Enzyme conversion of mulberry red pigments in a microfluidic aqueous two-phase system

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CONTNET

01 Introduction

Mulberry red pigment--natural edible pigment

Fresh mulberry 30%

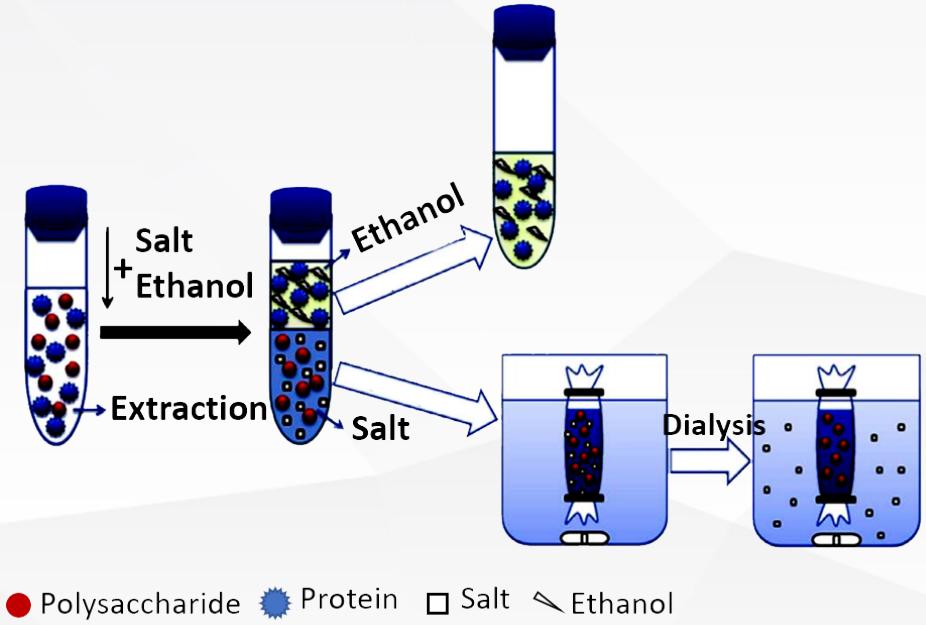
Rotten mulberry 60%

[1] Song H, et al. Nutrition Research, 2016, 36(7):710-718.

02 Methods

Aqueous two-phase enzyme catalysis system

Extraction, catalysis, separation



Disadvantages:

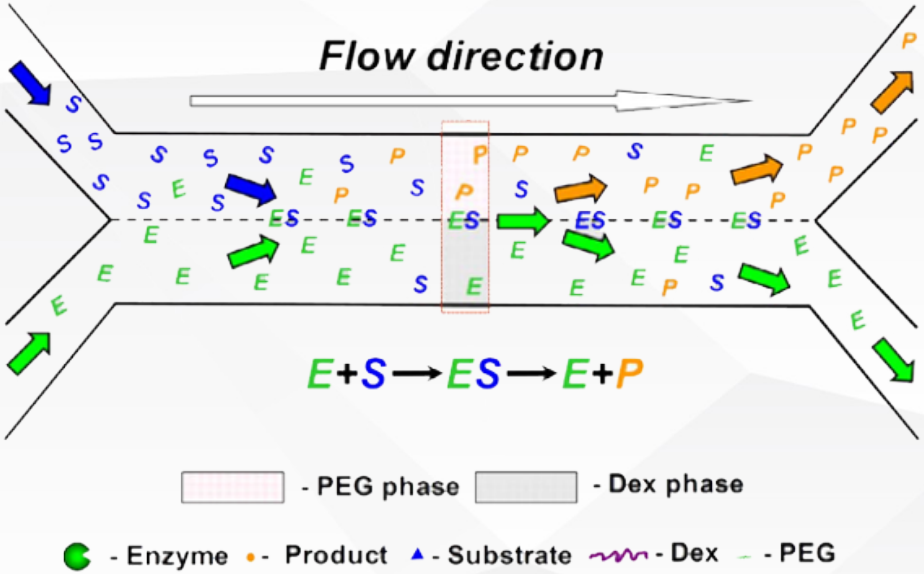
Long separation time
Easily emulsification

