30th Conference SSCHE, Proceedings on CD ROM, Tatranské Matliare (SK), 26 – 30 May, 2003

Comparative analysis of immobilization carriers for a endopolygalacturonase producing yeast strain

Catarina Almeida [1,2], Tomáš Brányik [1], Pedro Moradas-Ferreira[3], José Teixeira [1*]

1 – **CEB - Instituto de Biologia e Química Fina** Universidade do Minho, Campus de Gualtar 4710-057 BRAGA – PORTUGAL, Email: jateixeira@deb.uminho.pt
2 - **Instituto Superior de Ciências da Saúde-Sul** Quinta da Granja, 2829-511 MONTE DA CAPARICA – PORTUGAL
3 – **Instituto de Biologia Molecular e Celular** Rua do Campo Alegre, 823, 4150-180 PORTO - PORTUGAL **Instituto de Ciências Biomédicas Abel Salazar** Universidade do Porto, Largo Prof. Abel Salazar, 2 - P-4099-003 PORTO - PORTUGAL
(*Corresponding author)

Key words: Continuous reactors, immobilized yeast cells, pectinase

Introduction

Pectinases are a group of enzymes acting on pectin and other pectic substances found in vegetable tissues. Pectin consists of a α-1,4 polymer of D-galacturonic acid; the main chain is 60 – 90 % methylated and includes rhamnose units and side chains of arabinan, galactan and arabinogalactan. This complex polymer has a structural, scaffolding function in the primary cell wall and in the middle lamella of plant tissues (Naidu and Panda, 1998). In natural habitats, several microorganisms excrete different pectolytic enzymes to invade the cell walls and thus grow on the rich substrates found in plants. In the industrial world, pectinases have found use in any process that deals with extracting juices from fruits and vegetables and in the processing of plant tissues. Some examples are: fruit juice (apple, banana, mango, orange, guava…) clarification and viscosity reduction, as a preliminary grape treatment in wine industries, in tomato pulp extraction, in chocolate and tea fermentation, vegetable waste treatment, fiber degguming in textile industries and paper industries (Kashyap, e*t al.* 2001). *Aspergillus niger* is currently the only microorganism used for pectinase industrial production. It excretes a mixture of pectolytic enzymes (polygalacturonases, polymethylgalacturonases, pectin lyases and pectin esterases) together with other degrading enzymes such as arabinofuranosidases and amyloglucosidases (Blanco *et al*., 1999). In fact, commercial pectinase is a blend of enzymes. This can be useful due to the complexity of plant tissues and all the different chemical bonds to hydrolyse, but in some industrial cases a specific type of pectinase, or a specific blend is needed (Manachini *et al.*, 1988). The yeast strain *Kluyveromyces marxianus* CCT 3172 was selected from a cocoa fermentation as a good endopolygalacturonase producer (Schwan and Rose, 1994, Schwan *et al.,* 1997).

Continuous production of an endopolygalacturonase from yeast would be an interesting alternative to the current fungal batch production. To increase continuous reactors' productivity, high cell density systems can be used. Some carriers have been

successfully tested for yeasts and bacteria immobilization, namely porous materials such as porous glass and ceramic, synthetic polymers, cellulosic fibers and cellulose derivatives, activated charcoal, artificial polymers and gel matrixes as k-carrageenan, Ca and Ba alginate, and pectate (Hartmeier, 1988, Mensour *et al.* 1996, Pilkington *et al.*, 1999, Tata *et al.* 1999, Barranco-Florido *et al*. 2001, Navrátil *et al.* 2002).

In this work, a packed bed reactor (PBR) was chosen for pectinase production. Two carriers were tested for cell immobilization: a commercial porous silicate glass (Siran) and a recently tested cellulosic support, prepared from spent-grains, a by-product of the brewing industry. Although Siran has been used with satisfying results for cell and enzyme immobilization (Virkajärvi and Kronlöf, 1998, Castein, *et al.*, 1999, Srivastava and Onodera, 1998, Pérez *et al*., 1997, Racher and Griffiths, 1993), its high cost can be a drawback for industrial productions. The cellulosic carrier has already been tested with a brewing strain (*Saccharomyces uvarum*) and was found to be very efficient, having a high yeast loading capacity, together with an easy preparation, reusability, availability and an inert, non-toxic nature (Bányik *et al*., 2001, 2002).

Materials and methods

Media and strain

The wild type *Kluyveromyces marxianus* CCT 3172 used to inoculate both continuous reactors was pre-grown in 200 ml (in 500 ml Erlenmeyer flasks) semi-synthetic media at 30 ºC, 120 r. p. m. for 24 h.

The semi-synthetic medium (SS) for yeast growth included (g l^{-1}): 5 K_2HPO_4, 2 $(NH_4)_2SO_4$, 0,4 $MgSO_4.7H_2O$, 1 yeast extract, and different glucose concentrations (20, 40, 80).

