P-005	**Production of 6-APA for semi-synthetic antibiotics using covalent immobilization of penicillin G acylase onto grafted alginate beads**	

Elnashar, M.M. [#*]**; Ali, O.A.; Hassan, M.E.**
Biopolym & Nanobiotechnol Group, Centre Excellence Adv Sc, Nat Res Centre, Giza, Egypt
[#*]Corresponding author: magmel@gmail.com

INTRODUCTION AND OBJECTIVES

ß-lactam antibiotics - in particular penicillins and cephalosporines - represent one of the major biotechnology markets with annual sale of ~$ 15 billion. It represents ~65% of the total antibiotics market (Elander 2003). ß-lactam antibiotics alone constitute most of the world's antibiotics sale: $3x10^7$ kg/year out of a total $5x10^7$ kg/year produced world wide (De Souza 2005). Penicillin G acylase (PGA) hydrolyses penicillin G (PG) to produce 6-APA, which is the precursor of many antibiotics (e.g. ampicillin, pencillin K, pencilin F, amoxicillin). Therefore, the annual consumption of PGA is estimated to be in the range of 10-30 million tons which has replaced the multi step chemical conversion and made it cheap with an efficiency of ~80-90%.
In drug industries, enzymes are preferred to be immobilized on solid supports since they can be separated easily from the product. Moreover, they could be reused for tens of time, which saves time and money (Figure 1). Many carriers both organic and inorganic have been used for immobilization of PGA (Maria-Chong 2004). However, organic polymer carriers are most widely used for immobilization of enzymes (Wang 2007). Synthetic organic polymers enjoy high stability but are very expensive. In contrast natural organic polymers are cheap but with low resistance toward physical, chemical and microbial attack.

The aim of this work was to produce 6-APA via hydrolysis of PG using covalent immobilization of PGA onto chemically treated alginate beads. Uniform gel beads were prepared using the Encapsulator (Figure 2) and the beads were further grafted according to Elnashar (2009). The immobilization process has been optimized to reach its maximum loading capacity and the optimum conditions have been used for 15 times to prove the efficiency and durability of the immobilized enzyme.

MATERIALS & METHODS

Materials

Alginate has been purchased from Fluka. PGA lyophilized with specific activity of 17.04U/mg was purchased from CPC in Italy, and other chemicals were purchased from Sigma-Aldrich. Encapsulator for making uniform gel beads was bought from EncapBiosyStems Inc, Switzerland.

Preparation of grafted alginate gel beads

Alginate beads were prepared using the Encapsulator and gel beads were hardened according to Elnashar 2009 using polyethylenimine and glutaraldehyde. Grafted alginate beads were used to immobilize PGA covalently.

Immobilization, optimization and reusability of PGA

Grafted alginate beads were soaked in 60 U PGA prepared in 0.1 M phosphate buffer at pH7 for 0.5-24h. The optimum condition was further optimized by soaking the gel beads in different concentration of PGA (20-200 U) at the optimum time of soaking. The best result was used for reusability of the immobilized enzyme.

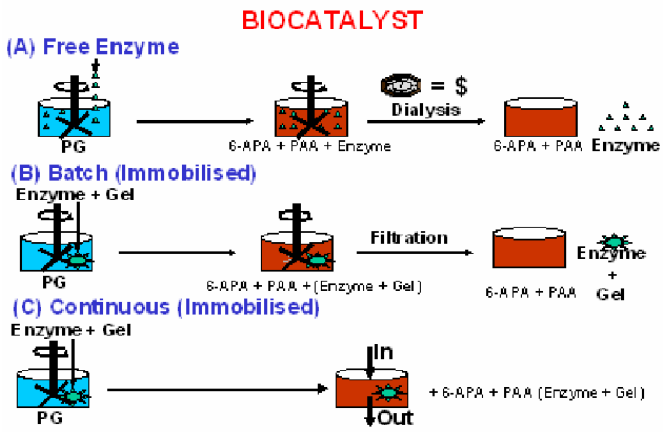

Fig. 1. Free and immobilized enzyme.

Fig. 2. Encapsulator for making uniform gel beads.

