	0.94 ± 0.65	2.90 ± 0.80
P. chrysosporium HD	0.75 ± 0.42	1.83 ± 0.56
P. chrysosporium BK	1.02 ± 0.34	2.21 ± 0.76
Aspergillus sp.	0.68 ± 0.14	14.83 ± 0.02

*Source-Brand, 1999; Brand *et al.*, 1999, 2000

The growth velocity of *Rhizopus* strains ranged from 0.94-2.19 $mm.h^{-1}$. The strains were capable to assimilate and metabolize the components present in the coffee husk producing biomass, which was characterized by radial growth. Best results were obtained with *R. arrhizus* 16179. The highest biomass (14.83 $mg.plate^{-1}$ in 92 hours) was

produced by *Aspergillus* sp., which presented a radial growth velocity of 0.68 $mm.h^{-1}$, showing great potential to degrade the toxic components of the substrate (Brand, 1999; Brand *et al.*, 2000).

4. Solid state fermentation

The strains of *Rhizopus* and *Phanerochaete* were maintained on PDA medium and that of *Aspergillus* on coffee husk extract agar. To prepare the inoculum, the strains were incubated for 10 days at 32, 35 and 28° C for the strains of *Rhizopus, Phanerochaete* and *Aspergillus* sp., respectively (Brand, 1999).

SSF was carried out in 250-ml Erlenmeyer flasks containing 20-g substrate. The experiments realized with selected strains of *Rhizopus, Phanerochaete* and *Aspergillus* were for the optimization of variables such as initial pH and moisture of the substrate, effect of addition of saline solution and effect of addition of water before autoclaving. For *Aspergillus* sp., which gave the best performance, surface response methodology was adapted to optimize various variables, which consisted of two experimental designs. The first one involved 2^{3-0} factors with four central points and one replicate resulting in a total of 22 experiments and the second one was a 3^{2-0} factors at three levels with one replicate that were significant by the analysis of the first study (Brand *et al.*, 1999, 2000).

SSF with *Rhizopus* LPB 79 showed that the addition of saline solution neither have any influence to degrade caffeine and tannins, nor resulted in better growth of the fungi. A 70 % moisture was the maximal water absorbing capacity of the substrate. The degradation rates obtained were 82 and 62% for caffeine and tannins, respectively under most suitable conditions. The pH of the medium influenced the metabolism of the mould for the growth and degradation of caffeine and tannins. SSF with *Phanerochaete chrysosporium* BK showed no beneficial effects by the addition of saline solution for the degradation of caffeine and tannins, although the growth of the micro-organism appeared to be better. The pH of the substrate influenced fungal metabolism to degrade toxic compounds present in coffee husk and maximum degradation was at pH 5.5 (70.8 and 45%, respectively for caffeine and tannins). The pH of the substrate also influenced the activity of *Aspergillus* sp. to degrade caffeine and tannins and under optimized

conditions, a 92% reduction in caffeine content of the coffee husk was achieved (Brand, 1999; Brand *et al.*, 1999, 2000).

5. Summary and conclusions

The strains of filamentous fungi belonging to *Rhizopus, Phanerochaete* and *Aspergillus* sp. were able to remove the anti-physiological factors, *viz.* caffeine and tannins from the coffee husk through their cultivation in SSF. The degree of degradation by each strain varied according to the fermentation conditions and was influenced by physical and chemical parameters during SSF. These experiments were realized without supplementation of any nutrients to the coffee husk medium, which proved that the fungal culture could utilize the nutrients available in the husk along with the toxic components of caffeine and tannins. Maximum caffeine degradation achieved was 92%, leaving only a very little quantity in the substrate. This study clearly demonstrated the potentialities in using filamentous fungal strains to degrade anti-physiological factors present in the coffee husk.

6. Futuristic Approach

There is need to translate and test the laboratory findings on a pilot-plant level. Studies should be carried out to test the feed-value of the fermented coffee husk for cattle and other live-stocks, including poultry and aquaculture. Fermented husk could be used alone as feed or as feed-supplement.

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