Carriers

The carriers used were porous silicate glass beads (Siran, SIKU012/05/120A, QVF Engineering, Mainz, Germany with bead diameters 2 to 3 mm and porosity 50 to 65 %) and a cellulosic support prepared from spent grains, a by-product of the brewing industry. Siran beads were washed in distilled water and autoclaved twice before the first use. The steps followed to obtain the carrier from spent grains are described in Bányik *et al*., (2001).

Enzyme assays

The endopolygalacturonase activity in the reactor effluent was assessed using the method described by Honda and co-workers (1982). On unit (U) is defined as 1 μmol of galacturonic acid released after 1 min of hydrolysis of polygalacturonic acid in the presence of the enzyme at 40 ^{o}C, pH 4.1.

Analytical methods

Glucose concentration was determined by the DNS method for reducing sugars quantification (Miller, 1954). For pectinase activity determination, the samples from reactors were centrifuged, filtered and then dialyzed with a 14 000 MWCO membrane against cold distilled water for 16 h. Lactose was used as a tracer for hydrodynamic studies.

Its concentration was determined using the specific enzymatic kit for detection of lactose and D-galactose from Boehringer Manheim / Roche.

Cells contact angle measurements

A solution of 20 g l^{-1} of agar and 10 % glycerol was cast on a microscope slide. Cell samples were taken from a continuous reactor outflow and washed with a solution with increasing ethanol concentration (10, 20 and 50 % (w/v)). 1 ml of a cell suspension in 50 % ethanol with a $Abs_{600\ nm} = 2.0$ was spread on the solidified agar and glycerol and allowed to dry. This step was repeated 4 times (Henriques *et al*. 2002). Contact angles were measured at room temperature using water, formamide and α-bromonaphtalene in a contact angle apparatus (Kruss-GmgH, Germany) by the sessile drop technique. The total surface tension (γ^{tot}) and its components (γ^{LW}, γ^{+}, γ^{-}, γ^{AB}), the values of the free energy of interaction between cells and water ΔG_{sws}^{tot} and the components (ΔG_{sws}^{LW}, ΔG_{sws}^{AB}) were calculated according to van Oss and co-workers (van Oss *et al*., 1988).

Scanning electron microscopy (SEM)

A sample of biocatalyst was taken from the CSTR reactor, washed with water and with a solution with increasing ethanol concentration (10, 25, 50, 75, 90, 100 %). It was allowed to dry for 5 days in an exicator and covered with a thin gold layer to allow for SEM observation.

Biomass quantification

The free biomass concentration at the reactors' outlet was measured by reading the absorbance of samples at 600 nm and then converting this value to dry weight per volume using an appropriate calibration curve.

At the end of the reactor's operation, samples of the biocatalyst were withdrawn from different heights of the fixed bed. For the spent grains, the carrier with adsorbed yeast cells was gently washed with 200 ml of distilled water. The resulting suspension was filtered and washed with water; the filter paper with carrier and immobilized cells was dried at 105°C for 16 h. A washing step (during 24 h at 120 r.p.m.) with a 3 % (w/v) NaOH solution released the attached biomass. After washing with distilled water and filtering, the biomass free carrier was dried at 105°C for 5 h. The biomass weight was calculated as being the weight difference of the dry carrier before and after the NaOH washing. (Brányik *et al*., 2002).

For the Siran carrier, the biocatalyst samples were dried for 48 h at 105°C and weighted. The biomass was then combusted at 550°C for 2 hours and the residual material was weighted. The biomass load (Xi) was calculated as the weight difference between the dry biocatalyst and the clean carrier. Biomass loads were expressed in $g_{biomass}\ g^{-1}_{carrier}$.

Bioreactor start-up and operation

Before operation, the reactor was sterilized with a hypochlorite solution (3 days). After this period of time, 10 reactors' volumes of sterile distilled water were used to wash

the column. The packed bed reactor (PBR) was a "Perspex" column, with height to internal diameter rate (H/Di) of 12 and an operation volume of 310 ml.

When using the spent-grains as cells support, 25 g of sterile dry carrier were aseptically inserted in the column and then inoculated with a pre-grown yeast culture. After 24 h of batch growth in the reactor, continuous operation started by feeding SS medium with 40 g l^{-1} glucose at the bottom of the column. A recycle rate of 40 ml h^{-1} was used during the entire operation time, by re-introducing a part of the outflow to the bottom of the column.

When Siran was used as carrier, approx. 150 g of sterilized dried beads where introduced into an Erlenmeyer flask with SS medium (20 g l^{-1} glucose) than inoculated with a pre-grown 100 ml culture. After 24 h of incubation at 30 ^{o}C, 120 r.p.m., the Siran beads were transferred into the reactor which was then filled with fresh SS medium (40 g l^{-1}). Continuous operation started 24 hours after the transfer. For this experiment the recycle rate was 200 ml h^{-1}.

