SUGARCANE BAGASSE AS SUPPORT FOR THE PRODUCTION OF COCONUT AROMA BY SOLID STATE FERMENTATION (SSF)

Manoela Pessanha da Penha,[a,]* Maria Helena Miguez da Rocha Leão,[b] and Selma Gomes Ferreira Leite [b]

Brazil is one of the major producers of sugarcane (*Saccharum officinarum*) in the world and consequently produces large quantities of waste such as sugarcane bagasse, which can be used as inert support for the production of aroma compounds by SSF. The aim of this study was to evaluate the centesimal composition and particle size distribution of sugar cane bagasse, as well as its applicability as support for the production of 6-pentyl-α-pyrone by SSF. Analyses were performed in triplicate to evaluate the levels of carbohydrates, proteins, lipids, and moisture in the waste. Also evaluated were the particle size distribution and morphology structure of the sugarcane bagasse. The aroma compound produced shows that the studied waste can be used for 6PP production by *Trichoderma harzianum* IOC 4042 by SSF process. By kinetic production of aroma it is concluded that the seventh day of fermentation yielded the largest production of the aroma compound, as published for other studies.

Keywords: Sugarcane bagasse; Agro-industrial waste; 6-pentyl-α-pyrone; Trichoderma harzianum; Solid state fermentation

*Contact information: a: Chemistry Institute, Federal University of Rio de Janeiro, Brazil; b: Department of Biochemistry Engineering, School of Chemistry, Federal University of Rio de Janeiro, Brazil. * Corresponding author: manoelapp@ufrj.br*

INTRODUCTION

There is an increasing global trend towards the efficient utilization of natural resources. The direct disposal of agro-industrial by-products as waste in the environment represents a major cause for environmental pollution and is also a significant loss of biomass that could be used for the production of different metabolites with added commercial value (Vendruscolo *et al.* 2007).

Agro-industrial residues are generally regarded as the best substrates for solid-state fermentation (SSF) processes, mainly due to their low cost. Nowadays, the world's concern with environmental issues reinforces the importance of using agro-industrial residue (Carrijo *et al.* 2002). Although product recovery and purification processes are more expensive when using natural supports, their use presumes a reduction in production costs, and it usually results in a much higher level of activity (Singhania *et al.* 2009).

The Brazilian economy is one of the most important economies of the world's agriculture, producing coffee, sugarcane, soybeans, cassava, fruits, *etc*. Almost all production is exported, which is an excellent contribution to the country's economic

development. However, this major production is responsible for generating a high amount of waste that causes serious environmental problems (Soccol and Vandenberghe 2003).

Wastes from the food and agricultural industries that are produced in large quantities and are rich in carbohydrates and other nutrients can serve as a substrate for the production of chemicals and enzymes by using the technique of solid state fermentation (Longo and Sanromán 2006). The nature of the solid substrate used is an important aspect. However the solid substrate not only supplies nutrients for microbial culture, but also serves as the physical support for the growth of microbial cells.

In recent years there has been an increasing trend toward the more efficient use of agro-industrial waste, including sugarcane bagasse. Various processes and products that use sugarcane bagasse as support have been reported. These include power generation, the production of paper, and the manufacture of products based on fermentation (Pandey *et al.* 2000).

The increase in cultivated lands in Brazil and worldwide is contributing to a rise in the quantity of by-products and waste from the agro-industry. Sugarcane bagasse contributes a significant proportion of the waste produced (Souza and Santos 2002). In comparison with other agro-industrial residues, sugarcane bagasse is considered a rich solar energy reserve, due to its high growth rate (about 80 t/ha in comparison with 1, 2, and 20 t/ha of wheat, and other plants and trees, respectively) and annual regeneration capacity (Pandey *et al.* 2000).

Sugarcane bagasse is one of the major cellulosic agro-industrial by-products of Brazil and is being used almost entirely as fuel for the sugar industry. In recent years there has been a tendency to use efficient agro-industrial waste such as sugarcane bagasse, not only as fuel, but also as a raw material for biotechnological processes, due to its lignocellulosic composition, which can be used for the metabolism of microorganisms to obtain products and metabolites of interest (Pandey *et al.* 2000).

