Preparation of xylo-oligosaccharides by deep eutectic solvent enhanced aqueous two-phase catalytic system

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Xylo-oligosaccharides (XOS) is a kind of oligosaccharide, with obvious benefits in reducing blood cholesterol, increasing calcium absorption, and maintaining gastrointestinal health and type II diabetes (Chen et al., 2016). At present, xylan-rich lignocelluloses are mainly prepared by chemical methods (alkali treatment, acid hydrolysis, hydrothermal method) and enzymatic hydrolysis (Liu et al., 2016). The acid hydrolysis is low in cost but difficult to separate the products generated by the reaction; the alkali treatment process requires a high concentration of alkali solution and high-temperature requirements (Singh et al., 2016). In comparison, the reaction process of the enzyme is mild, and no side reactions occur, which is suitable for practical application. Therefore, using an enzyme as a biocatalyst for the efficient production of oligosaccharides is a promising strategy.

Biphasic systems are systems made up of two immiscible components and are mainly used for the extraction and purification of downstream products, improving the current situation in homogeneous catalytic systems where the separation of product and catalyst is difficult, but not improving the environmental pollution caused by the use of organic solvents (Akama et al., 2002).The ATRP system, which uses water as the medium, has become a trend in recent years due to the low use of organic solvents, the mildness of the reaction steps and the environmental friendliness of the process. (Sun et al., 2018) The construction of aqueous catalytic systems for the reaction and separation of oligosaccharides can greatly improve the efficiency of industrial production and reduce the cost of post-reaction product separation (Chen et al., 2014). However, the construction of a biphasic system that separates the product oligosaccharides and the catalyst without destroying the xylanase activity is a difficult but highly relevant problem in practice.

Enzymes act as green catalysts in biochemical reactions and are able to speed up the reaction, therefore the selection of a suitable solvent is considered a key issue. Recently, the use of non-aqueous solvents has been used to replace aqueous solvents to facilitate the catalytic process of enzymes. DESs have attracted considerable interest as a green alternative to conventional solvents, not only because they are environmentally friendly, non-toxic and biodegradable organic compounds, but also their low cost, ease of production and several characteristics and properties (Harifi-Mood et al., 2018). Not only do DESs have specific properties compared to conventional organic solvents, but they are also non-destructive to enzyme activity. DESs have been reported to promote the catalytic efficiency and stability of laccase, cellulase and organophosphorus pesticide hydrolases. In the application of xylanase, there has been no research on enhancing enzyme stability and catalytic ability through deep eutectic solvents (Mann et al., 2009). Thus, the study of this aqueous two-phase catalytic system for strengthening xylanase by solvent system is very meaningful.

In this study, the relationship between different deep eutectic solvents and the catalytic capacity of xylanase was investigated and the deep eutectic solvent with the best effect was screened. On this basis, a deep eutectic solvent with salt and PEG catalytic separation system was constructed to prepare oligosaccharides.

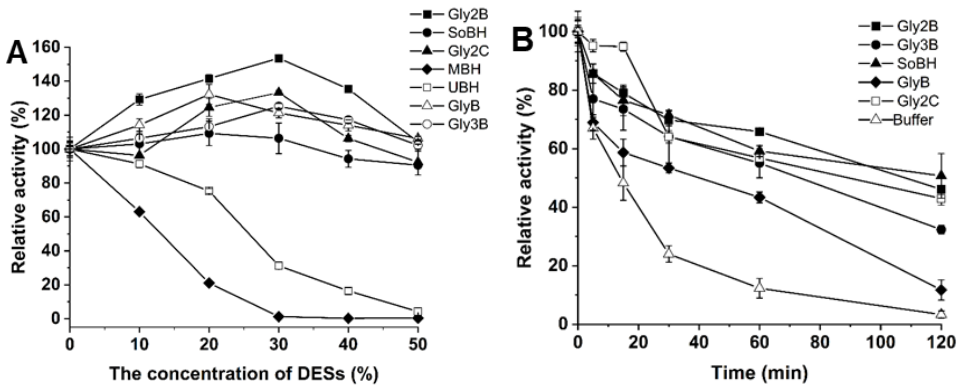

Figure.1 Effect of deep eutectic solvents on xylanase, (A) Catalytic capacity; (B) Temperature stability at 90 ℃.

Fig 1A shows deferent DES different compositions of DES had different effects on the catalytic capacity of xylanase, with UBH and MBH having a significant activity destruction of xylanase, while several other DES had an enzymatic activity enhancing effect on xylanase. Gly2B prepared by betaine and glycerol was the most effective, and the xylanase activity could be increased by 53.24% at 30% concentration of Gly2B. Fig 1B shows the different DES all improve the stability of xylanase and the hydrophobic environment better protects the active centre of xylanase, with betaine and sorbitol as hydrogen acceptors for SoBH and Gly2B being the most effective.

Table.1 Kinetic constants of xylanase in different solvents.

Solvent	K_m (mg/mL)	k_{cat} (/s)	V_{max} (U/mg)	k_{cat}/K_m (mL/s/mg)
Buffer	3.69±0.13 [a]	2530.28±273.49 [a]	4804.33±519.29 [a]	693.66±184 [a]
Gly2B	3.32±0.16 [b]	3105.53±143 [b]	5896.57±374 [b]	933.97±104 [b]

Table 1 shows that In Gly2B, the *K*m of xylanase was 13.45% lower and the catalytic efficiency was 24.63% higher than in the buffer. Therefore, the substrate affinity of xylanase is increased in the deep eutectic solvent, allowing for better catalysis.

In conclusion, deep eutectic solvents with different compositions were prepared, and the catalytic ability and stability of xylanase in different solutions with different concentrations were analyzed. On this basis, an aqueous two-phase catalytic system was constructed to prepare and separate xylo-oligosaccharides.

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