The dilution rate was considered to be D (h^{-1}) = volumetric feed rate / total working volume of the reactor. All the assays were performed at 25 ^{o}C.

Results and discussion

Cell immobilization

The cellulosic carrier from spent grains is both irregular in shape and non-homogeneous in chemical composition, originating "active sites" preferably colonized by yeasts (Brányik *et al*., 2001). Siran beads are composed of silicate glass with an open pore structure, relatively uniform in size but with an irregular orientation and shape (Fig 1).

Contact angles measured by the sessile drop technique (Henriques *et al.,* 2002) were used to calculate the yeast surface properties according to van Oss (1988, 1995). The values of total surface tension and free energy of interaction for *K. marxianus* CCT 3172 cells, for base-treated spent grains carrier (Brányik T., unpublished results) and for Siran (Nakari-Setälä *et al.,* 2002) are presented in Table 1. The high positive ΔG_{sws}^{tot} value found for the Siran carrier is associated with its surface hydrophilic character, and the negative value determined for the cellulosic carrier shows the presence of highly hydrophobic areas. From these values, the free energy of interaction between *K. marxianus* cells and the two different carriers was calculated. The values of ΔG_{cws}^{tot} =1.86±8.70 mJ m^{-2} (the wide error bar is associated with the non-uniform surface composition of the cellulosic carrier) for the interaction cells-water-spent grains and ΔG_{cws}^{tot} =55.5±5.0 mJ m^{-2} for cells-water-Siran showed an energetically less favourable adhesion between yeast cells and the surface of Siran beads. These results are in agreement with the observed behaviour of cells in the two different biocatalyst beds (Fig 1). The Siran carrier seems to immobilize cells only by spatial retention on its porous structure. It has an open pore matrix with an important presence of void spaces, allowing liquid motion and cell percolation through the fixed bed. This originates a difference in the biomass load at different heights in the column (for distances from the top of 0, 8, 18 and 31 cm biomass loads (X_i) of 0.072, 0.054, 0.065, and 0.143 g g^{-1}carrier were found at the end of reactors operation). As it was not possible to withdraw biocatalyst samples during reactors operation, four separated assays were performed using a smaller column and 1/10 of the Siran weight (approx. 15 g). A fixed

dilution rate of D = 0.33 h^{-1} was used and the four assays were stopped at 120, 240, 408 and 672 hours for immobilized biomass quantification. After 240 hours of continuous operation, biomass load reached a stationary value (Figure 2). From these experiments, it can be assumed that the immobilized biomass inside the PBR was nearly constant after the first 240 hours of reactor's operation.

When the cellulosic carrier was used, cells were attached to the irregular surface not only by retention inside fibres, threads and crevices, but also by cell - surface adhesion due to different interaction forces. In addition to that, the spent grains packing worked as a "filter layer" giving rise to zones of local accumulation of yeasts. The CO_2 bubbles formed during the experiment were to account for a sponge-like bed structure. The gas was periodically liberated through the top of the column thus mixing the packed bed and releasing parts of the biomass deposits. 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 showing a higher homogeneity in the bed colonisation than for the PBR with Siran.

Hydrodynamic studies (Residence time distribution)

Experiments were carried out to study the hydrodynamic behaviour inside the PBR for both tested carriers. Lactose was used as a tracer since this particular strain of *K. marxianus* is unable to metabolise it efficiently. Besides, glucose is not totally consumed during the experiments with the tracer, which acts as a catabolic repressor to lactose consumption.

A lactose concentration step was imposed at steady state conditions for both reactors. The residence time distribution is presented in Figures 3a and 3b. When the Siran carrier was used, the best fit for experimental tracer response seems to be an ideal CSTR. For the axial dispersion model, Peclet number is the fitting parameter, defined as uL/*D* (u being the linear velocity, L the height of the biocatalyst bed and *D* the axial dispersion coefficient); Pe = ∞ corresponds to ideal plug flow, and Pe = 0 to ideal mixed flow. The low Peclet (uL/*D*) value and the poor correlation obtained (Pe=1.07± 1.01 r^2=0.87) for the fitting with the axial dispersion model also suggest a nearly perfect mixing inside this biocatalyst bed. In fact, as already discussed above, this packed bed has an open pore matrix with a high void volume, which is likely to have a low resistance to mass transfer and fluid motion (Fig. 1).

Using spent grains as cell carrier, a good agreement was found both for the axial dispersion model with a Pe number of 5.51 ± 1.01 (r^2 = 0,98) and for a series of 3 CSTRs. From this, it can be implied that the reactor mixing is not negligible, however it has a lower extent than in the packed bed with Siran. The fibrous structure of the spent grains bed, working like a thick filter media, justifies the lower mixing found for the experiment with this cellulosic carrier.

