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Immobilization of *Bacillus firmus* strain 37 in inorganic matrix for cyclodextrin production

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Abstract

Cyclodextrins (CDs) are produced industrially from starch, using bacterial cyclodextrin glucanotransferases (CGTase). Cells of *Bacillus firmus* strain 37, immobilized by adsorption in different inorganic matrix, were used for β-CD production. The matrices were prepared by the sol–gel method and scanning electron microscopy observations showed that SiO_2/TiO_2 and SiO_2/MnO_2 were the most suitable matrices for effective immobilization. The operational stability of the immobilized cells was studied by repeated batch cultivation in four cycles of 144 h each. At the end of the second cycle, the cells immobilized in both matrices had maintained 60% of the β-CD production from the initial cycle, and a gradual fall in β-CD production occurred up to the fourth cycle, due to cell desorption from the matrices. Storage stability was studied for 7 months and the maximum β-CD production by immobilized cells in SiO_2/MnO_2 and SiO_2/TiO_2 occurred within 90 days of storage at 4 °C (4.4 ± 0.4 and 6.0 ± 0.3 mM β-CD, respectively). After 150 days of storage, they presented the same β-CD production as freshly prepared biocatalysts. When control cycles using free cells were carried out, they produced on average 3.0 ± 0.1 mM β-CD; hence, the immobilized cells showed a one-and-a-half to twofold increase in β-CD production compared to the free cells.

Keywords: Cyclodextrins; *Bacillus firmus*; Cell immobilization; Inorganic matrix; Operational stability

1. Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides containing six (α-CD), seven (β-CD), eight (γ-CD) or more glucopyranose units linked by α-(1,4) bonds, and are produced during degradation of starch by cyclodextrin glucanotransferases (CGTase) [1]. This enzyme is multifunctional, and its most specific reaction is the cyclization of maltooligosaccharides [2,3]. The CDs molecules contain a hydrophobic central cavity and a hydrophilic surface, and can form inclusion complexes with organic and inorganic molecules. They are extensively used in the food, cosmetics, pharmaceutical and agricultural industries [4,5].

Systems of immobilized cells have been applied for many biochemical processes and display several advantages, such as the repeated and prolonged use of cells, easy separation from the fermentation medium, higher reactor productivity, continuous fermentation with less sophisticated reactors and a low risk of contamination due to an increase in thermal stability [6–8]. In the biotransformation processes, the application of immobilized whole bacterial cells as a source of enzymes eliminates the need for them to be purified and, in some cases, the enzymes are more stable within the cell, which is their natural environment [9]. In addition, they provide a convenient system for obtaining products without involving the use of large fermenting reactors, labor-intensive and time-consuming centrifugations, and the handling of processing fluids, etc. [10,11].

In order to obtain a high level of activity and stability from the whole cells, various methods, including adsorption, covalent binding and gel entrapment, have been used for immobilization [12]. The oldest and easiest method is the adsorption of biocatalysts in macroscopic water-insoluble carriers, and a great selection of inorganic and organic materials have been used as support, including activated charcoal, aluminum oxide, diatoma-

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ceous earth, cellulose, porous glass and synthetic resin [10]. The immobilization method and the type of matrix are important factors, which affect the stability and the catalytic activity of the biocatalysts. The significant enzyme yields and operational stability of the biocatalysts indicate the possibility of continuous enzyme production and, therefore, its resulting products [9].

In recent years, the sol–gel method has gradually gained relevance; being widely used for the immobilization of many biomolecules, including enzymes, microbial cells and antibodies. One of the main advantages of this method is the non-involvement of high temperatures and harsh chemical reactions. The materials are characterized by high homogeneity and purity, high superficial area and porosity control, and well-modeled particles, which have greater thermostablity than organic molecules [12–14].

The production, characterization and immobilization of CGTase have been extensively described by various publications [4,15–22], however, very few research works are known which have used whole cells immobilization aiming at the production of the CGTase enzymes (*Bacillus circulans* ATCC 21783 [23–25], *Bacillus cereus* [17], *Bacillus amyloliquefaciens* [7] and *Bacillus agaradhaerens* [11]).

Considering our research group previous studies on the CGTase from *Bacillus firmus* strain 37, including the isolation of the microorganism, purification and characterization of its CGTase [15,16,21], optimization of the culture media for enzyme production [18] and immobilization of CGTase in chitosan, alumina and support-controlled-pore silica [19,20], this research study aimed at producing CDs from the *B. firmus* strain 37 immobilized in inorganic matrices, prepared by the sol–gel method.

