Recombinant *Escherichia coli* mutant strain producing GH 78 *α*-L-rhamnosidase for microfluidic biofilms catalysis

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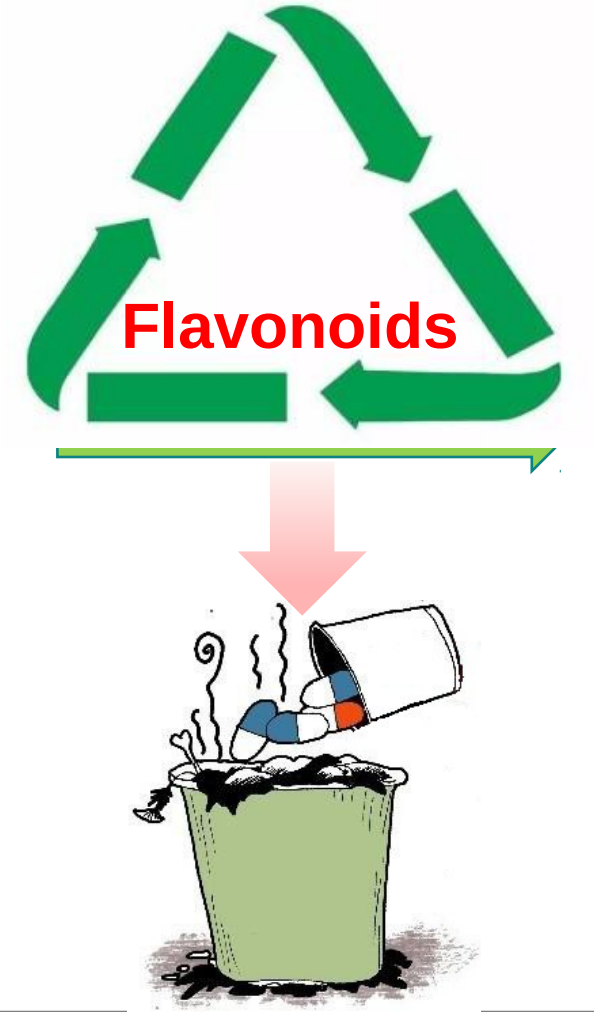
CONTENT

