Electronic Supplementary Information (ESI)

Wood-inspired High-efficiency Bioreactors with Hierarchical Porous Structures for Continuous Flow Reactions

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Experimental section

Preparation of engineering enzymes

The primers were designed based on the whole sequence of GH-3 β-glucosidase Bgl 3A gene of cyanobacteria thermophilus, and the β-glucosidase gene fragment was obtained from the genome of Cyanobacteria by PCR. The recombinant plasmid was then transfected into trans I-T1 receptor cells. The β-glucosidase expression vector with 6*His label at the C-terminal was extracted after the positive transformants were obtained by screening for Ampicillin resistance. The recombinant vector was transferred into Pichia pastoris GS115 competent cells for amplification and culture, and the expression was induced by BMGY and BMMY medium. Since Pichia pastoris itself does not secrete endogenous proteins, exogenous proteins are secreted after induction. The supernatant after culture is the crude enzyme solution of β-glucosidase labeled by His tag.

Simulation analysis of fluid flow in the reactor

Based on the SEM analysis results, a two-dimensional geometric simulation model is established. The model consisted of randomly sized channels (diameter ~20 μm), and the channel walls were added in the form of rectangular barriers. The pores (1-5 μm) were added to the channel walls according to the specific conditions. This model was regarded as a repeating unit of the reactor structure. The channel wall is set as the boundary condition, and the left and right sides are the inlet and outlet. Triangular mesh was used to divide the geometric area. The inlet velocity is adjusted according to the experiment, and the gravity part is ignored. The laminar physical field is used to simulate in COMSOL.

Determination of polydatin and resveratrol

The contents of polydatin and resveratrol were determined by waters alliance 2695HPLC system (Waters, Milford, MA, USA), equipped with quaternary pump, automatic sampler, vacuum degasser and waters 2998 PDA detector. Chromatographic separation was carried out on Sunfire C18 reversed-phase chromatographic column (150 mm×4.6 mm, 5μm). The flow rate is 1 mL/min, and the mobile phase is mixed with solvent A (methanol) and solvent B (0.1% formic acid): 0-

4 min, 20% A - 30% A; 4-10 min， 30% A - 65% A; 10-16 min, 65% A - 95% A; 16-20 min, 95% A - 20% A; 20-25 min, 20% A, injection volume 10 μL. The column temperature is set at 25 ℃.

