Immobilized urease applied in silkworm artificial feeding improves silk production

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Enzymes specifically reduce the activation energy of the reaction and subsequently increase the rate of biochemical reaction. Widely used in food industry, chemical industry, drug production, medicine, textile industry, agriculture and many other industries (Brena et al., 2013). In general, the main weakness of enzymes is their instability, which reduces their half-life in the case of temperature and pH fluctuations. However, enzyme immobilization technology offers opportunities to improve enzyme stability and reusability. In fact, the enhancement of enzyme thermal stability and pH stability is the most significant advantage of enzyme immobilization technology (Liu et al., 2013). Therefore, we can enhance the enzyme's tolerance to some harsh environments through immobilization techniques.

Artificial feed for silkworms has many potential applications, and they are important in sericulture. However, the problems of weak larvae and low silk protein synthesis efficiency of silkworm reared on artificial feed have not been solved (Dong et al, 2018). Studies have shown that although the formula feed imitates the composition of mulberry leaves and contains protein, fatty acids, carbohydrates, vitamins and minerals that meet the basic nutritional requirements, the formula feed cannot provide urease needed for nitrogen metabolism of silkworms, resulting in the accumulation of urea in the spinning stage (Hirayama et al., 2000). There are also studies and analysis f found that mulberry urea has a high similarity with other plant sources of urease, indicating that this enzyme is closely related to other plant ureases. However, the optimal pH value of mulberry leaf enzymes is 9.0, while the optimal pH value of most ureases isolated from plants and bacteria is neutral (Hirayama et al., 2000). Some studies have found that urea in silkworm can be converted into ammonia in the midgut by obtaining urease from mulberry leaves, the decomposed nitrogen source is also involved in the formation of silk (Jiang et al., 2015). Lack of urease will reduce silk yield. Therefore, how to add urease which is lacking in artificial feed is very important for silkworm rearing with artificial feed and has high economic value.

In this study, microspheres prepared with sodium alginate and calcium chloride were initially used to encapsulate jack bean urease. The enzyme properties were tested and it was found that the pH stability of jack bean urease after encapsulation was improved, indicating that the pH tolerance of jack bean urease under alkaline conditions could be improved through the encapsulation immobilization method.

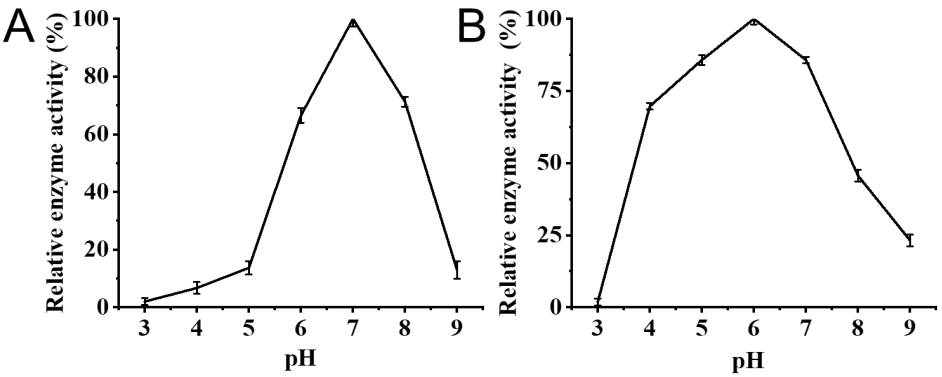

Figure 1. Enzymatic properties of jack bean urease

(A) The optimal pH of jack bean urease; (B) The pH stability of jack bean urease

Sodium alginate solution was mixed with urease and then drip-added to calcium chloride solution at a constant height through a syringe to prepare sodium alginate-$CaCl_2$ gel particles embedded with urease, as shown in Figure 2A. The pH stability of the embedded enzyme was also tested, as shown in Figure 2B. The results showed that the pH stability of urease was significantly improved after immobilization. Previous research has found that the study used sodium alginate microspheres coated with xylanase to improve pH tolerance and used them in poultry feed preparations (Kumar et al., 2017). Therefore, embedding improved the pH stability of urease compared to free enzymes.

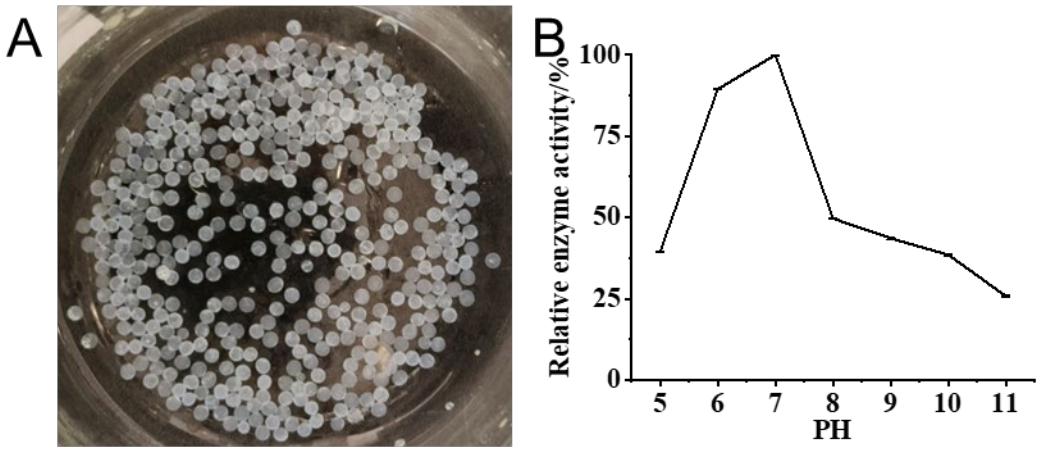

Figure 2. Morphology (A) and pH stability (B) of immobilized urease.

In this study, a kind of sodium alginate-$CaCl_2$ microsphere coated with jack bean urease was obtained. Through the encapsulation of nano-alginate microsphere, the stability of enzyme could be improved, and the physical and chemical properties of enzyme could be changed, so that it could adapt to higher environmental conditions while maintaining enzyme activity. This is obvious that enzyme could improve thermal stability, pH stability and storage stability through immobilization (Zahirinejad et al.,2021). Further research will be conducted on how to add it to the feed and its effects on silkworms after feeding.

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