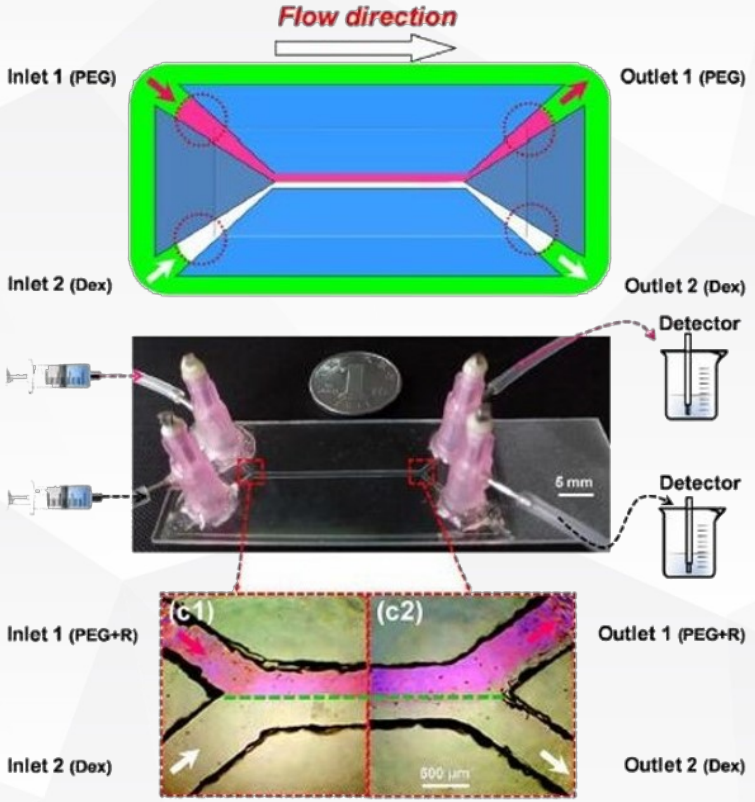
Microfluidic

[2] Krause J, et al. Journal of Chromatography A, 2015, 1391(1):72-79.

02 Methods

Microfluidic aqueous two-phase enzyme catalysis system





500 times higher than enzymatic rate in conventional reactor

[3] Meng S X, et al. Chemical Engineering Journal, 2017, 335.

