Brazilian Journal of Microbiology (2003) 34 (Suppl.1):102-104
ISSN 1517-8382

CAFFEINE DEGRADATION BY *RHIZOPUS DELEMAR* IN PACKED BED COLUMN BIOREACTOR USING COFFEE HUSK AS SUBSTRATE

Cristiane Vanessa Tagliari[1,2]*; Raquel K. Sanson[2]; André Zanette[2]; Telma Teixeira Franco[1]; Carlos Ricardo Soccol[2]

[1]Laboratório de Engenharia Bioquímica, Faculdade de Engenharia Química, Universidade Estadual de Campinas, Campinas, SP, Brasil. [2]Laboratório de Processos Biotecnológicos, Departamento de Engenharia Química, Universidade Federal do Paraná, Curitiba, PR, Brasil.

This paper corresponds to an "extended abstract" selected for oral presentation in the 22nd Brazilian Congress of Microbiology, held in Florianópolis, SC, Brazil, in November 17-20, 2003

ABSTRACT

Various microorganisms including bacteria, yeast and fungi can degrade caffeine. There are few publications about caffeine degradation pathway in filamentous fungi, mainly by solid-state fermentation (SSF). Studies were carried out on degradation of caffeine and their metabolites by filamentous fungi in SSF using coffee husk as substrate. The purpose of this work was to investigate the caffeine degradation pathway by *Rhizopus delemar* in packed bed column fermenter and to compare this degradation metabolism with glass flasks fermentation. The methylxanthines were quantified by HPLC analysis. The experiments were realized with the optimized conditions in previous experiments: pH 6.5, 28ºC, inoculation rate 10^6 spores/g substrate, aeration rate 60 mL/min and initial moisture 73%. Under these conditions, after 72 hous of fermentation was achieved only 0.19% of caffeine and 0.014% of theophylline in the coffee husk. The strain proved to be able for caffeine and theophylline degradation by SSF in packed bed column bioreactor.

Key words: decaffeination, fermentation, caffeine, theophylline, bioreactor, filamentous fungi.

INTRODUCTION

The caffeine (1,3,7-trimethylxanthine) is an alkaloid found in more than 60 plant species. Caffeine is, present with significant levels in coffee, tea, cocoa and Cola genera. The pharmacological effects of caffeine are well known: stimulation of central nervous system, toxicity when fed excessively, and mutagenicity on microorganisms (3).

Caffeine is considered toxic for many microorganisms; however, some microorganisms have the ability to grow in the presence of caffeine and the capacity to degrade the alkaloid. Several studies were carried out to investigate the use of caffeine, as a source of energy for microorganism growth (8). *Penicillium* and *Aspergillus* is the more frequent caffeine-degradation genera for fungi and *Pseudomonas* for bacteria (1,5).

Kurtzman and Shwimmer (7) first studied the degradation of this alkaloid using strains of *Penicillium roqueforti* and *Stemphylum sp*. The strains were able to degrade caffeine in a liquid medium containing caffeine concentrations below 19 g/L (7).

Roussos *et al*. (10) isolated strains from coffee and byproducts, which were mainly identified as *Penicillium* and *Aspergillus*. Eight of these strains showed great capacity to degrade caffeine in synthetic liquid medium without additional nitrogen source (10).

Brand *et al*. (2) isolated from coffee husk one strain of *Aspergillus niger* which is able to degraded 90% of caffeine and 57% of tannin by SSF (2).

These authors showed that the total degradation of caffeine by SSF was possible. However, in all fermentation studies

*Corresponding author. Laboratório de Processos Biotecnológicos, Departamento de Engenharia Química, Universidade Federal do Paraná. 81531-970, Curitiba, PR, Brasil. Fax: (+5541) 361-3195. E-mail: cristagliari@yahoo.com

involving caffeine degradation, no particular attention was given to its degradation products.

