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Influence of Medium Composition and pH on the Production of Polygalacturonases by *Aspergillus oryzae*

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ABSTRACT

A liquid medium containing wheat bran, salts and a source of inducer (pectin) was found to be suitable for the production of exo- and endo-polygalacturonases by Aspergillus oryzae *CCT3940. Induction of polygalacturonases by purified pectin was significantly higher than when rinds of citrus fruits were used as inducer.* A. oryzae *growth was favoured by pH close to 4, although a drop of pH to around 3 was needed for enzymes production. Afterwards, decreasing activities were observed with the normal increase in pH to near neutrality. The highest activities were achieved with an initial pH of 4 and controlled when it decreased to a value slightly below 3 (159 units endo-PG.mL^{-1} at 83 h and 45 units exo-PG.mL^{-1} at 64 h), being the loss in polygalacturonases activities strongly reduced at this condition. The best values of pH and temperature for the action of exo-PG (4.5/57ºC) and endo-PG (4.3/40ºC) were assessed.*

Key words: Pectinase, polygalacturonase, submerged process, production medium, pH

INTRODUCTION

Pectinolytic enzymes act on pectin, a complex polysaccharide which occurs mainly in the middle lamella of higher plants (Rombouts and Pilnik, 1980). Due to the great structural diversity of the pectin present in different plant tissues, pectinases have many different mechanisms of action and can be divided into two broad groups, de-polymerising enzymes which break α-1,4 linkages in the principal pectin chain such as polygalacturonase (poly-[1,4-α-D galacturonide] glycanohydrolase, E.C.3.2.1.15), pectin lyase (poly-[1,4-α-D methoxygalacturonide] lyase, E.C. 4.2.2.10) and pectate lyase (poly-[1,4-α-D galacturonide] lyase, E.C. 4.2.2.2) and de-methoxylating enzymes such as pectinesterase (pectin pectylhydrolase, E. C. 3.1.1.11) which de-esterify pectin from pectic acid by removing methoxyl residues.

Pectinases play an important role in the food industry, being used in the extraction and clarification of fruit juices and wines as well as the maceration of vegetables and fruits to facilitate the extraction of essential oils and the production of baby foods (Rombouts and Pilnik, 1980; Alkorta et al., 1998). Another important application for pectinases is the textile industry, especially in the treatment of natural fibers such as linen and ramie (Baracat et al., 1991).

Strains of the filamentous fungus *Aspergillus niger* are most often used in the commercial production of pectinases because they are classified as 'generally regarded as safe' (GRAS) which means that enzymes derived from them are acceptable for use in the food industry (Pariza and Foster, 1983).

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Other filamentous fungi which produce pectinases - *Aspergillus oryzae* (Maiorano, 1990; Dartora et al., 2002), *Penicillium italicum* and *Penicillium expansum* (Alkorta et al., 1998) - have been also studied. In previous works (Maiorano, 1990; Malvessi, 2000), *A. oryzae* CCT3940 showed higher endo-polygalacturonase activities when compared to different strains of *A. niger* .

Independent of the process used for the production of fungal pectinases, several aspects should be carefully considered, e.g. the selection of the production strain and the formulation of a balanced culture medium containing carbohydrates, nitrogen source, vitamins and mineral salts (Rombouts and Pilnik, 1980). In general, pectinases are induced enzymes, and because of this it is necessary to supplement the culture medium with pectin or with raw-materials rich in pectin, such as bagasse from sugar-beet, apples, sugarcane or citrus-fruit rinds (Maldonado et al., 1986; Solis-Pereira et al., 1996). Other important parameters involved in pectinase production are pH (Ueda et al., 1982; Galiotou-Panayotou et al., 1997), temperature (Bailey, 1990) and oxygen supply (Zetelaki-Horváth and Vas, 1981), all of which exert a great influence on the results of the process.

In this context, the objectives of the work described in this paper were to define a low-cost medium and to investigate the influence of pH on the production of polygalacturonases by *A. oryzae*, in batch submerged culture. Furthermore, the effect of pH and temperature on the activities of the polygalacturonases produced was assessed.

MATERIALS AND METHODS

Aspergillus oryzae CCT3940 (Universidade Estadual de Campinas, Brazil) was used in this study. The strain was plated onto glycerine agar (Maiorano, 1990) and incubated at 28 °C for five days and plates were stored at 4 °C until needed. Replicate cultures were made monthly.

