

Kitchen waste oil convert to biodiesel via microreactor with *Thermomyces lanuginosus* lipase-PNIPAAm

Can-Yang Shi ^{1, #}, Ya-Li Chai ^{1, #}, Yan Hu ¹, Jin-Zheng Wang ¹, Hai-cheng Yan¹, Jun Wang ^{1, 2, 3, 4}

¹School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212018, China

²Sericultural Research Institute, Chinese Academy of Agricultural Sciences, 212018 Zhenjiang, China

E-mail: wangjun@just.edu.cn (Prof. Dr. Jun. Wang)

These authors contributed equally to this work.



Introduction

Biodiesel is an important environmentally friendly biomass energy source and the kitchen waste oil has obvious duality of waste and resources. The traditional enzymatic method converting kitchen waste oil into FAME is environmentally friendly, but the catalysis which at the interface is limited. Lipase immobilization on conventional solid emulsifiers also limits the area of contact between the lipase and the oil. The lipase was grafted onto an emulsifying polymer for catalysis via microreactor to control the size of droplet was a Innovative approach.

Methods

Thermomyces lanuginosus lipase-poly (N-isopropylacrylamide) (TL-PNIPAAm) coupling biocatalyst was prepared by ATRP "Grafting-from" method. After the detection of characterization and enzymatic properties, the conjugates were used for the preparation of FAME from kitchen wasted oil via microreactor.

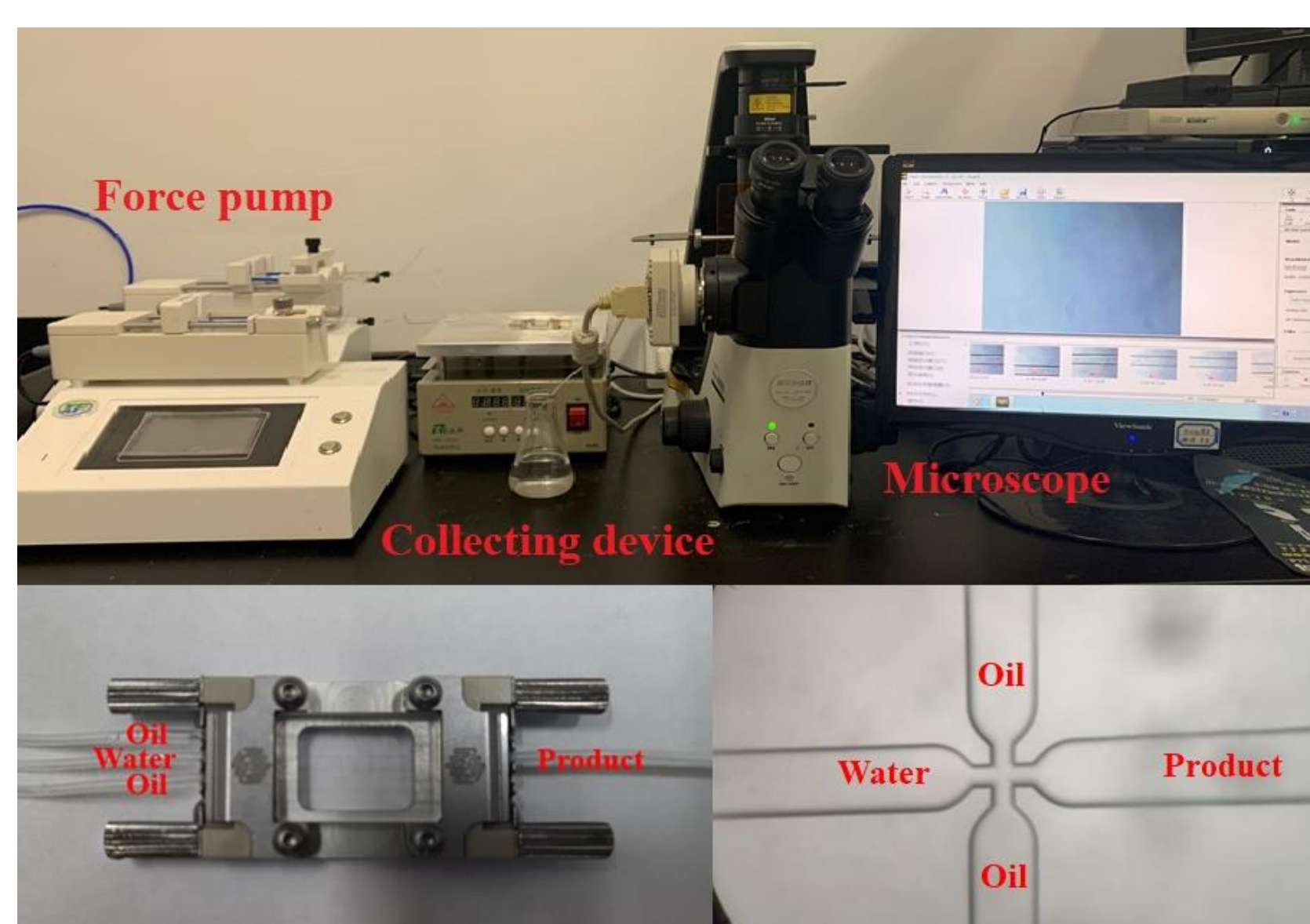


Fig. 1 W/O microreactor.