According to Hakil (4), theophylline is the major degradation product of caffeine by various filamentous fungi. The toxicity of theophylline in the system cardio-vascular and gastrointestinal is higher than caffeine. Lethal doses (LD_{50}) of caffeine and theophylline have been reported to be 200 mg/kg and 206 mg/kg respectively in rat (4).

The purpose of this work was to investigate the caffeine degradation pathway by filamentous fungi using SSF in packed bed column bioreactor.

MATERIALS AND METHODS

Microorganism and Substrate

Rhizopus delemar LPB 34 was maintained on coffee husk extract agar medium (CHEAM). Spores suspension was prepared after 10 days of culture on CHEAM at 32°C.

Coffee husk used as substrate contained approximately 7% proteins, 60% fibers, 2% fats, 10% minerals, 8% total sugars and 0.6% of caffeine.

Solid State Fermentation

The experiments were carried out with the previously optimized conditions: pH 6.5, 28°C, inoculation rate 10^6 espores/g substrate, airflow 60 mL/min and initial moisture 73%.

The packed bed column bioreactor (Fig. 1) consists of a glass column with lids at both the ends, which allows air flow control at the exit of the reactor (9). Each column had the capacity for 40 g of the substrate (dry weight basis). Two replicates of samples were collected for analysis every 24 h.

Sample Extraction

The fermented was submitted to mechanical agitation with water for 15 minutes and then was filtrated and centrifuged.

HPLC Analysis

The analyses of methylxanthines were performed on a Varian system with photodiode array detector (PDA) and Microsorb C18 column (4.6 x 250 mm). The eluents were methanol and water (24:76, v/v) with a flow rate of 1 mL/min, by a isocratic system.

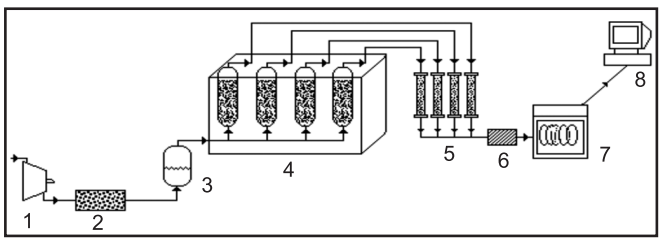

Figure 1. Packed bed column bioreactor. 1) Compressor, 2) air filter, 3) air saturation, 4) temperature control bath, 5) silica column, 6) distributor valve, 7) GC, 8) computer.

RESULTS AND DISCUSSION

For better evaluation of the degradation pathway of caffeine by *Rhizopus delemar* in coffee husk, the evolution of pH, moisture contends and the appearance of methylxanthines was monitored. The evolution of the methylxanthines quantified to more than 0.01% of dry material is shown in the Fig. 2.

During the experiment was observed that the pH increased from 6.0 to 9.0 after caffeine degradation, possibly due urea formation. The principal caffeine degradationproducts identified in the samples were the theophylline and 3-methylxanthine. This result is similar that observed in glass flasks and by Hakil (4) and Ina (6). First a 7-demethylation, giving theophylline from caffeine followed by a 1-demethylation leading to 3-methylxanthine from theophylline (Fig. 3).

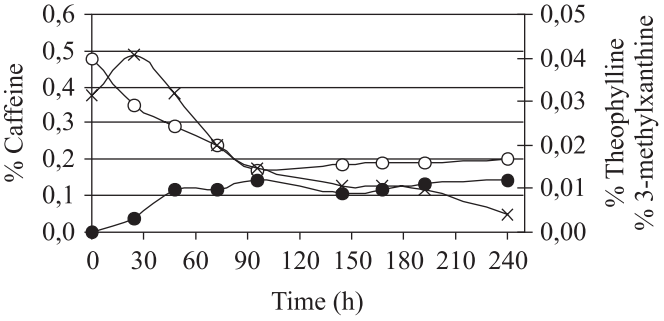

Figure 2. Methylxanthines degradation by *Rhizopus* in coffee husk during SSF in column bioreactor: ○ % caffeine, × % theophylline, ● % 3-methylxanthine.