Sugarcane bagasse contains approximately 50% cellulose and 25% hemicelluloses and lignin. Chemically, bagasse contains about 50% of α-cellulose, 30% of pentosans, and 2.4% ash. A large number of microorganisms, including bacteria, yeasts, and filamentous fungi have also been used in fermentation processes with sugarcane bagasse as support. Filamentous fungi, especially the basidiomycota, have been widely used and are preferred in the production of enzymes or protein enrichment (Pandey *et al.* 2000).

Various industrial waste such as cassava bagasse, sugarcane bagasse, apple pulp, potato waste, coffee pulp, and bark have been used as a substrate in SSF with different microorganisms for the production of aroma compounds and other substances (Soccol and Vandenberghe 2003). It is estimated that more than 100 aroma compounds are industrially produced by microbial fermentation (Medeiros *et al.* 2006). Fermentation is a promising biotechnological technique for the production of natural aroma compounds.

The production of natural aroma compounds by fermentation allows the recovery of natural food additives, which is desired by the consumer. Brazilian legislation classifies natural aroma as substances obtained by microbiological processes (Anvisa 2007). The Food and Drug Administration (FDA) defines natural aroma and specifies the type of substances generally regarded as safe for use as natural flavors including any substance that is extracted, distilled, or otherwise derived from plant or animal matter. It

may be produced either directly from the matter itself, or after it has been roasted, heated, or fermented (FDA 2011).

The chemical compound 6-Pentyl-α-pyrone (6-PP) has a characteristic coconut-like aroma. 6-PP is approved as a flavouring agent by JECFA (Joint Expert Committee on Food Additives) (FAO 2010). When it is produced by chemical synthesis, it requires seven reaction steps at high temperatures (490 °C), a difficult and costly process (Sahry-Bagnon *et al.* 2000). For this reason 6-PP produced by fermentation processes can be an interesting alternative to chemical synthesis.

The aim of this study was to evaluate the centesimal composition and morphological characteristics of sugarcane bagasse particles used as inert support for the production of aroma compound 6-pentyl-α-pyrone by solid state fermentation.

EXPERIMENTAL

Sugarcane Bagasse

Composition percentage

Sugarcane bagasse (*Saccharum officinarum*) was obtained in a local market for the production of sugarcane juice in Rio de Janeiro. The bagasse was subjected to a drying process at 60 ºC/24 h and ground in a granulator mill using knives and hammers, TREU ® (3 mm). Moisture levels (by dried until constant weight), lipids (direct extraction by Soxhlet apparatus), total sugars (phenol-sulfuric acid method), and protein (Kjeldahl method modificated) were determined, according to physico-chemical methods for food analysis (IAL 2005). The results were expressed in g/100 g (% w/w).

Particle size distribution of sugarcane bagasse

Particle size distribution of sugarcane bagasse was evaluated using a set of Granutest® sieves with openings measuring 0.3 mm, 0.15 mm, and 0.075 mm in diameter, at the macromolecular laboratory of Federal University of Rio de Janeiro (UFRJ).

Morphological analysis of sugarcane bagasse after SSF

A morphological analysis of the sugarcane bagasse after SSF was performed at the ceramic materials laboratory of the Military Institute of Engineering (IME) in Rio de Janeiro. For this analysis a JEOL® scanning electronic microscope (SEM) model JSM-5800LV was used. Initially, the bagasse sample was subjected to drying in an oven at 80 °C/24 h. The sample was then placed on metallic cylindrical holders called "stubs", measuring 10 mm in diameter and secured with double-sided adhesive tape. The sample in the stubs was then coated with gold and placed in the SEM.