2. Materials and methods

2.1. Microorganism and culture conditions

The microorganism used in the current study was *B. firmus* strain 37, isolated from soil cassava culture by Matioli et al. [15]. The microorganism was cultivated in solid medium, pH 10.3, containing (% p/V): soluble starch 1.0; polypeptone 0.5; yeast extract 0.5; K_2HPO_4 0.1; $MgSO_4{\cdot}7H_2O$ 0.02; Congo red dye 0.01; Na_2CO_3 1.0 and agar 1.5. The culture plates were incubated at 37 °C for 48 h, and the colonies were then transferred to 500 mL Erlenmeyer flasks containing 250 mL of liquid culture medium with the same composition as the plate medium, except for the presence of agar and dye. Incubation was carried out with 120 rpm orbital shaking at 37 °C for 48 h. The cells were harvested from the culture medium by centrifugation (2000 × *g*, 10 min, 4 °C) and later used for the immobilization procedures.

2.2. Matrices preparation and characterization

To immobilize the microorganism, five matrices were used: silica–titanium (SiO_2/TiO_2), silica–manganese (SiO_2/MnO_2), vanadium–titanium (V_2O_5/TiO_2), vanadium–silica (V_2O_5/SiO_2) and cellulose–titanium (cellulose/TiO_2). These matrices were prepared by the sol–gel method (general equation (1)), which basically involves hydrolysis reactions (Eq. (2)) and polycondensation by dehydration (Eq. (3)), or dealcoholation (Eq. (4)) of the alkoxides precursors, forming oxopolymers that are transformed into a three-dimensional network of oxides [26,27]:

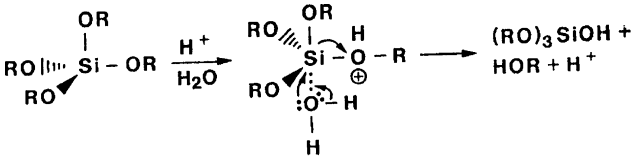

Fig. 1. Reaction of tetraethylortosilicate (TEOS) hydrolysis in water.

$$M(OR)_n + \frac{n}{2}H_2O \rightarrow MO_{n/2} + nROH \quad (1)$$

$$M(OR)_n + H_2O \rightarrow M(OR)_{n-1}(OH) + ROH \quad (2)$$

$$M(OR)_n + M(OR)_{n-1}(OH) \rightarrow M_2O(OR)_{2n-2} + ROH \quad (3)$$

$$2M(OR)_{n-1}(OH) \rightarrow M_2O(OR)_{2n-2} + H_2O \quad (4)$$

where M is the metal and $M(OR)_n$ is the alkoxide precursor.

The reactions of hydrolysis and condensation occur by nucleofilic substitution before the catalysts, which can be acid, basic or nucleofilic. The reaction of hydrolysis (for example, the tetraethylortosilicate—TEOS) under acid conditions involves the protonation of the alkoxide group followed by the water nucleofilic attack, leading to the formation of an intermediate pentacoordenate (Fig. 1) [26,27].

The SiO_2/TiO_2 matrix was prepared by adding 12.1 mL of an aqueous solution of HNO_3 0.85 mol/L in 250 mL of an ethanolic solution of TEOS 50% (v/v). The mixture remained under reflux and shaking at 80 °C for 150 min. Then, 21 mL of tetrabutoxide of titanium (TBOT) and 490 mL of ethanol were added and the preparation was kept shaking at room temperature for 2 h. 66 mL of an aqueous solution of HNO_3 0.6 mol/L were slowly added and allowed to rest for gelation. The formed xerogels were ground, dried at 110 °C for 24 h and sieved in order to obtain particles size between 75 and 250 μm diameter. The SiO_2/TiO_2 binary oxide obtained was calcinated at 500 °C under air flow [13].

Based in the method used for the synthesis of SiO_2/TiO_2, SiO_2/MnO_2 matrices were prepared, substituting TBOT for $MnCl_2$.

The V_2O_5/SiO_2 and V_2O_5/TiO_2 matrices were prepared according to the method described by Schneider et al. [28], using HCl to hydrolyze TEOS and HNO_3 for TBOT, respectively. After that, vanadium triisopropoxide (VOTIP) was added for polycondensation. For preparing the cellulose/TiO_2 matrix, according to Porto et al. [10], TBOT with cellulose, previously suspended in heptane, was used. The matrices tested had the same diameter range as that obtained for SiO_2/TiO_2.

