Inulin hydrolysis by inulinase immobilized in polyvinyl alcohol based hydrogel

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Introduction

Hydrogels are acknowledged as fitting materials for the immobilization of enzymes and whole cells, given their biocompatibility, low coefficient of friction, and high water content. The resulting biocatalysts are used in biotransformation systems with application in several sectors, among which food and feed industry (Walsh, 2007). Alongside their favourable characteristics, most hydrogels, namely those from natural polymers, often display low mechanical stability (Schlieker and Vorlop, 2006), and are furthermore prone to enzyme leakage (Cao, 2005), drawbacks that often restrain their wider application. Polyvinyl alcohol-based matrices have been shown to lead to particles with considerable mechanical stability, and thus present a suitable alternative to overcome the drawbacks of natural polymers while retaining the benefits inherent to their use (Schlieker and Vorlop, 2006). Several methods have been developed to form hydrogels from polyvinyl alcohol (PVA). One of these, developed by GeniaLab, Germany, has achieved commercial scale, and the lens-shaped particles yielded by this method have been used for the hydrolysis of sucrose and maltodextrins, which evidenced the potential of this approach in the sugar industry (Rebros et al., 2006, 2007). In the present work a modified procedure of the GeniaLab methodology, is used for the encapsulation of inulinase in PVA capsules, obtained from LentiKat® liquid upon extrusion into liquid polyethers. In this method, the PVA based hydrogel is extruded to low molecular weight polyethylene glycol (PEG) or polypropylene glicol (PPG), where gelification occurs instantaneously, in the form of hemispherical-like capsules. The procedure is quite simple and the PEG or PPG can be recycled for several times. The immobilized biocatalyst is used to yield fructose syrup through hydrolysis of inulin, a linear-linked fructose polymer that occurs as reserve substrate in Jerusalem artichoke, chicory or dahlia tubers (Ricca et al., 2007). Enzymatic hydrolysis of inulin is favored when matched to the chemical approach, since the later leads to the formation of unwanted by-products and colored compounds (Ricca et al., 2007). The use of an immobilized biocatalyst is mostly required in this particular application, for preventing product contamination with protein matter, while broadening the range of the mode of operation and often of operational conditions, namely temperature and pH, as well as commonly enhancing the stability of the biocatalyst. In this work the use of inulinase immobilized in PVA capsules obtained from extrusion into polyethers is evaluated and its performance in inulin hydrolysis is assessed and matched to the free enzyme. PEG 600 was selected as the most suitable polyether and although the immobilized system displayed diffusion limitations, full bioconversion of a 5.0 w/v inulin solution was obtained in a 24 hour run matching the performance of free inulinase. The

immobilized biocatalyst was successfully used in 20 consecutive batch runs with no significant decay in activity.

Material and methods

Fructozyme L, a commercial preparation of inulinase from *Aspergillus niger*, was provided by Sigma, polyethylene glycol (PEG) 200, 425 and 600, and polypropylene glycol (PPG) 425 e 2000 were from Fluka (Germany), LentiKat® liquid, a PVA-based material was from GeniaLab (Germany), and inulin from chicory (Fibruline Instant) was a kind gift from Cosucra (Belgium). All other chemical were of analytical grade from various suppliers.

Enzyme immobilization

In order to entrap inulinase in PVA, the LentiKat® liquid was heated at 95ºC and then cooled to 40ºC. The enzyme suspension (0.5 ml of a ten-fold, 100 mM acetate buffer, pH 4.5, diluted commercial preparation) was added to 10 ml of the LentiKat® liquid and mixed under magnetic stirring. The enzyme-rich solution was extruded through a needle (Therumo, 22 Gx1 1/4") into 150 ml of a given polyether, mildly magnetically stirred, where capsules are formed. After a 2-hour period, the hemispherical capsules were harvested, and thoroughly washed with acetate buffer, weighed after removal of excess buffer with qualitative filter paper, soaked in acetate buffer for about two hours, harvested and weighed again after removal of excess buffer with qualitative filter paper, and either immediately used for bioconversion runs or stored at 4ºC until use.

Enzyme assay and evaluation of operational stability

Bioconversion runs were performed at 50ºC, in 1.5 ml screw-capped magnetically stirred (500 rpm) reactors, filled with 1.0 ml of 100 mM acetate buffer (pH 4.5), containing 5.0% (w/v) of inulin and 50 mg of immobilized inulinase, or a similar amount of free enzyme. Samples (10 μl) were taken periodically and immediately assayed for reducing sugars. Operational stability was evaluated by promoting consecutive 24-hour batch runs, using PVA capsules obtained from extrusion in PEG 600, under the conditions described for the bioconversion runs. Samples were collected after 24 hours. After each run, the capsules were thoroughly washed with acetate buffer and immediately incubated in fresh inulin solution. All trials were performed in triplicates, at least.

