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Solid State Fermentation Process for Polygalacturonase Production Using Tray Bioreactor

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Polygalacturonase are pectinolytics enzymes that have technological, functional and biological applications in the food processing and plant-fungus interactions. These enzymes are in the group of most industrially applied, being pioneers in wines and fruit juices preparation. The aim of this work was polygalacturonase production by solid state fermentation process in a tray bioreactor, using cashew apple dry bagasse and *Aspergillus niger* CCT0916 microorganism. Utilizing a 2^2 factorial experimental design, it was observed the influence of temperature and substrate thickness on the response polygalacturonase activity, keeping constant the mass of moist substrate at 500 g during the experiments. The higher enzymatic activity predicted by the model (7 U/g), with 53 h of fermentation, was achieved under the conditions: moisture 50 %w.b, 10^6 spores/g inoculated spores concentration, 1.5 %(w/w) of ammonium sulphate as nitrogen source, 35 ºC fermentation temperature and 20 mm substrate thickness. This model was significant with 95 % confidence. It was observed that between the factors, temperature is the variable that most influence the process.

1. Introduction

Solid state fermentation process is defined as a process that occurs over a non-soluble material, acting as support and nutrients source, with small quantity of water, under the action of fermenting agent (Couto and Sanromàn, 2006). Although there are many projects to industrial bioreactors, these have a limited extent for this type of process.

There are several types of reactors used in solid state fermentation process. Among them it has the tray type reactor, where substrate is spread over the trays forming a thin layer with a few centimetres of depth. According to Pandey (2004), this type of reactor is limited due to mass transfer and heat. Thus, these reactors can develop large internal temperature gradients and gas concentration with substrate height above of 40 mm.

Despite the difficulties, the use of semi-solid medium can be advantageous because it allows the use of agro-industrial residues as substrate, which is abundant raw material and low cost at Brazil. Several agro-industrial wastes have been used in enzymes production by solid state fermentation. Cashew apple is one of them, being rich in sugars, organic acids and fibre. Brazil produced in 2009 approximately 1.9 Mt/y of cashew fruit. The number obtained for cashew apple exploitation account 10 % of the total produced (Oliveira and Ipiranga, 2009).

Among many bioproducts found in literature obtained by solid state fermentation, pectinases are enzymes group that degrade pectic substances. Pectinases are widely used in food industry, especially in the fruit juice industry to reduce viscosity, improve the efficiency of filtration and clarification. This

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enzyme alone is responsible for on quarter of food enzymes production in the world (Gomes et al., 2011).

This work is a continuation of work done previously. Alcântara and Silva (2011) studied the optimization of solid state fermentation process for polygalacturonases production, in laboratory scale with 10 g of humid medium, using cashew apple dry bagasse as substrate and *Aspergillus niger* CCT0916. There was verifying the influence of inoculated spores' number, initial moisture content of medium, sulphate ammonium concentration, as nitrogen source, and fermentation temperature. It was observed that maximum polygalacturonase activity (33 U/g) was obtained with 50 %(w.b), 10^6 spores per gram of humid medium, 1.5 %(w/w) of ammonium sulphate and 35 ºC at 29 h of fermentation. The model for 29 h of fermentation was statistically significant with 95 % confidence. Moisture is variable that most influence the process, having a positive effect on response, as fermentation temperature. Inoculated spores concentration resulted in negative effect and ammonium sulphate concentration do not show any effect on polygalacturonase activity.

With the aim of scaling up of a solid state fermentation process, this work proposes polygalacturonase production in a tray bioreactor, using cashew apple dry bagasse and *Aspergillus niger* CCT0916 microorganism. It was used the 2^2 factorial experimental design, observing influence of fermentation temperature and substrate thickness on the response polygalacturonase activity, keeping constant the mass of moist substrate at 500 g during the experiments.

2. Method and Materials

2.1 Substrate

Cashew apple bagasse was obtained from fresh cashew fruit acquired at Empresa de Abastecimento de Serviços Agrícolas (Empasa) in Campina Grande City, Brazil. First, cashew nut was removed. Next, apple was triturated and pressed to separate the juice. Humid bagasse was dried with air renewal and circulation at 55 ºC. After drying process, bagasse was ground in TECNAL knife mill. The physico-chemical analysis of bagasse showed that this has the following characteristics: moisture 14.7 %(w.b), pH 4.15, reducing sugar 31.12 g/100g, solid soluble 36.67 ºBrix and pectin 10.67 %calcium pectate. As the particle size, 64 % of bagasse was retained in 20 mesh sieve, corresponding to 0.85 mm.

2.2 Fermentative process

The microorganism used was *Aspergillus niger* CCT0916, donated by Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, Fortaleza – Brazil). Spore concentration was 10^6 spores per gram of humid medium.

Substrate was hydrated with distilled water to obtain 50 %(w.b) of initial moisture content and it was diluted 1.5 %(w/w) of ammonium sulphate concentration in this volume. On polypropylene trays (Figure 1), it was weighed 500 g of sterilized humidified medium. After spore inoculation, medium was incubated at temperature by experimental design for 77 h.