Production medium

Wheat bran (WB) medium (Maiorano, 1990) was used as the basal media, which contained ($g.L^{-1}$): wheat bran (MOINHO NORDESTE, Antonio Prado, Brazil), 40; citric pectin (DELAWARE, Porto Alegre, Brazil), 10; yeast extract (SIGMA, St. Louis, USA), 0.5; $(NH_4)_2SO_4$, 5.0; KH_2PO_4, 2.5; $MgSO_4$, 0.5; $FeSO_4.7H_2O$, $6.3x10^{-5}$; $ZnSO_4$; $6.2x10^{-5}$; $MnSO_4$, $1.0x10^{-6}$. The pH was adjusted to 4 before autoclaving at 121°C for 20 minutes.

To determine the effect of wheat bran on pectinase production, medium was supplemented with 10, 20, 40 or 60 $g.L^{-1}$ of wheat bran, while keeping the other media components constant.

In another experiment, citric pectin was replaced by the dried rinds of *Citrus latifolia* Tanaka (Tahiti lime) or *Citrus deliciosa* Tenore (tangerine) containing about 30% (w/w, dry weight basis) pectin (Fogarty and Kelly, 1983) at 30, 60 and 90 $g.L^{-1}$. Other media components were as for the standard WB media.

The effect of yeast extract on endo-PG activity production was evaluated by adding SIGMA yeast extract and the less refined, cheaper, PRODEX yeast extract (PRODESA, Valinhos, Brazil) in the production medium (0.025 to 0.5 $g.L^{-1}$). Other media components were as for the standard WB medium.

Cultivation was carried out in Erlenmeyer flasks or in a bioreactor and in both cases the inoculum consisted of a suspension of *A. oryzae* CCT3940 spores to give $1x10^5$ $spores.mL^{-1}$ after inoculation. The influence of different media compositions and initial pH (2, 3, 4, 5, 6 and 7) on the production of endo-PG was evaluated in 500 mL Erlenmeyer flasks containing 100 mL of inoculated WB incubated at 28 °C in a shaker (CERTOMAT U, B. BRAUN BIOTECH, Melsungen, Germany) at 300 rpm. Samples were collected from 24 to 144 h, centrifuged for 20 min at 10000 rpm and stored at 4 °C for subsequent analysis. The results for the different media were compared in terms of endo-PG activity, which was assayed as described below.

Studies on the effect of pH on the production of exo- and endo-PG were carried out in a 5-litre BIOSTAT B (BRAUN BIOTECH, Melsungen, Germany) with a working volume of 3.5 L of WB medium containing 10 $g.L^{-1}$ DELAWARE citric pectin. Agitation was at 300 rpm and aeration at 2 $L.min^{-1}$. Dissolved oxygen concentration (pO_2) was kept at a minimum of 30% of saturation. Four pH conditions were compared: an initial pH of 4 with no control during the experiment; an initial pH of 4 with the pH being maintained by the automatic addition of 2N HCL or 3M NaOH; an initial pH of 3 with the automatic maintenance of the pH at this level; and an initial pH of 4 which was allowed to drop to pH 2.7 at which it was automatically maintained until the end of the process.

In all cases, samples were collected periodically, centrifuged at 10000 rpm for 20 min and stored at 4°C for subsequent evaluation of free reducing sugars (RS), total reducing sugars (TRS) and exo- and endo-PG.

The influence of pH (3.5 to 5.5) and temperature (20 to 70 °C) on exo- and endo-PG activities was evaluated using the crude enzyme preparation produced after 96 h cultivation in flasks (described above). For these assays, the samples of culture media were diluted when necessary, the diluent being acetic acid-acetate buffer 0.05 M for experiments involving endo-PG and 0.1 M for exo-PG, at the suitable pH values. The temperature and pH values that gave the highest enzyme activities were combined and used in the repeat experiments to optimise the enzyme assays.

Free reducing sugars were quantified by the dinitrosalicylic acid (DNS) method (Miller, 1959), and total reducing sugars by an adaptation of the method proposed by Bittman (1974), based on the hydrolysis in 1.5M H_2SO_4 of the material pelleted by centrifugation followed by neutralisation with 4N NaOH, clarification with $K_4F_6(CN)_6.3H_2O$ (150 $g.L^{-1}$) and $CuSO_4.5H_2O$ (300 $g.L^{-1}$) and the subsequent quantification of the reducing sugars liberated by the DNS method (Miller, 1959).

The endo-PG activity was determined by measuring the reduction of viscosity of a standard pectin solution. For this estimation 3.2 mL of diluted sample were mixed with 14.8 mL of a 1% (w/v) solution of citric pectin (DELAWARE, Porto Alegre, Brazil) in 0.05 M acetic acid-acetate buffer (pH 4) and the reaction mixture incubated at 30°C for 30 min, after which the viscosity was measured in a DV-II$^+$ viscometer (BROOKFIELD ENGINEERING, Middleboro, USA). One unit of endo-PG activity was defined as the quantity of enzyme which caused a 50% reduction in viscosity of the reaction mixture after 30 min incubation at 30°C (Gainvors et al., 2000).