Solid State Fermentation

To confirm the application of sugarcane bagasse analyzed as a fermentation substrate, a solid state fermentation process using *Trichoderma harzianum* 4042 for the production of aroma compound 6-pentyl-α-pyrone was used. The process was conducted for 9 days and analyzed on the 3rd, 5th, 7th, and 9th days for the production of 6-PP.

Microorganism and cultivation method

Trichoderma harzianum IOC 4042 was obtained from the fungi culture collection of Oswaldo Cruz Institute (CCFF-IOC/Fiocruz, Brazil). The microorganism was grown and periodically placed in tubes containing potato dextrose agar, $CaCO_3$ (0.02%), and $MgSO_4 \cdot 7H_2O$ (0.02%) and stored at 4 ºC (Ramos *et al.* 2009). The spores produced by *Trichoderma harzianum* IOC 4042 microorganism were suspended by growing them at 28 ºC for seven days. The spores were resuspended in a sterile saline solution (0.9% NaCl) and quantified in a Neubauer chamber.

Conditions of fermentative process

Solid state fermentation was done using sugarcane bagasse as inert support in Erlenmeyer flasks of 250 mL with 4.5 g of substrate humidified with nutrient solution with concentration (g/L) of glucose (30), $(NH_4)_2SO_4$ (0.943), $MgSO_4 \cdot 7H_2O$ (1.5), KH_2PO_4 (1.0), KCl (0.5), $CaCl_2 \cdot 2H_2O$ (0.008), $FeSO_4 \cdot 7H_2O$ (0.01), and $ZnSO_4 \cdot 7H_2O$ (0.001), and 1 mL of spore suspension (2.0×10^6 CFU/mL). The flasks were covered with cotton and kept stationary at 28 ºC. Both the substrate and nutrient solution were autoclaved at 121 ºC for 15 min. The 6-PP concentration was determined after seven days of cultivation. Moisture level was determined by dry weight, at 80 ºC for 24 h (Ramos *et al.* 2008).

Analytical Procedures

Extraction of 6-PP

Headspace solid-phase microextraction (HS-SPME) was used for the determination of 6-pentyl-α-pyrone by gas chromatography (GC). Extractions were performed using a manual SPME holder with silica fused fiber coating with 100 µm polydimethylsiloxane (PDMS) (Supelco, Bellefonte, PA, USA). Samples contained 1 g and 20 mL of NaCl solution 25% (w/v) and were placed in a 40 mL vial that was hermetically sealed with a polypropylene hole cap and PTFE/silicone septa (Supelco, Bellefonte, PA, USA). These samples were placed on a magnetic plate and constantly agitated in a water bath at 79 ºC. The equilibrium time was 2 min, and the fiber was exposed to a sample headspace for 29 min (extraction time). Thermal desorption of analytes from the fiber coating occurred in a GC injector at 250 ºC for 4 min (Ramos *et al.* 2009).

Chromatographic analysis

Analyses CG were performed by gas chromatography (Varian CP3800, Palo Alto, CA, USA), equipped with a flame ionization detector. Analytes were separated on a Carbowax 20M column (internal diameter: 0.53 mm, length: 30 m, film thickness: 1 µm). Split mode was applied with a split ratio of 10. The column was maintained at 60 ºC followed by a slope-wise increment of 10 ºC/min until 240 ºC. Injector and detector temperatures were 250 ºC and 260 ºC, respectively. Hydrogen was used as a carrier gas with a flow rate of 4.0 mL/min.

Quantification was done by comparing the peak area with a calibration curve obtained from triplicate analyses of sugarcane bagasse (0.1 g) impregnated with 6-PP standard solutions. Aroma extraction and quantification were performed by SPME and

chromatographic analysis as previously described in *Extraction of 6-PP and Chromatographic analysis.*

Standard solutions (concentrations of 10, 25, 50, 100, 150, and 200 mg/L) were prepared by dissolving the lactone (6-amyl-2-pyrone, TCI) in an ethanol-distilled water solution (1%). The R^2 of the calibration curve obtained was equal to 0.9954, indicating a suitable accommodation of the linear equation to the experimental data. The regression equation (y = 1214,1x + 259, with y = area chromatogram peak and x = aroma concentration in ppm) obtained could be used for quantifying the production of 6-PP in SSF.