03 Results

8.4 s

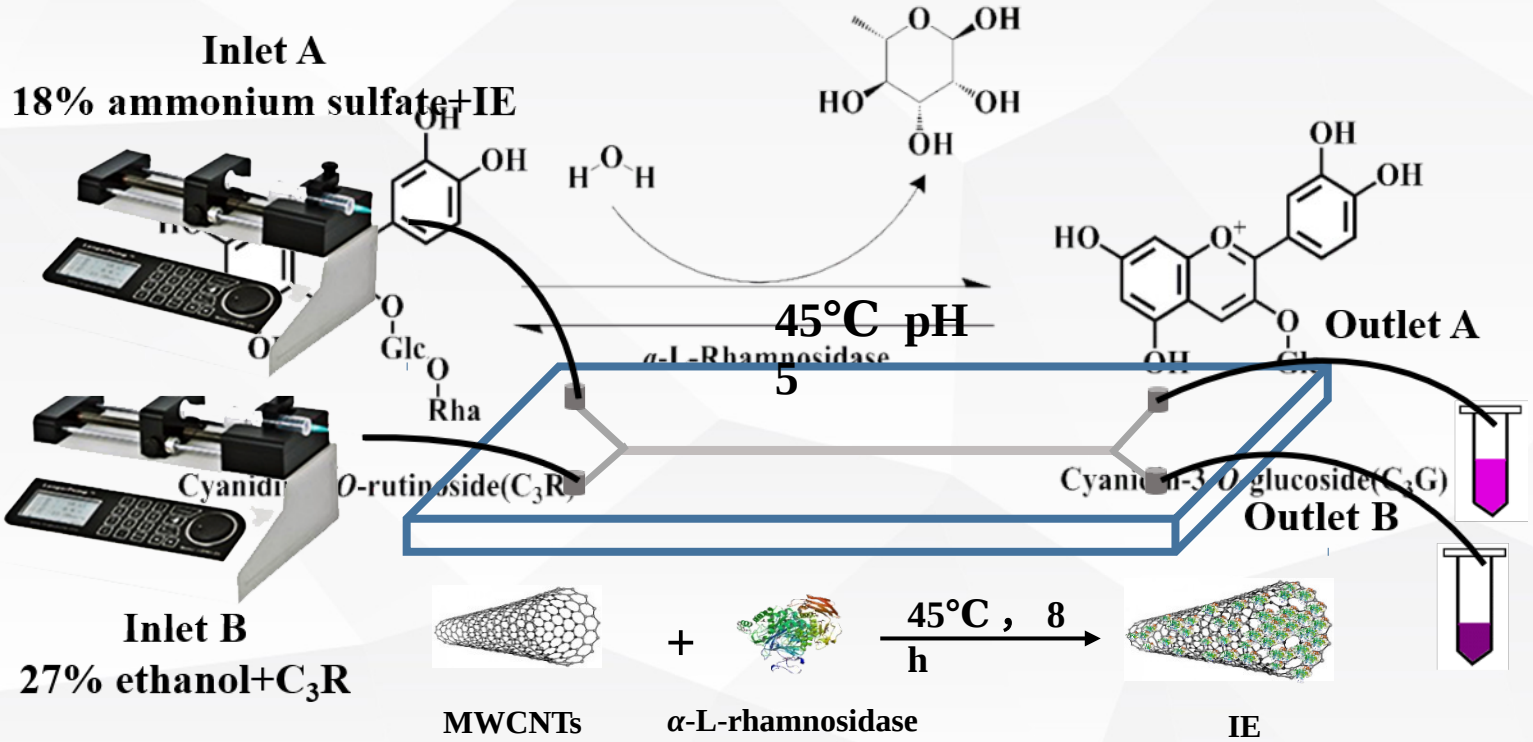


Fig. 1 Diagram of microfluidic aqueous two-phase device.

HPLC-PDA-ESI-MS

03 Results

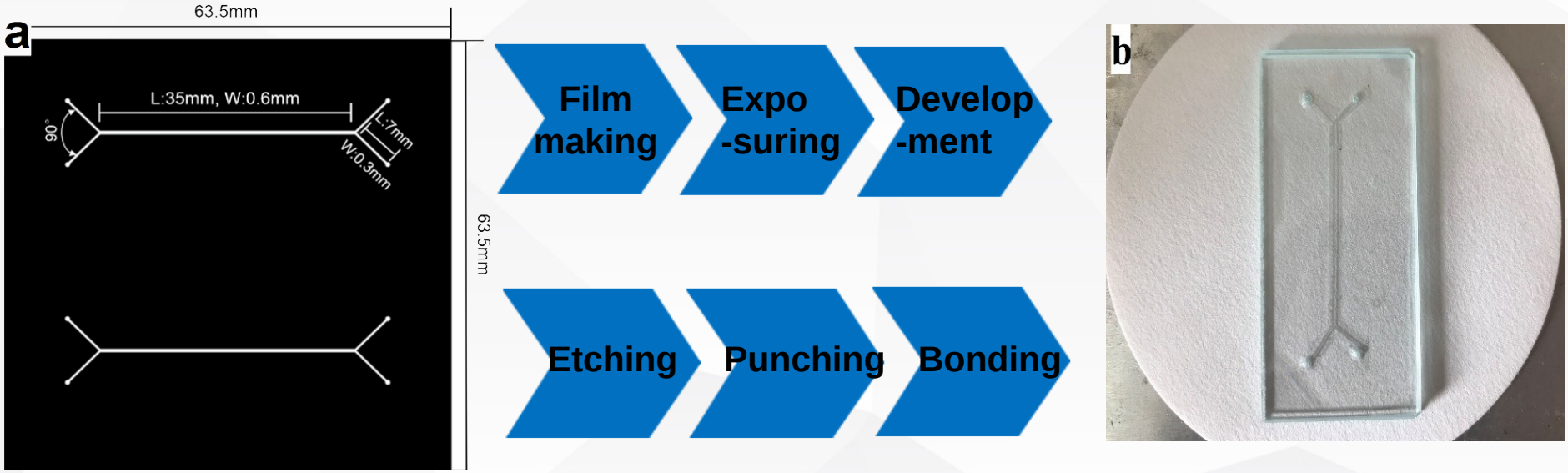


Fig.2 Design and manufacture of double Y-branched microfluidic chip.
(a) Design figure of double Y-type microfluidic chip; (b) Real figure of double Y-branched microfluidic chip.