For the evaluation of exo-PG, 50 µL of diluted sample of culture media were added to 2 mL of a 0.25% (w/v) of polygalacturonic acid (SIGMA) in 0.05 M acetic acid-acetate buffer (pH 4) and the reaction mixture incubated at 35°C for 30 min, after which the quantity of reducing sugars liberated was measured by the Somogyi (1952) method, using D-galacturonic acid (SIGMA) as a standard. One unit of exo-PG activity was defined as the quantity of enzyme which liberated 1 µmole of reducing sugar, measured as galacturonic acid, from polygalacturonic acid . mL^{-1} culture media . $minute^{-1}$ (Couri, 1993).

Maximum specific growth rates ($\mu_{X,m}$) in the bioreactor experiments were calculated as suggested by Malvessi (2000). In bioreactor runs, M_{O2} was determined by integration of the area under the oxygen demand curves using the dynamic method of Wang et al. (1979) and process time. To calculate $\mu_{X,m}$ for the exponential growth phase the following equation was used:

$$\mu_{X,\,m} = \frac{d\,(\ln M_{O2})}{dt}$$

RESULTS AND DISCUSSION

The results of the experiments to evaluate the effects of the inducer (pectin) concentration on the production of endo-PG are shown in Fig. 1, where a comparison is made between WB medium without inducer and WB media with 5, 10, 20 or 30 $g.L^{-1}$ of pectin. In the absence of pectin, very low endo-PG activities were detected, which demonstrated the need for an inducer to stimulate endo-PG production in processes using *A. oryzae* CCT3940. In the presence of 5 $g.L^{-1}$ of pectin, endo-PG activity reached 125 $U.mL^{-1}$ after 120 h cultivation. A maximum endo-PG value of 177 $U.mL^{-1}$ was attained with 10 $g.L^{-1}$ of pectin, again after 120 h. Using 20 $g.L^{-1}$ of pectin, an endo-PG peak was observed at 72 h, but at 96 h there was no significant difference with 10 and 20 $g.L^{-1}$ of pectin. At 30 $g.L^{-1}$ pectin, there was a decrease in endo-PG activity, although this might have been due to the increase in viscosity of the medium that resulted in difficulty in maintaining a homogenous culture and oxygen transfer. The peak values for endo-PG coincided with low pH values, between 3,5 - 4,5 (data not shown).

The Tahiti lime and tangerine rinds used as endo-PG inducers resulted in a maximum of 50 $U.mL^{-1}$ after 96 h when the rinds were present at 30 $g.L^{-1}$. With higher concentrations, the viscosity of the culture medium increased significantly, which could have been the reason for the insignificant endo-PG activities obtained at higher concentrations of rind. These results indicated that other inducers, having less effect on the rheology of the medium, needed to be tested as a cheaper alternative to purified pectin.

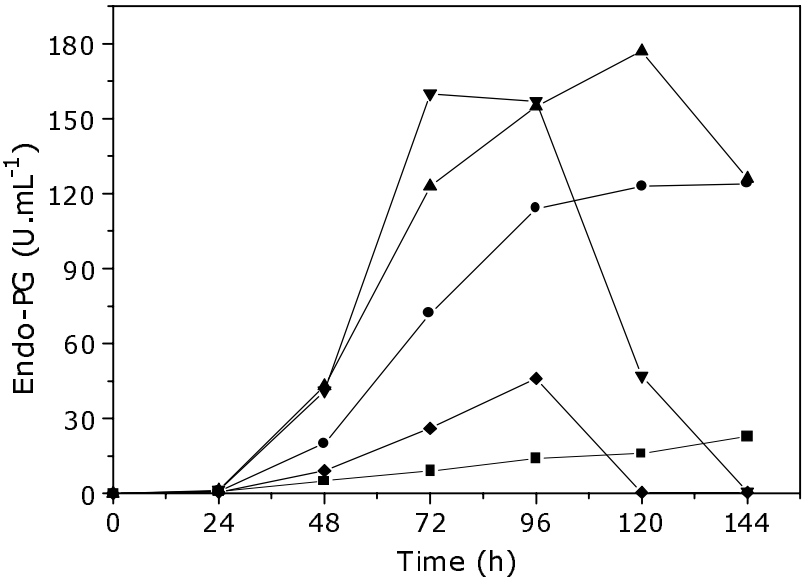

Figure 1 - Variation in endo-polygalacturonase activity with time during the cultivation of *Aspergillus oryzae* CCT3940 in shaken flasks containing wheat bran media with different pectin concentrations. (■) 0 $g.L^{-1}$; (●) 5 $g.L^{-1}$; (▲) 10 $g.L^{-1}$; (▼) 20 $g.L^{-1}$; (◆) 30 $g.L^{-1}$

