Preparation of xylose oligosaccharides from the degradation of agricultural waste by carrier-free immobilized enzymes

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Xylooligosaccharides(XOS) is a new type of green additive, as a prebiotic, can directly enter the intestine and be preferentially used by probiotics such as Bifidobacteria and rapidly probiotics, thereby inhibiting the growth of harmful bacteria, so it can improve the intestinal microecology, regulate human physiological functions, and prevent constipation, diarrhea and other diseases (Liu et al., 2018). XOS also promotes the body's absorption of calcium. These excellent properties make it widely used in baked goods, health foods, beverage products, preserved fruit preserves, etc.(Aacharyet al., 2011). In addition, XOS can also be applied to medicine, agriculture, cosmetics and other fields (Surek et al., 2017). Agricultural wastes such as corn cobs, wheat bran, mulberry branches, bean stalks, etc., are high in xylans. But most of them have been wasted. If a suitable way that degrade xylan into xylon oligosaccharides such as xylosaccharides, xylanose and so on can be found, it will not only increase economic value, but also be environmentally friendly.

At present, the methods of degrading xylans are usually physical, chemical, and biological enzymes (Teng et al., 2010; Fan et al., 2012). Among them, the biological enzyme is the main method and development direction of the industrial production of XOS due to its advantages of mild reaction conditions, easy control of products, high XOS conversion rate, few by-products and environmental friendliness. As a complex structure of heteropolysaccharides, its hydrolysis requires the synergy of a variety of enzymes, of which xylanase is the main chain of hydrolyzed xylan, which is the key enzyme for the preparation of xylose oligosaccharides. However, due to the influence of temperature, pH, enzyme concentration, solvent and other factors, the catalytic activity of free enzymes is very low or even easily inactivated. In order to improve the stability and catalytic activity of xylanase, the technique of immobilized enzyme solved this problem well and greatly promoted the binding of enzymes to substrates. With the in-depth study of immobilized enzymes, there are still some problems to be solved, such as the active center failed to effectively contact the substrate, and the difficult of recover immobilized enzymes. Compared with this method, the carrier-free immobilized enzyme has many advantages: without expensive carrier, it greatly reduces in cost; this method is increasing the specific surface area of the immobilized enzyme, increasing the contact area with the substrate, and thus improving the enzyme activity; the carrier-free immobilized enzyme is connected through covalent bonds, and the fixed enzyme molecules are not easy to lose in use, which increases the number of reuses.

Conjugates of proteins and polymer molecules are another highly concerned approach to the technology of vector-free immobilized enzymes. Studies have shown that the regulatory effect of polymer molecules on enzyme activity is closely related not only to the structure and properties of polymer molecules and the types of chain delivery reagents(Murata et al., 2013), but also to the size of the polymer molecules(Yang et al., 2016). However, the use of polymer molecules of different hydrophobicity to regulate the mechanism of xylanase activity has not been explored. Therefore, this paper will systematically explore the effects of several polymers with different chain lengths on xylanase activity.

In this study, the xylanases was used to synthesize polymer graft XYL containing different alkyl side chains by ATRP technology, and on this basis, the effect of polymer grafting on the molecular structure of XYL was systematically investigated. Then the understanding of the mechanism of regulating the XYL enzyme activity mechanism of polymer molecular side chain structure regulation was deepened by the determination of the activity parameters and the stability of polymer graft XYL enzyme, thereby rationally screening the monomer compound molecular synthesis polymer graft XYL provide useful references.

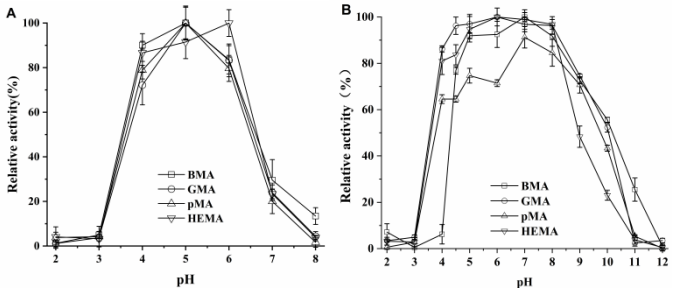

Fig 1: (A) Optimal pH of XYL-BMA, XYL-GMA, XYL-pMA and XYL-HEMA; (B) pH stability of XYL-BMA, XYL-GMA, XYL-pMA and XYL-HEMA

Fig 1(A) shows the results of the XYL-BMA, XYL-GMA, XYL-pMA and XYL-HEMA. There is little different in optimal pH of these conjugates and the optimal pH of XYL-HEMA conjugate is 6 while others' were all 5. Fig 1(B) shows that the relative activity of enzyme remained above 60 % at pH range 4-10 via using polymers of different chain lengths committed to modifying xylanases.

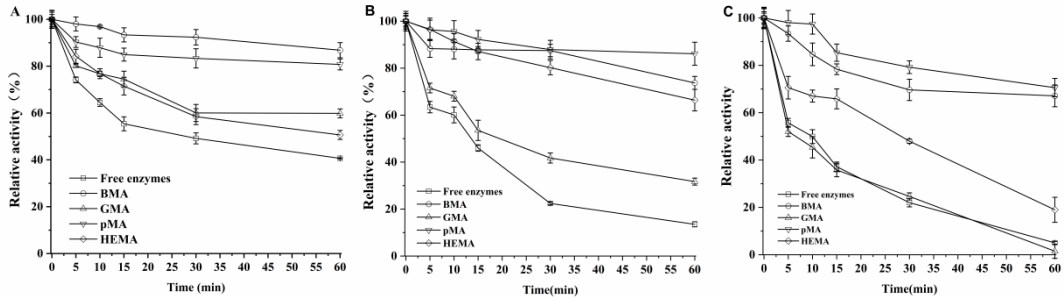

Fig 2: (A) Temperature stability of free XYL, XYL-BMA, XYL-GMA, XYL-pMA and XYL-HEMA in 50℃; (B) Temperature stability of free XYL, XYL-BMA, XYL-GMA, XYL-pMA and XYL-HEMA in 80℃; (C) Temperature stability of free XYL, XYL-BMA, XYL-GMA, XYL-pMA and XYL-HEMA in 90℃.

Fig 2 shows that the connection of polymers greatly improved the thermal stability of enzymes compared with free enzyme, which may be attributed to the encapsulation and protection of enzyme activity centers by polymers. Moreover, the polymer with longer chain length enables the enzyme to maintain high residual activity for a long time at higher temperature. At 50℃, the stability of the BMA grafted enzyme is particularly excellent, and it may be that the side chain group of butyl methacrylate is larger, which can better protect the enzyme after graft polymerization. And at 80 and 90℃, the stability of pMA is better. It can be shown in Fig 2 (B) and (C) that the remain activity of XYL-pMA can keep over 80% in 80℃ and 70% in 90℃ after incubation 60 min.

In conclusion, Polymers with different hydrophobicity are used to modify xylanase, which greatly improves the thermal stability of xylanase and makes it more widely used in industrial degradation of agricultural waste to xylooligosaccharides.

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