Study on the preparation of xylooligosaccharides by deep eutectic solvent enhanced aqueous two-phase xylanase catalytic reaction separation coupling system

*Liu Yang[1] , Shi Can-yang[1] , Shuai You[1,2], Gong Lu-chan[1,2] , Jun Wang [1,2]**

[1]*School* of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212100, PR China
[2]Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212100, PR China
E-mail: wangjun@just.edu.cn (Prof. Dr. Jun. Wang)

Abstract

As a new food additive, xylooligosaccharides are widely used in intestinal health care, immune regulation, blood glucose and lipid regulation and other fields. At present, xylanase-catalyzed hydrolysis of xylan is an effective method for preparing xylooligosaccharides. However, due to the disadvantages of enzyme inactivation, poor stability and low reusability, its practical application is limited. Two-phase interface biocatalysis is an efficient processing technology integrating separation and catalysis, which can realize the simple and efficient recovery of biocatalysts and improve their reusability. A large number of studies have shown that the composition of deep eutectic solvent as the medium improves the catalytic performance of biocatalysts, while the application of aqueous two-phase catalytic reaction separation coupling system composed of deep eutectic solvent as the medium to enhance xylanase catalysis is still blank.

Methods

Eight deep eutectic solvents were prepared and characterized by using betaine and choline chloride as hydrogen bond acceptors, ethylene glycol, glycerol, citric acid, malic acid and sorbitol as hydrogen bond donors. Using xylanase activity as an indicator, the effect of the above hydrogen bond acceptor and donor pairing on the catalytic performance of xylanase was investigated, and the hydrogen bond acceptor donor combination suitable for xylanase was screened. On this basis, a deep eutectic solvent + Na_2SO_4 / PEG aqueous two-phase system was constructed with deep eutectic solvent as co-solvent, and the influencing factors of xylanase partition coefficient in this system were explored in order to construct a xylanase aqueous two-phase catalytic reaction separation coupling system, aiming to improve the reuse of xylanase, improve the biocatalytic efficiency, and increase its economic benefits.

Results & Discussion

Fig.1 shows that Gly2B aqueous solution could improve the activity and temperature stability of xylanase. When the concentration of Gly2B aqueous solution was 30 % (w / w), the xylanase activity could be increased by 51.2 %. When the xylanase was incubated at 90 ° C for 1 h, the xylanase in Gly2B aqueous solution still retained 42.76 % of the relative activity, which was 3 times higher than that of the control group (10.36 %).

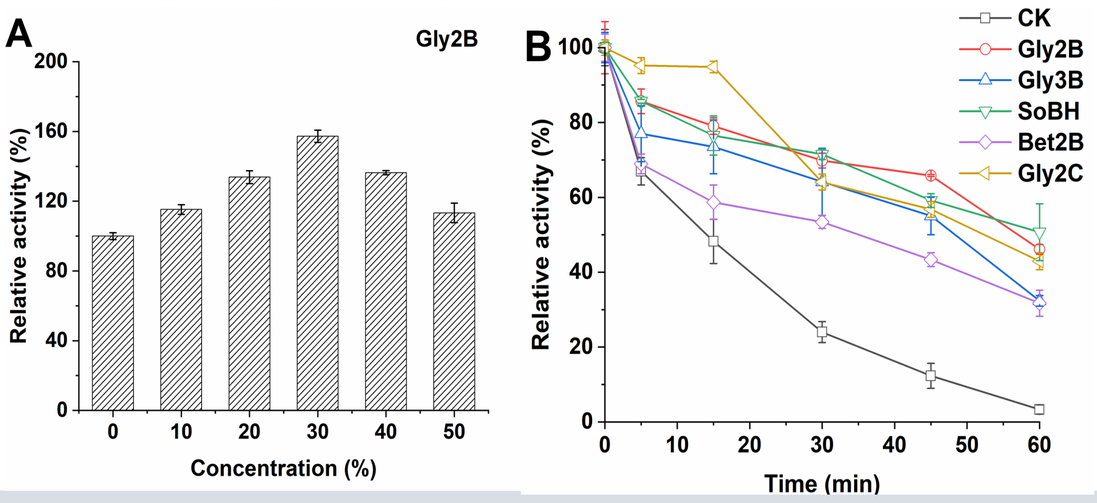

Fig.1 Effect of Gly2B aqueous solution on xylanase activity and Stability. (A) Enzyme activity; (B) Temperature stability at 90 ° C.

Gly2B+ Na_2SO_4 / PEG aqueous two-phase system was constructed with deep eutectic solvent as co-solvent, the effect of PEG molecular weight on the partition coefficient of xylanase was investigated.