The effect of the concentration of wheat bran and yeast extract on endo-PG activity in the process was evaluated in 96 h shake-flask tests. With 10, 20 and 40 $g.L^{-1}$ of wheat bran, increasing endo-PG activities were achieved (46, 86 and 171 $U.mL^{-1}$, respectively). With 60 $g.L^{-1}$, although a good activity was attained (150 $U.mL^{-1}$), the rheology of the medium indicated that serious mass transfer and manipulation problems would occur in high-volume cultivation, and because of this we recommend the use of 40 $g.L^{-1}$ of wheat bran.

Yeast extract did not exert any marked influence on the production of enzyme, with about 170 units endo-PG . mL^{-1}, after 96 h cultivation, being attained for both types of yeast extract tested. It seemed that the high concentration of wheat bran in the medium might have provided the various amino acids, vitamins and B-complex factors necessary for *A. oryzae*.

The strong effect of pH on the production of endo-PG was clearly observed in flask cultures, where pH values of 2-7 were tested (Fig. 2). The maximum endo-PG activity occurred with an initial pH of 4, activity reaching 170 $U.mL^{-1}$ at 120 h. When the initial pH was 5, maximum endo-PG was 135 $U.mL^{-1}$ after 96 h of cultivation, while in medium with an initial pH of 3, there was a gradual increase in endo-PG activity, which reached a maximum of 125 $U.mL^{-1}$ after 144 h of cultivation. The lowest level of endo-PG activity was detected when the initial pH was 6 (endo-PG activity of 103 $U.mL^{-1}$ at 72 h). There was no appreciable endo-PG activity in the flasks containing media at an initial pH of 2 or 7.

In interpreting these results both mycelial growth and pH variation should be considered because there was clear evidence that the growth of *Aspergillus* strains was best at about pH 4 while the highest endo-PG activities occurred at pH of about 3 (Maldonado et al., 1986 e Galiotou-Panayotou et al., 1997). At pH values of 2-3, there was less cell growth, and this endo-PG activity was also lower, while at pH values of 4, 5 and 6 the pH was favourable for mycelial growth in the first 24 h which resulted in elevated peak endo-PG activities. When the pH was 7, there was a decrease in pH to pH 3.5 in 48 h, which might have favoured fungal growth, although this minimum value was still too high for endo-PG formation. Following the process time when the maximum endo-PG activities were achieved, a drop occurred in cultures with initial pH values of

4 to 7, this drop possibly being related to the increase in pH to values which could have favoured the action of proteases, which might have been released to the medium, with the capacity to degrade pectinases present in the cultures. Similar effect was previously described by Kusters van Sorensen et al. (1992) for pectin-lyase produced by *Aspergillus niger*.