RESULTS AND DISCUSSION

Enzyme loading time

In this experiment, 60 U of free PGA was incubated with 1g of grafted alginate beads for 0.5-24h. The results in Figure 3 showed that maximum loading of PGA (31 U/g gel beads) has been achieved after 24 h. However, there is no marked change in the loading capacity between 16 h & 24 h. Accordingly, 16 h of soaking (29.4 U/g gel beads) was selected for further experiments.

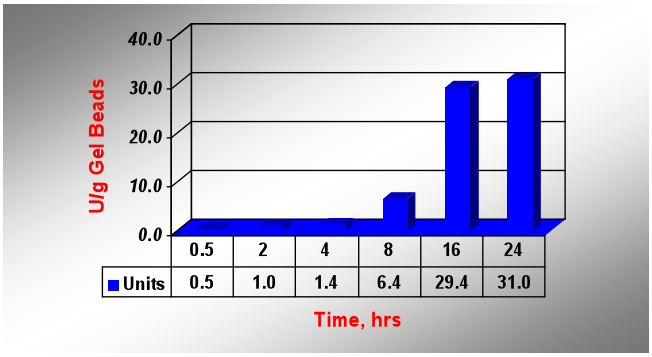

Fig. 3. Effect of enzyme loading time on the immobilization capacity.

Enzyme loading concentration

PGA at different concentrations (20-200U) was incubated for 16h with 1g alginate beads. The results in Figure 4 showed that the amount of immobilized enzyme was gradually increased by increasing the enzyme concentration from 20 U to 160 U, where 70 U/g gel beads was immobilized, after which a plateau was almost achieved. These results are ***seven folds*** that obtained by the authors in a previous work using grafted carrageenan gel beads (Elnashar 2008).

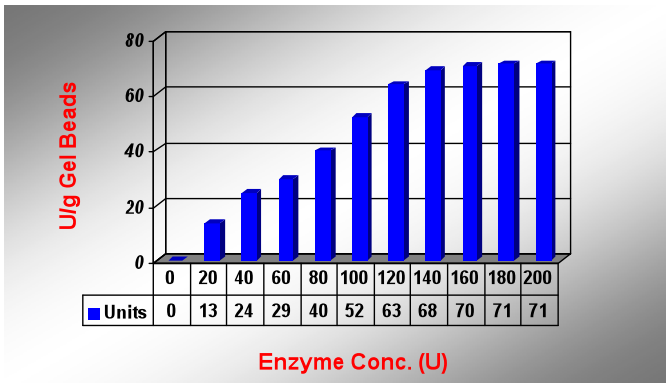

Fig. 4. Effect of PGA concentration on the enzyme loading capacity.

Operational stability

The best formulation of 70 U/g gel beads has been incubated with 9 ml of equal amounts of paradimethylamino benzaldehyde (PDAB) with PG for 5 min. Results showed gradual decrease of the relative enzyme activity by reuses to reach around 80% of the enzyme activity after 15 reuses. The decrease in enzyme activity might be attributed to inactivation of the enzyme due to continuous use (Elnashar 2009).

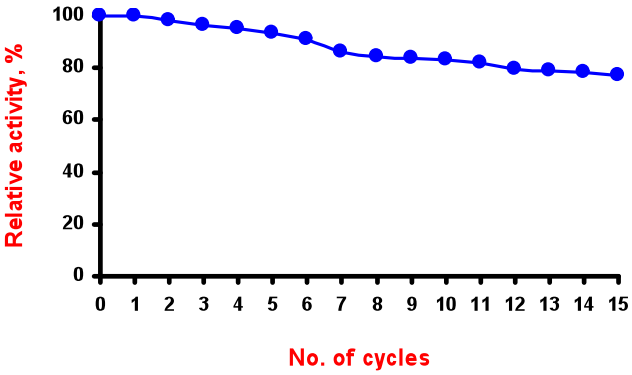

Fig. 6. Reusability of immobilized enzyme.

CONCLUSIONS

Production of uniform alginate gel beads using the Encapsulator.

Grafted alginate beads can immobilize 71 U/g PGA which is 7 folds that of grafted carrageenan gel beads (Elnashar 2008).

The reusability test was applicable up to 15 cycles with retention of ~80% of the immobilized enzyme activity.

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