P-008 **Solving lactose health and environmental problems using immobilized lactase on bio-polymeric beads**

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INTRODUCTION AND OBJECTIVES

Half of the entire productions of dairy by products (whey) are disposed of which represents a problem to the environment and especially the marine life as the acidity of the water increases killing living organisms. On the other hand, by product itself contains 4-5 % lactose, thus finding a technology to transform lactose into more profitable product such as glucose will be of particular interest to the food industry (Novalin 2005). Moreover, the consumption of foods with a content of lactose is problematic for almost *70%* of the world population suffering with lactose intolerance. Unfortunately, there is *''no cure to lactose intolerance''* (Elnashar 2009 a).

This environmental catastrophe together with the relatively low sweetness and solubility of lactose, have lead to an increasing interest in the development of industrial processes to hydrolyze the lactose contained in dairy products. As an advantage, the products of hydrolysis, glucose and galactose, are sweeter, more soluble, and more digestible than lactose (Elnashar 2009 a). In this work, we immobilized lactase on grafted carrageenan beads prepared using the Encapsulator (Figure 1) according to our patent by Elnashar (2009 b). Immobilization is more useful as it saves time and money as the immobilized enzyme could be reused for tens of time rather than the free enzyme, which is only used for one time.

MATERIALS & METHODS

Materials

Carrageenan has been purchased from Fluka. Lactase from Aspergillus oryzae (E.C.232-864-1), 11 U/mg, and other chemicals were purchased from Sigma-Aldrich. Encapsulator for making uniform gel beads was bought from EncapBioSystems Inc., Switzerland.

Preparation of κ-Carrageenan gel beads

κ-Carrageenan gel was prepared as previously reported by Elnashar (2009b) by dissolving 2.5% (w/v) carrageenan in distilled water at 70 °C using an overhead mechanical stirrer until complete dissolution had occurred. Then beads formed by using Encapsulator (Figure 1). The carrageenan gel disks were hardened using 0.3 M KCl for 3 h as a control (Moon 1991) and with 4% (w/v) polyethyleneimine (PEI) at pH 9.5 for 3 h. The gel disks were separated from the PEI solution then thoroughly washed with distilled water and soaked in 2.5% (v/v) glutaraldehyde solution for 3 h (Elnashar 2009 b).

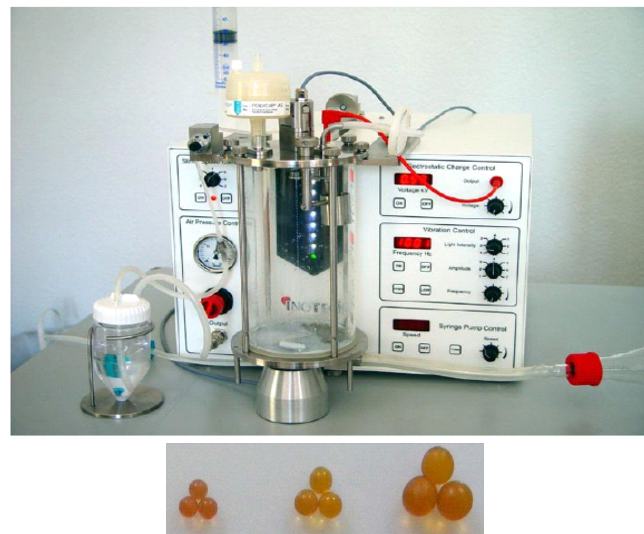

Figure 1: Encapsulator for making gel beads.

Conversion of lactose to glucose

Free and immobilized enzyme was incubated into 10 ml of 200 mM lactose at pH 4.5 and 37 °C. Samples were withdrawn at interval times from 30 min to 5 h and analyzed for glucose content. (Figure 2&4)

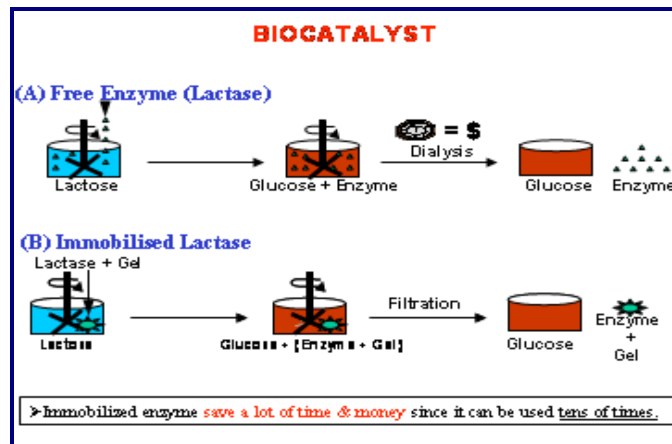

Figure 2. Free and immobilized enzymes.