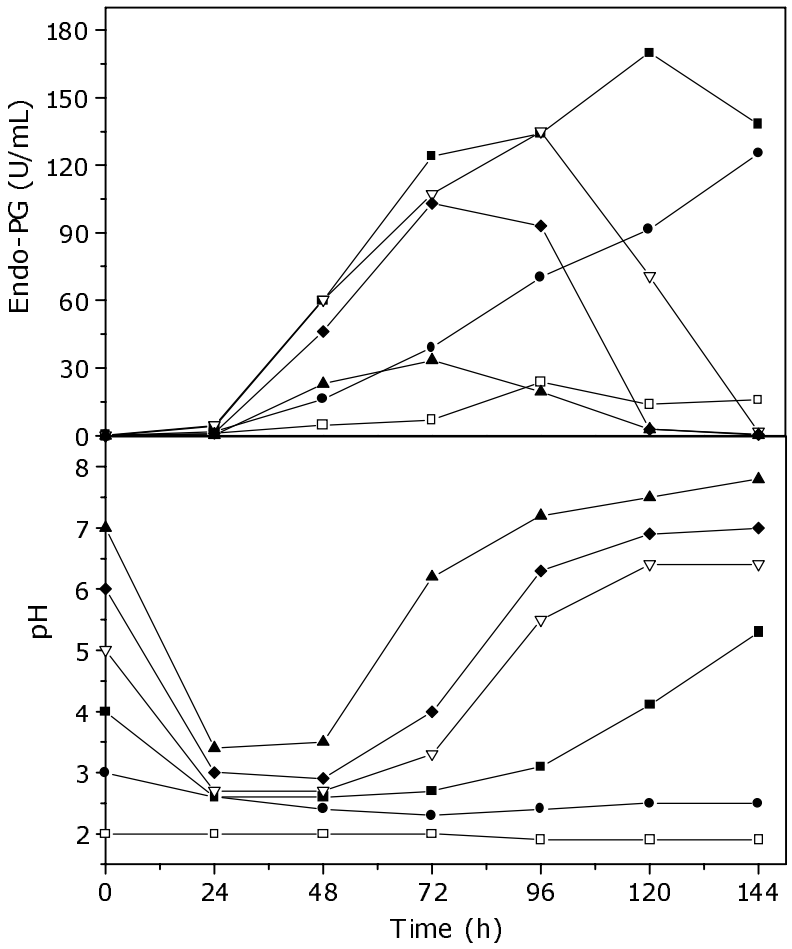

Figure 2 - Variation in endo-polygalacturonase activity and pH with time during the cultivation of *Aspergillus oryzae* CCT3940 in shaken flasks containing wheat bran media with 10 $g.L^{-1}$ of pectin at different initial pH values. ( ) pH 2; (●) pH 3; (■) pH 4; (∇) pH 5; (◆) pH 6; (▲) pH 7

The results on the effect of pH on the formation of exo- and endo-PG are summarised in Table 1. It can be seen that the best yields and productivities for both enzymes occurred in the experiments in which the pH freely decreased from 4 to approximately 3, as observed in flasks experiments. The $\mu_{X,m}$ values were highest when the initial pH was 4 or the pH was automatically adjusted to this value.

Fig. 3 shows the kinetic profile of the bioreactor experiment in which the initial pH was 4 and not controlled throughout the process. In this experiment, we obtained maximum endo-PG activities of 134 $U.mL^{-1}$ at 97 h (similar to what occurred in flask culture) and a maximum exo-PG activity of 54 $U.mL^{-1}$ also at 97 h. After this, there was a rapid drop in activities for both enzymes.

Table 1 - General results for the cultivation of *Aspergillus oryzae* CCT3940 in a 5-litre aerated bioreactor using wheat bran medium, at 28°C.

	pH 4, no control	**pH 4, constant**	**pH 3, constant**	**pH 4, controlled at pH 2.7**
$\mu_{X,m}$ (h^{-1})	0.20	0.30	0.15	0.20
Endo-PG $_{máx}$ ($U.mL^{-1}$)	134	4.5	64	159
Exo-PG $_{máx}$ ($U.mL^{-1}$)	54	3.5	22	45
$Y_{endo\text{-}PG/ART}$ ($U.g^{-1}$)	5394	385	3162	4869
$Y_{exo\text{-}PG/ART}$ ($U.g^{-1}$)	12182	487	7780	16737
$p_{endo\text{-}PG}$ ($U.mL^{-1}.h^{-1}$)	1,38	0,05	0,41	1,92
$p_{exo\text{-}PG}$ ($U.mL^{-1}.h^{-1}$)	0,56	0,09	0,34	0,70

$\mu_{X,m}$, maximum specific growth rate; Endo-PG_{max}, Exo-PG_{max}, maximum enzymes activities; $Y_{exo\text{-}PG/TRS,}$ $Y_{endo\text{-}PG/TRS}$, exo and endo-PG yields at maximum enzymes activities; $p_{exo\text{-}PG,}$ $p_{endo\text{-}PG,}$ exo and endo-PG productivities at maximum enzymes activity.