Statistical Analysis

The results of the centesimal composition and size distribution of sugarcane bagasse particles were analyzed by descriptive statistics, with tabular and graphical description of experimentally obtained data by mean and standard deviation.

For quantifying concentrations of 6-PP in SSF, a calibration curve was prepared. Estimated values for concentrations of aroma compound were obtained by means of a fitted model, described by the equation of the line obtained by linear regression using the Microsoft Office Excel 2007 version.

RESULTS AND DISCUSSION

Sugarcane bagasse has low nutritional value, which occurs with other lignocellulosic residues, and is rich in cell wall matter (Souza and Santos 2002). Table 1 shows values of macronutrients (% w/w) found in the sugarcane bagasse used as inert support for fermentation in this work.

Table 1. Chemical Composition of Sugarcane Bagasse

Sugarcane bagasse	Composition (%)
Moisture	17.3 ± 0.35
Total sugars (in glucose)	30.9 ± 0.15
Protein	1.8 ± 0.33
Lipids	0.7 ± 0.15

The moisture level of sugarcane bagasse was 17.3% (w/w). Low moisture content is associated with the process used to obtain it, since it is ground and subjected to drying when it is to be used in fermentation. Filamentous fungi grow best in environments with moisture content of around 50% to 70% (Pandey *et al*. 2000). This is above the values obtained in the analysis of bagasse (17.3%). Please note that, in order to grow the fungus in this project, the bagasse was saturated with a nutrient solution which increased the percentage of moisture in the medium.

The content of lipids was 0.7% (w/w), which is less than the value found by Sales-Campos *et al.* (2010) (1.36%). In fermentation, certain types of lipids can serve as a precursor to compound 6-PP produced by *Trichoderma harzianum*, since the lactone may be originally from successive β-oxidation of saturated and unsaturated hydroxy acids

(Bonnarme *et al.* 1997). However the low percentage of lipids in agro-industrial waste was not an impediment to the development of the microorganism and 6-PP production.

Carbohydrates are 30.9% (w/w) of the residue analyzed. Pereira *et al.* (2009) found a total sugar content of 16.4% in unprocessed bagasse. Glucose can be used by the microorganism in fermentation, which shows potential in the use of residue as support for the process, since the filamentous fungus grows rapidly under different substrates using monosaccharide, disaccharide, and polysaccharide complexes, well as purines, pyrimidines, amino acids, aldehydes, and organic acids as carbon and energy sources, producing a broad spectrum of metabolites (Mendoza 2009).

The protein content of bagasse was 1.7% (w/w). Pereira *et al.* (2009) and Carvalho *et al.* (2009) found amounts of 2.0% and 2.4% crude protein for sugarcane bagasse, respectively. The crude protein content in treated material has been frequently raised by the use of chemical additives such as urea and ammonia anhydrous, which have a high content of non-protein nitrogen (Carvalho *et al.* 2009).

The analysis of macronutrients in sugarcane bagasse confirms the interest in using it as a substrate for fermentation in the solid state, mainly due to the content of glucose present. It is also due to the fact that this support can be easily degraded by microorganisms such as filamentous fungi of the genus *Trichoderma*, which have the ability to utilize lignocellulosic wastes and therefore produce various compounds of interest in the food, pharmaceutical, and textile industries.

The size of the substrate particles is of particular importance when characterizing the substrate and evaluating the capacity of the system to interchange with microbial growth and the transfer of heat and mass during the SSF process. Granulometry influences the diffusion of gases and heat through fermentation, ensuring the growth of microorganisms and the retention of non volatile compounds produced, even in a solid matrix (Pandey 2003).