Operational Stability

The reusability of immobilized enzyme was studied using the modified gel disks. Immobilized enzyme was incubated into 10 ml of 200 mM lactose at pH 4.5 and 37C for 3 h, and the substrate solution was assayed for glucose content determination. The same gel disks were then washed with distilled water and re-incubated with another substrate solution; this procedure was repeated 15 times and the starting operational activity was considered as 100% relative activity.

RESULTS AND DISCUSSION

Maximum Loading Efficiency

The data shown in Figure 3 are revealing a maximum lactase loading and percent enzyme efficiency of 27 U/g gel beads (90%, enzyme loading efficiency).

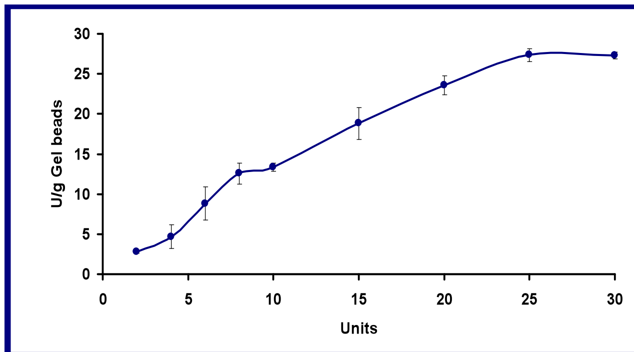

Figure 3. Lactase immobilization

Full conversion of lactose, using free and immobilized enzyme

The free enzyme reached a plateau and conversion of 80% of lactose after 2.5 h, whereas the immobilized enzyme reached 60-65 % of conversion at that time and increased to 100% after 3-4 h as shown in (Figure 4). The decrease in substrate conversion after 3 h could be regarded to diffusion limitation (Novalin 2005).

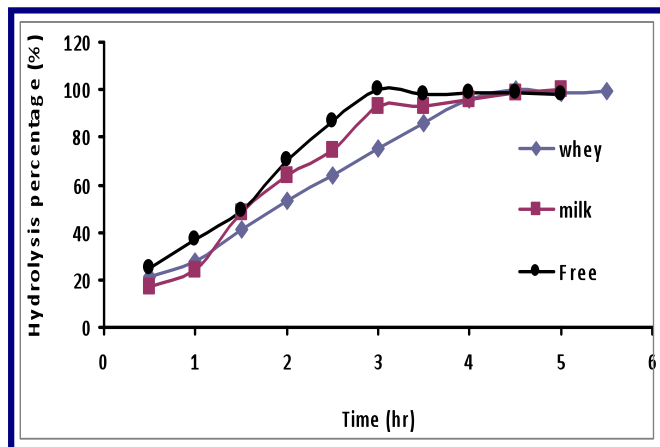

Figure 4. Conversion of lactose to glucose using free and immobilized enzyme.

Operational stability of immobilized lactase

The data shown in Figure 5 indicates that immobilized lactase retained around 80% and 50% of its relative activity by the 20th use, using milk and whey, respectively. The loss in activity was attributed to inactivation of the enzyme due to continuous use (Elnashar 2009a).

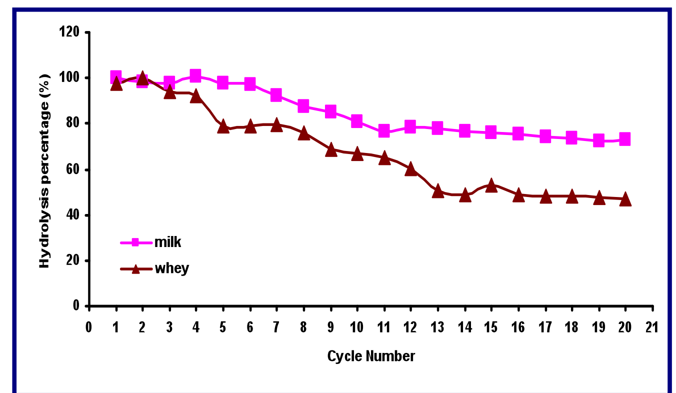

Figure 5. Reusability of enzyme using lactose in milk and whey.

CONCLUSION

- Production of carrageenan gel-beads using the Encapsulator.
- Immobilized lactase enzyme can be reused more than 20 times.
- Maximum lactose conversion in whey and milk could be achieved within 3-4 h using both free and immobilized enzyme.
- Maximum enzyme loading is 27 U/g gel beads with 90% enzyme loading efficiency.

ACKNOWLEDGEMENT

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