Table.1 Effect of molecular weight of PEG on the partition coefficient of xylanase

Molecular weight (kDa)	Concentration of PEG (%, w/w)	Concentration of Na_2SO_4 (%, w/w)	K_X	R	Y_X (%)
2	10	10	1.06±0.05[a]	0.33±0.12[a]	73.96±2.39[a]
4	10	10	0.93±0.04[b]	0.30±0.11[a]	77.97±1.12[b]
6	10	10	0.23±0.04[c]	0.35±0.09[a]	93.35±1.89[c]
8	10	10	0.19±0.03[c]	0.35±0.09[a]	93.94±1.28[c]
20	10	10	0.21±0.03[c]	0.34±0.08[a]	93.25±2.39[c]

Table.1 shows that the distribution of xylanase with the increase of PEG molecular weight. tended to salt phase. By adjusting the molecular weight of PEG, its distribution coefficient can be affected. As the molecular weight of PEG increased, the partition coefficient of the enzyme increased. When the molecular weight of PEG reached 6 kDa, the recovery rate of xylanase in salt phase reached 93.35 %, which was 26 % higher than that of 2 kDa PEG (73.96 %). Therefore, the aqueous two-phase system constructed in this paper can separate and enrich xylanase, which is expected to achieve efficient recycling of xylanase.

The reuse of xylanase in the aqueous two-phase system and homogeneous system constructed in this paper was detected at pH 6, 60 ° C.

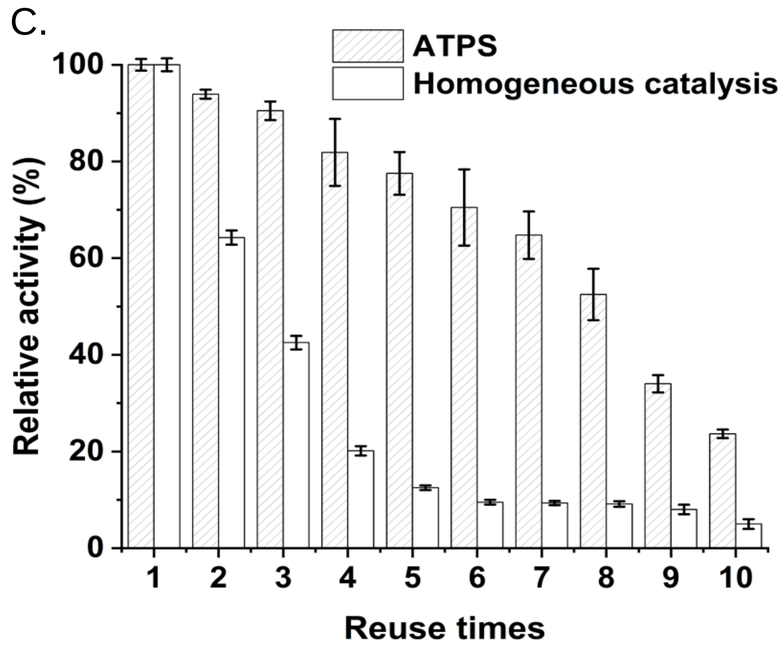

Fig.2 Reuse time of xylanase for the two-phase aqueous catalytic reaction separation coupled to the homogeneous catalytic system

Fig.2 shows that the reuse times of xylanase in the aqueous two-phase catalytic system were significantly higher than those in the homogeneous catalytic system (p<0.05). When reused for 7 times, the residual activity of xylanase in the aqueous two-phase catalytic system was 64.75 %, which was 6.5 times of the residual activity (10%) of xylanase in the homogeneous phase after repeated use for 7 times (p<0.05).

Conclusion

The eutectic solvent composed of alcohol hydrogen bond receptors has a significant effect on the catalytic activity and temperature stability of xylanase. The constructed ”Gly2B + Na_2SO_4 / PEG” aqueous two-phase separation system can efficiently separate and enrich xylanase, it can significantly improve the reuse of xylanase.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (21978121)
Postgraduate Research Practice Inno-& vation Program of Jiangsu Province (KYCX22_3763).

References

[1] Delorme A E, Andanson J M, Verney. International Journal of Biological Macromolecules, 2020, 163:919-926.
[2] Jiménez V P, Valle G S, Alia T I, et al. *Food Bioprod. Process*, 2020, 123: 238-250.
[3] Tebyanian K H. Biointerface Research in Applied Chemistry, 2020, 10(5):6488-6497.