A recycle was used in both situations but in the case of the spent grains bed it's volumetric flow was five times lower than the one used with Siran to avoid fluidisation of the lighter spent grains carrier. This fact can also account for the differences in the mixing characteristics.

Pectinase production

During the operation of both packed-bed reactors, the free biomass concentration, glucose concentration and pectinase activity were measured at the reactors outlet (Fig. 4 for the PBR with spent grains; the data for the PBR with Siran is not shown). Similar values were found for pectinase activities: in the PBR with spent grains the values oscillated between 2.45 U ml^{-1} and 7.82 U ml^{-1}, while in the PBR with Siran the pectinase activities ranged from 3.08 U ml^{-1} to 7.72 U ml^{-1}.

Using the PBR with spent grains, the volumetric productivity (P_V) values range from 0.61 to 0.98 U ml^{-1} h^{-1} and increase with the dilution rate (Figure 5). For the Siran packed reactor, productivity also increased with the dilution rate and ranged from 0.39 to 1.68 U ml^{-1} h^{-1}(Figure 5). The highest value of P_V in the PBR (1.68 U ml^{-1} h^{-1}) was found for the Siran bed working with a D = 0.298 h^{-1} and an inlet sugar concentration of 40 g l^{-1}.

When the specific pectinase production rate (q_P) and the specific glucose consumption rate (q_S) were plotted against D (Sousa *et al.,* 1994), the same tendency of linear increase was found (Figure 6). As no samples were taken from the packed bed throughout the operation time, the values of the immobilized biomass were estimated using the biomass accumulation trend from Figure 2. In the case of the Siran bed after the first 240 h of continuous operation the average biomass concentration was considered constant and equal to 34 g l^{-1} reactor volume. A similar behavior of the bed colonization was assumed for the spent grains, and therefore the biomass concentration was considered also constant (18.5 g l^{-1}) after 240 h of the reactor's operation.

For the spent grains PBR, the q_S and q_P values found using S_{in} = 40 g l^{-1} and S_{in} = 80 g l^{-1} are similar, indicating that there is a limitation for glucose conversion to pectinase in this column. In fact, increasing D with high glucose concentrations in the inlet is useless, since it results in a higher sugar concentration at the outlet. This was not noticeable for S_{in} = 20 g l^{-1} with the tested dilution rates.

In the Siran PBR glucose total consumption was achieved only at a D = 0.105 h^{-1} and S_{in} = 40 g l^{-1}. Using the same dilution rates and the same inlet sugar concentration (S_{in}) of 40 g l^{-1}, the q_S of the cells in the spent grains bed is slightly higher than for yeast immobilized in Siran which consequently results in a higher pectinase production rate (q_p) in the immobilized system with spent grains. Although the packed bed with Siran has a higher biomass concentration, its non-uniform distribution throughout the column is possibly the reason for this difference. In fact, the bottom of the reactor (about 15 to 20 % in terms of reactor volume) had a large and, in some places, compact yeast accumulation (estimated as 25 to 30 % of the total biomass in the column) that probably imposed a high mass transfer resistance for glucose and pectinase. The values found for q_S at S_{in} = 80 g l^{-1} are higher than for S_{in} = 40 g l^{-1}, but this difference was not, as should be expected, reflected in the q_P values, which are lower for S_{in} = 80 g l^{-1}. This contradictory results can also be a consequence of the uneven biomass distribution in the Siran column. As the assays with S_{in} = 80 g l^{-1} were performed after the ones with S_{in} = 40 g l^{-1}, the biomass accumulation at the bottom of the column was increased and these clustered cells are probably less efficient in converting sugar to pectinase due to nutrient restrictions imposed by the high mass transfer resistance.

Both carriers can be successfully used for cell immobilization although, to increase the biomass loads and improve its distribution throughout the column, the surface of the Siran carrier should be activated. In fact, a pre-treatment with trimethylchlorosilane

favorably changes its surface properties for yeast attachment, as a six-fold decrease is found for the free energy of interaction (ΔG_{sws}^{tot}) (Nakari-Setälä *et al*., 2002).

From the performed assays, it can be concluded that the best results for pectinase production can be achieved using a high X_i in the column and a high D, together with total glucose consumption.

The better biomass distribution throughout the column obtained in the spent grains PBR and the advantages related to this available by-product from brewing industries make it a suitable option as a cell carrier.

References

Barranco-Florido, E., Garcia-Garibay M., Gomez-Ruiz, L., Azaola, A., Immobilization system of *Kluyveromyces marxianus* cells in barium alginate for inulin hydrolysis. Process Biochem., **37** 513-519 (2001).