Gene sequence of engineered strain

GGCTGAAGCTTACGTAAACAGGCATCGCCAGGTTATCGGCAGGAATCACCTCAATCATCAC

CATCGCAGCCAAAGAGAACTTGCCTATTCACCCCCCTCTTATCCCTCACCATGGATGGACCC

GAAGGCTGCAGAGTGGCAGAATGCCTACGCCAAGGCCAAGGCGTTCGTTTCCAAACTGACC

CTCCTCGAGAAGGTCAACCTGACAACTGGCGTTGGTTGGGCATCTGAGCAGTGTGTGGGAA

ACACCGGGTCTGTTCCCCGTCTGGGCCTTCGCGGCTTGTGCCTTCACGACTCTCCCCTGGGG

ATTCGAGGTAGCGACTACAACTCGGCCTTCCCCTCGGGCCAGACGACTGCGGCCACCTTCG

ACAGGATTCTTATGTACCGTCGGGGCTACGCCATGGGGAAGGAAGCAAAGGGCAAAGGGA

TTAACGTCCTGCTCGGGCCGGTGGCTGGCCCCCTTGGTCGCATGCCTGCTGCTGGTAGAAA

CTGGGAAGGGTTCTCGCCGGATCCTGTCCTTACTGGCGTTGGCATGGCTGAGACGATTAAG

GGGATCCAGGATGCGGGTGTCGTTGCTTGCGCAAAGCACTTGATTGGAAACGAGCAGGAG

CATTTCCGGCAGGCAGGCGAGGACTACGGATTCAACATCACCGAGGCCTTGTCCTCCAACA

TCGACGACAAGACCATGCATGAACTGTACTTGTGGCCCTTCGTAGACGCCGTGCGCGCCGG

TGTAGGCTCCGTCATGTGCGCCTACACCCAAGTCAACAACTCGTACTCTTGCCAGAACTCGT

ACCTCTTGAACTACTTGCTCAAGAATGAGCTTGGGTTCCAGGGTTTCGTTATGAGCGACTGG

CAGGCTCAGCACGCAGGCGTTTCCGCCGCGGTTGCAGGTCTCGACATGTCCATGCCCGGAG

ATACCACGTTTAACACTGGTGTCAGTTTCTGGGGAGCAAACCTCACTCTGGCTGTGCTCAAC

GGGACTGTCCCGCGTTACCGCATTGACGATATGGCCATGCGAATCATGGCTGCCTTCTTCAA

AGTCCACCCGGATATTCACCTCGACCCTGTCAACTTCTCGTTCTGGACTCGCCAGACATACG

GCCCCCTTCATTGGCGCGCCGGGGATGGCTATCAGCAGGTCAACTTTCACGTCGATGTTCG

GGAGGATCATCACTTGCTCATCCGGGAGATTGCCGCCAAGGGGACTGTCCTGCTCAAGAAC

ACCGGCGGTCTTCCTTTGAAGAAGCCCAAGTTTATCGCTGTCATTGGTGACGACGCCGGACC

AAACCCCAATGGTCCCAACAGCTGCGACGACCGCGGCTGCAACAACGGCACCCTTGCCATG

GGCTGGGGCTCGGGAACGGCCAACTTCCCGTATCTCATTACCCCAGACGCTGCCCTCCAGG

CACGGGCCCTCCGAGACGGCACCCGCTATGAGAGCGTGCTGACCAACTACGCCCTGGAGAA

GACCGAGACTCTCGTGTCCCAGGCCAACGCGACGGCGATTGTCTTTGTCAATGCGAACTCG

GGTGAGGGGTACACAAGGGTTGACGGCAATATGGGCGACCGCAAGAATCTGACCCTCTGG

GGGAACGGCGACGATCTGATCAAGAACGTCTCCAGCTGGTGCTCAAACACCATTGTCGTCA

TCCACTCGACAGGCCCCGTCTTGCTGACAGACTGGTACAACAGCCCCAACATCACCGCCATC

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CGTCTTTATCGACTACCGCTACTTCGACAGAGAGGATACGCCCGTCATCTACGAATTCGGAC

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CCCTACAAGCCGACCACGGGCAAGACGACACCCGCGCCCACCTTTGGCCAGGATGATGCAT

ATGGCTATACTGGCTCCGAAAAATATCTCTTTCCCCCAAGCCCCTTCCGCCGCATCACGGATT

ACATTTACCCATACCTCAACTCGACCGACCCACACGAGGCATCGCTAGACCCGCACTATGGT

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CACGGCCGACATCACCAACATGGGATCCGTCACGGGCGACGAGGTGCCGCAGCTGTACGT

GTCCCTCGGCGGGCCCCAGGATCCCAAGGTTGTTTTGCGCGGGTTCGATCGGTTGAGAGAG

ATCGGGCCGGGAGAGACGCGGCAGTTCATGGCCCGCCTTACGAGGCGCGATTTGAGTAAT

TGGGATCCGGTTCTTCAGGATTGGGTGGTGGGTGGACATAAGAAGACGGTGTTTGTGGGG

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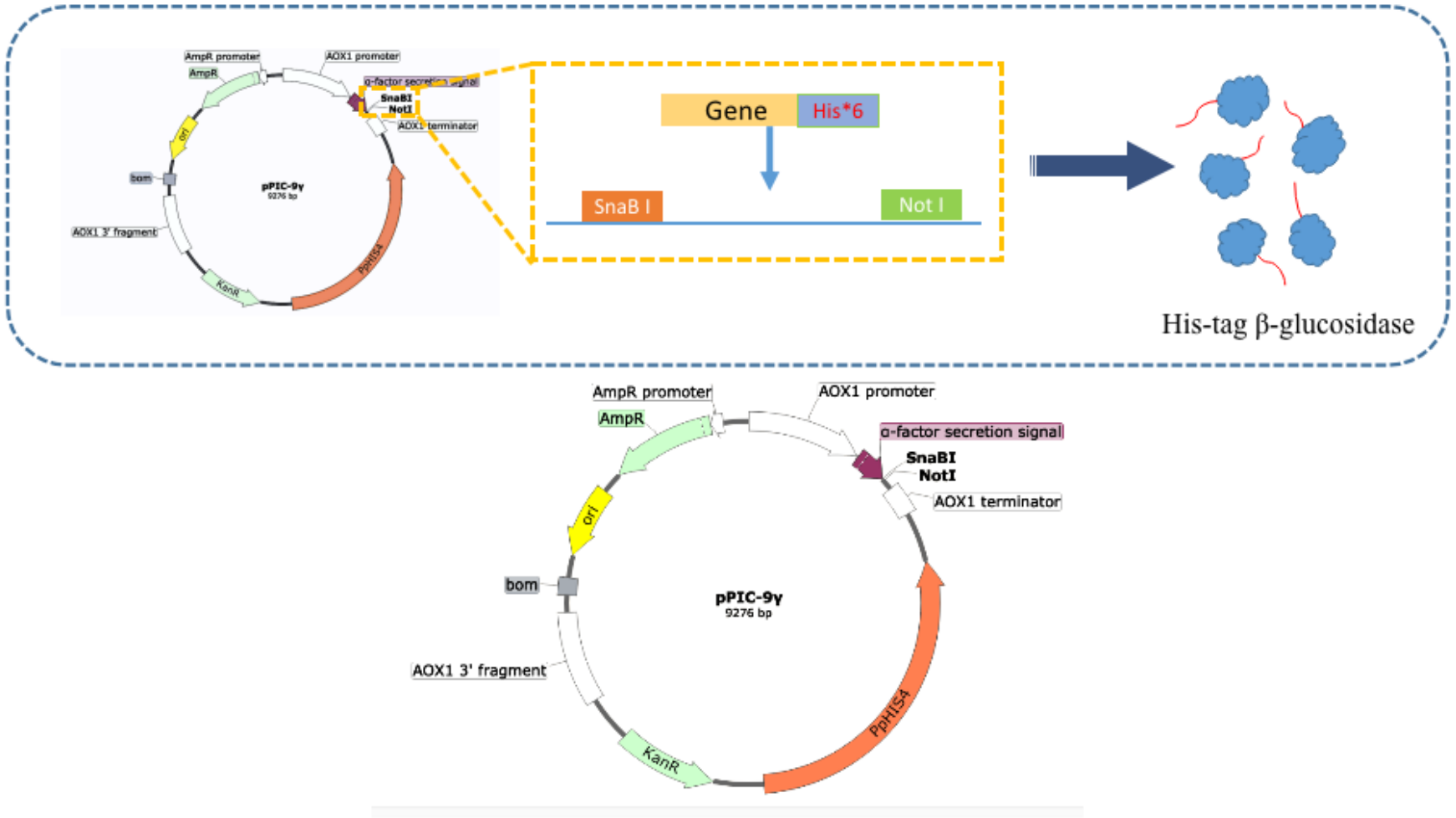

