P-015 **PVA and sol-gel carriers for entrapment of β-galactosidase for production of galacto-oligosaccharides**

Jovanovic-Malinovska R. [1#], Fernandes P [2,3]., Winkelhausen E. [1] and Fonseca L. [2,3 *]
[1] Food Technol & Biotechnol, Univ SS Cyril & Methodius, Skopje, R. Macedonia [2] Bioeng, [3] IBB, Cent for Biological & Chem Eng, Inst Sup Técnico, Univ Técnica de Lisboa, Portugal
* Supervisor # Contact email : rruzzica@yahoo.com

INTRODUCTION AND OBJECTIVES

In the last 20 years the immobilization of ß-galactosidase on different surfaces has gained a lot of attention in the food industry (Grosova et al., 2008). ß-galactosidase (EC 3.2.1.23) catalyzes not only the hydrolysis of lactose but also the transgalactosylation reaction to produce galacto-oligosaccharides (GOS) (Park and Oh, 2010). In the cheese industry lactose is a waste, which causes several economical and environmental problems. Approximately 47% of the whey produced annually worldwide is disposed off (Novalin et al., 2005). Therefore, conversion of lactose into a highly valuable product such as GOS is of high interest to the food industry. GOS, known as *Bifidus growth factor*, because of the selective stimulation of bifidobacteria in the low intestine, have many health benefits (Tomomatsu et al., 1994), and have wide commercial applications as prebiotic food ingredients and dietary supplements.

Despite the abundant reports on β-galactosidase immobilization, most are referred to lactose hydrolysis. This study focuses on developing a method for immobilization of β-galactosidase in PVA and sol-gel carriers, investigating the properties of immobilized enzyme and the possibility of using the immobilized enzyme system for the synthesis of GOS from lactose. The β-galactosidase from *Aspergillus oryzae* was chosen as a model enzyme for this study.

MATERIALS AND METHODS

Materials

Commercial preparation of ß-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae* (8U/mg solid, 125000U), lactose and tetramethoxysilane (TMOS) were obtained from Sigma-Aldrich. Lentikats® was obtained from Genialab. All other reagents were of analytical grade from different sources.

Enzyme immobilization

The enzyme preparation (10 mg/ml) was diluted in 100 mM acetate buffer pH 4.5. Immobilization in PVA was performed according to the protocol provided by GeniaLab, adding 0.2 ml of the diluted enzyme preparation in 1 ml LentiKat® liquid. The resulting solution was extruded to Petri dishes. After dehydration, under 30ºC, to 30 % (w/w) of the original weight, to allow for gelation, the lenses were incubated in 100 ml of a 15 g/l solution of LentiKat®stabilizer for two hours at room temperature. The lenses were then washed and stored in 100 mM acetate buffer pH 4.5 at 4ºC until use. Immobilization in sol-gel was based on the work by Bernardino et al., 2009. Solution containing 100 µL TMOS and 40 µL HCl (10 mM) was sonicated in a Transsonic T 460 sonicating water bath for 10 min until the hydrolysis reaction was complete. In a typical immobilization procedure 160 µL of enzyme (10 mg/ml) was suspended in the sol solution. To obtain micro particles, 300 µL of the sol-gel solution with enzyme was immediately added to 6 mL of 150 mM AOT/isooctane solution, before gelation. The resulting mixture was vortexed for 1 min, washed twice with 100 mM acetate buffer and aged at room temperature under controlled water activity (a_w = 0.75), during 1 week. The micro-particles obtained were suspended in 1 mL of the acetate buffer and stored at 4ºC until use.

Enzyme activity

For ß-galactosidase activity assay, immobilized enzyme was added to lactose solution and samples were collected along the time. The product released in the assay, glucose, was determined spectrophotometrically. One ß -galactosidase unit (U) was defined as the amount of immobilized enzyme catalyzing the release of 1 µmol of glucose per min per mg of protein at pH 4.5 and 40ºC.

GOS synthesis in a batch process

Batch conversions were performed using 6 mL of lactose solution (40% w/v lactose in 100 mM acetate buffer, pH 4.5). The substrate solution was incubated with free and immobilized β-galactosidase in PVA and sol-gel at 40°C for 12 h. Samples were taken at appropriate time intervals and analyzed for sugar content by high performance liquid chromatography (HPLC). The concentration of released protein was determined based on a Bradford method.

Reusability of the immobilized enzyme

In order to test the reusability, immobilized enzyme was incubated with 5% w/v lactose in 100 mM acetate buffer, pH 4.5 at 40°C, and enzyme activity was measured. After each reaction cycle the pellet containing immobilized enzyme was recovered by centrifugation and reused for another reaction cycle.

RESULTS AND DISSCUSION

Effect of pH and temperature on enzyme activity

Fig. 1 demonstrates the pH and temperature activity profiles of the free and immobilized β-galactosidase. The optimum pH of the enzyme was 4.5 for free and en-

trapped enzyme in PVA and sol-gel carriers. The pH profile of the immobilized β-galactosidase displayed an improved stability on both sides of the optimum pH values, in comparison to that in the free form, which means that the immobilization method preserved enzyme activity in a wider range.