The middle main channel is 3.5 cm length and 600 *μ*m width
The double Y-branch is 7 mm length and 300 *μ*m width

03 Results

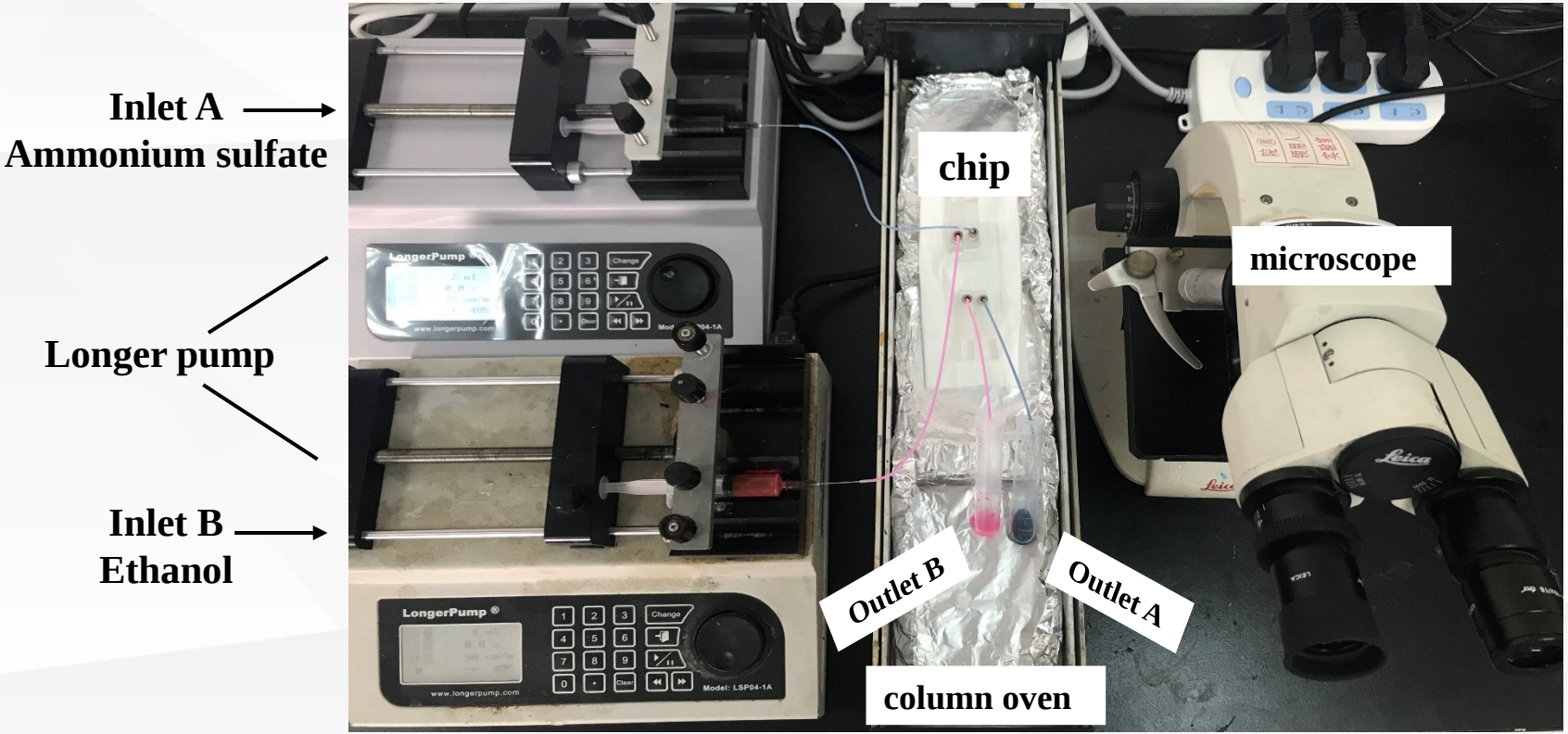


Fig. 3 Physical diagram of microfluidic aqueous two-phase device.