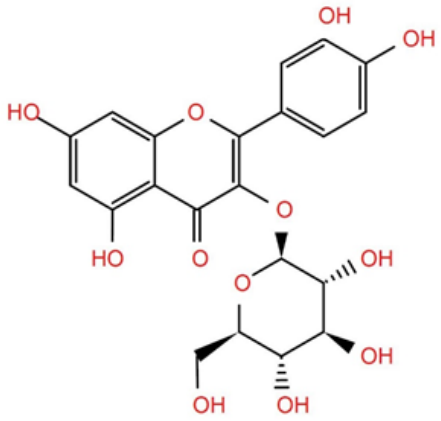
1. Introduction

Flavonoids sources

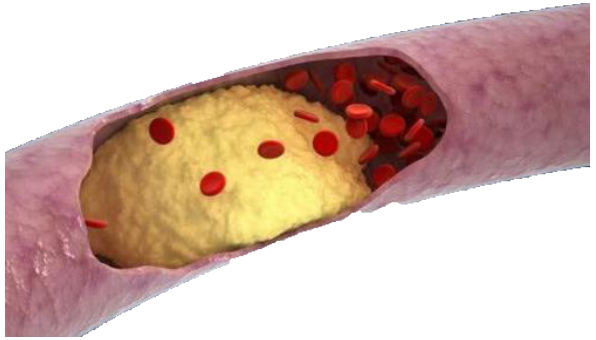


Waste resources

High-value product prepared by biocatalysis



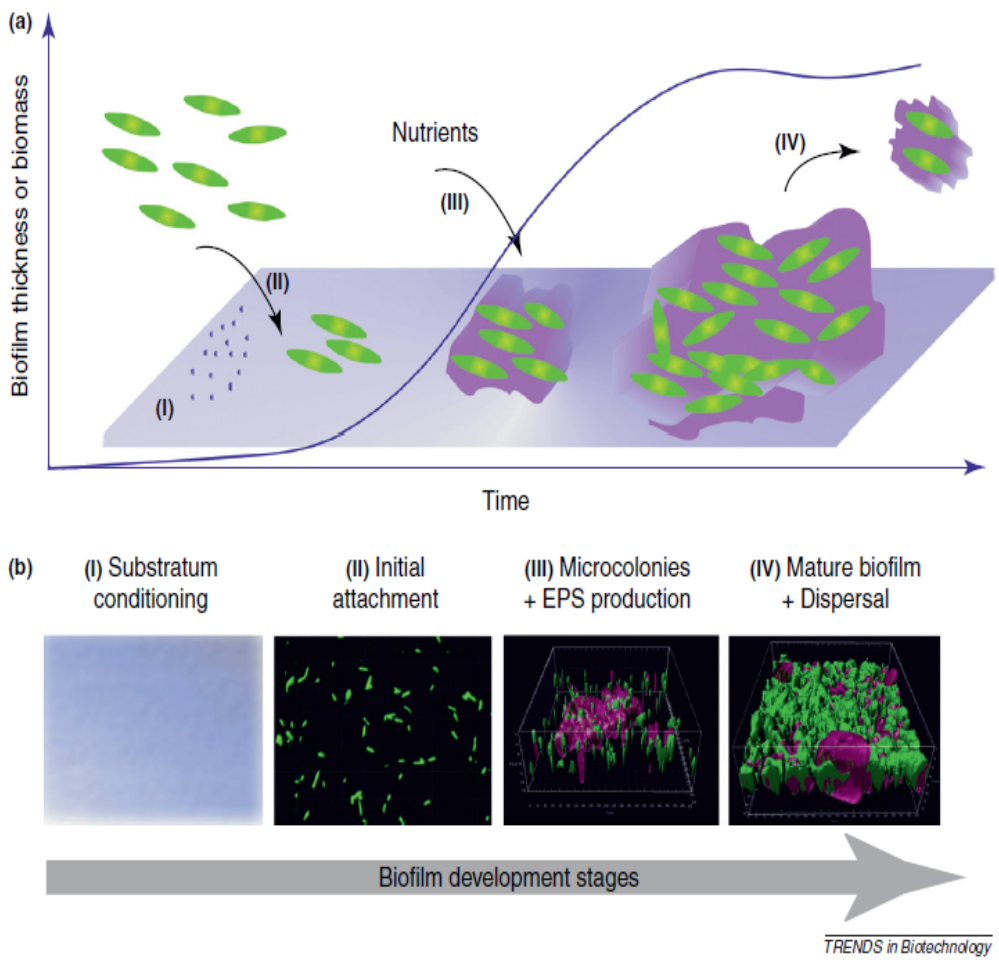
Isoquercitrin

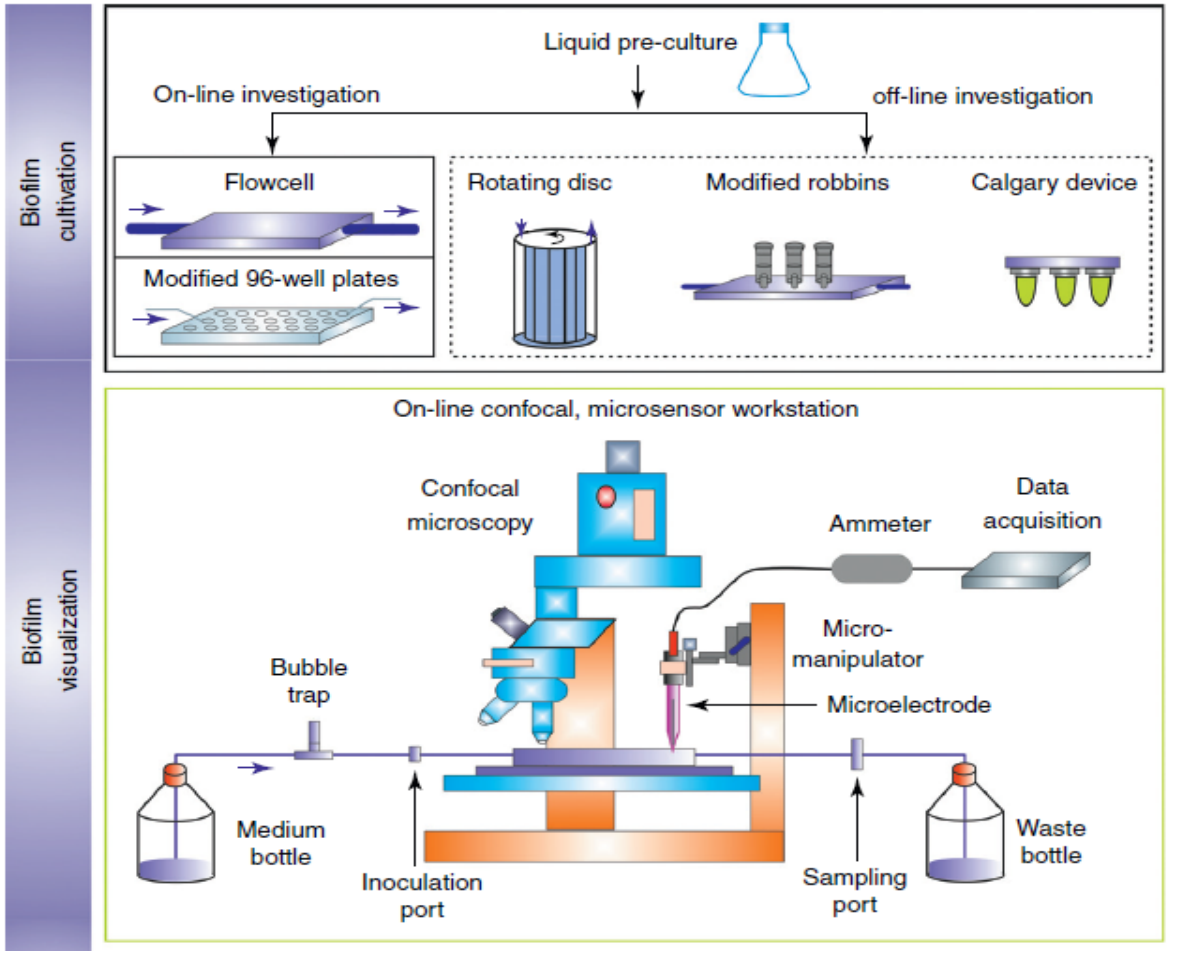


How to enhance production ?

[1] Junior A G , et al. J Ethnopharmacol, 2011, 134(2): 215.