Results & Discussion

The pH and temperature stability of the conjugate were improved compared with that of the free enzyme. The interfacial catalysis efficiency of the microreactor was 5.5 times higher than that of the traditional reaction system. The microfluidic with TL-PNIPAAm conjugates was successfully constructed in the reactor.

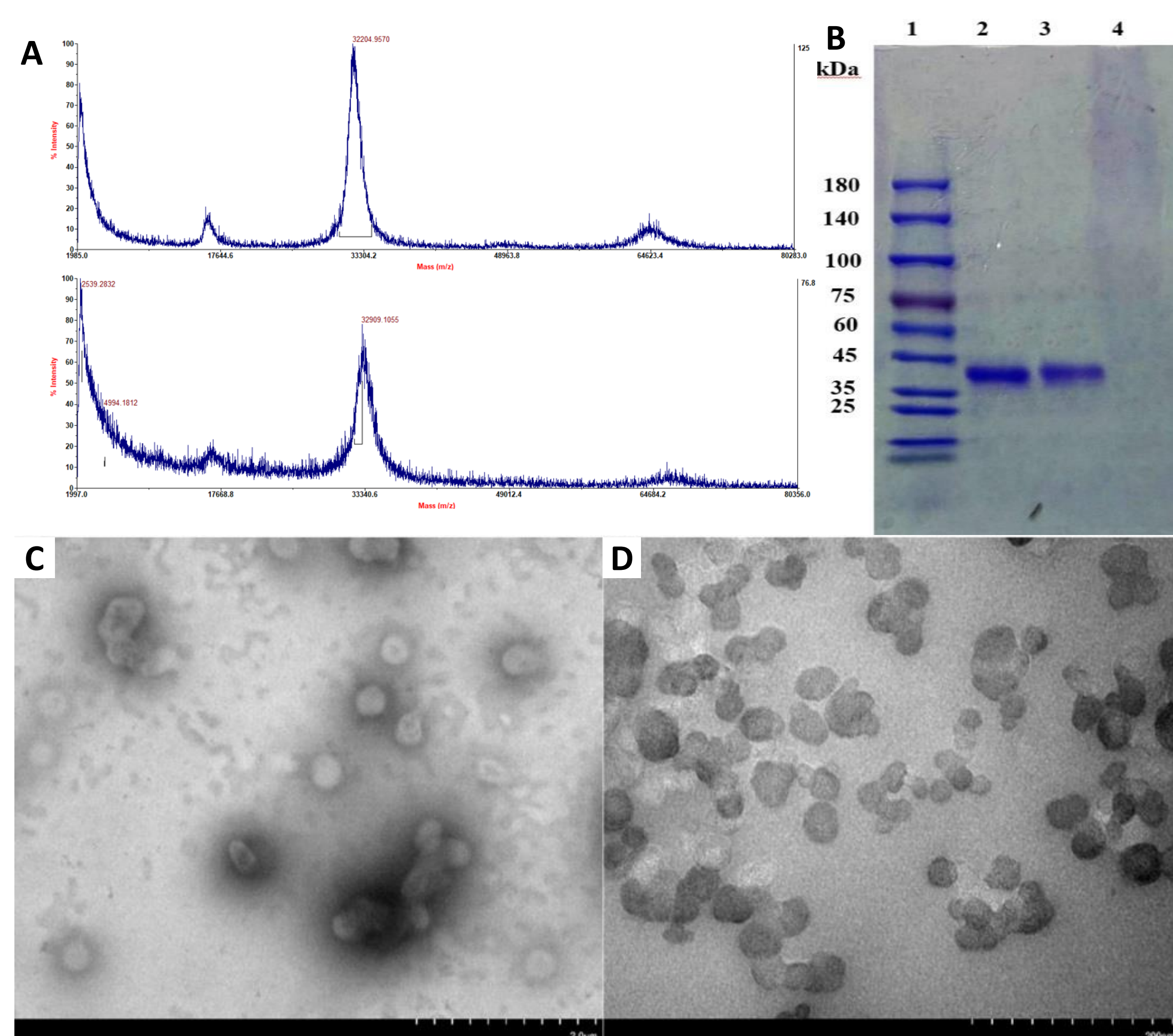


Fig. 2 Characterization of the free lipase and the TL-PNIPAAm conjugates. (A) MALDI-TOF MS (B) SDS-PAGE (C) (D) TEM.