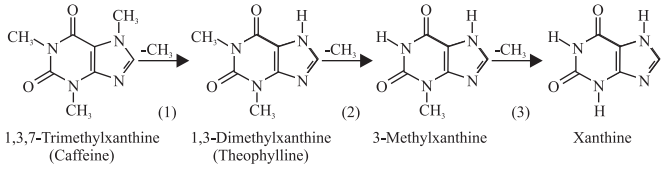

Figure 3. First steps in the degradation pathway of caffeine by *Rhizopus delemar*.

CONCLUSION

We can conclude that the first steps of caffeine degradation by *Rhizopus* delemar LPB 34 by solid state fermentation of coffee husk consist of demethylation reactions. This result is the same showed by others filamentous fungi but differs from observations made in the bacterial and human metabolism.

ACKNOWLEDGMENTS

We thank FAPESP, European Union and CNPQ for the financial support.

RESUMO

Degradação de cafeína por *Rhizopus delemar* em biorreator de colunas usando casca de café como substrato

Diversos microrganismos incluindo bactérias, fungos e leveduras são capazes de assimilar a cafeína de meios sintéticos ou de resíduos de café. Existem poucos trabalhos sobre a via de degradação da cafeína em fungos filamentosos, principalmente por fermentação no estado sólido (FES). Estudos de degradação da cafeína por fungos filamentosos em FES usando casca de café como substrato vêm sendo realizados. O objetivo deste trabalho foi investigar a via de degradação da cafeína por *Rhizopus delemar* em biorreator de colunas aeradas e comparar este metabolismo de degradação com o da fermentação em frascos de vidro. As metilxantinas foram quantificadas por análises em HPLC. Os experimentos foram realizados com as condições otimizadas previamente: pH 6,5, 28°C, 10^6 espores/g substrato, vazão de ar 60mL/min e 73% de umidade inicial. Após 90 horas de fermentação, 65% da cafeína foi reduzida, resultando 0,19% de cafeína e 0,014% de teofilina na casca de café. Esta cepa provou ter habilidade para degradar cafeína e teofilina por FES em biorreator de colunas.

Palavras chave: descafeinação, fermentação, cafeína, teofilina, biorreator, fungos filamentosos

REFERENCES

1. Asano, Y.; Komeda, T.; Amada, H. Microbial production of theobromine from caffeine. *Biosci. Biotech. Biochem.*, 57(8):1286-1289, 1993.
2. Brand, D.; Pandey, A.; Roussos, S.; Soccol, C.R. *Enzyme Microb. Technol.*, 27:127-133, 2000.
3. Denis, S.; Augur, C.; Marin, B.; Roussos S. *Biotechnol. Techn.,* 12(5):359-362, 1998.
4. Hakil, M. France, 1999. (PhD thesis Franche-Comté University).
5. Hakil, M.; Voisinet, F.; Viniegra-gonzales, G.; Augur, C. *Process Biochem.,* 35(1):103-109, 1999.
6. Ina, K. Biochemical studies of caffeine. *Nippon Nogeikagaku Kaishi,* 45(8):378-380, 1971.
7. Kurtzman, R.H.; Shwimmer, S. Caffeine removal from growth media by microorganisms. *Experientia*, 127:481-482, 1971.
8. Mazzafera, P. *Review in Scientia Agricola*, 59(4):815-821, 1994.
9. Pandey, A.; Soccol, C.; Rodrigues-Leon, J.; Nigam., P. Solid State Fermentation in Biotechnology. Fundamentals and Applications. New Delhi., 2001.
10. Roussos, S.; Hannibal, L.; Aquiahuatl, M.A.; Trejo Hernandez, M.R.; Marakis, S. *J. Food Sci. Technol.*, 31:316-319, 1994.