Figure 1: Tray bioreactor

Enzyme extraction for the fermented complex was performed by adding 5.0 mL/g of fermented medium using 200 mM acetate buffer pH 4.5. The samples were the left in water bath for 1 hour at 30 ºC and filtered on Wattman 1 paper filter.
One unit of polygalacturonase activity was defined as the amount of enzyme that releases 1 µmol of galacturonic acid per minute of reaction at 35 ºC for 30 min.
A 2^2 factorial experimental design (Table 1) was conducted with 3 experiments at centre point to determine the influence of temperature fermentation (T_b) and substrate thickness (C) on the response polygalacturonase activity (PG).

Table 1: Concentration and tests from factorial design

Tests	Variables	
	C (mm)	T_b (ºC)
1	20 (-1)	25 (-1)
2	60 (+1)	25 (-1)
3	20 (-1)	35 (+1)
4	60 (+1)	35 (+1)
5	40 (0)	30 (0)
6	40 (0)	30 (0)
7	40 (0)	30 (0)

Model validation was done using Test F. This test shows the ratio between calculated F and tabulated F. When this ratio is greater than 1, the regression is statistically significant. For a statistically also predictive, value of this ratio must be greater than 4 (Rodrigues and Iemma, 2005).
Determination coefficient R^2 was also used as parameters to verify the fit between curve and experimental points. The maximum possible value is 1, meaning that there is no waste and all variation around the mean is explained by the regression.

3. Results and Discussion

Table 2 shows the values obtained for polygalacturonase activity (PG) for each test of factorial experimental design. The highest polygalacturonase activity 11.68 U/g was found with 40 mm of substrate thickness and 30 ºC of temperature at 21 h fermentation time.

Table 2: Polygalacturonase activities (PG) obtained with execution of experimental design

Tests	Fermentation time (h)						
	5	21	29	45	53	69	77
1	0	0	0	1.20	2.14	6.52	3.81
2	0	0.46	0.95	4.64	4.07	9.18	5.06
3	1.68	1.66	2.55	5.60	6.70	6.53	5.84
4	0	0.78	0	6.71	6.46	3.71	2.16
5	3.15	**11.68**	9.79	5.87	4.66	5.14	5.64
6	0	2.35	3.45	5.69	5.53	3.30	2.66
7	0	1.91	0.64	2.99	3.92	2.79	1.08

From the regression of polygalacturonase activity data, it was built a first order models to represent them with 95 % of confidence. Table 3 shows the empirical models for each fermentation time. The model that represents 53 h of fermentation was considered statistically significant as the coefficients in bold in the equations.

Table 3: Empirical models for polygalacturonase activity in each fermentation time

Empirical models	R^2	Teste F
$PG5 = 0.69 - 0.42C + 0.42T_b - 0.42CT_b$	0.2309	0.032
$PG21 = 2.69 + 0.10C + 0.50T_b - 0.34CT_b$	0.0149	0.002
$PG29 = 2.48 - 0.40C + 0.40T_b - 0.88CT_b$	0.0600	0.007
$PG45 = \mathbf{4.67} + 1.14C + 1.62T_b - 0.58CT_b$	0.7598	0.341
$PG53 = \mathbf{4.78} + 0.42C + \mathbf{1.74T_b} - 0.54CT_b$	0.9129	1.130
$PG69 = \mathbf{5.31} - 0.04C - 1.36T_b - 1.37CT_b$	0.4842	0.101
$PG77 = \mathbf{3.75} - 0.61C - 0.22T_b - 1.23CT_b$	0.3776	0.065

Figure 2 shows the profile of curve that represents the synergistic effects of fermentation temperature (T_b) and substrate thickness (C) on polygalacturonase activity response (PG).

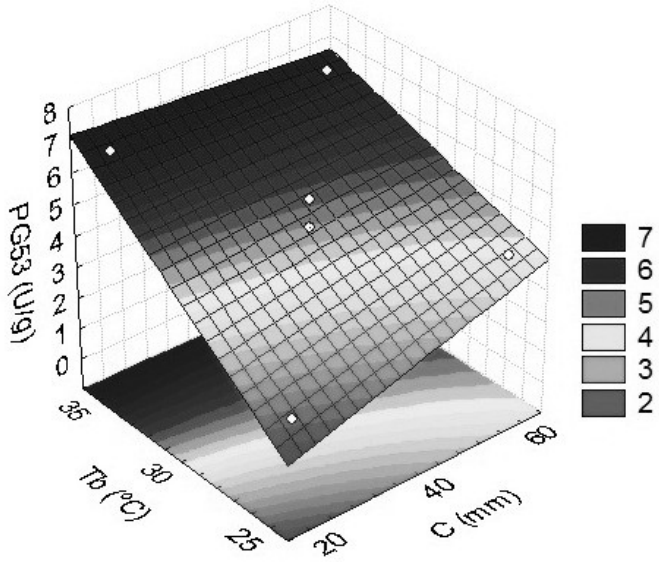

Figure 2: Surface response for polygalacturonase activity (PG) at 53 hours of fermentation: influence of fermentation temperature (T_b) and substrate thickness (C)

It is observed that fermentation temperature has greater influence on polygalacturonase activity (Figure 3). The highest activity calculated by the model (7 U/g) was achieved under conditions: 35 ºC fermentation temperature and 20 mm substrate thickness. It is observed through the response surface (Figure 2) that temperature and substrate thickness have positive effect on polygalacturonase activity, within the studied concentrations range and fermentation time of 53 h.