Scheme S1 Scheme diagram of vector plasmid and engineering strain preparation

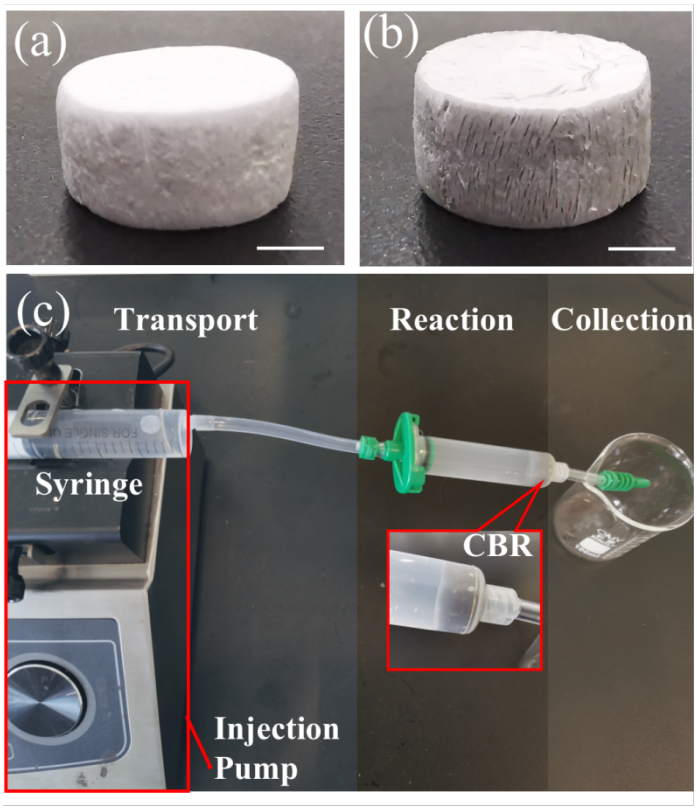

Fig. S1 The apparent image of the reactor: (a) CE; (b) CE-NiO. Scale bars: 5 mm; (c) Construction of the bioreactor

The prepared cellulose material is a cylinder with a diameter of 15 mm and a height of 10 mm. CE-NiO with immobilized enzyme was filled into the canister and connected to the syringe through a hose. The injection pump pushed the reaction liquid in the syringe into the reactor for catalytic reaction, and the liquid released was the product. The canister with the cellulose material will be placed in a controlled temperature container for temperature control.

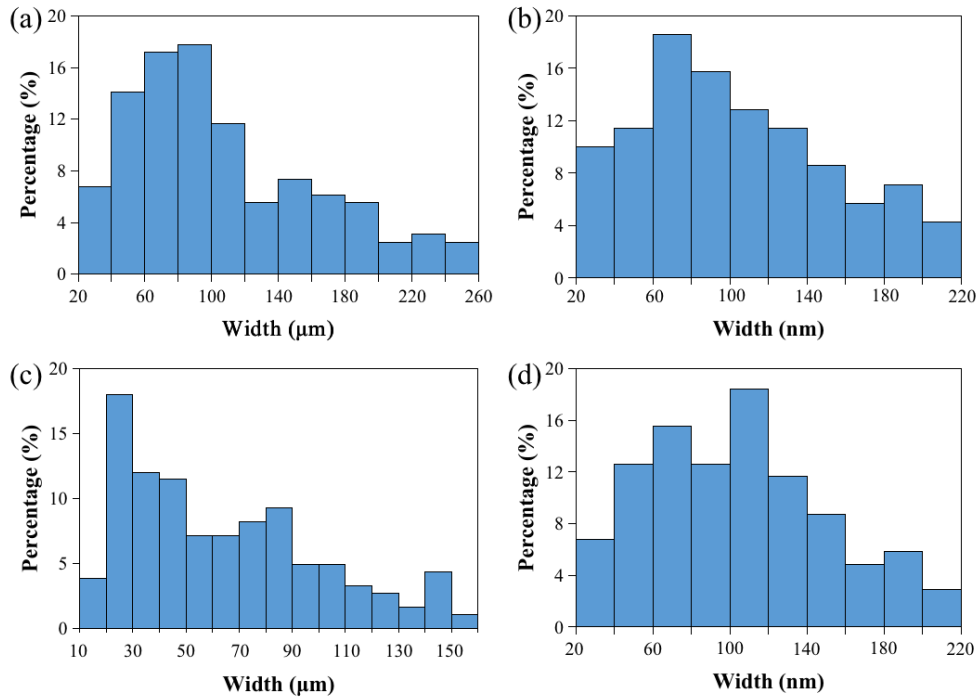

Fig. S2 Pore size analysis: (a) Channel diameter of CE; (b) Inner wall pore diameter of CE; (c) Channel diameter of CE-NiO; (d) Inner wall pore diameter of CE-NiO