03 Results

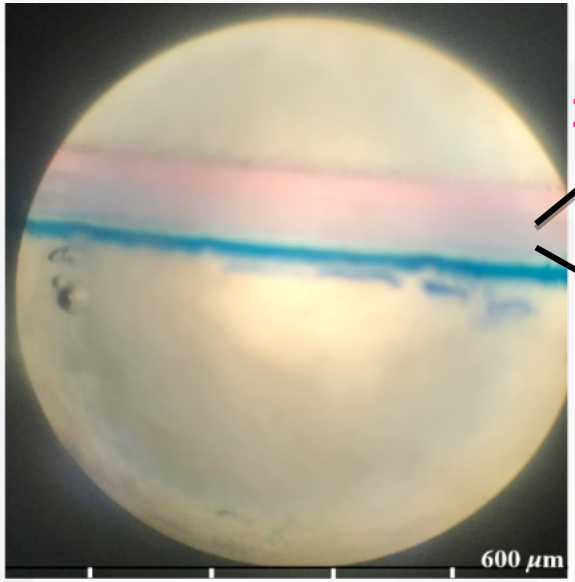


Fig. 4 Photograph of ATPS in microchannel under microscope.

18% Ethanol and Rhodamine B
11.5 μL/min

27% Ammonium sulfate and malachite green
15.5 μL/min

$$Re = \frac{dv\rho}{\mu}$$

$Re < 2000$

parallel flow formation

Tab. 1 Calculation of Re in microchannel.

Substance	Width (μm)	Flow rate (μL/min)	Density (kg/m³)	Viscosity (Pa·s)	*Re*
Ethanol	900	11.5	7.89×10^{2}	1.074×10^{-3}	1.11×10^{-3}
Ammonium sulfate	900	15.5	1.42×10^{3}	2×10^{-3}	1.48×10^{-3}

03 Results

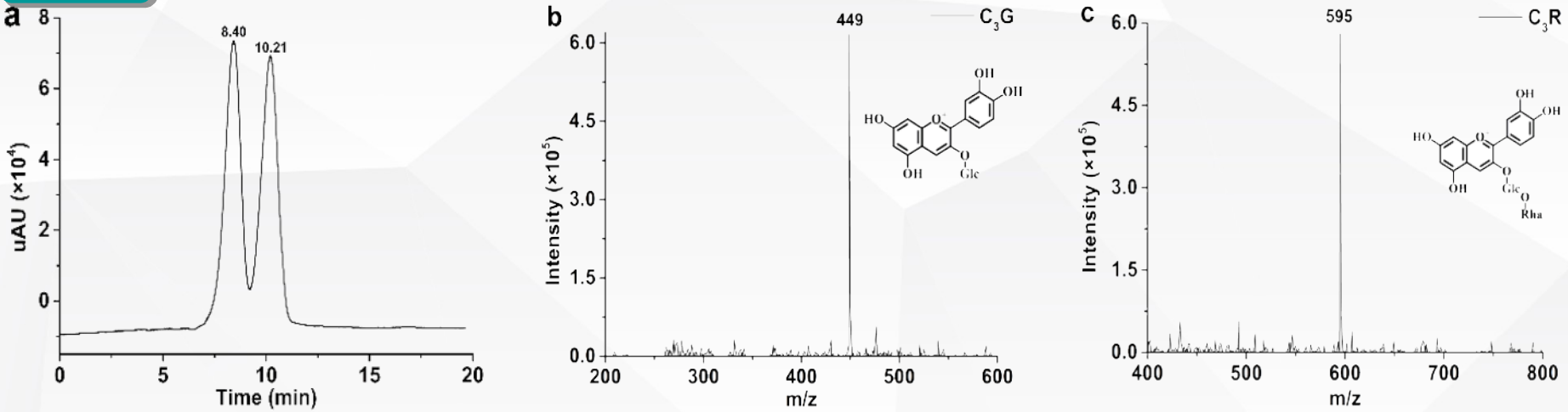
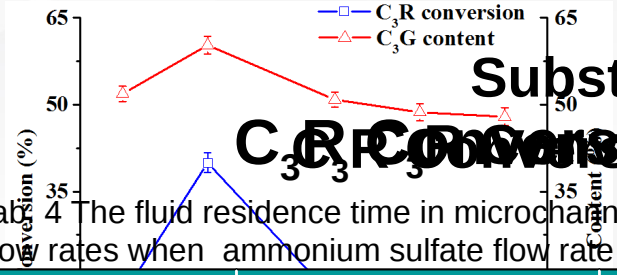