Biofilms as living catalysts

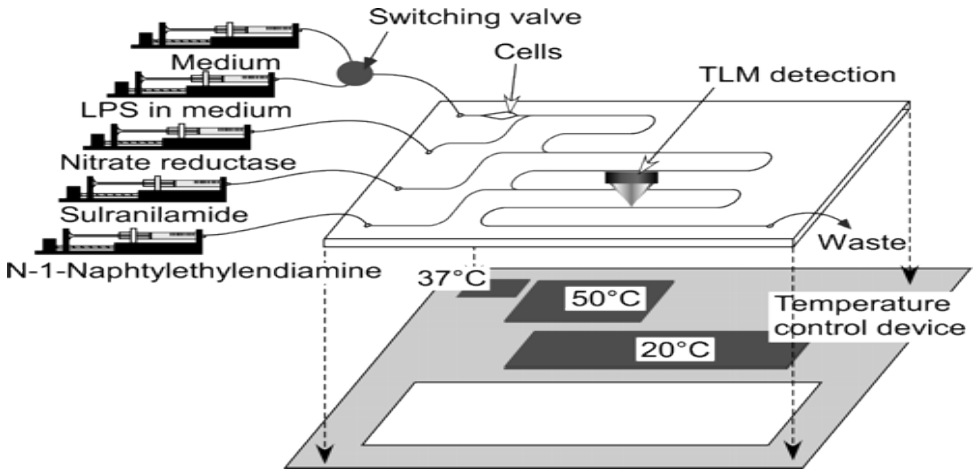


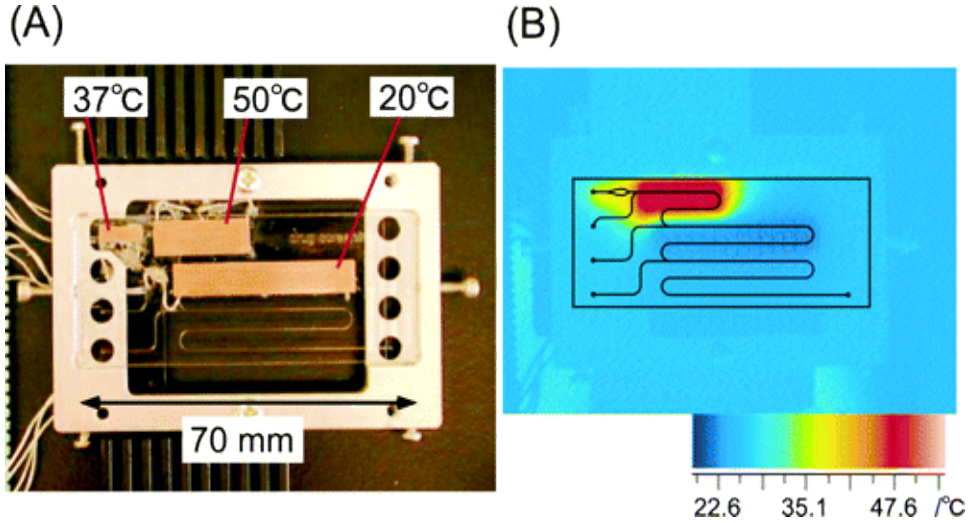


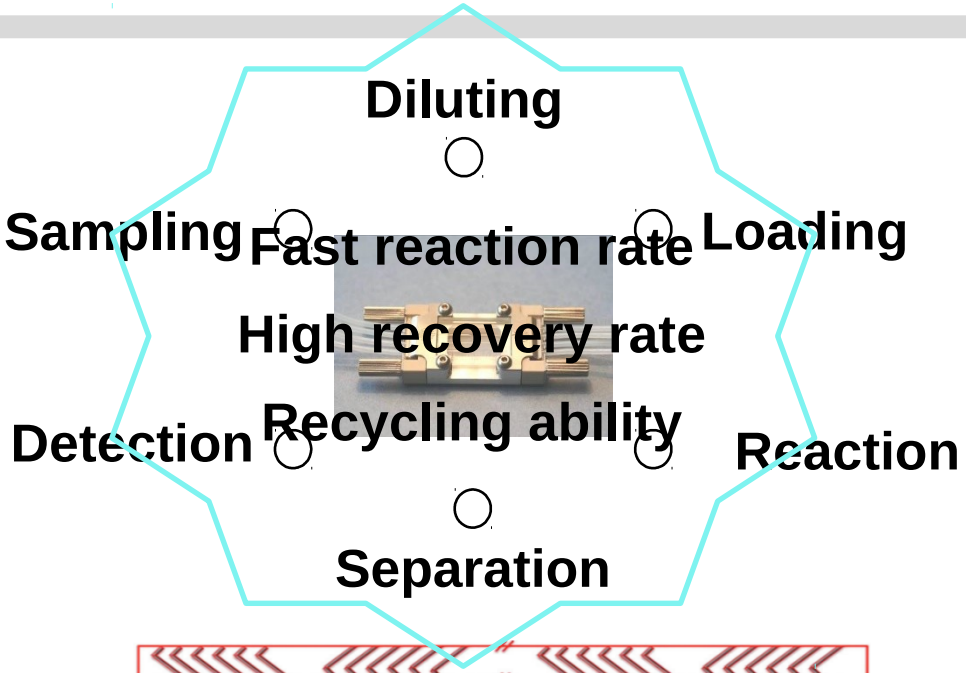
Biofilms are resilient to a wide variety of environmental stresses.
This inherited robustness has been exploited mainly for bioremediation.

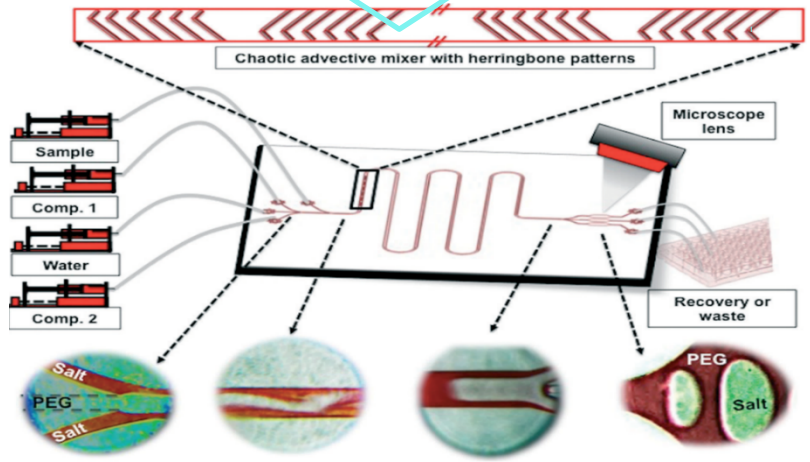
[2] Halan B , et al. Trends in Biotechnology, 2012, 30(9): 453-465.

Microfluidic biofilms catalysis technology









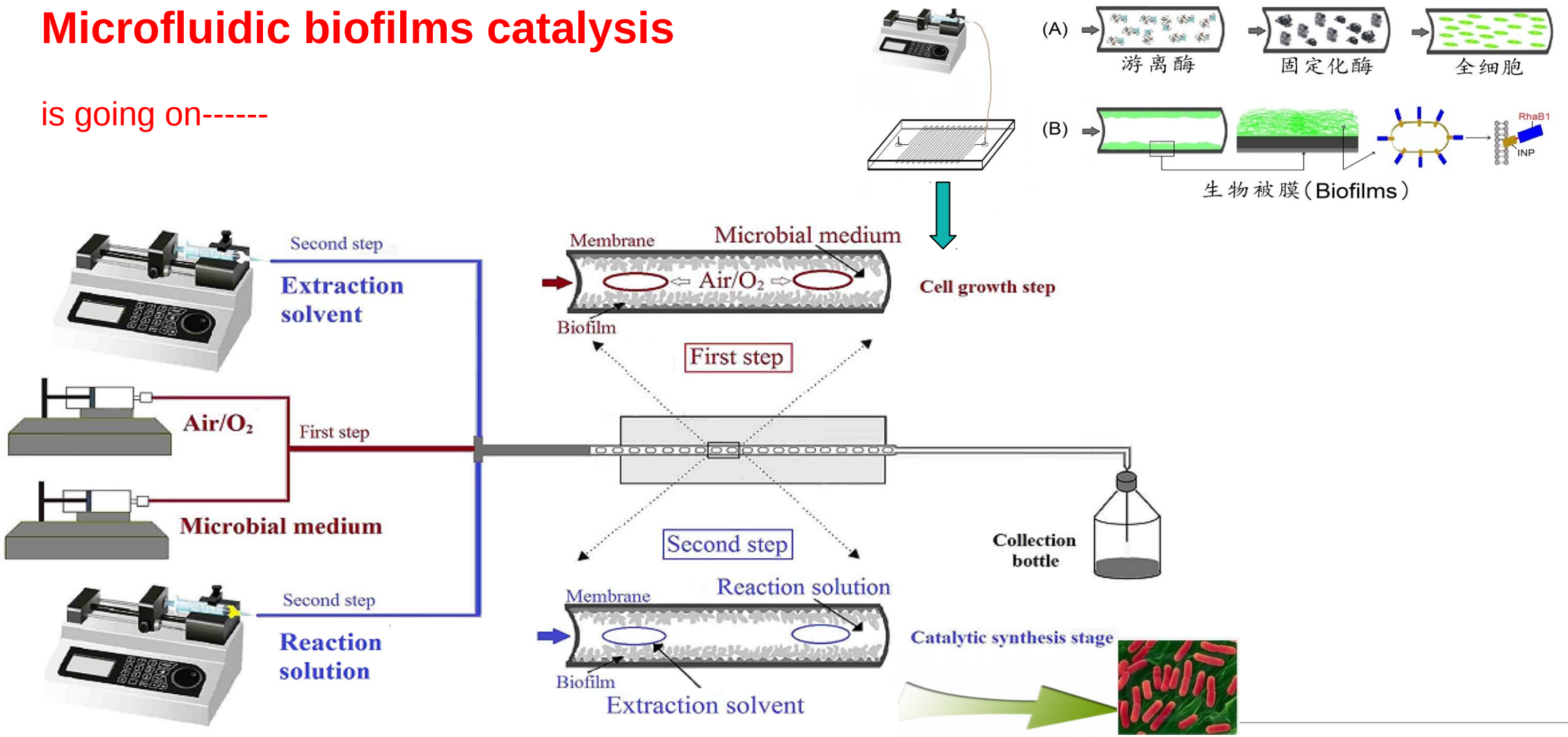
[3] Vázquez-Villegas P, et al. Lab on A Chip, 2016, 16(14): 2662.