Analytical methods

Quantification of reducing sugars was performed by the DNS method (Miller, 1959). Quantification of protein was performed by the BCA method (Smith et al., 1985) using a commercial kit from Pierce. Particles of immobilized biocatalyst were dissolved in distilled water heated at 70ºC, prior to protein quantification. Liquid supernatants resulting form the immobilization procedures were also assayed for protein levels. The standard deviation from these determinations did not exceed 8%, except if stated otherwise.

Results and Discussion

Extrusion of containing inulinase into each of the polyether liquids assayed yielded roughly hemispherical capsules, with about 3 mm diameter (Figure 1), but for PPG 2000, where the drops of LentiKat® tended to aggregate once fallen in the polyether. The catalytic activity of the capsules formed under the different polyethers did not differ significantly (Figure 2). Since those formed under PEG 600 slightly outperformed the remaining polyethers trialed, they were chosen for further work.

Figure 1: Typical shape of PVA capsules obtained following extrusion polyethers. The capsules depicted were obtained in in PEG 600.

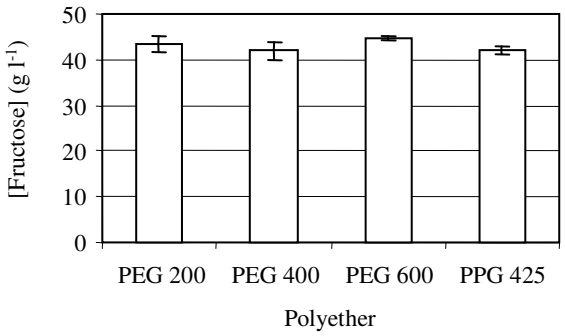

Figure 2: Fructose yield obtained after a 24 hour batch run using PVA capsules obtained after extrusion under different polyethers. Bioconversion runs were performed at 50ºC and pH 4.5.

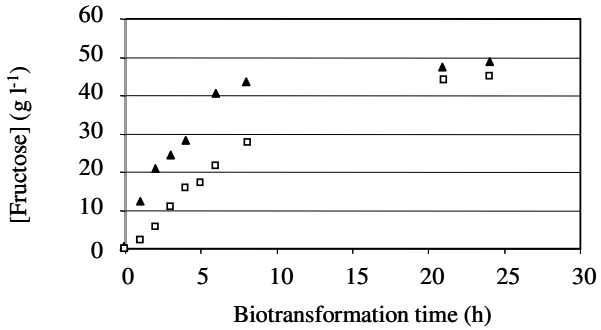

Figure 3: Time course of a typical bioconversion run of a 5.0 % inulin solution, at 50ºC and pH 4.5. Similar amounts of enzyme were used, in the free (▲) and immobilized (□) form

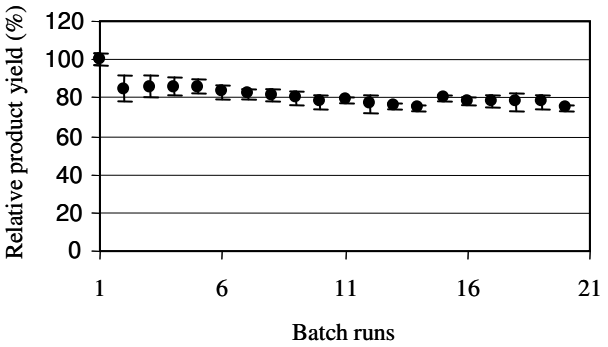

Figure 4: Repeated batch hydrolysis of a 5.0 % (w/v) solution of inulin with immobilized inulinase. Reactions were performed at 50 ºC and pH 4.5. Final product yield in the first run was about 50 g l^{-1}.

The performance of the biotransformation system was somehow hampered by diffusion limitations, as suggested by the trend of the time course of the bioconversion run, a feature that is not totally surprising given the bulky nature of the substrate molecule (Figure 3). The immobilized form of the biocatalyst was successfully in 20 consecutive batch runs with no significant decrease in the final product yield throughout the runs (Figure 4). The capsules were apparently not affected by mechanical or thermal induced stress resulting from the operational conditions. PVA lenticular particles obtained from Lentikat® liquid through a standard procedure from Genialab (http://www.geniaLab.de/download/tt-english.pdf) have been shown to be sensitive to long term operation at temperatures above 55ºC (Rebros et al, 2006, 2007) a feature also observed with the capsules used in this work (data not shown).

Conclusions

Inulinase was effectively immobilized in PVA capsules obtained by extruding Lentikat® liquid into polyether liquids, particularly when PEG 600 was used as recipient. Diffusion limitations were evidenced in the immobilized bioconversion system when matched to its free counterpart, yet roughly full bioconversion of a 5.0 % (w/v) inulin solution was obtained in the former system after a 24-hour batch run, which furthermore displayed significant operational stability. The data presented are clearly suggestive of the potential of the immobilized system presented in larger scale.

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