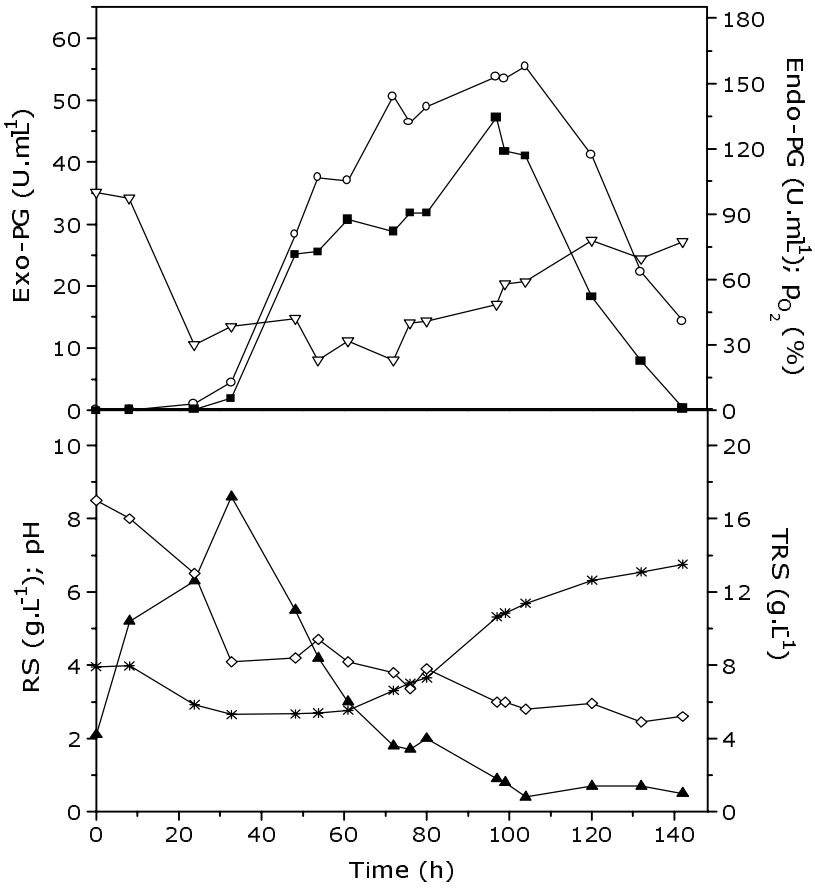

Figure 3 - Variation in exo and endo-polygalacturonase activities, dissolved oxygen concentration (p_{O2}), reducing sugars (RS), total reducing sugars (TRS) and pH with time during the cultivation of *Aspergillus oryzae* CCT3940 in bioreactor using wheat bran medium with 10 $g.L^{-1}$ pectin and a non-controlled initial pH of 4. (■) endo-PG; (o) exo-PG; (∇) p_{O2}; (▲) RS; (◊) TRS; (✳) pH.

In the early stages of cultivation, the concentration of reducing sugars was about 2g.L^{-1}, attaining a maximum concentration of ca. 9 g.L^{-1} at 30-40 h. This was probably due to the hydrolysis of starch present in the wheat bran by amylases excreted by *A. oryzae*, although further work would be needed to confirm this hypothesis. In this case, the accumulation of reducing sugars could be explained by the fact that the rate of starch hydrolysis was greater than the consumption of sugars by the low *A. oryzae* concentration. The minimum concentration of reducing sugars occurred at 100-110 h, coinciding with the formation of enzymes. Total reducing sugars decreased up to 80-100 h of process, attaining a plateau of ca. 6 g.L^{-1} (Fig. 3). According to Bertolin et al. (2001), the method of Miller may overestimate the amount of TRS in wheat bran. As such, the concentration of residual TRS in bioreactor experiments most probably originates from reducing compounds, which cannot be metabolised by *A. oryzae*, resulting from the analytical method used.

The dissolved oxygen concentration, automatically controlled at 30% of saturation 20 h after inoculation started to rise again at about 70 h due to the reduction in the metabolic activity. In the experiments in which the pH was controlled at 4 during the total process, increased fungal biomass production was observed in the early part of the process, as indicated by the $\mu_{X,m}$ value of 0.30 h^{-1} (Table 1). Later, however, there was intense oxygen consumption, with a rapid fall in the concentration of dissolved oxygen, which remained below 30% of saturation during a significant proportion (15-50 h) of the process. At the end of the process (as indicated by the levels of reducing sugars and total reducing sugars and by interruption in the formation of pectinases) the dissolved oxygen again began to rise. These experiments gave insignificant results in terms of exo- and endo-PG activities as compared to the previously described experiment (Table 1), this being clearly related to the fact that production of these enzymes normally occurred at low pH values.

The experiments in which the pH was controlled at pH 3 showed that this pH was inadequate for fungal growth, as demonstrated by the fact that the $\mu_{X,m}$ value was 0.15 h^{-1}. The endo-PG activity increased during the process, but reached 64 U.mL^{-1} after 154 h, while the exo-PG activity remained practically unaltered from 63 h onwards at 22 U.mL^{-1} (Table 1). In this condition we observed that the exo- and endo-PG activities, although inferior to those previously obtained, did not present the characteristic reduction in the final hours of cultivation. The highest concentration of reducing sugars occurred after 40 h cultivation, and was followed by drop in the reducing sugar concentration that remained at about 2 g.L^{-1} until the interruption of the process. The concentration of TRS dropped slowly up to about 120 h, which was followed by a steep rise to about 7 g.L^{-1} at which it remained until the end of the process. The concentration of dissolved oxygen reached a minimum saturation of 30 % after 30 h cultivation until about 65 h when it started to rise again.