[4] Qi L, et al. Analytical & Bioanalytical Chemistry, 2015, 407(13): 3617-3625.

Microfluidic biocatalysis (Our 2.0 Edition)

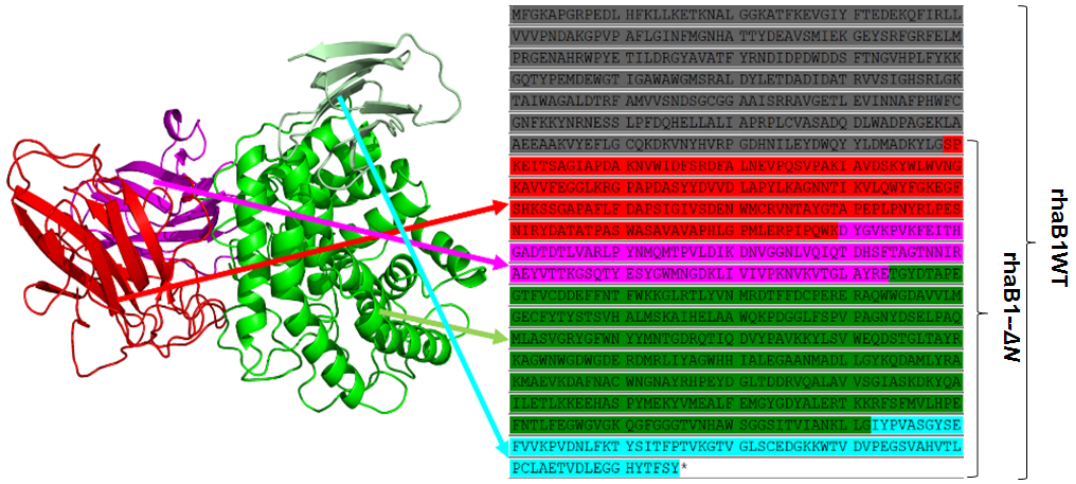
Microfluidic biofilms catalysis

is going on------



[5] Wang J*, et al. International Journal of Molecular Sciences. 2018, 19(9), 2590.

2. Methods

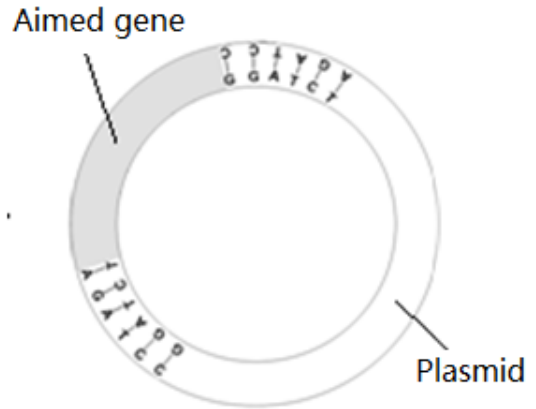


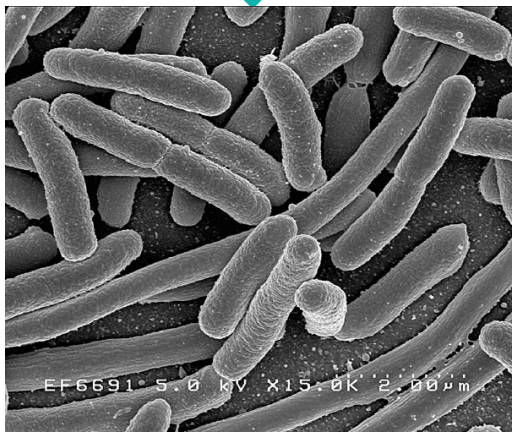
Truncated geng ***rhaB1-ΔN***
Fluorescent protein gene ***EGFP***