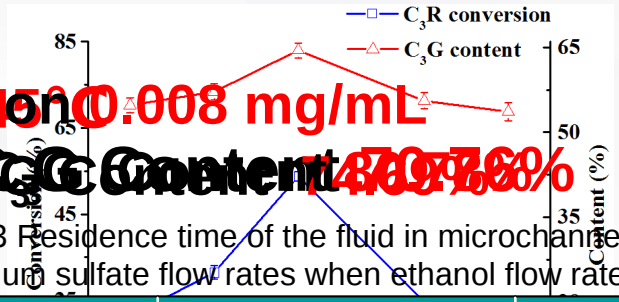
Fig. 5 HPLC–PDA-MS/MS of the product in microfluidic aqueous two-phase enzyme catalytic system.
(a) HPLC chromatograms of mulberry red pigment; (b) Mass chromatogram of C_3G; (c) Mass chromatogram of C_3R.

Tab. 2 HPLC-PDA-ESI-MS data of mulberry red pigment.

Peak	Rt (min)	$[M]^+$ (m/z)	λ_{max} (nm)	Content
1	8.40	449	280,513	C_3G
2	10.21	595	280,513	C_3R

[4] Diaconeasa Z, et al. Plant Foods for Human Nutrition, 72(4), 404-410 (2017).





Substrate concentration 0.008 mg/mL

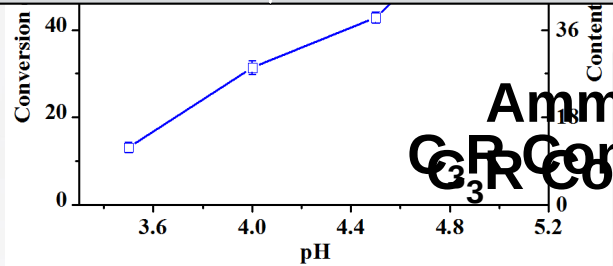
C_3R Conversion 70.99% C_3G Content 70.76%

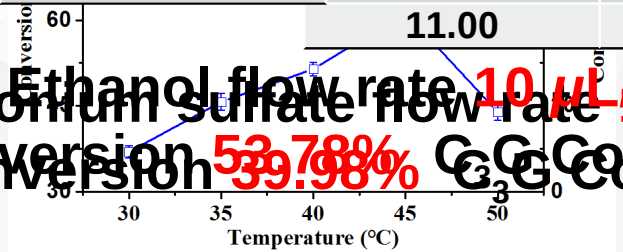
Tab. 4 The fluid residence time in microchannel at ethanol different flow rates when ammonium sulfate flow rate fixed at 14.5 μL/min.

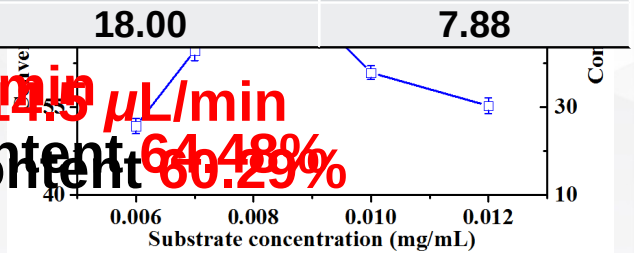
Ethanol flow rate (μL/min)	Ammonium sulfate flow rate (μL/min)	Residence time τ (s)
8.00	14.50	10.50
8.50	14.50	10.12
9.00	14.50	9.77
10.00	14.50	8.59
11.00	14.50	8.33
11.50	14.50	8.10
12.00	14.50	7.88
12.50	14.50	7.46

Tab. 3 Residence time of the fluid in microchannel at different ammonium sulfate flow rates when ethanol flow rate at 11 μL/min.