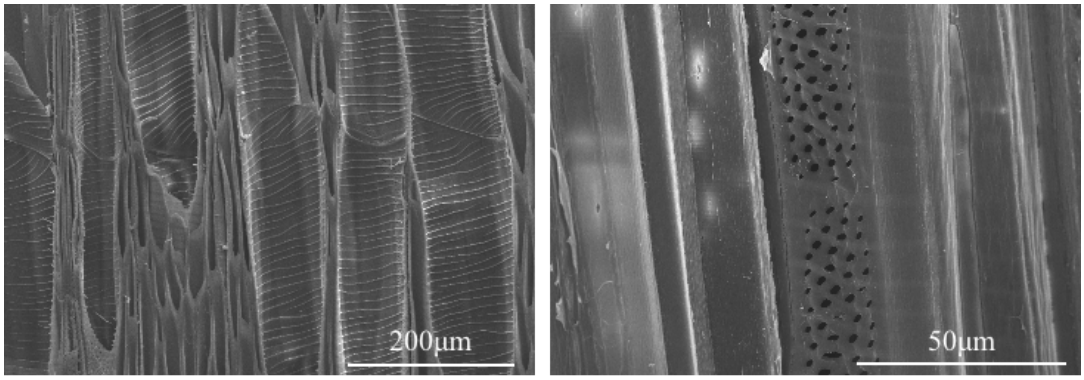

Fig. S3 SEM image of natural basswood after delignification

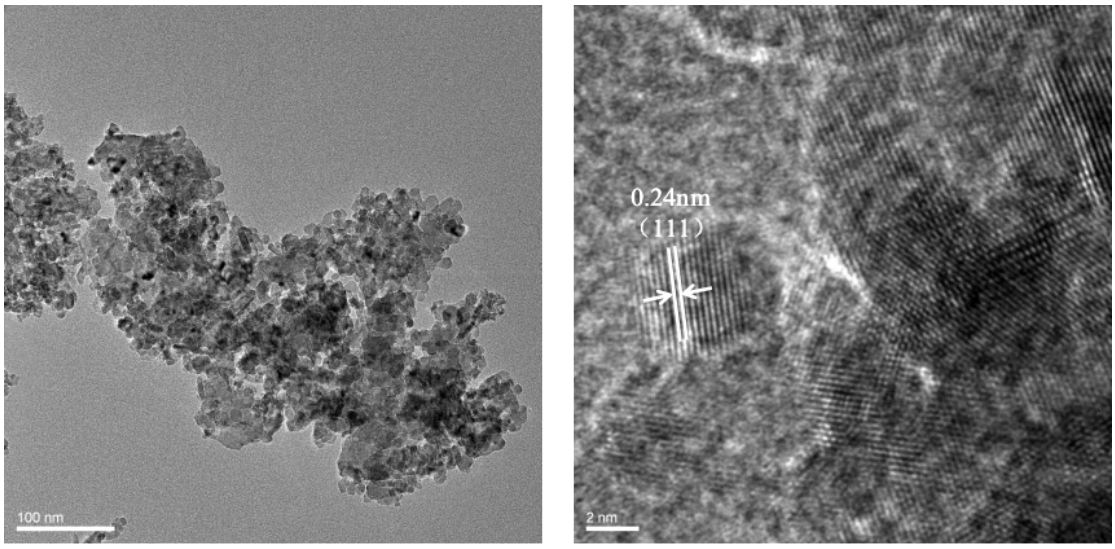

Fig. S4 TEM image of NiO NPs

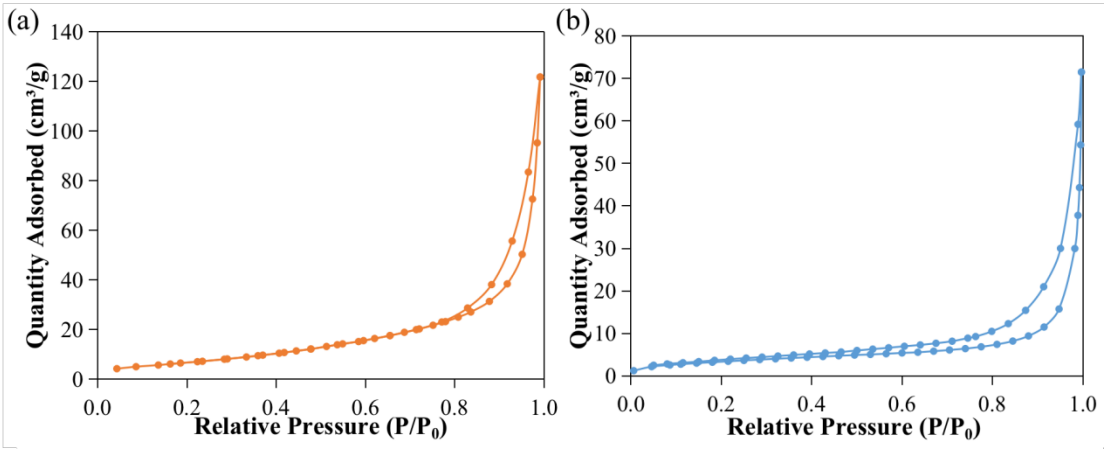

Fig. S5 Nitrogen adsorption-desorption isotherm diagram: (a) CE; (b) CE-NiO

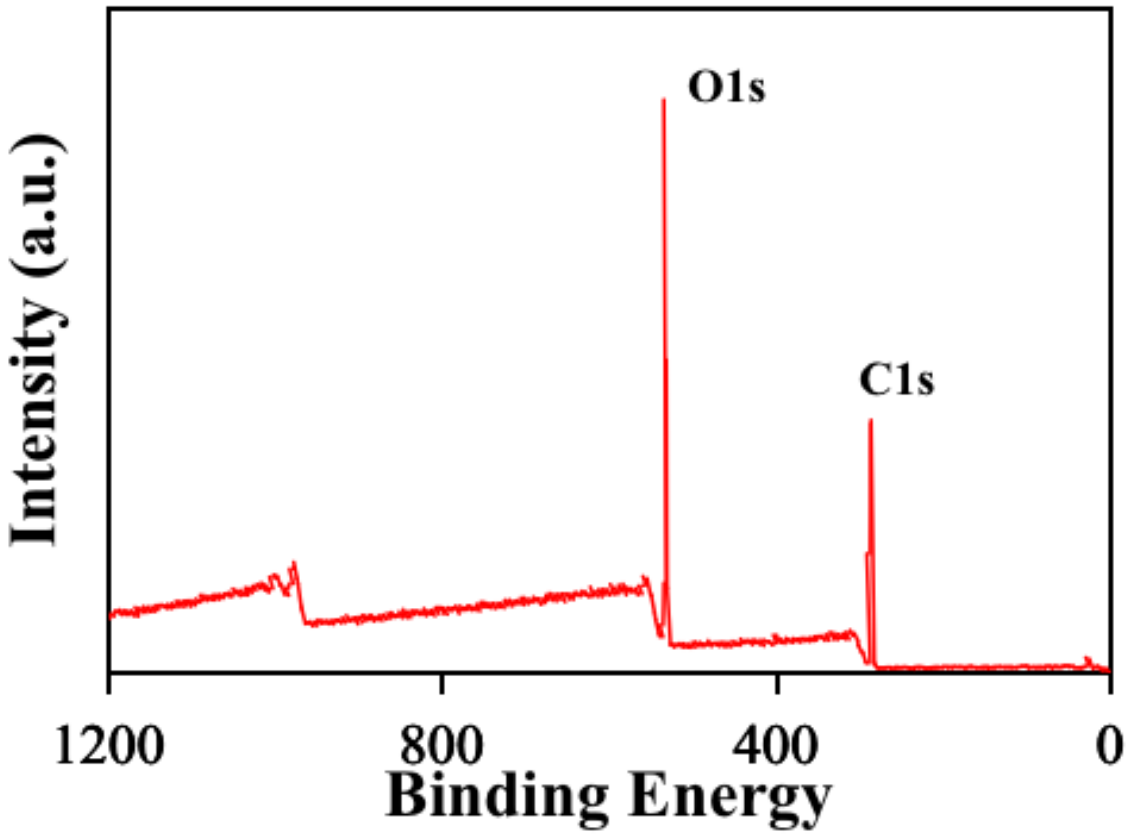

Fig. S6 XPS spectra of CE

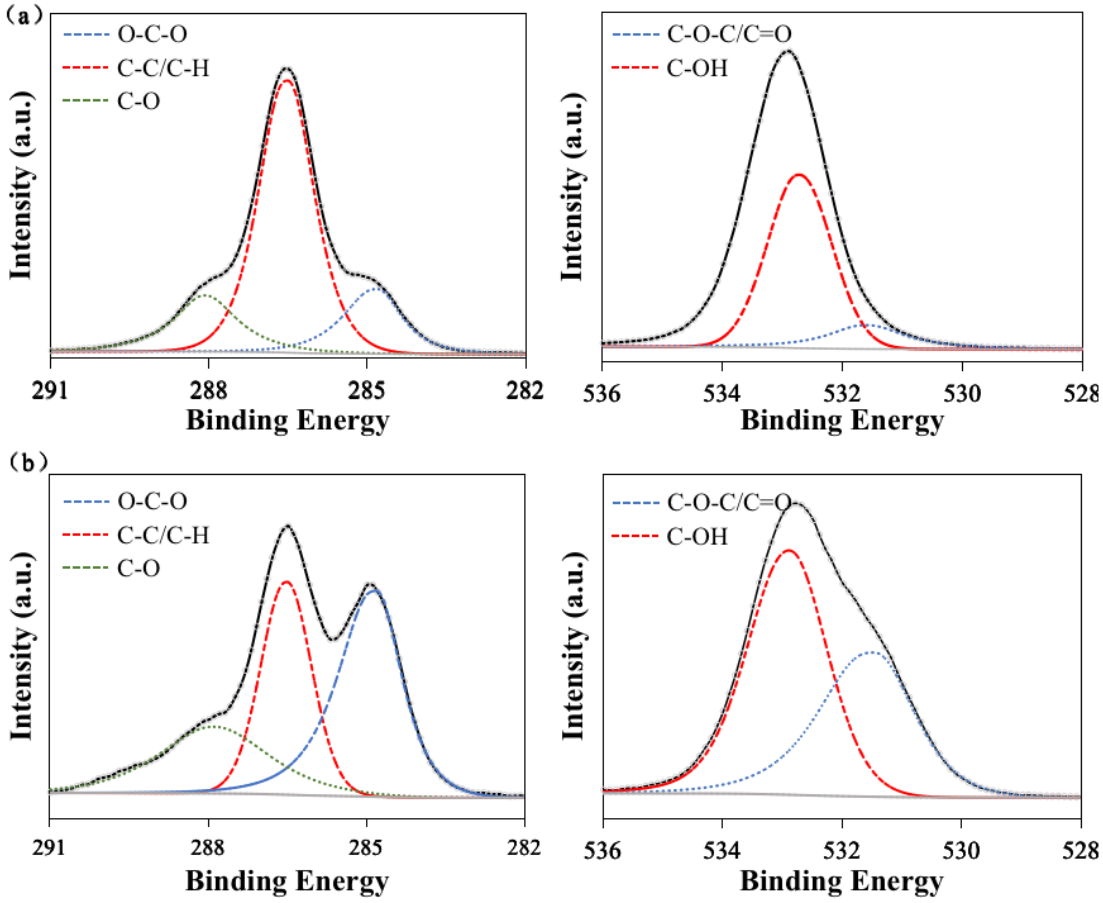

Fig. S7 XPS spectrum analysis of different materials (a) CE; (b) CE-NiO

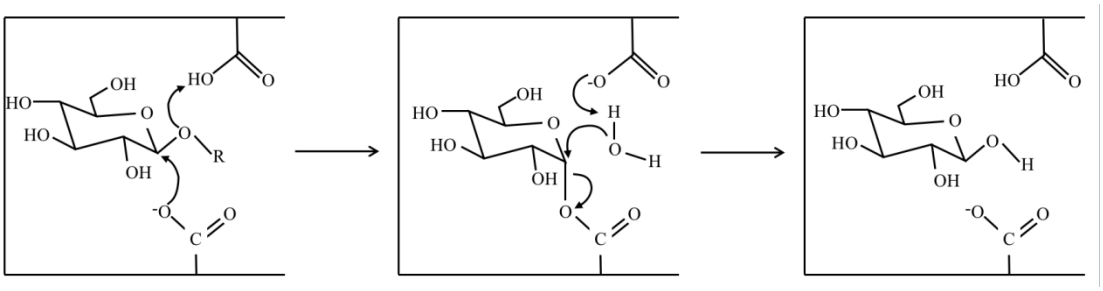

Fig. S8 Mechanism of reaction catalyzed by β-glucosidase