Blanco, P., Sieiro, C., Villa, T. G., Production of pectic enzymes in yeasts. FEMS Microbiol. Lett., **175**, 1-9 (1999).

Brányik, T., Vicente, A. A., Machado Cruz, J. M., Teixeira, J. A., Continuous primary beer fermentation with brewing yeast immobilized on spent grains. J Inst Brew **108** (4) 410-415 (2002).

Brányik, T., Vicente, A.A., Machado Cruz, J.M., Teixeira, J. A. Spent grains - a new support for brewing yeast immobilisation. Biotechnol. Lett. 23:1073-1078 (2001).

Canstein, H., Li, Y., Timmis, K.N., Deckwer, W.-D., Wagner-Dobler, I., Removal of mercury from chloralkali electrolysis wastewater bu mercury-resistant *Pseudomonas putida*, Appl. Environ. Microbiol. **65** (12) 5279-5284 (1999)

Hartmeier, W., Immobilized Biocatalysts – An Introduction, Springer Verlag ed. (1988).

Honda, S., Nishimura, Y.: Takahashi, M., Chiba, H., Kakehi, K., A manual method for the spectrophotometric determination of reducing carbohydrates with 2-cyanoacetamide, Anal. Biochem. **119**, 1, 194-199 (1982).

Henriques, M., Gasparetto, K., Azevedo, J., Oliveira, R., Experimental methodology to quantify *Candida albicans* cell surface hydrophobicity. Biotechnol. Lett. **24**, 1111-1115 (2002).

Kashyap, D.R., Vohra, P.K., Chopra, S. and Tewari, R., Applications of pectinases in the commercial sector: a review. Bioresource Technol.77, 215-227 (2001).

Manachini, P. L., Parani, C., Fortina, M. G., Pectic enzymes from *Aspergillus pullulans* LV10. Enzyme Microbiol. and Technol., **10** (11), 682-685 (1988).

Mensour, N. A., Margaritis, A., Briens, C. L., Pilkington, H., Russel, I., Application of immobilized yeasts in the brewing industry. In: **Wijffels R. H., Buitelaar, R. M., Bucke,**

C., Tramper, J., eds, "Immmobilised Cells: Basics and Applications" Amsterdam, Elsevier Science B. V., 661-671 (1996).

Miller, G. L., Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. **31**, 426-428 (1959).

Naidu, G. S. N. , Panda, T., Production of pectolytic enzymes –a review. Bioprocess Eng. **19**, 355-361 (1998).

Nakari-Setälä, T., Azeredo, J., Henriques, M., Oliveira, R., Teixeira, J., Linder, M., Penttilä, Expression of a fungal hydrophobin in *Saccharomyces cerevisiae* cell wall: effect on cell surface properties and immobilization, Appl. Environ. Microbiol. **68** (7) 3385-3391 (2002).

Navrátil, M., Gemeiner, P., Klein, J., Sturdik, E., Malovikova, A., Nahalka, J., Vikartovska, A., Domeny, Z., Smogrovicova, D., Properties of hydrogel materials used for entrapment of microbial cells in production of fermented beverages. Art. Cells, Blood Subs., and Immob. Biotech. **30**, 3, 199-218 (2002).

Pérez, M., Romero, L.I., Nebot, E., Sales, D., Colonisation of porous sintered-glass support in anaerobic thermophilic bioreactors. Bioresource Technol, **59**, 177-183 (1997).

Pilkington, H., Margaritis, A., Mensour, N., Sobczak, Hancock, I., Russel I., Kappa-carrageenan gel immobilisation of lager brewing yeast. J. I. Brewing **105**, 6, 398 404 (1999).

Racher, A.J., Griffiths, J.B., Investigation of parameters affecting a fixed bed bioreactor process for recombinant cell lines Cytotechnology;**13** (2):125-31 (1993).

Schwan, R.F., Cooper R.M., Wheals, A.E., Endopolygalacturonase secretion by *Kluyveromyces marxianus* and other cocoa pulp-degrading yeasts. Enzyme Microb. Technol. 21, 234-244 (1997).

Schwan, R.F., Rose, A.H., Polygalacturonase production by *Kluyveromyces marxianus*: Effect of medium composition. J. Appl. Bacteriol. 76, 62-67 (1994).
Sousa, M. L., Mota, M., Teixeira, J.A., Influence of operational parameters on the start up of a flocculation airlift bioreactor . Colloids and Surf. B: Biointerfaces 2, 181-188 (1994).

Srivastava, P., Onodera, R. J., A comparative evaluation of Cephalosporin C production using various immobilization modes. Gen. Appl. Microbiol **44**(2), 113-117. (1998).