The results shows that the TL-PNIPAAm conjugates were successfully prepared.

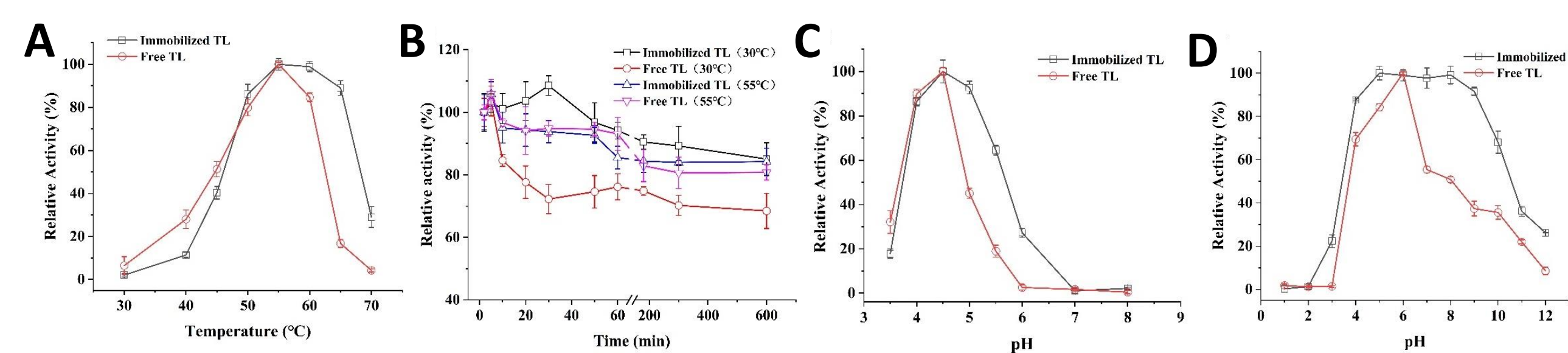


Fig. 2 Enzymatic properties analysis of free TL and TL-PNIPAAm conjugates. (A) The optimal temperature of TL and conjugates; (B) The temperature stability of TL and conjugates; (C) The optimal pH of TL and conjugates; (D) The pH stability of TL and conjugates.

The results shows that the relative activity of TL-PNIPAAm conjugates waere 20% more than free lipase after 10 hours of treatment at 55 °C . More than 80% of the relative activity of theTL-PNIPAAm conjugates is in the pH range of 4-9, which is much wider than the pH6 of the free enzyme.

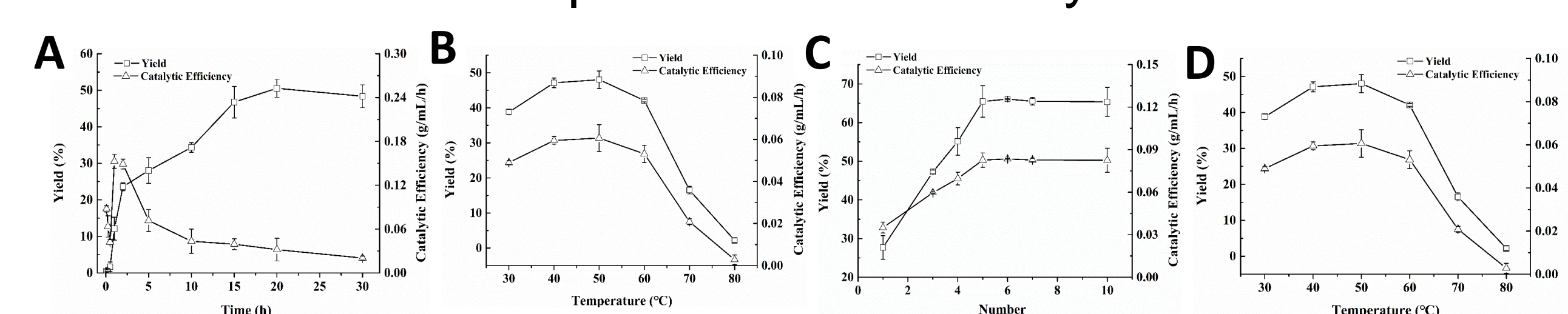


Fig. 3 Optimization in conventional catalytic systems (A) time (B) temperature (C) alcohol to oil ratio (D)methanol addition times