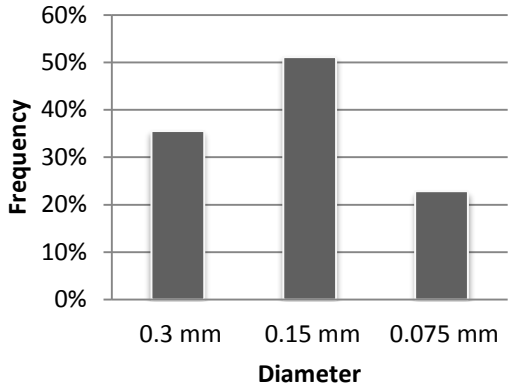

Fig. 1. Particle size distribution of sugarcane bagasse

The bagasse analyzed showed that 51% of the particles were 0.15 mm (see Fig. 1). The rest were distributed between 36% and 23% and retained in sieves with diameters of 0.3 mm and 0.075 mm, respectively. According to the bagasse particle size analysis performed by Souza *et al.* (2005), 35% of the bagasse analyzed was retained in sieves of 1.705 mm in diameter, which is higher than that obtained in this study.

Microscopic heterogeneity of the substrate, once considered the weakest point of SSF, is considered today to be its main strength in increasing production efficiency and promoting changes in microbial physiology. However, the transfer of oxygen among the particles of the system can become a problem when using extremely fine-textured substrate (Santos *et al.* 2006). So, it is important to analyze the size of the particles that compose the substrate used in fermentation.

The size of approximately 20% of the particles were smaller than 0.075 mm, which shows that the diameter of 80% of the sugarcane bagasse particles were larger than 0.15 mm. Smaller particles provide increased surface area for microbial attacks, but on the other hand, they tend to compact easily and therefore compromise the respiration and aeration system (Souza *et al.* 2007). The support must not compact during SSF, so the air necessary for microbial development can pass through the spaces in the fermentation system (Pinto *et al.* 2006).

The presence of pores in the structure of the material is also interesting, since it helps aerate the support and facilitates the availability of water necessary for microbial metabolism. Growth of *T. harzianum* IOC4042 mycelia was observed on the surface of sugarcane bagasse after fermentation on the 7th day (Fig. 2).