Tata, M., Bower, P., Bromberg, S., Duncombe, D., Fehring, J., Lau, V. V., Ryder, D., Stassi, P., Immobilized yeast bioreactor systems for continuous beer fermentation Biotechnol. Prog. **15**,1, 105-113 (1999).

van Oss, C. J., Chaudhury, M. K. Good R. J., Interfacial Lifshitz-van der Walls and polar interaction in macroscopic systems. Chem. Rev. **88**, 927-941, (1988).

van Oss, C. J., Hydrophobicity of biosurfaces – origin, quantitative determination and interaction energies. Colloids and Surfaces B: Biointerfaces **5**, 91-110 (1995).

Virkajärvi, I., Kronlöf, J., Long-term stability of immobilized yeast columns in primary fermentation. J. Am. Soc. Brew. Chem., **56** (2) 70-75 (1998).

Figures and Table

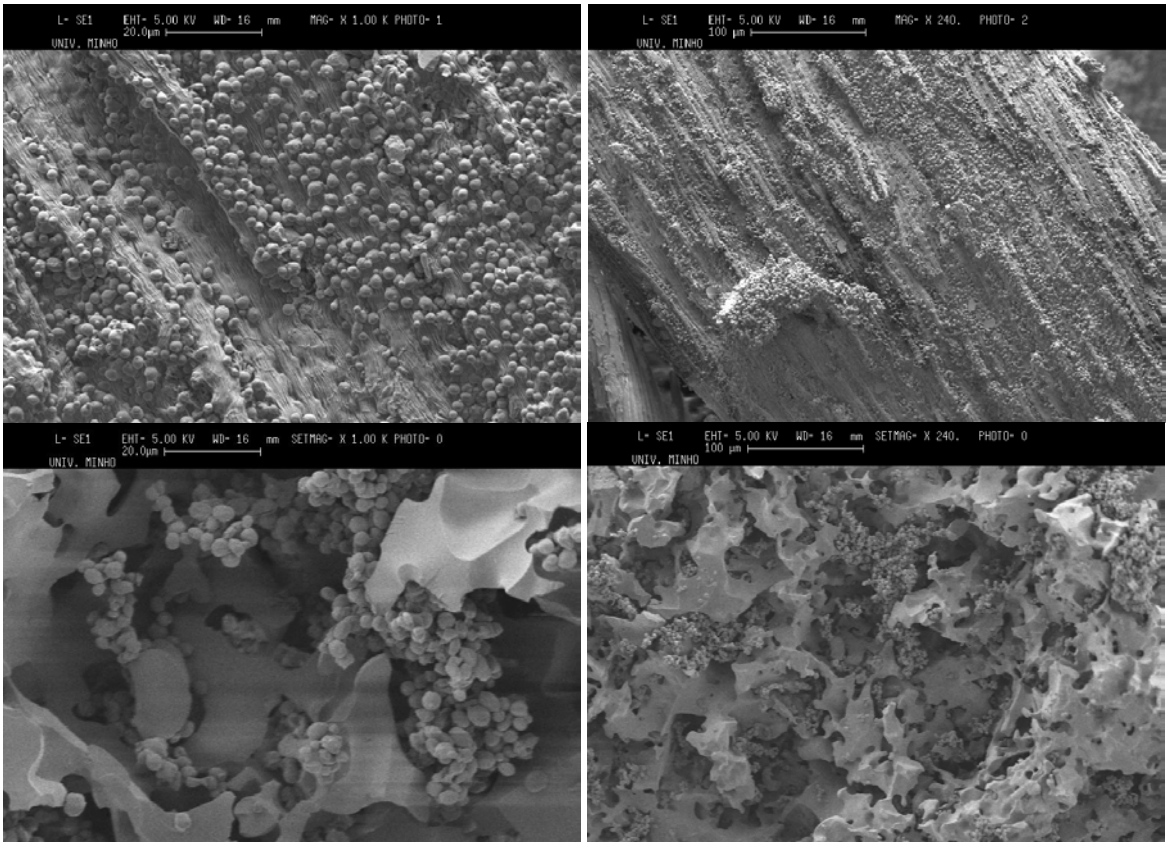


Figure 1 - SEM photos. Top images - cells on spent grains. Bottom images – cells on Siran beads. Left images- bar corresponds to 20 µm Right images– bar corresponds to 100 µm.