β-glucosidase (EC3.2.1.21) can hydrolyze the binding of the terminal non-reducing β-D-glucose bond, while releasing β-D-glucose and the corresponding ligand[1]. The constructed β-glucosidase gene is derived from thermophilic fungus Talaromyces leycettanus JCM12802 and belongs to the GH3 family based on the similarity of amino acid sequence and conserved domain[2]. Enzymes in this family generally hydrolyze the glycoside substrate through a retention mechanism involving two key active site carboxylic acid residues[3, 4]. As shown in Fig. S8, the nucleophile of the enzyme attacks the oxygen atom on the glycosidic bond of the substrate with the help of the carboxyl side chain, and covalently connects with it to form the enzyme substrate transition state. Then the acid-base carboxyl group performs acid catalysis, provides protons to promote the departure of the substrate ligand, and finally performs base catalysis to activate one water molecule to attack the transition state, reacts with it to cut the glycosidic bond, and releases β-monosaccharide products and enzymes.

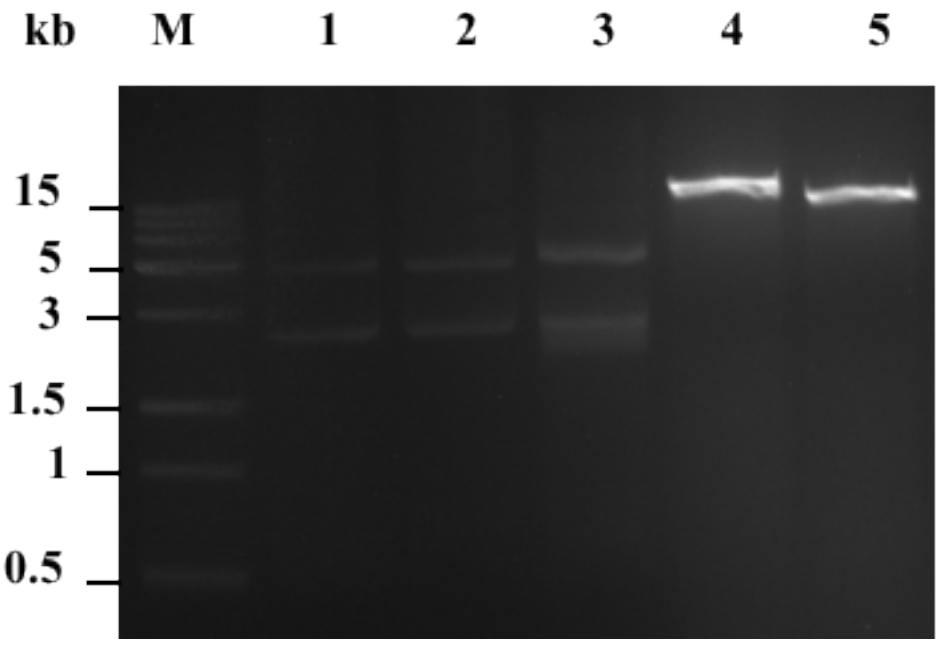

Fig. S9 DNA electrophoresis of objective gene amplification (Lane 1-3) and enzyme digestion plasmid (Lane 4-5)

