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Agar-calcium pectinate beads as a covalent immobilization support

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Enzymes play an important role in food industries. For instance, the enzyme β-D-galactosidase (β-gal), which catalyzes the hydrolysis of lactose, is critical to the industry of lactose free dairy products that are required by the lactose intolerant people. However, enzymes suffer from their low stability. The separation of soluble enzymes from the reaction mixture is also tedious and expensive. Thus, industrial enzymes should be immobilized onto solid supports as being attached to such solid supports will facilitate the removal of these enzymes from the reaction mixture. Furthermore, the immobilization process is known to enhance the stability of the enzymes. Calcium pectinate (CP) was recently employed as a solid support to covalently immobilize enzymes. Nevertheless, CP suffers from instability in the presence of cations. This instability has limited the activation of the CP beads for the covalent immobilization of enzymes as the first step of the activation process comprised reacting with the cationic poly amino compound, polyethyleneimine (PEI). Agar was added to pectin during the preparation of the calcium pectinate (CP) beads in order to overcome the CP's instability in the presence of cations. The agar-CP beads managed to retain their integrity in presence of PEI and this enabled a more efficient activation process. The significant positive effect imparted by the addition of agar was also confirmed from the ANOVA of the Box-Behnken Design (BBD). The BBD was employed to investigate the optimum agar concentration and also the optimum PEI concentration and pH that would allow for the attainment of the highest observed activity of the immobilized β-gal. The optimized agar-CP beads immobilized a whole of 7.96±0.18U β-gal/g beads. Moreover, these beads retained 86.84±0.37% of their initial activity after 13 reusability cycles which proved their operational stability.

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