rhaB1-ΔN-EGFP

SDS-PAGE analysis

Enzyme activity analysis

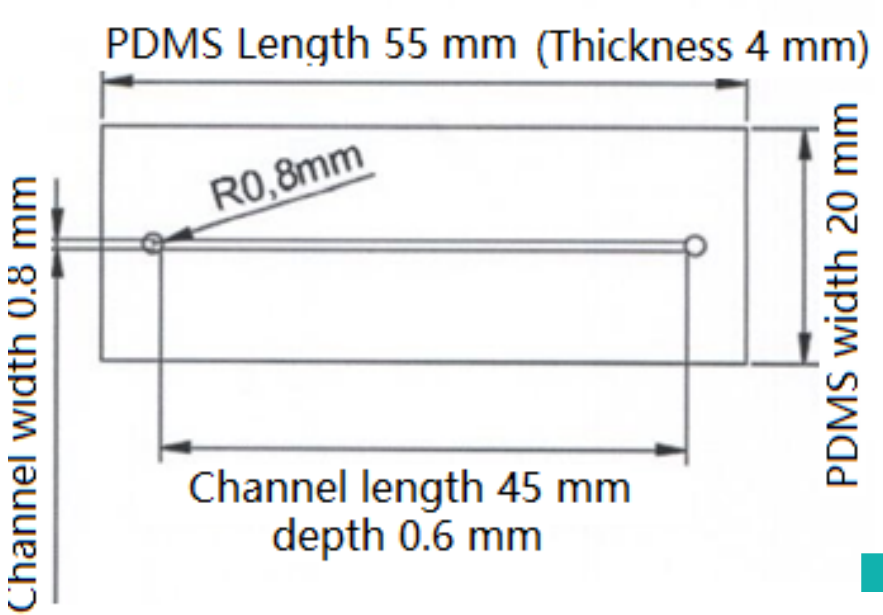


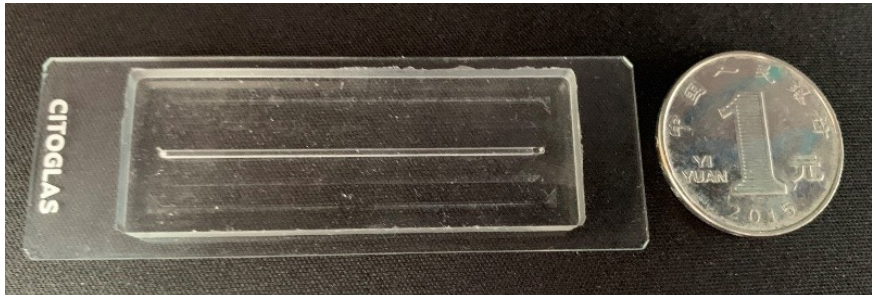


Recombinant *E. coli* BL21-pET28a-*rhaB1-ΔN-EGFP*

The geng rhaB1 provided by Prof. Dr. Wolfgang Streit, UH, Germany

Self-made microreactors





PDMS microchip

Microfluidic biofilm catalytic system

3. Results

A new catalyst rhaB1-ΔN-EGFP

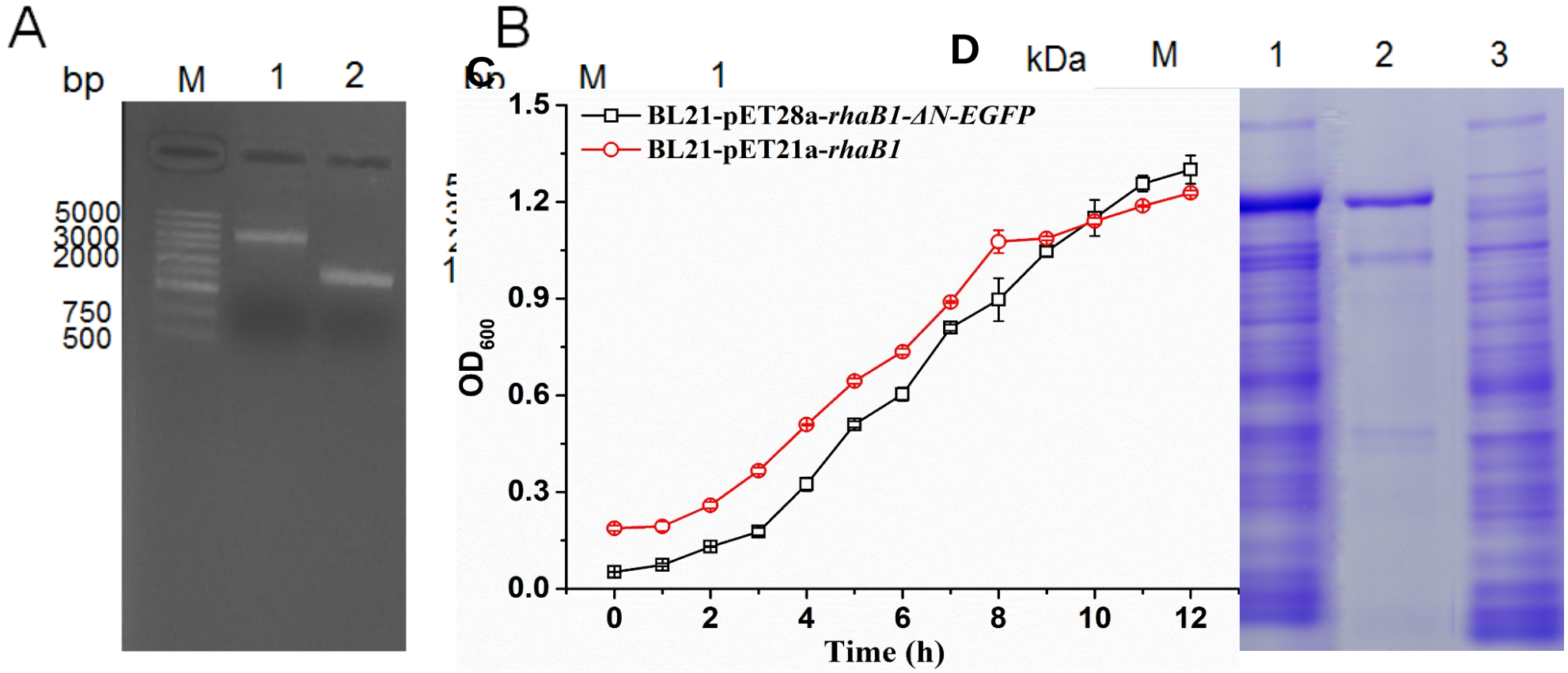
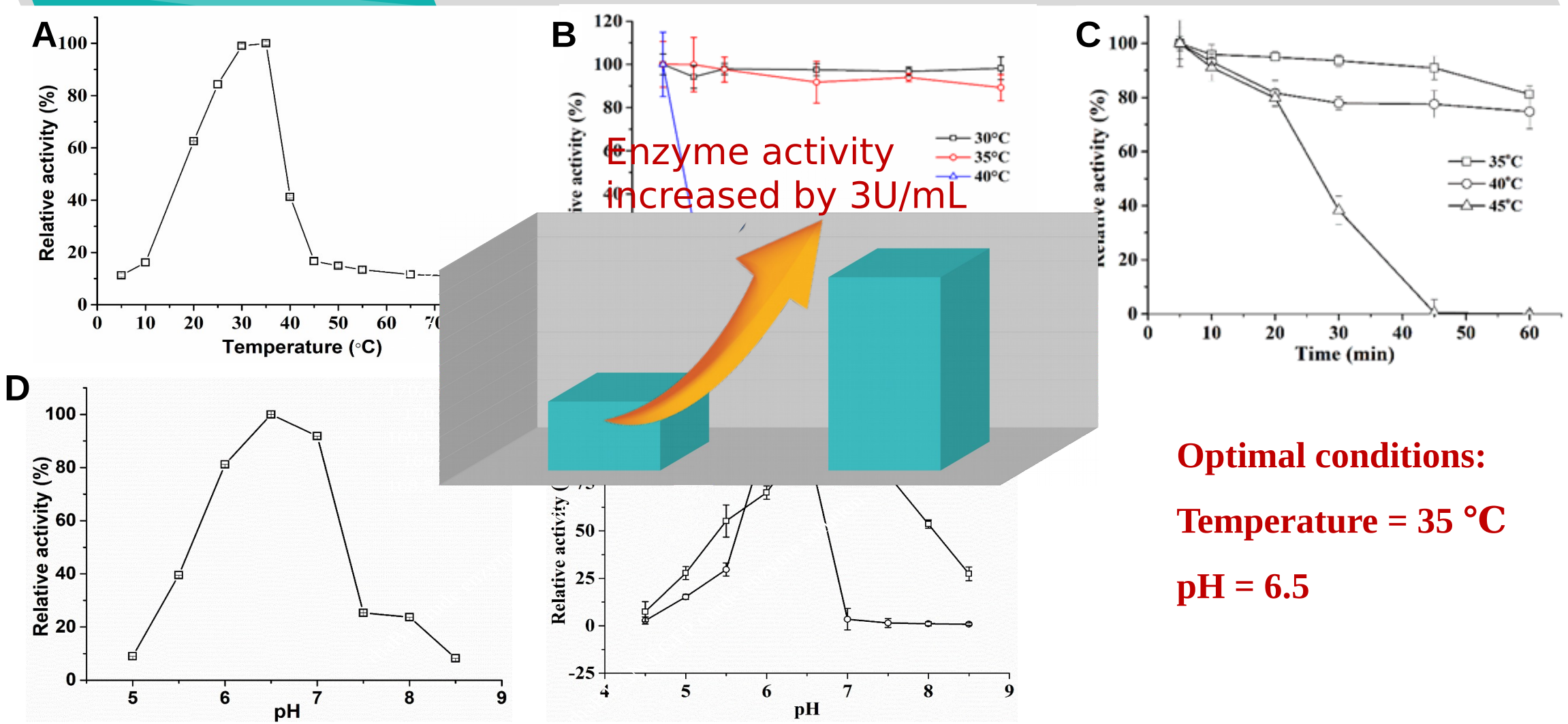