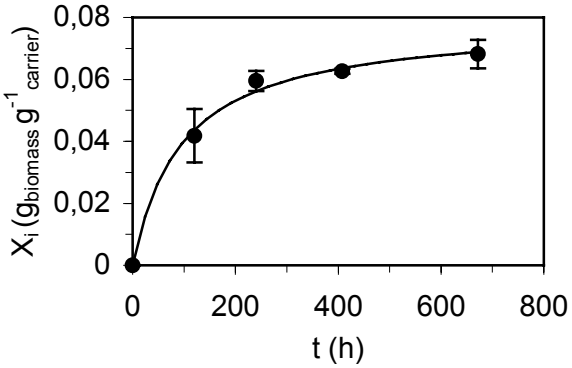
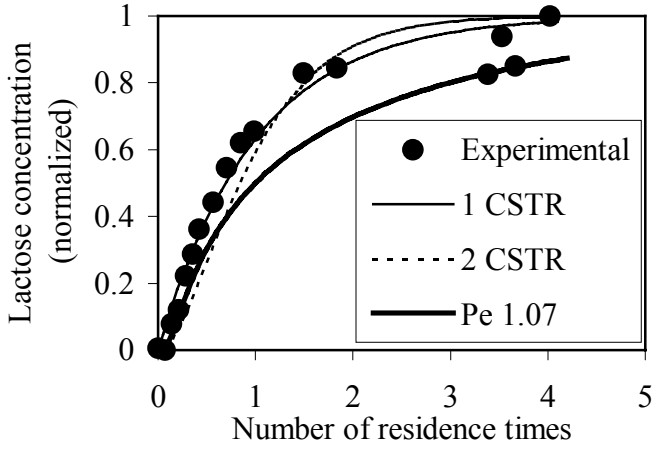
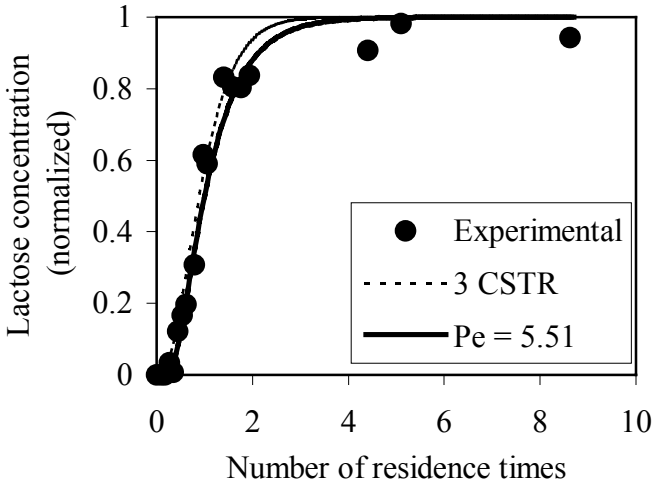


Figure 2 – Immobilized biomass load (X_i) in the Siran packed bed (assays with the smaller column reactors).



a)



b)

Figure 3 - Tracer response and model fitting for the packed bed with Siran (a)) and for the packed bed with spent grains (b)).

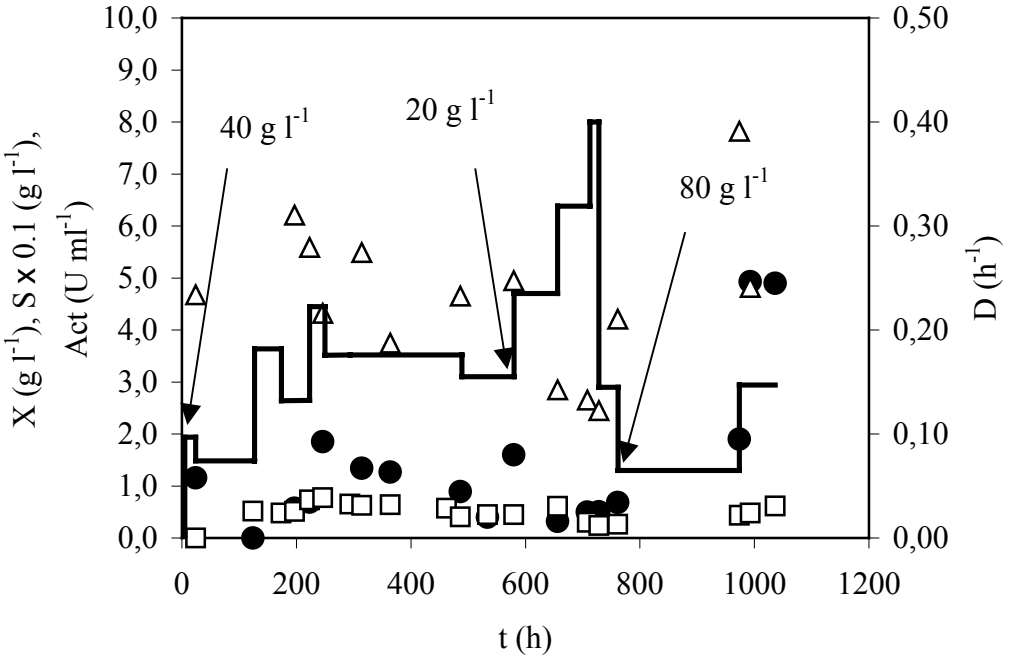


Figure 4 - Evolution of glucose concentration S (circles), free biomass concentration X (squares), pectinase activity Act (triangles), and dilution rate D (—) during PBR operation with spent grains. The arrows mark the changes in the inlet glucose concentration.