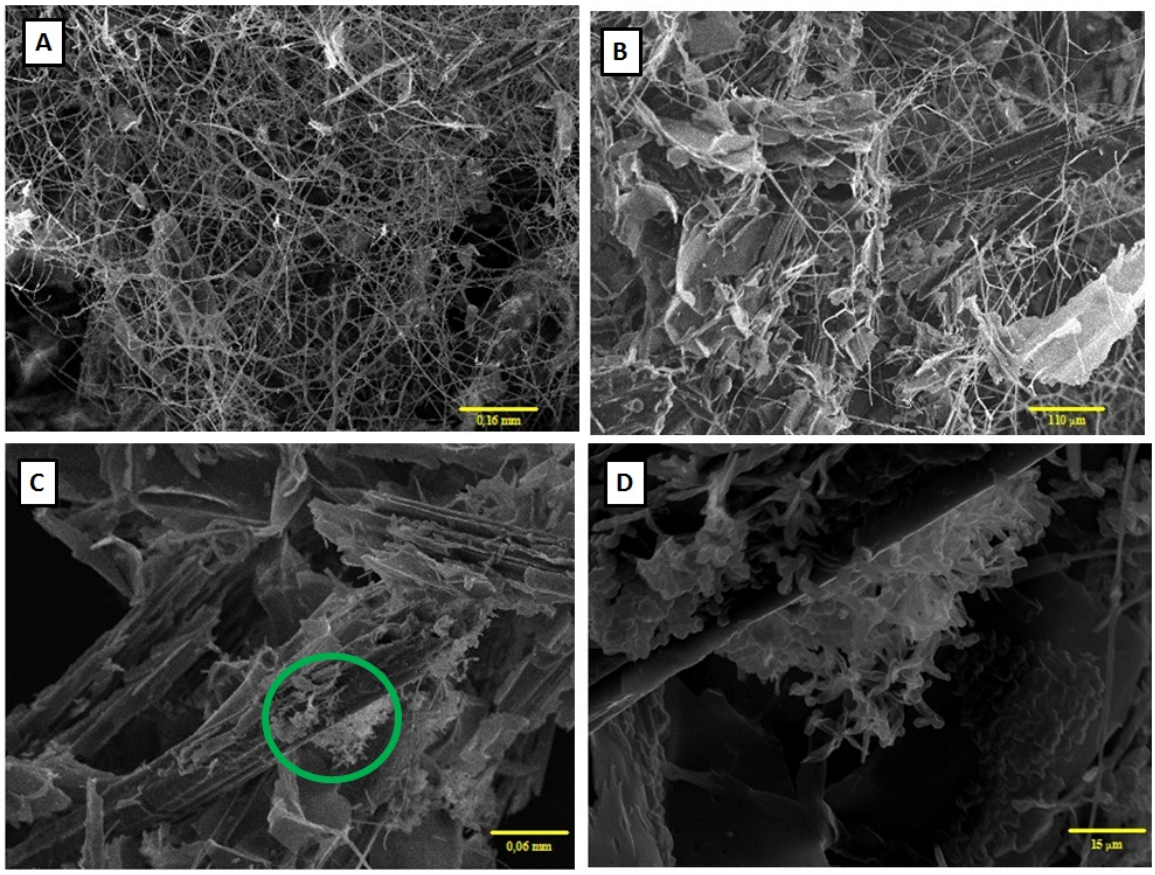

Fig. 2. Scanning electron microscopy showing details of colonization of sugarcane bagasse by *T. harzianum* IOC4042 7 days after inoculation for SSF. Micrograph D is a high magnification of the green circle area presented in photograph C. (A) x100 (B) x160 (C) x300 (D) x1200

The highest concentration of the substance was obtained on the 7th day of fermentation (Fig. 4), 93 ppm (0.093 mg/g dry weight), which agreed with other studies that also showed the highest production of 6PP on the seventh day (Sarhy-Bagnon *et al.* 2000; Ramos *et al.* 2009; Ladeira *et al.* 2010).

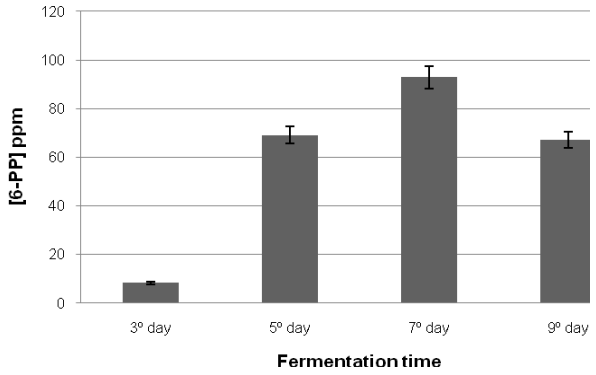

Fig. 3. Production of 6-pentyl-α-pyrone by *T. harzianum* 4042 in sugarcane bagasse

Fungi of the genus *Trichoderma* seem to have a mechanism to detoxify the 6-PP compound by the hydroxylation of the C1 and C5 carbons. The existence of a method such as this to detoxify 6-PP suggests that the substance cannot remain for long periods in the environment in which it was produced, therefore resulting in the decreased concentration of the aroma compound after the 7th day of fermentation (Daoubi *et al.* 2009).

Increased production of 6-PP by SSF using *T. harzianum* was achieved after ten days of fermentation (2800 ppm – 2.8 mg/g), using sugarcane bagasse as support (Sarhy-Bagnon *et al.* 2000). Ladeira *et al.* (2010) obtained a value of 254 ppm (0.254 mg/g), using the same support and fermentation condition, but with another strain of *T. harzianum*.

Ramos *et al.* (2009) already studied the aroma production by this strain isolated from soil. These authors obtained satisfactory results, showing that *T. harzianum* IOC 4042 and *T. harzianum* IOC 4040 can produce coconut aroma with SSF using other agro-industrial residue as support, with a difference marginally significant between the strains.