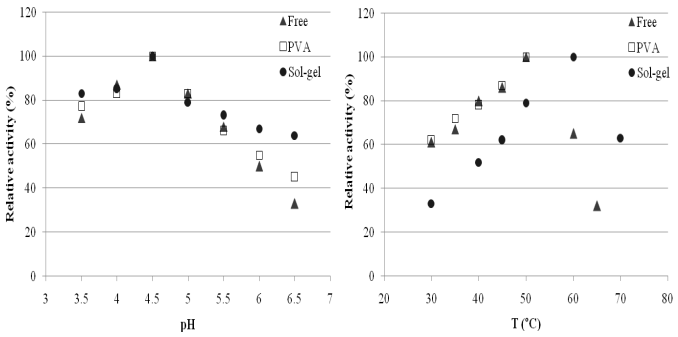

Figure 1: Influence of pH and T on the activity of free (closed triangles), PVA (squares) and sol-gel (closed dots) immobilized forms of β-galactosidase. Bioconversion runs were carried out at 40°C (pH assay) and pH 4.5 (temperature (assay).

Immobilization temperature is one of the important parameters for sufficient activity of immobilized enzyme. As seen from Fig.1, immobilized enzyme activity was affected by immobilization temperature. The temperature optimum was increased from 50°C for free and immobilized enzyme in PVA, to 60°C for the immobilized enzyme in sol-gel. Furthermore, sol-gel immobilized β-galactosidase retained higher fractions of the catalytic activity at higher temperatures (63% enzyme activity at 70°C). On the other hand, it displayed lower activity at lower temperatures, as compared to free and PVA immobilized β-galactosidase.

Reusability of immobilized β-galactosidase

The reusability of the immobilized β-galactosidase has been shown in Fig.2. PVA and sol-gel entrapped enzyme showed respectively 61 and 95% of the initial activity after their 7th repeated use.

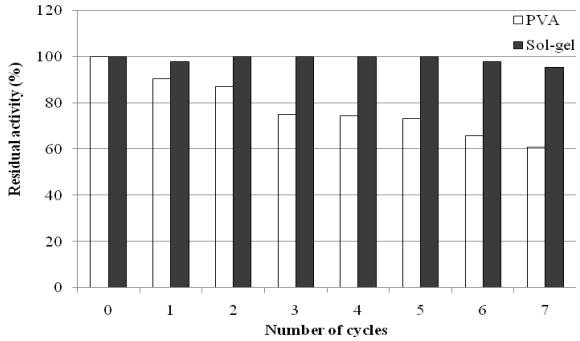

Figure 2: Reusability of immobilized β-galactosidase in PVA (open bars) and sol-gel (closed bars).

Formation of GOS by free and immobilized β-galactosidase in PVA and sol-gel carriers

The formation of GOS catalyzed by free and PVA or sol-gel immobilized β-galactosidase after 12 h is shown in Fig.3. The total GOS yield was 23, 31 and 22% for the free enzyme and immobilized in PVA and sol-gel carriers, respectively. These maximal values corresponded to lactose conversion of 57, 44 and 52% for the free enzyme and immobilized in PVA and sol-gel carriers.

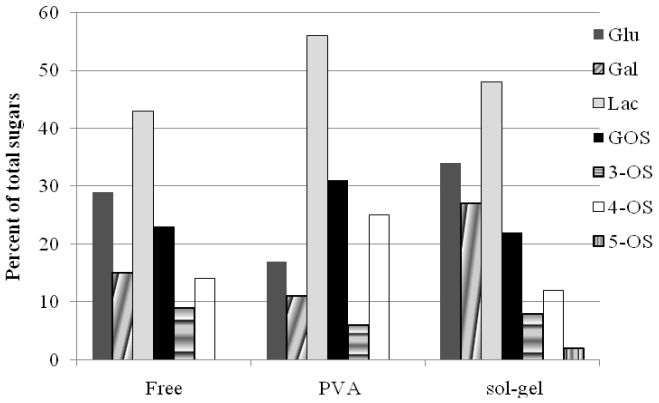

Figure 3: Lactose conversion with free and immobilized β-galactosidase. Glu, glucose; Gal, galactose; Lac, lactose; GOS, total GOS; 3-OS, trisaccharides; 4-OS, tetrasaccharides; 5-OS, pentasaccharides.

As shown in Fig.3, 4-OS also dominated over the other types of GOS formed in the reaction. Larger GOS, 5-OS, were produced only with immobilized β-galactosidase in sol-gel. On the other hand, high glucose and galactose yields were observed with sol-gel immobilized β-galactosidase, which indicated that hydrolysis reaction dominated the transgalactosylation reaction.

CONCLUSION

β-galactosidase from *Aspegillus oryzae* was successfully immobilized on the PVA and sol-gel carriers. Sol-gel carrier showed lower GOS yield despite better activity characteristics. Having selected PVA as carrier, the further experiments should focus on enhancing the reusability and optimizing the conditions for GOS production.

REFERENCES

- Bernardino, S. et al. (2009) *A new biocatalyst: Penicillin G acylase immobilized in sol-gel*. Biotechnology Journal 4 (5) 695–702
- Grosova, Z. et al. (2008) *Perspectives and applications of immobilised B-galactosidase in food industry - a review.* Czech Journal of Food Sciences 26 1-14.
- Novalin, S.et al. (2005) *A new innovative process to produce lactose reduced skim milk.* Journal of Biotechnology 119, 212-218.
- Park, A.R. et al. (2010) *Galacto-oligosaccharide production using microbial ß-galactosidase: current state and perspectives.* Applied Microbiology Biotechnology 85, 1279-1286
- Tomomatsu, H. (1994) *Health Effects of Oligosaccharides.* Food Technology 48, 61-65