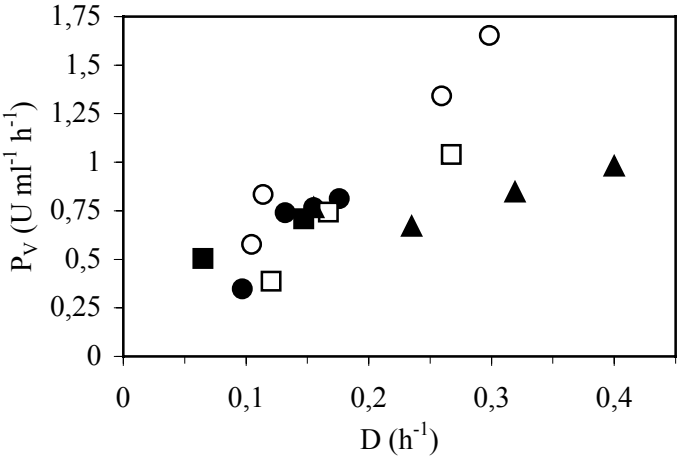
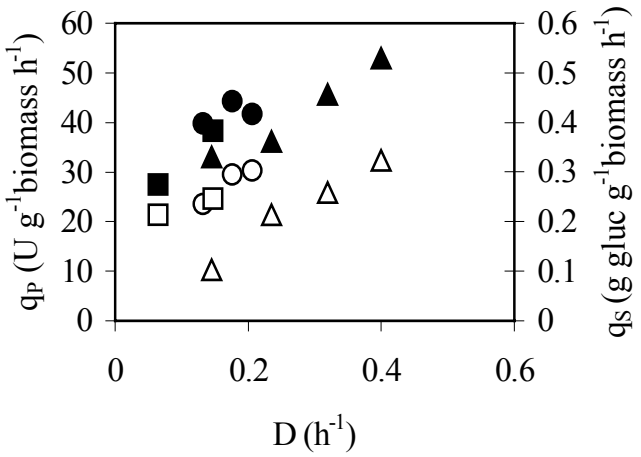
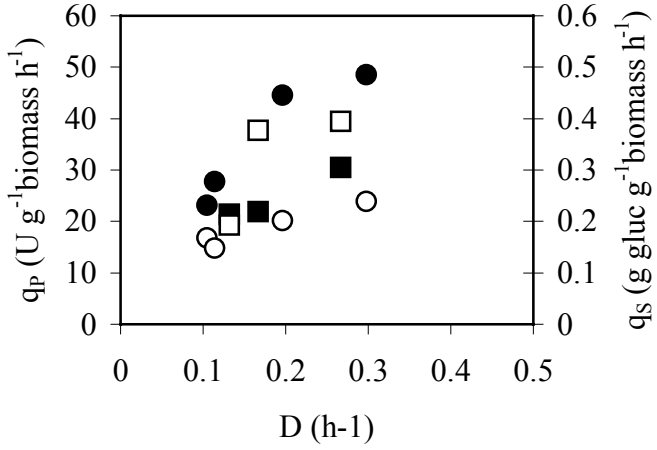


Figure 5 - Pectinase volumetric productivity (P_V) for different inlet concentrations of glucose. For PBR with spent grains the data are shown as filled symbols and for PBR with Siran data as empty symbols. Triangles correspond to S_{in} = 20 g l^{-1}, circles to S_{in} = 40 g l^{-1}, squares to S_{in} = 80 g l^{-1}.



a)



b)

Figure 6 – Effect of the dilution rate on the specific glucose consumption rate (q_S) and specific pectinase production rate (q_P) for a) the spent grains PBR and b) the Siran PBR at different inlet glucose concentrations. Empty symbols refer to q_S and filled symbols to q_P. Triangles are used for S_{in} = 20 g l-1, circles for S_{in} = 40 g l-1, and squares for S_{in} = 80 g l^{-1}.

Table 1 – Surface tension γ^{tot} and free energy of interaction for cells *K. marxianus* CCT 3172 grown in continuous culture (ΔG_{cwc}^{tot}), for the base treated spent grains carrier and for Siran (ΔG_{sws}^{tot}). All values in mJ m^{-2}.

	γ^{tot}	ΔG_{sws}^{tot}	ΔG_{cwc}^{to}
Cells from continuous culture	61.0	-	22.9
Base treated carrier	41.5	-57.7	-
Siran (Nakari-Setälä, *et al*. 2002)	56.1	119.8	-