Mulberry red pigment with high purity produced by whole-cell bioconversion in an aqueous two-phase system

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Abstract

Large numbers of mulberry fruits are discarded every year, owing to imperfect preservation technology. However, cyanidin-3-*O*-glucoside (C_3G) in defective mulberries, is safe and nontoxic, and is recognized as a suitable source of anthocyanins in nature. Therefore, a novel aqueous two-phase system (ATPS) with whole-cell biocatalyst was constructed to enhance the C_3G content in red pigments from defective mulberry fruits for the first time. The cyanidin-3-*O*-rutinoside (C_3R) conversion was 66.82% within 1.5h, and the purity of C_3G in the product was 84.2% with an increase of 24%. High recovery efficiency of C_3G as much as 96% was obtained. Overall, a kind of high-quality red mulberry pigments was prepared using the innovative system quickly and effectively, and would be of great significance for reuse of bioresources.

Methods

In this paper, glycosylation directed transformation is the main research content. The reaction was separated and purified by PEG /inorganic salt aqueous two-phase system with whole cells expressing *α*-L-rhamnosidase as catalyst. A new high-quality mulberry red pigment with a purity of C_3G close to 85% was prepared.

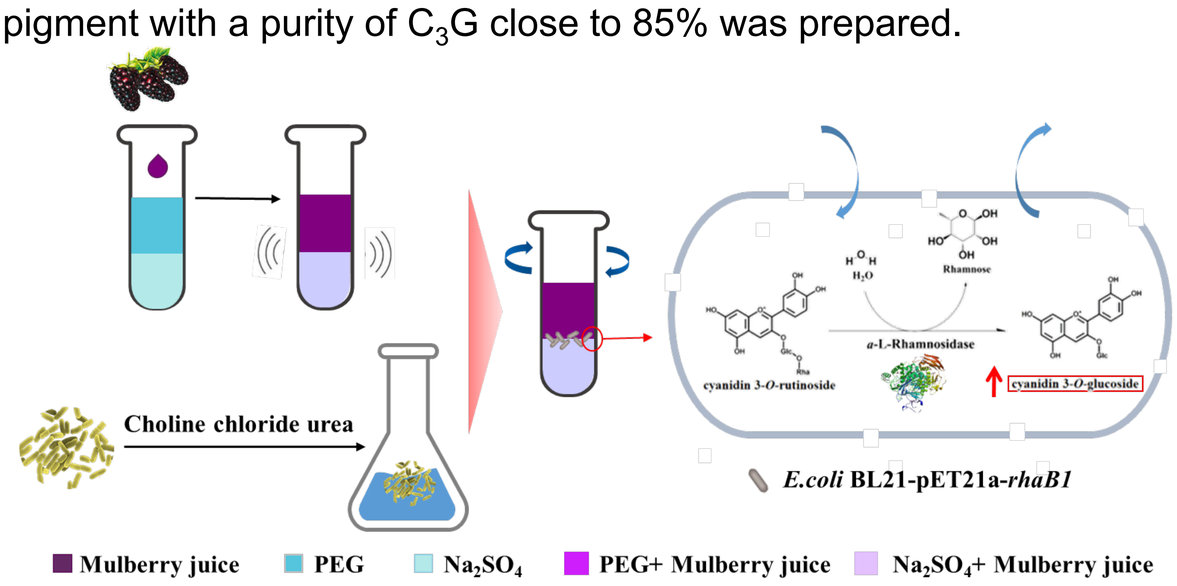

Fig.1 The whole cell catalytic reaction in aqueous two-phase system.

Results & Discussion

Fig.2 shows that mulberry red pigment has good stability in PEG / Na_2SO_4 aqueous two-phase system. The content of mulberry red pigment remained above 80% after 4 h at 40 ℃. The results provided a mild environment for the biotransformation of mulberry red pigment in the two-phase system, and laid a foundation for the realization of the coupling of reaction and separation.

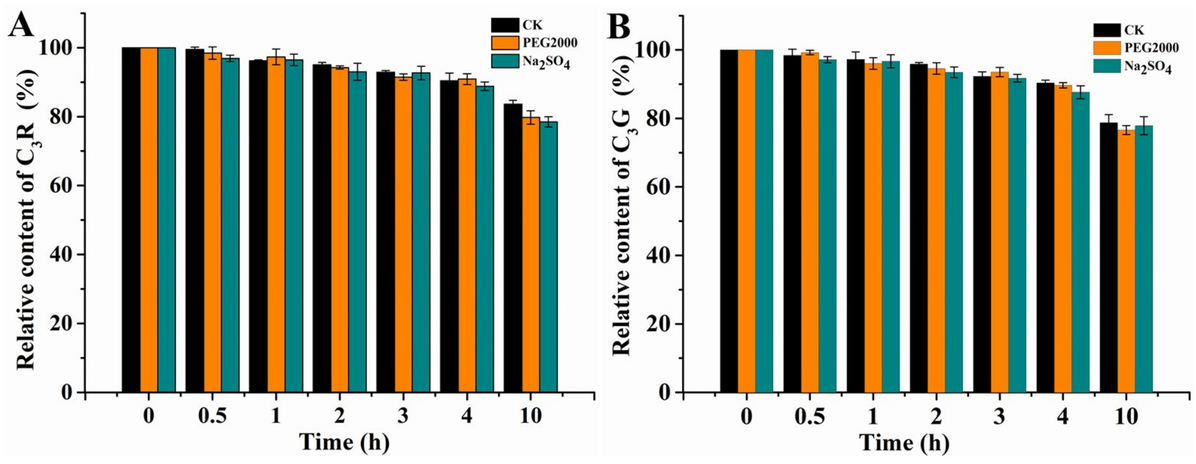

Fig.2 The stability of mulberry red pigment in PEG/Na_2SO_4 aqueous two-phase system. (A) C_3G; (B) C_3R