In the experiments in which the initial pH value of 4 was allowed to drop to pH 2.7 and then automatically maintained at this value for the rest of the process the $\mu_{X,m}$ value of 0.20 h^{-1} was inferior to the $\mu_{X,m}$ value of 0.30 h^{-1} which occurred when the pH was maintained at pH 4 throughout the process, and it is reasonable to assume that fungal growth was prejudiced after 24 h when the drop in pH occurred. As shown in Fig. 4, the highest concentration of reducing sugars in this condition occurred after about 40 h of cultivation, with a subsequent decrease up to about 70 h followed by a final phase in which the concentration remained approximately constant. Regarding total reducing sugars, these were consumed up to 70 h after which there was the characteristic plateau already discussed. As also shown in Fig. 4, the maximum endo-PG activity was 159 U.mL^{-1} and occurred after 83 h of cultivation. After this peak value there was a tendency to maintain this activity for over 50 h, the opposite to what happened in the experiments in which the pH was not controlled. The exo-PG activity reached a maximum value of about 45 U.mL^{-1} after 64 h of cultivation, and there was practically no variation in exo-PG activity until the interruption of the process.

The results of these experiments are coherent with the hypothesis discussed previously regarding the possible degradation of polygalacturonase by proteases at higher pH values, suggesting that the maintenance of a low pH will limit this effect. This finding indicated the possibility of maintaining high levels of enzyme activities even after the total consumption of substrate, preserving the product prior to downstream processing.

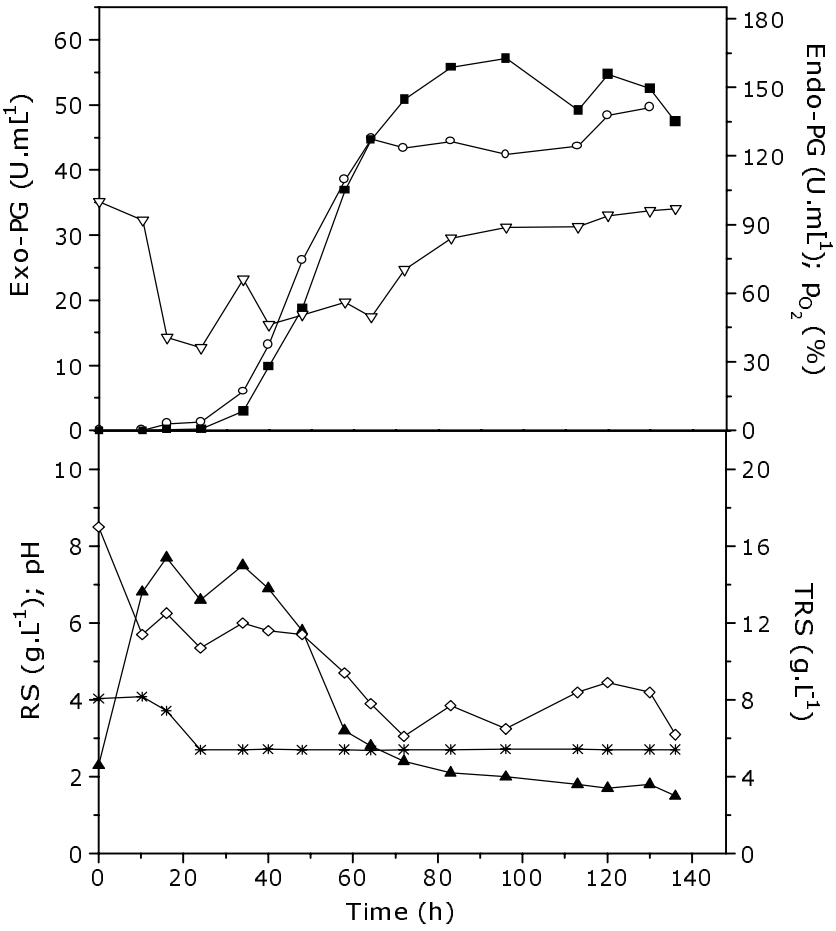

Figure 4 - Variation in exo and endo-polygalacturonase activities, dissolved oxygen concentration (p_{O2}), reducing sugars (RS), total reducing sugars (TRS) and pH with time during the cultivation of *Aspergillus oryzae* CCT3940 in bioreactor using wheat bran medium with 10 $g.L^{-1}$ pectin and a initial pH of 4 which was maintained at constant pH after reaching pH 2.7. (■) endo-PG; (o) exo-PG; (∇) p_{O2}; (▲) RS; (◊) TRS; (✳) pH

The data obtained from the method used for endo-PG activity determination is strongly dependent on the type of pectin used to prepare the substrate solution. As such, in terms of endo-PG, the results presented in this work could not be compared with others described in the literature. With respect to exo-PG however, our results could be compared to the maximum exo-PG activity of ca. 30 $U.mL^{-1}$ reported by Couri et al. (2000) and Taragano et al. (1997), both working with wheat bran media and *A. niger* strains, although significant differences in the experimental conditions were observed in those works.

