Polymer modification improves the stability of xylanase-polymer conjugates as biological interface catalysts in agricultural waste biorefinery

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Key words: immobilized enzyme; biodiesel; polymer; lipase; grafting-from.
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Agricultural wastes such as mulberry, wheat bran and corn silk are usually burned to destroy the environment and cause waste. In fact, these agricultural wastes contain large amounts of xylan that can be degraded to xylooligosaccharides (Cano et al., 2007). Xylooligosaccharides is a mixture of xylooligosaccharides with xylobiose and xylotriose as the main components, which is obtained from the hydrolysis of β-1,4 glycosidic bond of xylan by endo-xylanase with xylan as the substrate (Santibanez et al., 2021). Xylooligosaccharides also have a strong ability to proliferate intestinal probiotics, meanwhile, its energy value is approximately zero, which can play a role in low energy food. It is also available for patients with diabetes, obesity and hyperglycemia. Thus, Xylooligosaccharides is a widely used in many fields such as food and health products and were called ideal additive for functional foods(Ayyappan et al., 2016).

At present, the production of Xylooligosaccharidesis more inclined to enzymatic hydrolysis due to the secondary reaction occurs during the acid hydrolysis process, resulting in harmful by-prodcts and harming the environment (Quinones et al., 2015). The specificity of enzyme and the temperature of reaction make enzymatic hydrolysis effectively avoid the shortcomings of acid hydrolysis and become a more popular technology than acid hydrolysis (Hu, et al, 2022). However, it is difficult to separate the products and catalysts in the conventional enzymatic hydrolysis system, which largely causes the waste of enzymes and products (Jafari et al. 2020). Pickring interfacial biocatalysis (PIB) provide solutions to these deficiencies. Meantime, the enzyme - polymer conjugate is the double identity of catalyst and interface stabilizer in PIB system (Sun et al., 2018). However, the effect of polymer properties on the stability of xylanase activity has not been studied. Herein, polymers with different properties such as hydrophilicity, hydrophobicity, carrying positive and negative charges are used to modify xylanases (XYL) by ATRP “grafting-from” method (Vazquez et al., 2000). The detection of enzymatic properties can compare the changes of enzyme activity and stability caused by different properties of polymers, and help to select high-quality polymers and suitable grafting number.

The group screen of XYL. Conjugates were observed as broad bands at high molecular weight. The broad band of enzyme-protein conjugates is due mainly to the attachment of uncharged polymer chains, which hinder movement of the sample. And with the increase of polymer connection, the molecular bands migrate to the polymer bands. The results of SDS-PAGE show the probably molecular weight of free XYL, XYL MI. Compared to unmodified lipase, the XYL MI showed a slight shift to higher molecular weight which proved it successfully connected to the amino.

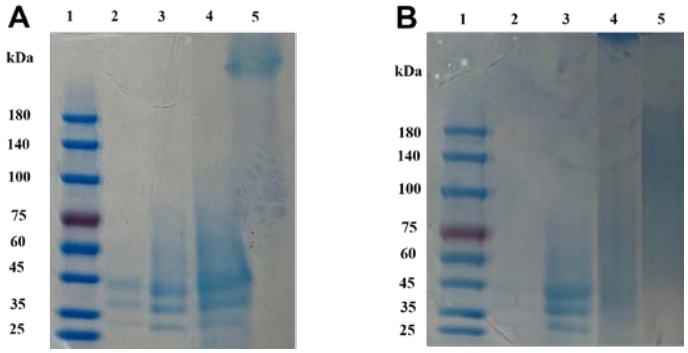

Figure.1 SDS-PAGE of free XYL, XYL MI and XYL-polymer conjugates, (A) line 1: marker; line 2: free XYL; line 3: XYL MI; line 4: XYL-PNIPAAm50, line 5: XYL-PNIPAAm200; (B) line 1: marker; line 2: free XYL; line 3: XYL MI; line 4: XYL-PDMAPMA50, line 5: XYL-PDMAPMA200.

The results of enzyme activity show that the connection of NIPAAm and PDMAPMA improved the temperature stability of the enzyme, and the increase was positively correlated with the grafting amount. Interestingly, PDMAPMA200 can increase the optimum pH value of the enzyme from 4.5 to 6, and only increases to 5 while the number of connections at 50, which should be related to the positive charge carried by PDMAPMA.

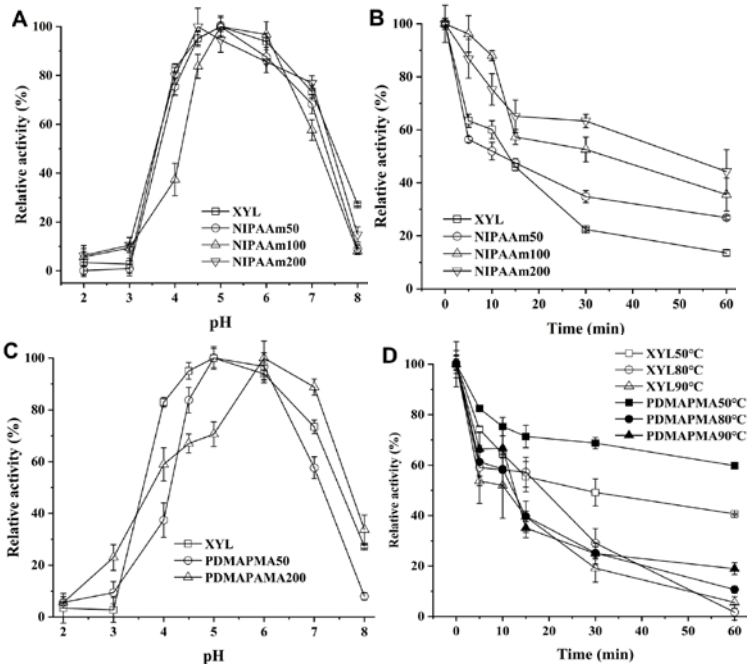

Figure 2 (A) pH activity of XYL, XYL-NIPAAm50, XYL-NIPAAm100, XYL-NIPAAm200; (B) Temperature activity in 80℃ of XYL, XYL-NIPAAm50, XYL-NIPAAm100, XYL-NIPAAm200; (C) pH activity of XYL, XYL-PDMAPMA50, XYL-PDMAPMA200; (D) Temperature activity in 50℃, 80℃ and 90℃ of XYL, XYL-PDMAPMA200.

In conclusion, an ATRP "graft-self" method was developed to conjugate the polymer onto XYL, Next, different properties of polymer connections cause different effects will be explored. It is significant to degrade xylan from agricultural wastes to prepare xylooligosaccharides.

Acknowledgements: This work was supported by National Natural Science Foundation of China (grant number 21978121), the Natural Science Foundation of Jiangsu Province (BK20190957), and the 333 High-level Talent Training Project of Jiangsu Province (BRA2019281).

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