The five matrices were characterized according to their specific surface area (S_0), measured by the Brunauer, Emmett and Teller (BET) multipoint technique [29]; average volume pores (V_p), determined by mercurium intrusion technique [30]; and average diameter pores (D_p).

2.3. Immobilization procedure

Different amounts of *B. firmus* strain 37 whole cells, with a known wet-cell weight (8.0 g dry cells/100 g wet cells), were used for each of the immobilization procedures. One point two grams of wet-weight cells was used for the initial evaluation of microorganism immobilization in the five matrices, 1.2–3.0 g for studying the effect of the initial biomass concentration, and 2.4 g for the stability tests.

Cell immobilization was carried out with 50 mL of sterile distillate water, *B. firmus* strain 37 wet cells and the appropriate sterilized matrix (0.6 g). The suspension was shaken at 120 rpm and kept at 28 °C during 12 h.

2.4. Biocatalyst characterization

The biocatalyst produced by the immobilization of *B. firmus* strain 37 cells was characterized by determining its activity for the production of CDs and its storage and operational stability. All the experiments were carried out in duplicate.

The cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2, with different concentrations of initial biomass, were centrifuged ($800 \times g$, 3 min, 4 °C), washed with sterile distillate water and submitted to the CD production cycle.

The method described by Matioli et al. [15] for CDs production using purified CGTase was adapted in this research study. For each cycle of CD production, the culture medium was constituted of 50 mL of maltodextrine 10% (w/v) in Tris–HCl buffer, pH 8.0, 50 mM and $CaCl_2$ 5 mM. The assay was performed at 50 °C, with shaking of 120 rpm during 144 h. Aliquots of 1 mL were drawn periodically (0, 6, 12, 24, 36, 48, 72, 96, 120 and 144 h), diluted in 1 mL of distillate water and submitted to boiling for posterior dosage of β-CD produced. At the end of the cycle, the CGTase activity in the reaction medium was determined according to the method described by Matioli et al. [15]. One unit (U) of enzyme activity was defined as the amount of enzyme that produces 1 μmol β-CD/min under the assay conditions.

To determine the operational stability, four cycles of CDs production were carried out and after each cycle, the cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2 were removed from the production medium by centrifugation ($800 \times g$, 3 min, 4 °C), washed with sterile distillate water and kept at 4 °C until re-utilization. For each cycle a new CD production medium was prepared and the CGTase activity was determined.

In order to evaluate the storage stability, the cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2 were divided into five aliquots and kept at 4 °C. The aliquots were periodically sampled and submitted to the CD production cycles (zero time, 60, 90, 150 and 210 days of storage) with no re-utilization. Control cycles with free cells were also carried out in this assay.

To verify the possibility of cell desorption in SiO_2/TiO_2 and SiO_2/MnO_2 matrices and to analyze the chance of microbial growth after the immobilization in the matrices, the total protein content was determined [31] in newly immobilized samples and after the final cycle of CD production.

2.5. Analytical methods

2.5.1. Scanning electron microscopy (SEM)

The immobilized cells were placed in Tris–HCl buffer, pH 8.0, 50 mM and $CaCl_2$ 5 mM, containing 2.5% of glutaraldehyde, for 24 h. After this time, the supernatant solution was discarded and the immobilized cells were washed in aqueous ethanolic solution 30, 50, 70, 90 and 100%. The material was kept in absolute ethanol for further dehydration in a supercritical fluid extraction system using CO_2 under high pressure. The same procedure was carried out with free cells. To obtain the micrographs, a scanning electron microscope (Shimadzu, model SS 550) with an accelerating voltage of 10 kV was used. The samples of immobilized and free cells were put on the surface of a double face conductive tape and gold coated.

2.5.2. β-CD determination

The β-CD concentration was measured by the dye-extinction colorimetric method using phenolphthalein solution [22]. The assay was performed by mixing 0.5 mL of sample containing β-CD with 2.5 mL of a phenolphthalein working solution 0.06 M, containing carbonate–bicarbonate buffer 0.12 M, pH 10.5 and the absorbance was read at 550 nm. For the blank, the sample was substituted by distillate water. The phenolphthalein working solution was prepared at the dosage moment from a stock solution of phenolphthalein 3 mM in 95% ethanol (2 mL of phenolphthalein stock solution, 20 mL of carbonate–bicarbonate buffer 0.6 M, pH 10.5, and the volume completed to 100 mL with distilled water). The concentration of β-CD was calculated with Eq. (5), obtained by determination of the equilibrium constant ($K_{\beta\text{-CD}}$) for the formation of an inclusion complex of β-CD with phenolphthalein. This constant was determined by non-linear regression of Eq. (6) to a series of absorbance data in function of standard concentration of β-CD ($(0–1) \times 10^{-3}$ M) prepared in distillate water