It is important to explain that the threshold of the 6-PP compound is 150 ppb (Franco 2003), so production above this threshold, as found in this study and mentioned by other authors, is desirable and interesting from the biotechnological point of view.

This study reinforces the use of sugarcane bagasse as support for the production of aroma, as shown by Ladeira *et al.* (2010), which optimized the nutritive solution and resulted in substantial production on the same day of fermentation (7^{th} day), as reported in this study.

CONCLUSIONS

1. Sugarcane bagasse has the potential to be used as support for aroma compound production by SSF. The favorable effect of the bagasse can be attributed to the

macronutrients and morphology of the bagasse, which improved growth and microbial metabolism.

2. Using sugarcane bagasse as support, *Trichoderma harzianum* IOC4042 produced 93 ppm of 6-PP on the 7th day of fermentation, reinforcing an interesting application of agro-industrial residue in biotechnology.

ACKNOWLEDGMENTS

The authors are grateful for the support of the CNPq, a FAPERJ, a FUJB, Alexandre Guedes Torres, Ph.D., Cristina Andrade Tristão, Ph.D., Elisabete de Paula Barros, Ph.D., Rafaela Cristina Barata Alves, MS, and Carolina Montes Durões de Souza, BS.

REFERENCES CITED

Anvisa – Agência Nacional de Vigilância Sanitária. (2007). RDC nº 2, de 15 de janeiro de 2007. *Regulamento técnico sobre aditivos aromatizantes*. DOU

Bonnarme, P., Djian, A., Latrasse, A., Feron, G., Giniès, C., Durand, A., and Le Quéré, J.-L. (1997). "Production of 6-pentyl-α-pyrone by *Trichoderma* sp. from vegetable oils," *Journal of Biotechnology* 56, 143-150.

Carrijo, O. A., Liz, R. S., and Makishima, N. (2002). "Fibra da casca do coco verde como substrato agrícola," *Hortic. Bras.* 20(4), 533-535.

Carvalho, G. G. P., Cavali, J., Fernandes, F. E. P., Rosa, L. O., Olivindo, C. S., Porto, M. O., Pires, A. J. V., and Garcia, R. (2009). "Composição química e digestibilidade da matéria seca do bagaço de cana-de-açúcar tratado com óxido de cálcio," *Arq. Bras. Med. Vet. Zootec.* 61(6), 1346-1352.

Daoubi, M., Pinedo-Rivilla, C., Rubio, M. B., Hermosa, R., Mone, E., Aleu, J., and Collado, I. G. (2009). "Hemisynthesis and absolute configuration of novel 6-pentyl-2H-pyran-2-one derivates from *Trichoderma spp*," *Tetrahedron* 65, 4834-4840.

FDA – Food and Drug Administration. (2011). Code of Federal Regulations, "Foods; labeling of spices, flavorings, colorings and chemical preservatives," v.6. Sec. 101.22

FAO – Food and Agriculture Organization of United Nations (2010). Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives, 73rd Meeting.

IAL–Instituto Adolfo Lutz. (2005), *Métodos Físico-Químicos para Análises de Alimentos*, 4th Ed., Instituto Aldolfo Lutz, São Paulo, 1018 pp.

Longo, M. A., and Sanromán, M. A. (2006). "Production of food aroma compounds: Microbial and enzymatic methodologies," *Food Technol. Biotechnol.* 44(3), 35-353.

Ladeira, N. C., Peixoto, V. J., Penha, M. P., Barros, E. B. P., and Leite, S. G. F. (2010). "Optimization of 6-pentyl-alpha-pyrone production by solid state fermentation using sugarcane bagasse as residue," *BioResources* 5(4), 2297-2306.