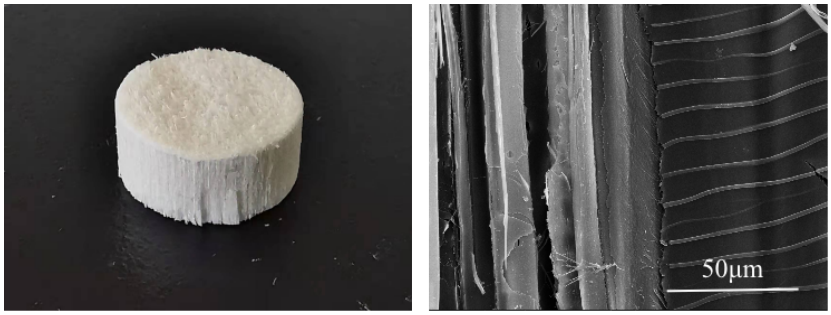

Fig. S10 The appearance and SEM image of natural basswood after delignification

Fig. S11 The appearance and SEM image of disordered porous sponge

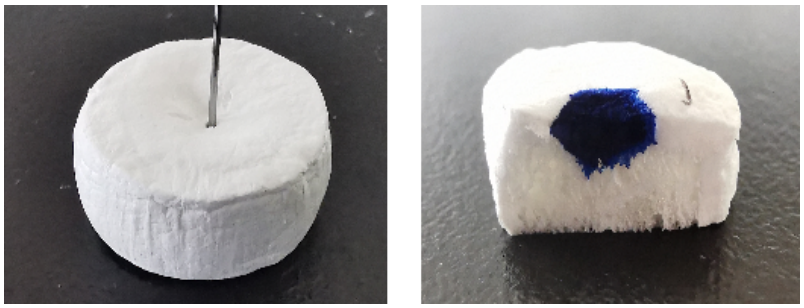

Fig. S12 Methylene blue permeation in CE

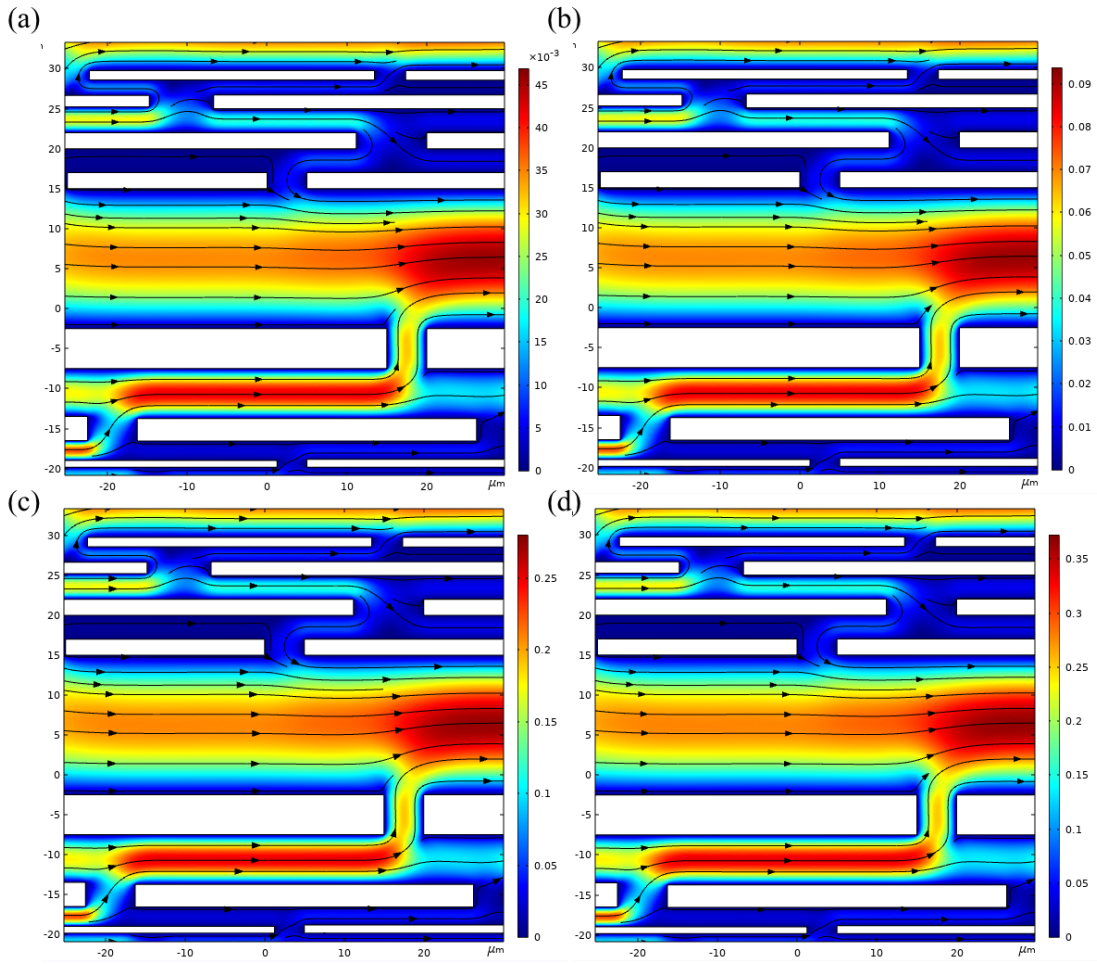

Fig. S13 Simulation diagram of fluid flow in directional channel reactor with porous inner wall (number of pores: 9) at different speeds: (a) 0.02 m/s; (b) 0.04 m/s; (c) 0.12 m/s; (d) 0.16 m/s

