Continuous production of pectinase by immobilized yeast cells on spent grains

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Pectinases are a group of enzymes responsible for the hydrolysis of pectic materials found in plants. According to the specific pectic substrate and catalytic activity, pectinases include pectin-lyases, exopolygalacturonases, endopolygalacturonases, pectinesterases… They are important industrial enzymes, used in food and beverages industries for cloud point stabilization in juices, to increase the pulp extraction from fruits and vegetables, in cocoa beans fermentation and for soluble tea preparations. More recently, they have been used in textile industries, for degumming of fibre crops, in wastewater treatment, or paper industries [1]. Currently, *Aspergillus niger* is the preferred source of industrial pectinases.

A yeast strain excreting endopolygalacturonase (*Kluyveromyces marxianus* CCT 3172, found in cocoa fermentations in Brazil (2, 3)) was used in this work to study the possibility of continuous production of this enzyme. It is a feasible and interesting alternative to fungi batch production essentially due to the specificity of the type of pectinase excreted by *K. marx.* CCT 3172, to the lower broth viscosity and to the consequent easier downstream operations.

In order to increase the reactors' productivity, a cellulosic carrier obtained from barley spent grains (a by-product from brewing industries) was tested as an immobilization support. Previous experimental work with *Saccharomyces uvarum* showed that this carrier was very efficient due to its high yeast loading capacity, easy preparation, reusability, availability and to its inert, non-toxic nature (4, 5). Two types of reactors were studied for pectinase production using glucose as carbon and energy source – a continuous stirred tank reactor (CSTR) and a packed bed reactor (PBR) with a recycled flow.

Results and Discussion

Cell immobilization was achieved by cell-carrier adhesion, cell-cell attachment and by spatial retention of cells inside pores and crevices of the supports' surface. Beside these mechanisms, the sponge-like structure created inside the PBR worked as a filter layer allowing for local accumulations of yeast biomass visible with naked eye. The maximum immobilized biomass load obtained for the strain *Kluyveromyces marxianus* CCT 3172 inside the CSTR was 0.320 $g_{biomass}\ g^{-1}_{carrier}$. At the end of the operation time, the PBR reactor had a biomass load of 0.247 $g\ g^{-1}$ carrier at the bottom and 0.204 $g\ g^{-1}$ at the top.

The pectinase productivity was calculated from assays carried out in both reactors. During the operation, the free biomass concentration, glucose concentration and pectinase activity were measured at the reactors outlet. As it is shown in the Figure, the CSTR pectinase productivity values are very low for all the dilution rates and glucose concentrations on the inlet. Although this difference in productivity values obtained for CSTR and PBR was not expected, some reasons could be found to explain it. It has been reported for several *Kluyveromyces* and *Saccharomyces* strains that the production of endopolygalacturonase depends upon the dissolved oxygen concentration in the culture media. Wimborne and Rickard (6) found that under anaerobic conditions, the enzyme was produced, but at 60 % of oxygen saturation its production was completely repressed.

Cruz-Guerreiro and co-workers (7) studied the strain *K. marxianus* CDBB-L-278 and found that a dissolved oxygen concentration of 3.3 mg l^{-1} was the threshold for repression of endoPG production, regardless of the culture temperature. Although the broth inside the CSTR had no direct air sparging, a sterile air inlet was used at its headspace to avoid contamination. Together with the mechanical stirring, this possibly led to a higher dissolved oxygen concentration in the CSTR than in the PBR.

For the PBR productivity values, a linear increase with the dilution rate was found for both inlet sugar concentrations tested (Figure). This same tendency was observed for the specific pectinase production rate (q_P in U g^{-1} biomass h^{-1}) and in the specific glucose consumption rate (q_S in g consumed glucose g^{-1} biomass h^{-1}). The highest value for pectinase volumetric productivity (P_V = 0.98 U $ml^{-1}h^{-1}$) was achieved in the PBR for a D = 0.40 h^{-1}, a glucose concentration on the inlet of S_{in} = 20 g l^{-1} and a biomass load in the support of X_i = 0.225 g g^{-1}.

The *K. marxianus* CCT 3172 wild type strain was isolated from a cocoa fermentation, where its activity was related to the breakdown of the pectic materials on the surface of cocoa beans (2). Its optimal environment should therefore be a culture over a solid lattice, with no hydrodynamic stress and a very low oxygen concentration. These conditions are to a certain extent similar to those found inside the PBR.

The highest volumetric productivity value is 10 times higher than the one obtained for the *K. marxianus* CCT 3172 strain in a non-aerated batch culture (Pereira M., unpublished work).

It was possible to conclude that a high dilution rate, together with a high biomass load and complete glucose consumption are the optimal conditions for continuous endopolygalacturonase production.

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Figure Pectinase volumetric productivity for different inlet concentrations of glucose, S_{in}. PBR data are shown as filled symbols and CSTR data as empty symbols. Triangles correspond to S_{in} = 10 g l^{-1}, squares to S_{in} = 20 g l^{-1}, diamonds to S_{in} = 30 g l^{-1}, circles to S_{in} = 40 g l^{-1}.