Ethanol flow rate (μL/min)	Ammonium sulfate flow rate (μL/min)	Residence time τ (s)
11.00	13.50	10.50
11.00	14.00	10.12
11.00	14.50	9.77
11.00	16.00	8.86
11.00	16.50	8.59
11.00	17.00	8.33
11.00	17.50	8.10
11.00	18.00	7.88







Ethanol flow rate 10 μL/min Ammonium Sulfate flow rate 14.5 μL/min

C_3R Conversion 53.78% C_3G Content 64.48%

C_3R Conversion 39.98% C_3G Content 60.29%

Fig. 6 Effect of conditionsons on C_3R conversion rate and C_3G content.

03 Results

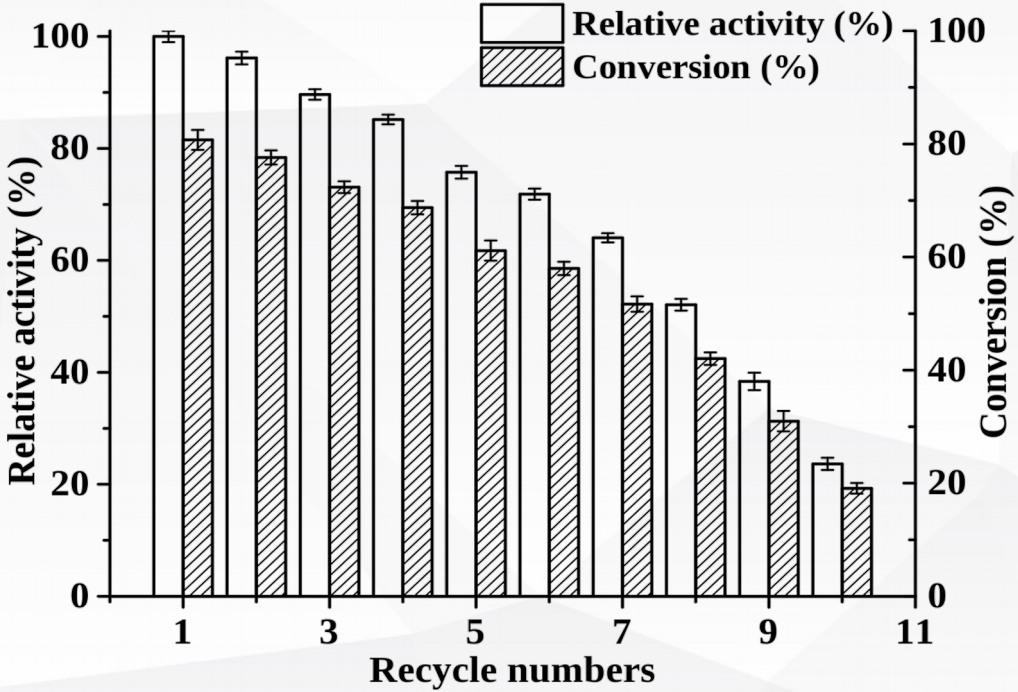


Fig. 7 Reuse of immobilized enzyme.