$$C_{\beta\text{-CD}} = 0.3\left(1 - \frac{A_{550}}{A_{0/550}}\right)\left(1 + 1.0813\frac{A_{0/550}}{A_{550}}\right) \quad (5)$$

where $C_{\beta\text{-CD}}$ is the concentration of β-CD (mM), A_{550} and $A_{0/550}$ are the absorbances of samples and blank, respectively.

$$C_{\beta\text{-CD}} = a\left(1 - \frac{A_{550}}{A_{0/550}}\right)\left(1 + \frac{A_{0/550}}{K_{\beta\text{-CD}}aA_{550}}\right) \quad (6)$$

where a is the total concentration of phenolphthalein in the assay cuvette (5×10^{-5} M).

Firstly the concentrations of standard β-CD were corrected, taking into account the dilution performed in the cuvette (1:6) and the equilibrium constant ($K_{\beta\text{-CD}}$) was calculated using the Quasi–Newton method, resulting in $21627.10 \pm 85.61\ M^{-1}$, for the confidence interval of 95%. Eq. (5) was obtained by the substitution of $K_{\beta\text{-CD}}$ and a value in Eq. (6) and multiplying this equation by a factor equal to 6000 that is related to the dilution in the assay procedure and the conversion from molar in the cuvette to give millimolar of β-CD in the sample.

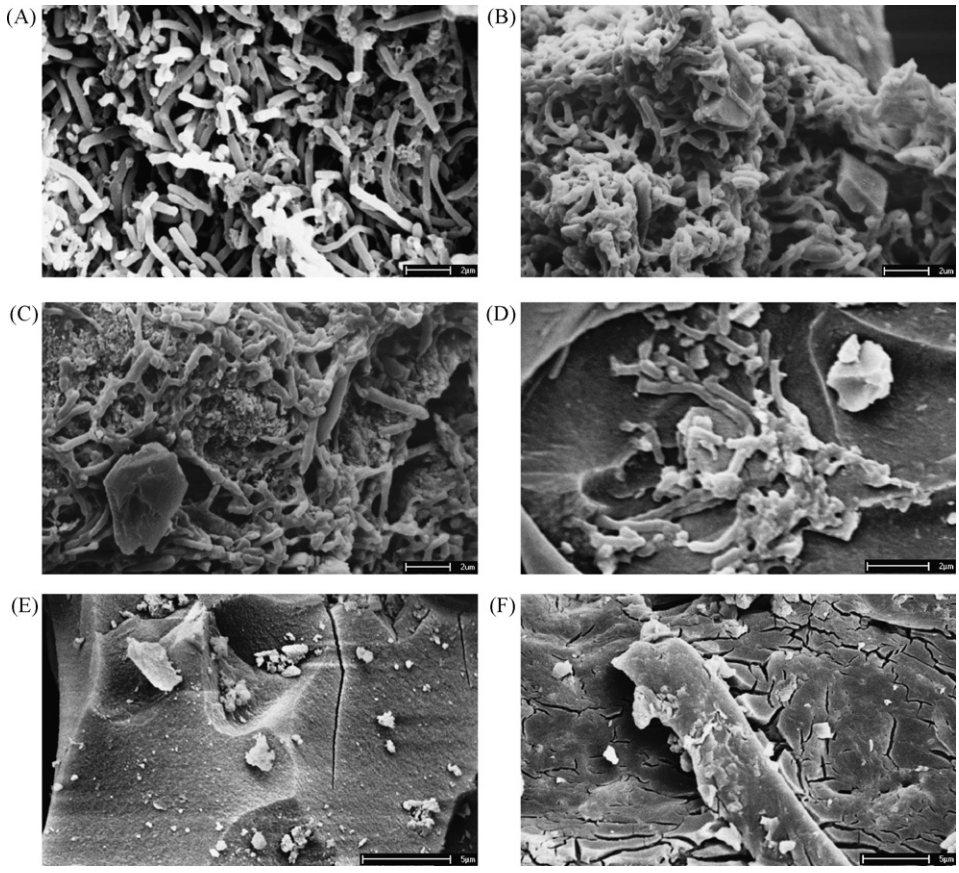

Fig. 2. Scanning electron micrographs. Free cells (A). Immobilized cells: SiO_2/TiO_2 (B), SiO_2/MnO_2 (C), V_2O_5/TiO_2 (D), V_2O_5/SiO_2 (E) and cellulose/TiO_2 (F).