Medeiros, A. B. P., Pandey, A., Vandenberghe, L. P. S., Pastore, G. M., and Soccol, C. R. (2006). "Production and recovery of aroma compounds produced by solid-state

fermentation using different adsorbents," *Food Technol. Biotechnol.* 44(1), 47-51.
Mendoza, D. P. G. (2009). "Variações do secretoma de *Trichoderma harzianum* em resposta a diferentes fontes de carbono," Dissertação de Mestrado, Univ. Brasília.
Pandey, A. (2003). "Solid-state fermentation," *Biochemical Engineering J.* 13, 81-84.
Pandey, A., Soccol, C. R., Nigam, P., and Soccol, V. T. (2000). "Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse," *Biores. Tech.* 74, 69-80.
Pereira, R. C., Evangelista, A. R., and Muniz, J. A. (2009). "Evaluation of sugar cane bagasse subjected to haying and ensiling," *Ciênc. Agrotec.* 33(6), 1649-1654.
Pinto, G. A. S., Brito, E. S., Silva, F. L. H. Da, Santos, S. F. M., and Macedo, G. R. (2006). "Fermentação em estado sólido: Uma alternativa para o aproveitamento e valorização de resíduos agroindustriais," *Revista de Química Indust.* 74(724), 17-20.
Ramos, A. S., Fiaux, S. B., and Leite, S. G. F. (2008). "Production of 6-pentyl-α-pyrone by T*richoderma harzianum* in solid-state fermentation," *Braz. J. Microbiol.* 39(4), 712-717.
Ramos, A. S., Martins, P. S. O., Fiaux, S. B., and Leite, S. G. F. (2009). "Microextração em fase sólida de 6-pentil-α-pirona produzida por fermentação em estado sólido," *Ciênc. Tecnol. Aliment.* 29(3), 523-528.
Sales-Campos, C., Araujo, L. M., Minhoni, M. T. A., and Andrade, M. C. N. (2010). "Análise físico-química e composição nutricional da matéria-prima de substratos pré e pós cultivo de *Pleurotus ostreatus*," *Interciencia* 35(1), 70-76.
Santos, D. T., Sarrouh, B. F., Santos, J. C., Pérez, V. H., and Silva, S. S. (2006). "Potencialidades e aplicações da fermentação semi-sólida em biotecnologia," *Janus* n. 4, 164-183.
Sarhy-Bagnon, V., Lozano, P., Saucedo Castañeda, G., and Roussos, S. (2000). "Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* in liquid and solid state cultures," *Process Biochem.* 36(1-2), 103- 109.
Singhania, R. R., Patel, A. K., Soccol, C. R., and Pandey, A. (2009). "Recent advances in solid-state fermentation," *Biochemical Engineering Journal* 44, 13-18.
Soccol, C. R., and Vandenberghe, L. P. S. (2003). "Overview of applied solid-state fermentation in Brazil," *Biochemical Engineering Journal* 13, 205-218.
Souza, R. L. A., Amorim, B. C., Silva, F. L. H. da, and Conrado, L. S. (2007). "Caracterização do resíduo seco do maracujá para utilização em fermentação semi-sólida," In: *Simpósio Nacional de Bioprocessos*, 16, Curitiba, 1-8.
Souza, T. C., Ferreira, C. A., Brandão, P. C., Hori, C. E., and Romanielo, L. L. (2005). "Avaliação do uso de biomassa como adsorvente para a separação de contaminantes orgânicos em efluentes líquidos," *VI Congresso Brasileiro de Engenharia Química em Iniciação Científica*.
Souza, O., and Santos, I. E. (2002). "Aproveitamento do bagaço de cana-de-açúcar pelos ruminantes," *Comun. Técnico: Ministério da Agricultura, Pecuária e Abastecimento*.
Vendruscolo, F., Koch, F., Pitol, L. O., and Ninow, J. L. (2007). "Produção de proteína unicelular a partir do bagaço de maçã utilizando fermentação em estado sólido," *Revista Brasileira de Tecnologia Agroindustrial* 1(1), 53-57.

Article submitted: May 28, 2011; Peer review completed: Aug. 3, 2011; Revised version received: Dec. 11, 2011; Further revisions: Mar. 30, 2012; Accepted: April 17, 2012.