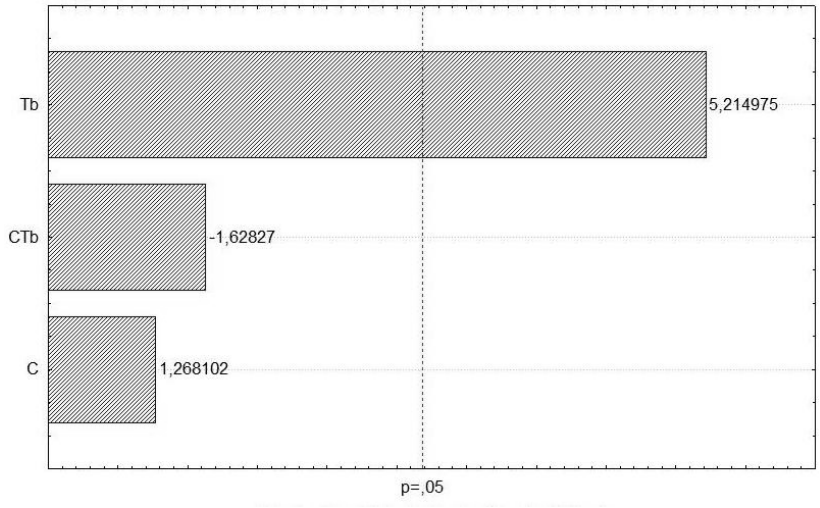

Figure 3: Pareto graphic for polygalacturonase activity (PG) at 53 hours of fermentation

According Pinto et al. (2006), temperature is also considered a critical factor due to the accumulation of metabolic heat generated during fermentation, directly affecting microorganism germination and product formation.

Comparing the development of fermentation between tray reactor (substrate mass of 500 g) and laboratory-scale fermentation (substrate mass of 10 g), described by Alcântara and Silva (2011), it is observed that maximum activities were obtained with temperatures and times fermentation different. In addition, polygalacturonase activity achieved with tray reactor was three times lower.

When the subject is solid state fermentation, a large number of works have been developed in laboratory scale, which is relatively easy task to control certain enzyme production parameters. On this scale, many enzyme productions by solid state fermentation process showed very promising. However, extrapolating these processes to pilot-scale bioreactors, which contains higher amount of substrate, the difficulties in fermentation conduct are revealed. Thus, in scale up bioreactors, substrate bed often reach above temperatures of ideal process, due difficulties to remove heat. So there is a possibility that these temperatures are denaturing the enzymes during fermentation (Santos et al., 2004).

4. Conclusion

The highest polygalacturonase activity 11.68 U/g was found with 40 mm of substrate thickness and 30 ºC of temperature at 21 h fermentation time. However, only the model for 53 h of fermentation was statistically significant. In this fermentation time, it was observed that temperature and substrate thickness have positive effect on polygalacturonase activity and fermentation temperature has greater influence on response.

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References

Alcântara S.R., Silva F.L.H., 2011, Influence of spores concentration, moisture, ammonium sulphate concentration and temperature on polygalacturonase production using cashew apple in the solid state fermentation process. Chemical Engineering Transactions, 24, 949-954, DOI: 10.3303/CET1124159.

Couto S.R., Sanromàn M.A., 2006, Application of solid state fermentation to food industry – A review. Journal of Food Engineering, 76, 291-302.

Gomes J., Zeni J., Cence K., Toniazzo G., Treichel H., Valduga E., 2011, Evaluation of production and characterization of polygalacturonase by *Aspergillus niger* ATCC 9642. Food and Bioproducts Processing, 89, 281-287.

Pandey A., 2004, Concise Encyclopedia of Bioresource Technology. Food Products Press, New York, USA.

Rodrigues M.I., Iemma A.F., 2005, Experimental design and optimization of processes: a strategy of sequential planning (In Portugese). Casa do Pão Editora, São Paulo, Brazil.

Santos M.M., Rosa A.S., Dal'Boit S., Mitchell D.A., Krieger N., 2004, Thermal denaturation: is solid-state fermentation really a good technology for the production of enzymes? Bioresource Technology, 93, 261-268.

Oliveira L.G.L., Ipiranga A.S.R., 2009, Sustainability and innovation in the productive chain of cashew in Ceará (In Portugese). Revista Eletrônica de Gestão Organizacional, 7, 252-272.

Pinto G.A.S., Brito E.S., Silva F.L.H., Santos S.F.M., Macedo G.R., 2006, Solid state fermentation: an alternative to the use and exploitation of agro-industrial residues (In Portugese). Revista Química Industrial, 74, 17-20.