3. Results and discussion

3.1. Matrices characterization and microbial cells immobilization

The immobilization of the microorganism *B. firmus* strain 37 in the five matrices studied was evaluated through the micrographs obtained by SEM. The bacterium size was estimated as 0.5 μm × 5.0 μm (Fig. 2A).

Table 1 shows the results of the specific surface area (S_0), average volume (V_p) and average diameter (D_p) of the pores of the five matrices studied. Considering that the determined D_p are in average around 1800 times smaller than the bacterium under study, it is possible to conclude that there was no possibility of microorganism penetration inside the matrices, meaning that the cells immobilization occurred by adsorption on their surface.

Table 1
Specific surface area (S_0), average volume (V_p) and average diameter (D_p) of the matrices pores

Matrices	S_0 (m^2/g)	V_p (mL/g)	D_p (μm)
SiO_2/TiO_2	503	0.35	0.0028
SiO_2/MnO_2	454	0.16	0.0014
V_2O_5/TiO_2	436	0.10	0.0009
V_2O_5/SiO_2	468	0.20	0.0017
Cellulose/TiO_2	48	0.08	0.0066

The micrographs allowed observing that there was good adsorption of the bacterium in SiO_2/TiO_2 and SiO_2/MnO_2 matrices, a satisfactory adsorption in V_2O_5/TiO_2 matrix and practically no bacterium attached the V_2O_5/SiO_2 and cellulose/TiO_2 matrices (Fig. 2B–F). Therefore, SiO_2/TiO_2 and SiO_2/MnO_2 matrices were selected to continue the study.

3.2. Initial biomass effect

The amount of immobilized cell biomass in the support was a very important parameter which influenced the production of β-CD by the immobilized cells. For the SiO_2/TiO_2 matrix, the use of an initial biomass load from 1.2 to 3.0 g provided an increase in β-CD production of 20%, reaching 13.4 ± 0.2 mM (Fig. 3A). For the immobilized cells in SiO_2/MnO_2 β-CD production was 25% higher when the amount of initial biomass, increased from 1.2 to 1.8 g, and the production of β-CD remained practically constant when 2.4 and 3.0 g of the cells were used, reaching 17.9 ± 0.1 mM of β-CD produced (Fig. 3B).

The specific CGTase productivity showed a decrease from 60.8 to 29.3 and 77.5–38.6 U/g wet cells h when the initial biomass was raised from 1.2 to 3.0 g for the immobilized cells in SiO_2/TiO_2 and SiO_2/MnO_2, respectively, suggesting that biofilm layers are produced on the external surface of the matrix particles and that the activity is limited to only the biocatalyst located in the outer biofilm layer, which comes into contact with the substrate more easily. For the CGTase of *Bacillus agarad-*