Immobilized enzyme can be reused **7** times

Relative enzyme activity is higher than 60%

03 Results

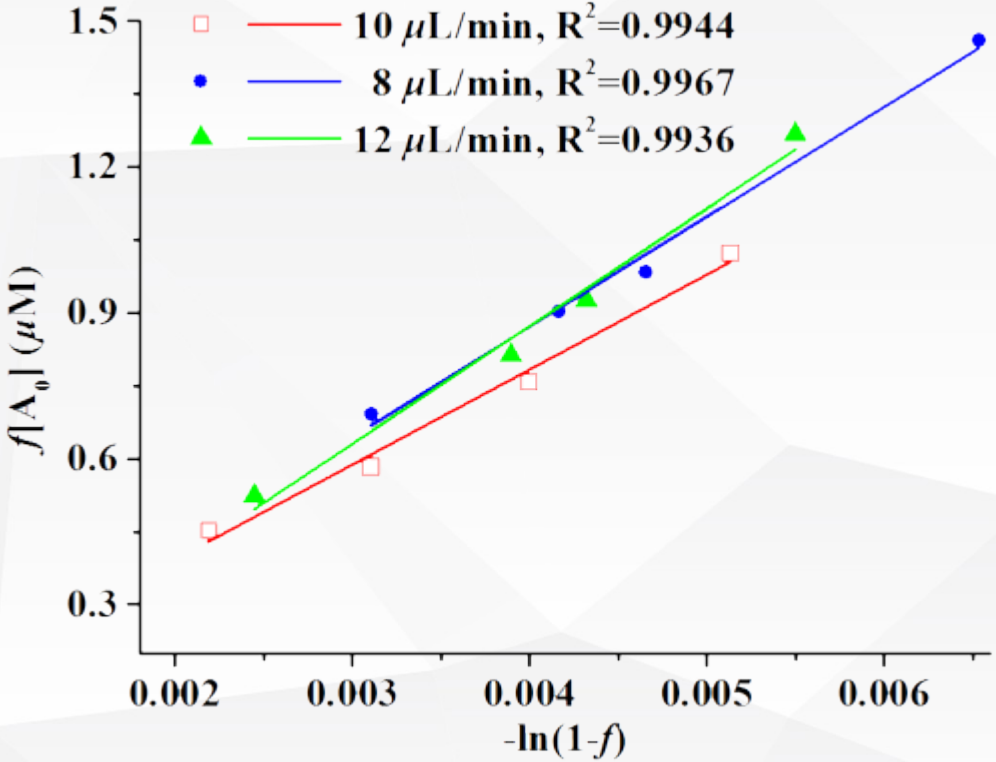


Fig. 8 Lilly-Hornby plots for immobilized enzyme in photopatterned microchannel.

$$f[A_0] = \frac{C}{Q} + K_m ln(1-f)$$

Tab. 5 Different *Km* of ethanol flow rates when ammonium sulfate flow rate fixed at 14.5 μL/min

Ethanol flow rate (μL/min)	*Km* (μM)
12	242.53
8	226.14
10	**195.39**

04 Conclusion

1. The size of **double Y-branched** microfluidic chips: middle main channel: 35 mm×0.6 mm × 0.15 mm, double Y-branch: 7 mm×0.3 mm×0.15 mm.

2. Under the optimal conditions of ammonium sulfate flow rate of **14.50 μL/min**, ethanol flow rate of **10 μL/min**, pH **5**, temperature **45°C** and substrate concentration of **0.008 mg/mL**, the conversion of C_3R and the content of C_3G were **82%** and **81%**, respectively.

3. The immobilized enzyme could be reused **7** times, the relative enzyme activity was stabilized at more than 60% and the C_3R conversion rate was maintained at more than 50%.

4. When the ethanol flow rate was **10 μL/min**, the Km value was the lowest and the enzyme and substrate concentration had the highest affinity.

05 Acknowledgement

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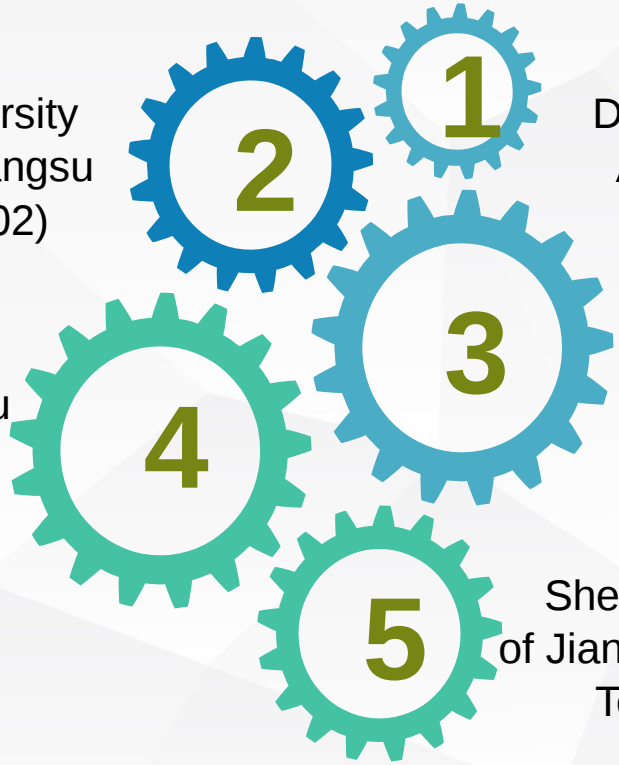



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