Fig. 1 Expression of rhaB1-ΔN-EGFP.

(A) Fragment of *rhaB1-ΔN* (2076 bp) and *EGFP* (720 bp) was amplified by PCR with template; (B) Recombinant plasmids was extracted from pET28a-*rhaB1-ΔN-EGFP*; (C) Strain growth in a flask; (D) SDS-PAGE of rhaB1-ΔN-EGFP (103 kDa). (M) protein Maker, (1) induced expression of rhaB1-ΔN-EGFP, (2) Purified rhaB1-ΔN-EGFP, (3) BL21-pET28a.

Enzyme activity assays of rhaB1-ΔN-EGFP



Optimal conditions:

Temperature = 35 °C

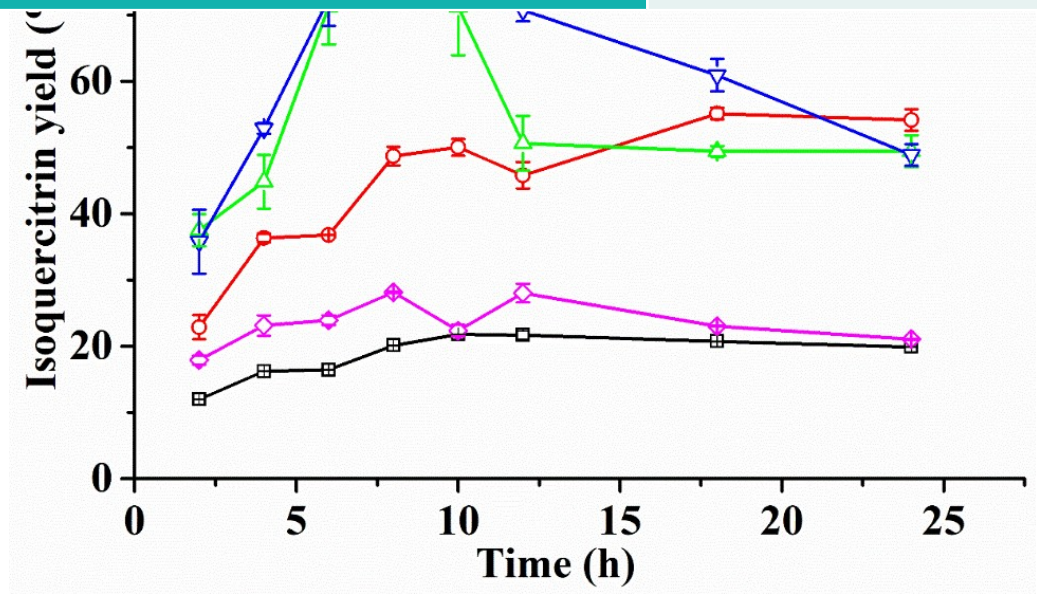
pH = 6.5

Fig. 2 Enzyme activity assays of rhaB1-ΔN-EGFP and rhaB1.

Isoquercitrin production catalyzed by rhaB1-ΔN-EGFP_

Table 1 Comparison of rhaB1 and rhaB1-ΔN-EGFP catalysts performance for rutin hydrolysis.

Free enzyme	Temperature (°C)	pH	Time (h)	Yield (%)
rhaB1	35	5.0	10	98.3±3.8
rhaB1-ΔN-EGFP	40	6.5	10	92.9±4.4