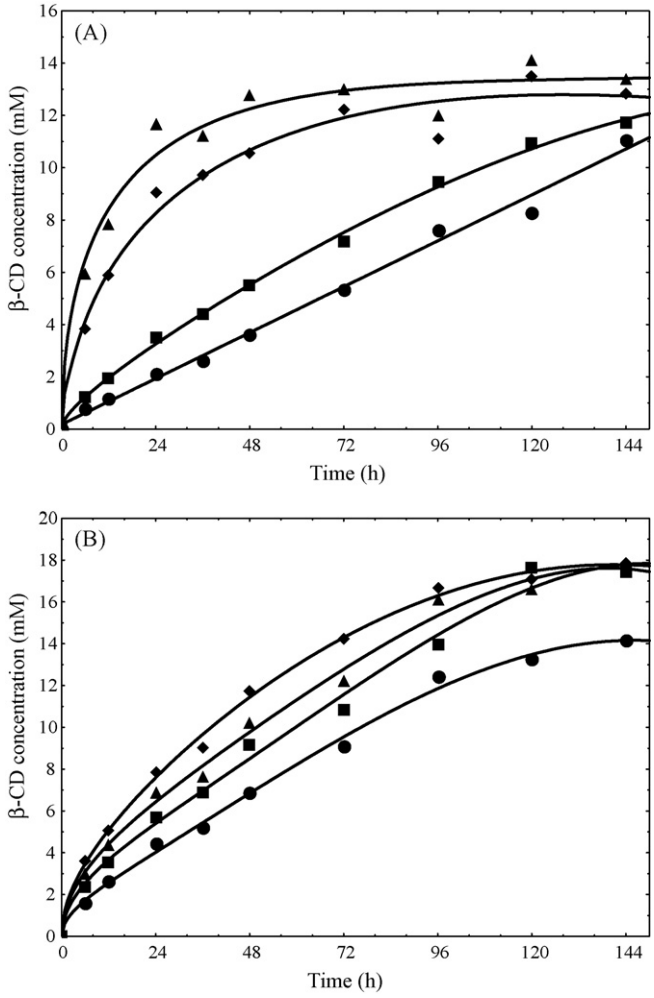

Fig. 3. Initial biomass effect on β-CD production by immobilized cells of *B. firmus* strain 37. (A) SiO_2/TiO_2 and (B) SiO_2/MnO_2 (● 1.2 g; ■ 1.8 g; ♦ 2.4 g and ▲ 3.0 g of immobilized cells). Conditions: maltodextrine 10% (w/v) in Tris–HCl buffer, pH 8.0, 50 mM and $CaCl_2$ 5 mM, 50 °C and 120 rpm.

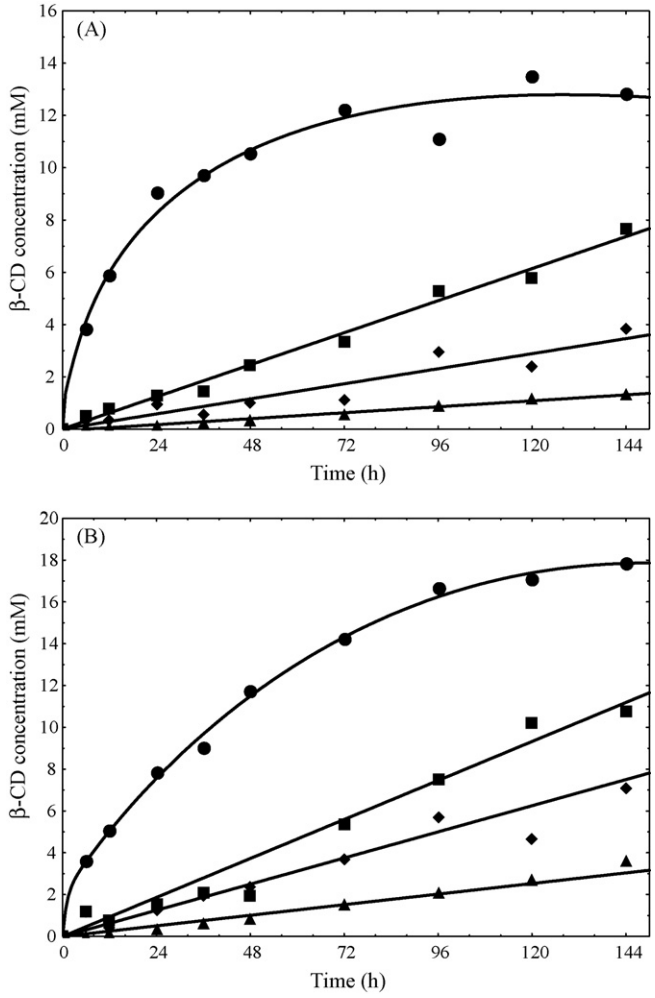

Fig. 4. Operational stability of the immobilized cells of *B. firmus* strain 37. (A) SiO_2/TiO_2 and (B) SiO_2/MnO_2 (● first cycle; ■ second cycle; ♦ third cycle and ▲ fourth cycle). Conditions: maltodextrine 10% (w/v) in Tris–HCl buffer, pH 8.0, 50 mM and $CaCl_2$ 5 mM, 50 °C and 120 rpm.

haerens LS-3C immobilized in polyvinyl alcohol-cryogel, no significant difference was observed among the different beads with respect to their specific activity. Initial cell loading of 1, 3 and 10% (w/v) after activation gave a specific activity of 2.2, 2.5 and 2.8 mg β-CD production/h g beads, respectively [11].