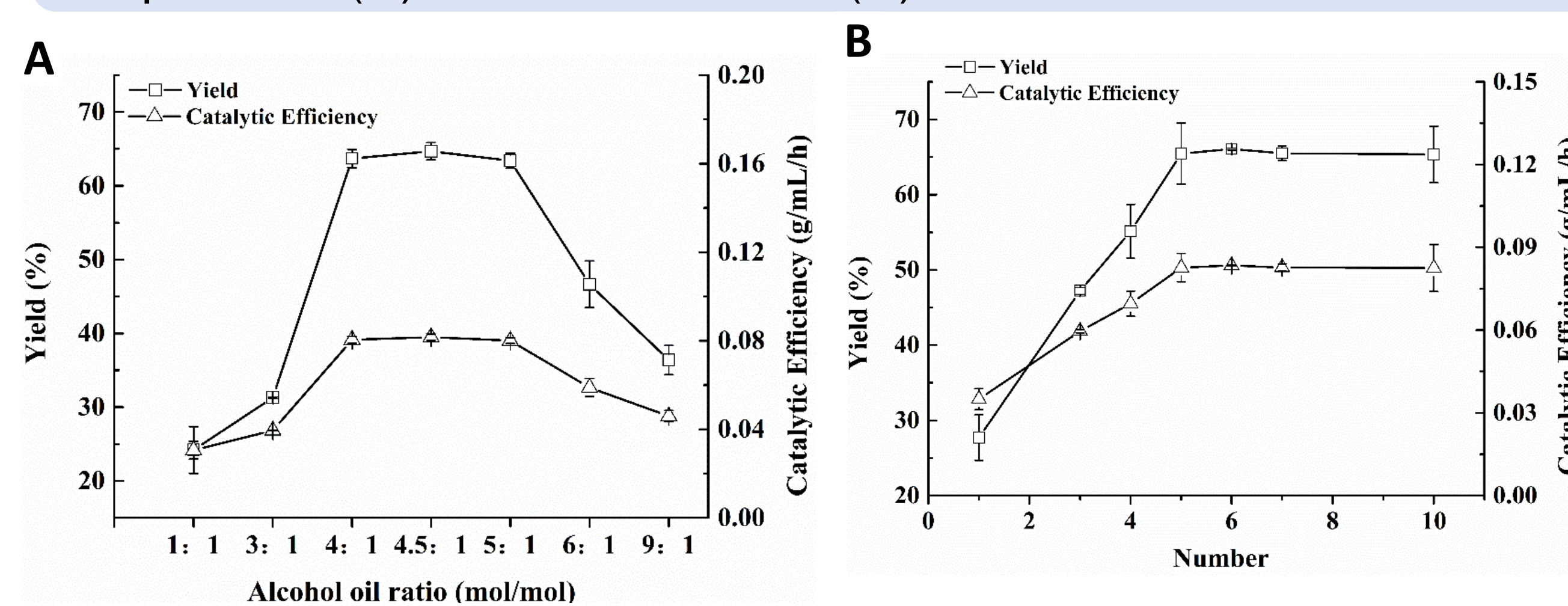


Fig. 4 Optimization of catalytic process for microfluidic catalytic system (A) temperature (B) Flow rate ratio between oil and water phases

The results shows that The interfacial catalysis efficiency of the microreactor was 5.5 times higher than that of the traditional reaction system. The microfluidic with TL-PNIPAAm conjugates was successfully constructed in the reactor.

Conclusions

- The optimum reaction conditions of enzyme were as follows: at 55 °C, pH = 5.5, The temperature and pH stability TL-PNIPAAm conjugates get improved.
- The optimum reaction conditions of Conventional catalytic system were 40 °C, 20 h, oil-methanol ratio 4.5 and flow with 5 times. The best yield was 68.2% and catalytic efficiency was 40mg/ml/h.
- When the flow rate ratio of continuous phase to dispersed phase is greater than 1.75, the microfluidics O/W droplet formation state is stable and the size is uniform. The catalytic efficiency is 50, 5 times that of conventional system

Acknowledgements

- The Natural Science Foundation of China (21978121).
- The Natural Science Foundation of Jiangsu Province (BK20190957) Talent Peaks Project of Jiangsu Province (2015-NY-021).
- 333 High-level Talent Training Project of Jiangsu Province (BRA2019281).

References

- [1] Sun, Z., Glebe, U., Charan, et al. Angewandte Chemie International Edition, 57(42), 13810-13814(2018).
- [2] Gebreyohannes, A. Y., Dharmjeet, M., et al. Bioresource Technology, S485285433 (2018). Kovaliov, M., Cheng, CS, et al. POLYM CHEM-UK, 9(37), 4651-4659(2018).