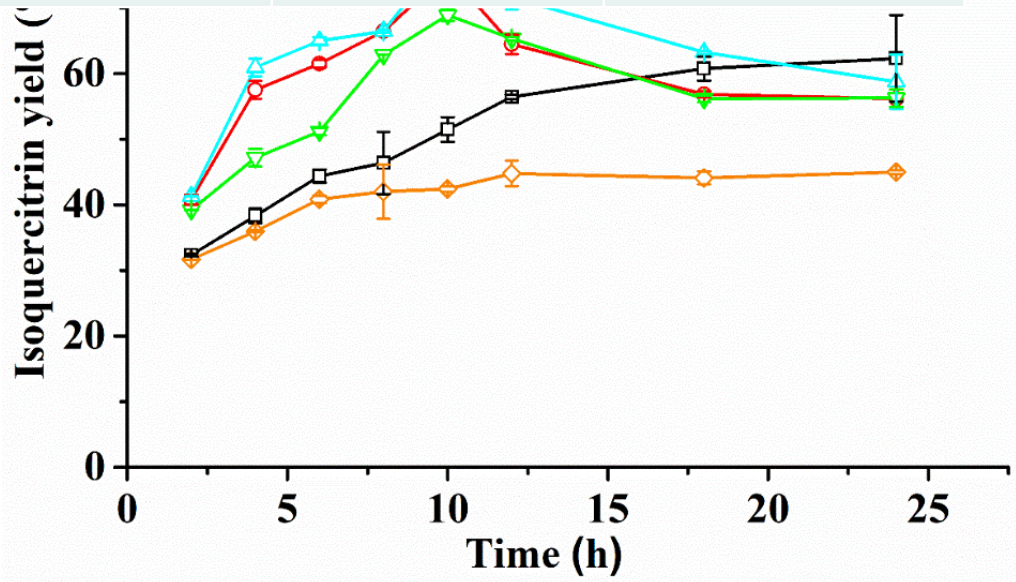


Fig. 3 Effect of pH and temperature on isoquercitrin yield

Construction and formation of microfluidic biofilms_

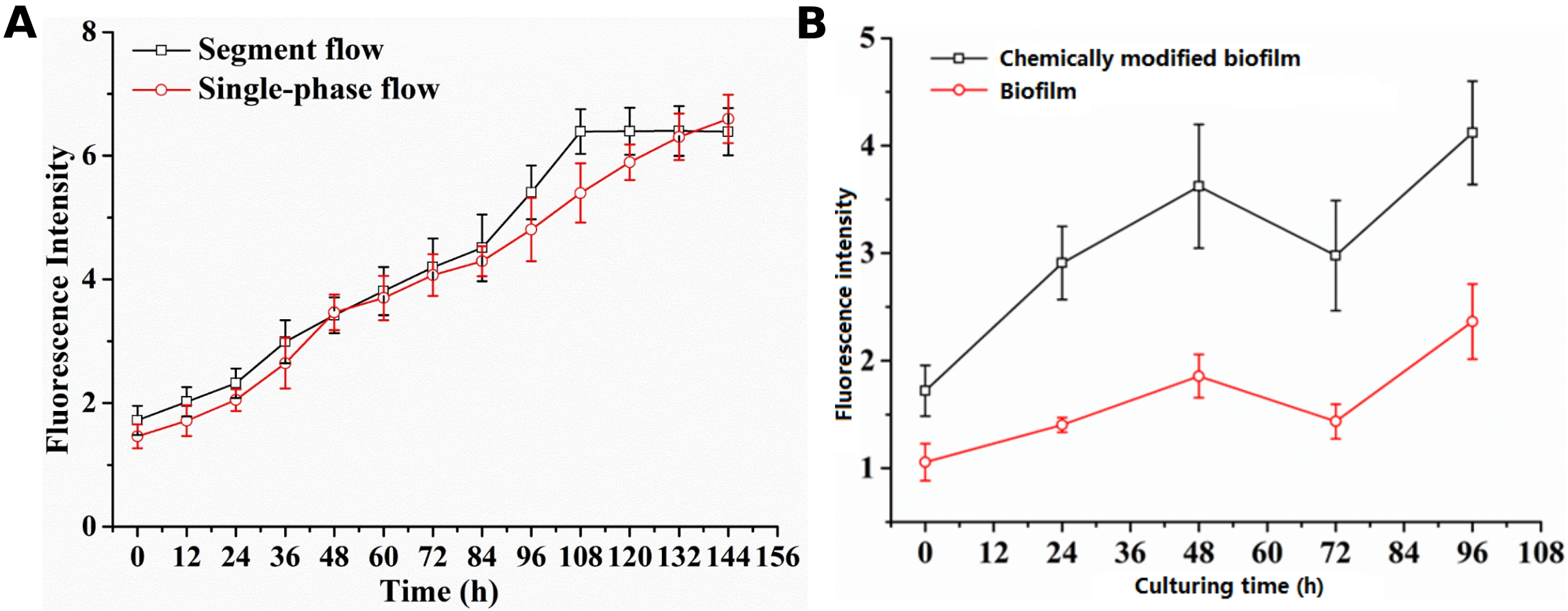


Fig. 4 Construction and formation of microfluidic biofilm.
(A) Culture method; (B) Surface chemically modification.

Culture parameters of microfluidic biofilms_

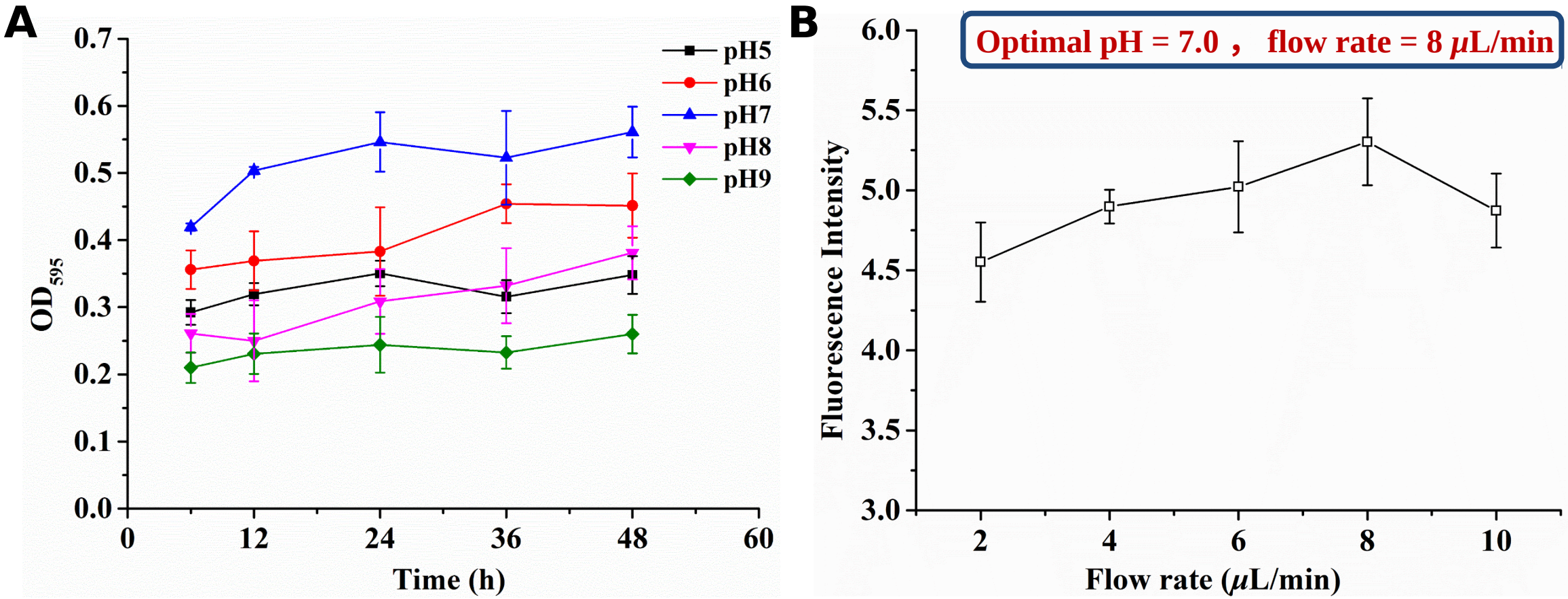


Fig. 5 Effects of different flow rates and pH on the growth of biofilm.
(A) pH; (B) Flow rate.