Fig.3 shows The optimal conditions of "PEG2000/Na_2SO_4" aqueous two-phase whole cell catalytic reaction, as follows: 15% PEG2000, 10% sodium sulfate, 14% mulberry juice (C_3R concentration was 0.171 mg/mL) and 7.5% (37.5 mg/mL) whole-cell biocatalyst and pure water. The system was reacted at 45 ℃, and pH 4.5. Within 1.5 h, C_3R conversion was 66.82 % and the purity of C_3G in the product was 84.2% with an increase of 24%.

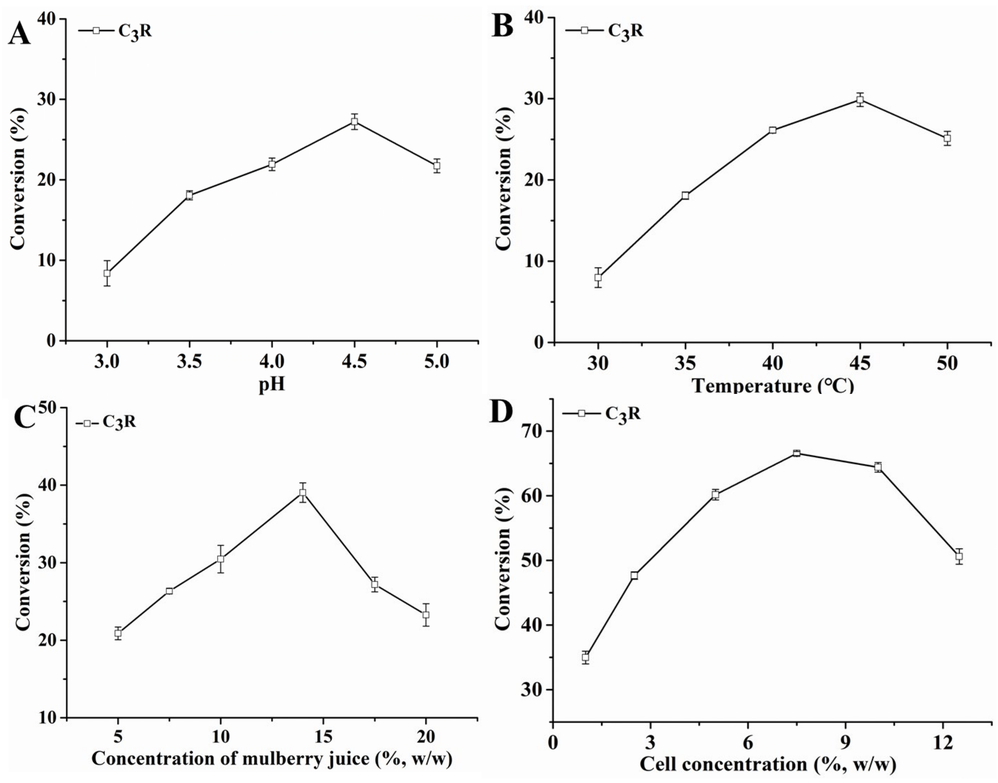

Fig.3 The effect of pH (A), temperature (B), concentration of mulberry juice(C) and cell concentration (D), on the conversion of C_3R in the aqueous two-phase system.

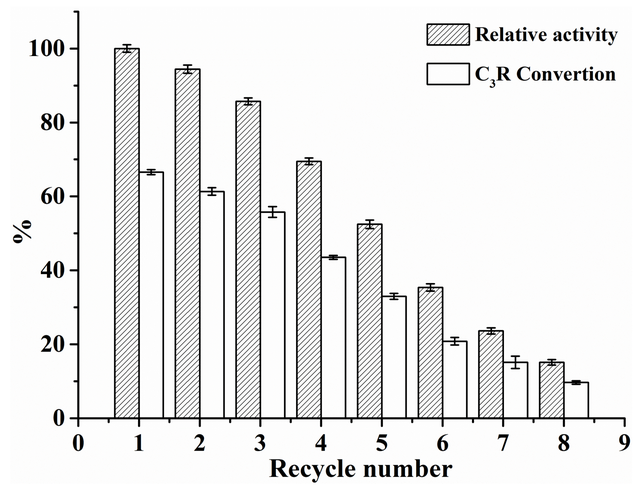

Fig.4 The cycles and relative enzyme activity of whole-cell catalyst in aqueous two phases

Fig.4 shows the reusability of the whole-cell biocatalyst in aqueous two phases was high, and it could be reused 5 times, maintaining a relative activity over 50%.

Conclusion

After optimization, the purity of C_3G reached the maximum of 84.2%,which was 24% higher than the original. A new aqueous two phase reaction system was constructed to reuse the defective mulberries, and its separation characteristics and reaction rules were analyzed, which conforms to the significance of green chemistry.

Acknowledgement

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