The influence of pH and temperature on the activities of both polygalacturonases were also assessed. In experiments with different pH values highest endo-PG activities were found at pH values of about 4.7 and, as shown in Fig. 5, the measured activities were more than 50 % higher than they were at pH 4.0, the pH normally used in evaluating endo-PG activities.

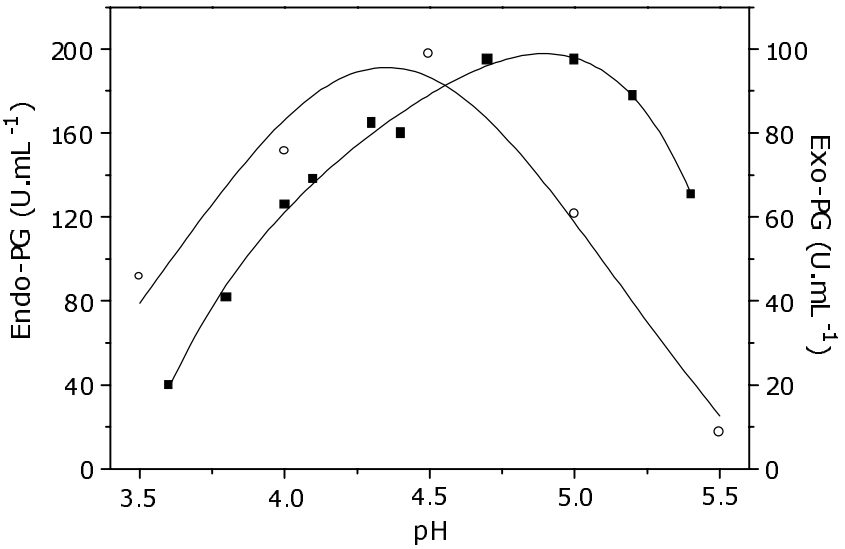

Figure 5 - Variation in endo-polygalacturonase (■) and exo-polygalacturonase (o) activities of *Aspergillus oryzae* CCT3940 with pH, at 30 and 35 °C respectively.

As also shown in Fig. 5, the response of exo-PG activity to changes in pH was clearly more accentuated than the response of endo-PG activity, a fact which was evidenced by a peak of 99U.mL^{-1} at pH 4.5 under the standardised conditions used in the experiment.

In relation to the effect of temperature on the activity of endo-PG, between 35 and 40 °C (at pH 4.0) a peak in activity of 250 U.mL^{-1} was seen, although above 40 °C there was a decrease in activity with total loss of catalytic activity at 60 °C. The activity of exo-PG was highest in the range 55-60 °C and attained about 150 U.mL^{-1}, although there was a high reduction in activity above 60 °C.

In further experiments with endo-PG the best conditions of pH and temperature were combined by using pH 4.3 and 40 °C and attained a maximum endo-PG activity of 320 U.mL^{-1}, significantly higher than that obtained using the standard conditions (ca. 180 U.mL^{-1}). It was interesting to note that at 40 °C the highest endo-PG activities occurred at pH values lower than at 30 °C, which could have been related to the stability of endo-PG at these temperatures and pH values, something that needed further study. For exo-PG, we obtained an activity of 198 U.mL^{-1} at 57 °C and pH 4.5, which was a significant increase in comparison with that found under normal conditions (ca. 76 U.mL^{-1}). It seemed that, as in the case of endo-PG, more studies are needed on the stability of exo-PG in relation to pH and temperature in which the time during which the assay is carried out is also considered.

RESUMO

Um meio líquido contendo farelo de trigo, sais e fonte de indutor (pectina) foi definido para a produção de exo e endo-poligalacturonases por *Aspergillus oryzae* CCT3940. A indução por pectina purificada foi significativamente maior que a observada com cascas de cítricos. O crescimento de *A. oryzae* é favorecido por valores de pH próximos a 4, embora uma queda até valor em torno de 3 seja necessária para a produção das enzimas. Posteriormente, atividades decrescentes foram observadas com a subida normal do pH até próximo à neutralidade. As maiores atividades foram alcançadas quando o processo foi iniciado em pH 4 e controlado quando decresceu até níveis ligeiramente abaixo de 3 (159 unidades.mL^{-1} para endo-PG, em 83 h, e 45 unidades.mL^{-1}, em 64 h, para exo-PG), com a perda de atividades sendo drasticamente reduzida. Os melhores valores de pH e temperatura para a ação de exo-PG (4,5/57°C) e endo-PG (4,3/40ºC) foram estimados.

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