3.3. Operational stability

One of the acknowledged advantages of cell immobilization is the ease of cell separation from the production medium, for a possible re-utilization and the greater operational stability given by the number of cycles, which the immobilized cells can be used, without significant loss of activity [23,32,33]. In this study, the operational stability was determined by using 2.4 g of initial biomass in four repeated cycles of 144 h each. For the cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2 the best result was obtained in the first cycle (Fig. 4), reaching 12.9 ± 0.4 and 17.9 ± 0.1 mM of β-CD, respectively. At the end of the second cycle (288 h test), the cells immobilized in both matrices kept 60% of the β-CD production at the initial cycle. A gradual fall in β-CD production occurred up to the fourth cycle (576 h test), and β-CD production reduced to 10% of the production of the first cycle for the SiO_2/TiO_2 matrix and 20% for the SiO_2/MnO_2 matrix. The enzymatic activity obtained in the first cycle for CGTase of cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2 matrices was 84.4 and 117.3 U/mL, respectively. It has decreased gradually for each successive cycle, following the same rate observed for β-CD production.

In Fig. 4, the first cycle is described by a curve-shaped almost asymptotic and further cycles by straight-lines. It is possible that the differences in β-CD production in the cycles may be due to weakly bound cells in the matrices that desorbed in the course of the first cycle, thus remaining for the cells of further cycles that were more strongly bound to the matrices. Protein determinations were carried out, and confirmed the cell desorption. The protein content of the recently immobilized cells in the SiO_2/TiO_2 and SiO_2/MnO_2 matrices was 0.17 and 0.40 g nitrogen/100 g of sample, respectively, and after the end of a CD production cycle there was an average reduction of 14%.

The repeated use of the immobilized *Mycobacterium* sp. NRRL B-3805 [33] and *Pseudomonas putida* [34] cells on Celite

supports did not prove to be effective due to cell desorption, suggesting that the interactions between support and cells are too weak. On the other hand the use of chrysotile as an effective immobilization matrix for mycobacterial cells displayed a significant operational stability, since catalytic activity remained apparently unchanged for a 30-day period. The stability is related not only to product yield, but to mechanical integrity of the support as well [35].

For *B. agaradhaerens* LS-3C immobilized cells, after the ninth cycle (270 h of test), the CGTase activity was maintained at 70% of the initial rate [11]. A good operational stability was observed with *B. amyloliquefaciens*, immobilized in calcium alginate, which was studied during 14 cycles of 24 h each, keeping 80% of the initial activity [7]. Vassileva et al. [23] studied the operational stability of *B. circulans* ATCC 21783 immobilized in agar during 5 cycles of 48 h each. There was a gradual increase of CGTase activity until the fourth cycle, reaching 210 U/mL, meaning a 14% higher enzyme concentration than the initial activity. However, in the fifth cycle the enzyme activity suffered a reduction of 10% (189 U/mL).

3.4. Storage stability

The long viability and continuous metabolic activity are also advantages for using immobilized cells [6]. The storage stability was investigated using 2.4 g of initial biomass and the cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2 were maintained at 4 °C for 210 days. The maximum of β-CD production occurred with the cells stored for 90 days, giving 6.0 ± 0.3 and 4.4 ± 0.4 mM of β-CD for the cells immobilized in SiO_2/TiO_2 and SiO_2/MnO_2, respectively (Fig. 5). Therefore, the results obtained showed that the immobilized cells were quite stable and, after 150 days of storage, they did not present any reduction of β-CD production, in relation to newly made biocatalyst. The long viability of immobilized cells may be due to their different protein, nucleic acid and inorganic substance compositions, compared to the free cells [7]. Also, according to Jamuna and Ramakrishna [6], in the case of *Bacillus* immobilized cells, the induced stress conditions imposed by the immobilization can alter the synthesis mechanism of the enzyme produced. However, definitive evidence regarding this statement is lacking in previous literature and warrants further studies.

In the storage stability test, control cycles using free cells were also carried out, producing in average 3.0 ± 0.1 mM of β-CD. Therefore, the immobilized cells showed a relative production 2-fold higher for the immobilized cells in SiO_2/TiO_2 and 1.5-fold higher for SiO_2/MnO_2. It is possible suggest that the highest capacity of β-CD production was not a consequence of the increase in the amount of immobilized microbial cells, considering that there was an average reduction of 14% of the total protein content in newly immobilized samples and after the final cycle of CD production. Also, immobilized cells of *Methylomonas* sp. strain GYJ3, immobilized by sol–gel entrapment in sodium silicate, presented an enzymatic activity 1.5-fold higher than the free cells [12].