Characterization of microfluidic biofilms by LSCM_

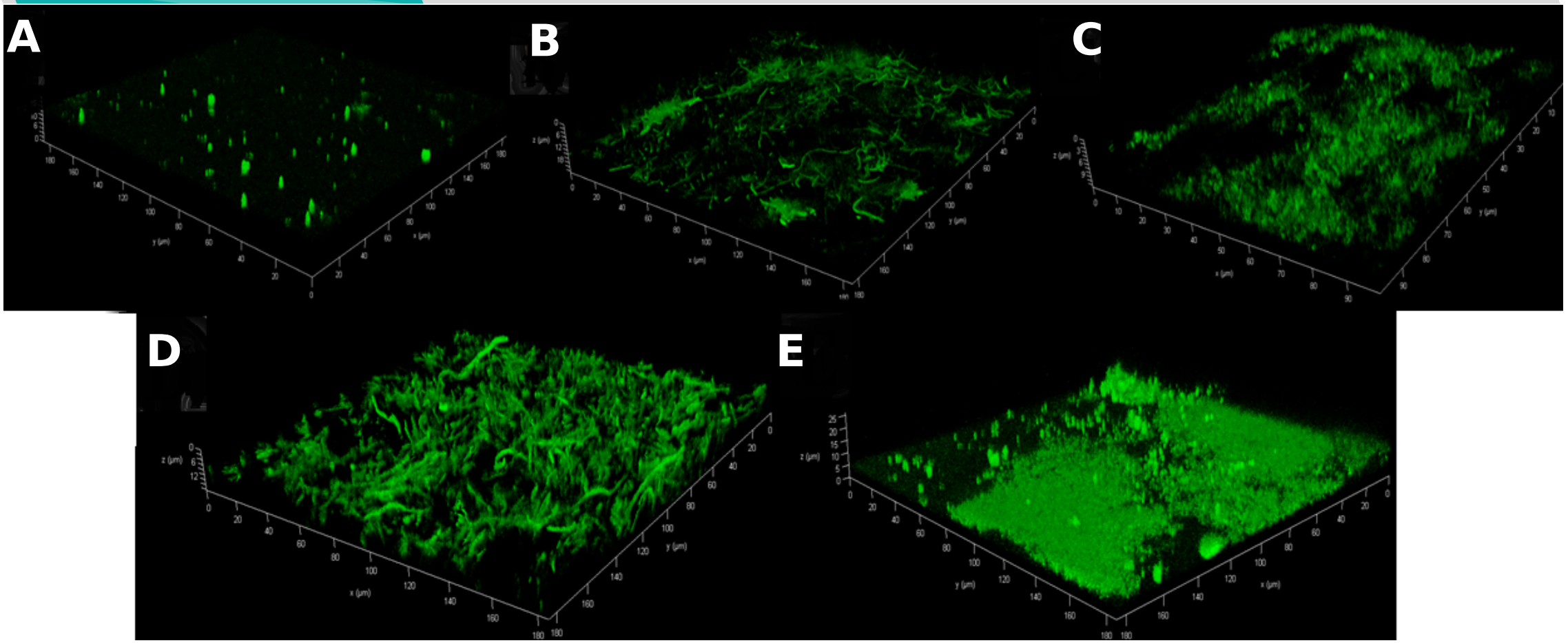


Fig. 6 Laser scanning confocal microscopy observation of bacterial biofilm growth in microchannels. (A) adsorption growth for 2 h; (B) single channel culture for 24 h; (C) single channel culture for 48 h; (D) sectional flow culture for 24 h; (E) Growth chart of 48 h in subsection flow culture.

Catalytic process of microfluidic biofilms

0.79 $\mu g/L_{tube}/d$

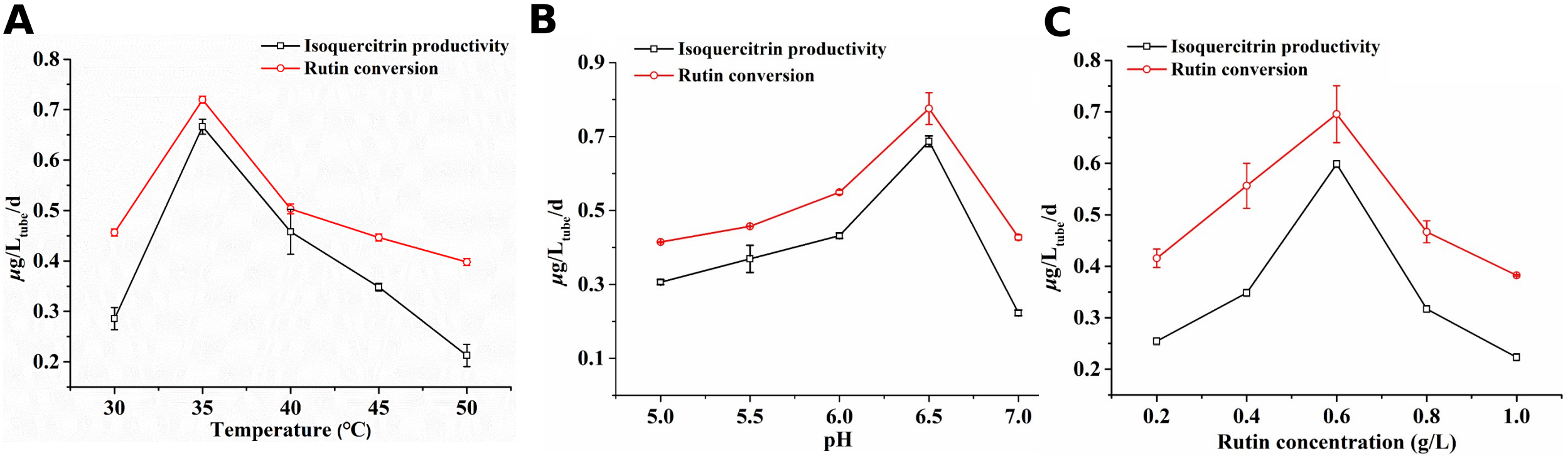


Fig 8 Effect of temperature (A), pH (B) and rutin concentration (C) on the isoquercitrin productivity and rutin conversion in the microfluidicbiofilm reactor

Temperature **35 ℃**

pH **6.5**

Rutin concentration **0.6 g/L**

4. Conclusions

➢1. The recombinant strain BL21-pET28a-*rhaB1-ΔN-EGFP* was successfully constructed and produced a new enzyme rhaB1-ΔN-EGFP.

➢2. rhaB1-ΔN-EGFP showed 95% relative activity after treatment for 60 min at the optimum temperature of 35 ℃, showing good thermal stability.

➢3. Using free enzyme rhaB1-ΔN-EGFP to catalysis the hydrolysis of rutin, the optimum temperature and pH value were 40 ℃ and 6.5, and the maximum yield of isoquercitrin was 92.9±4.4%.

➢4. The fluorescence intensity of the biofilm increased by 74% after 24 hours under segmental flow, and the biofilms exhibited compact and flat characteristics under the fluid force.

➢5. The yield of isoquercitrin reached **0.79 μg/L$_{tube}$/d** when the substrate rutin concentration was 0.6 g/L, the reaction temperature was 35 ℃, and the pH was 6.5.

Acknowledgments

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Thank you very much for your kind attention!

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