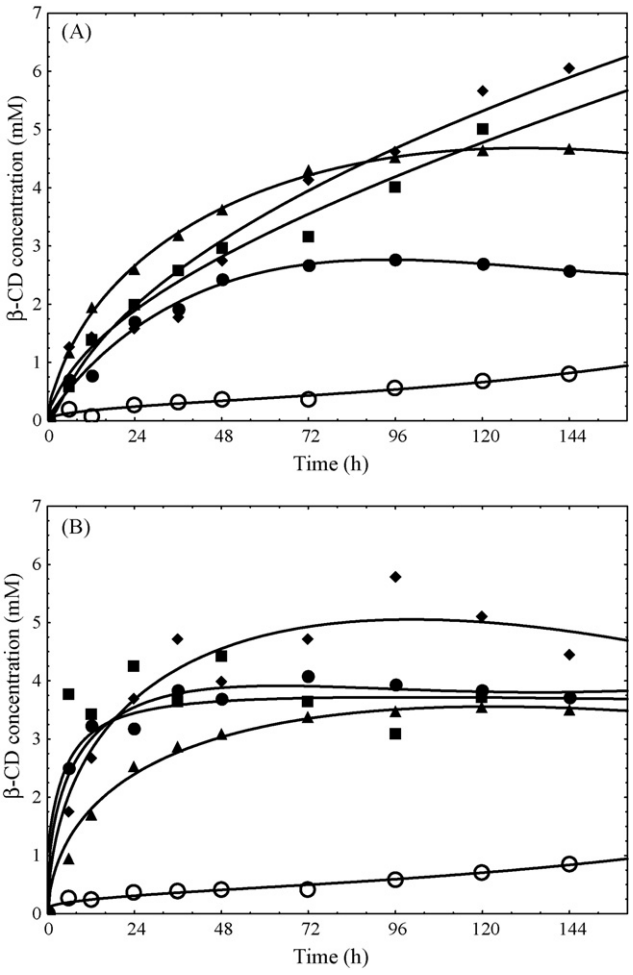

Fig. 5. Storage stability of immobilized cells of *B. firmus* strain 37. (A) SiO_2/TiO_2 and (B) SiO_2/MnO_2. Storage time: ● 0 day; ■ 60 days; ♦ 90 days; ▲ 150 days and ◯ 210 days. Conditions: maltodextrine 10% (w/v) in Tris–HCl buffer, pH 8.0, 50 mM and $CaCl_2$ 5 mM, 50 °C and 120 rpm.

4. Conclusion

Systems of immobilized *B. firmus* strain 37 cells for CGTase production with sol–gel matrices have been developed. However, the present research study also showed the possibility of direct CD production by immobilized cells in inorganic matrices. The sol–gel preparation method of matrices showed to be appropriate and a good adsorption of *B. firmus* strain 37 occurred in SiO_2/TiO_2 and SiO_2/MnO_2 matrices.

The SiO_2/MnO_2 matrix presented advantages compared to SiO_2/TiO_2 matrix while studying the effect of initial biomass, as the use of 1.8 g of biomass was sufficient to reach the highest β-CD production (17.9 ± 0.1 mM), while for the SiO_2/TiO_2 matrix the use of 3.0 g of biomass was necessary to reach a β-CD production 25% smaller.

Operational stability tests demonstrated that both matrices maintained good β-CD production up to the end of the second cycle (288 h test) and a gradual fall in β-CD production occurred up to the fourth cycle, due to cell desorption from the matrices. Storage stability tests showed that the cells immobilized in SiO_2/TiO_2 presented a β-CD production 36% higher

than for the SiO_2/MnO_2 matrix and, when compared to free cells, the SiO_2/TiO_2 biocatalyst performance showed a twofold improvement. When these control cycles using free cells were carried out, no significant loss of activity occurred; therefore, the decay observed for the immobilized cells in the operational stability tests can be ascribed to cell leakage from the matrices. The choice of SiO_2/TiO_2 or SiO_2/MnO_2 matrices depends on the aim of the use, since both present distinct characteristics and advantages.

CD production from immobilized cells in inorganic matrices prepared by the sol–gel method proved to be as feasible as the production achieved by Martins et al. [11] using PVA-cryogel entrapped cells. The same inference was observed when Tardioli et al. [22] used enzyme immobilized in glyoxyl-agarose. Furthermore, if gram-scale reactions are the objective, the use of large fermentation reactors is not always recommended. Thus, whole-cell immobilization is a good alternative to overcome these problems, allowing several months of storage and thus behaving as a normal reagent [10].

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