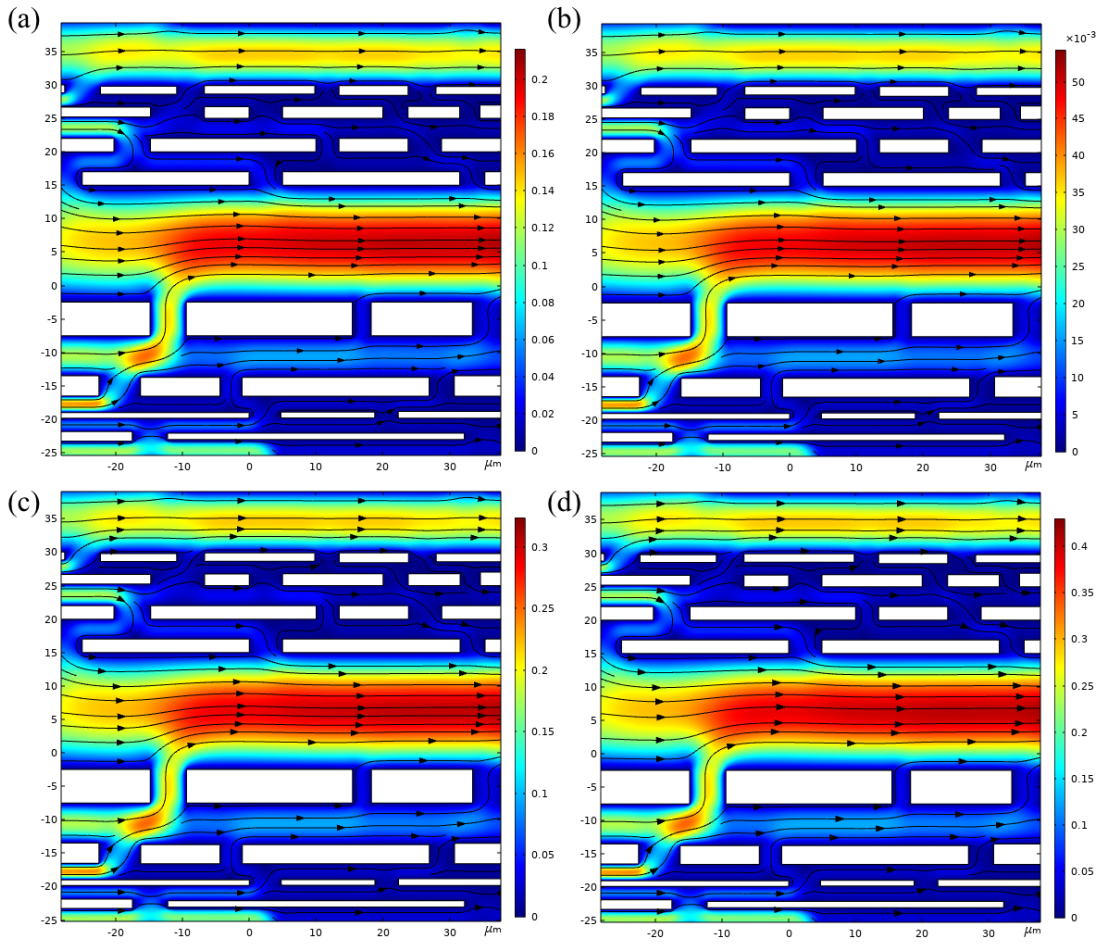

Fig. S14 Simulation diagram of fluid flow in directional channel reactor with more pores in the inner wall (number of pores: 22) at different speed: (a) 0.08 m/s; (b) 0.02 m/s; (c) 0.12 m/s; (d) 0.16 m/s

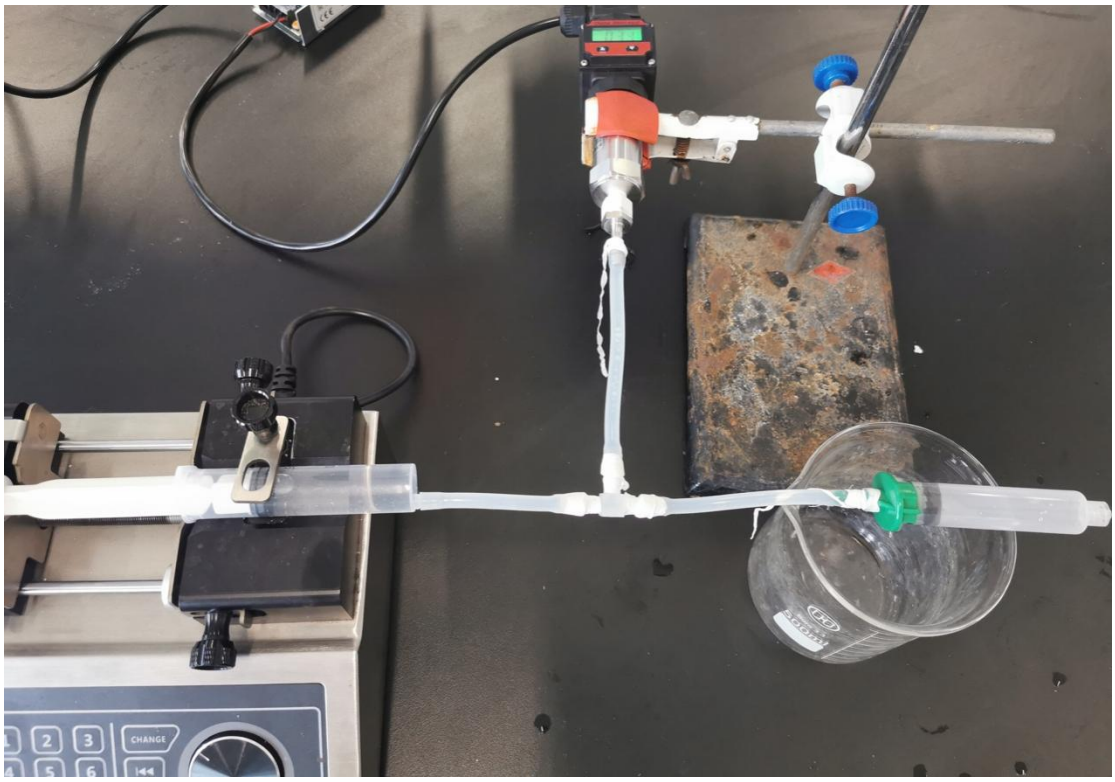

Fig. S15 Schematic diagram of fluid pressure test device

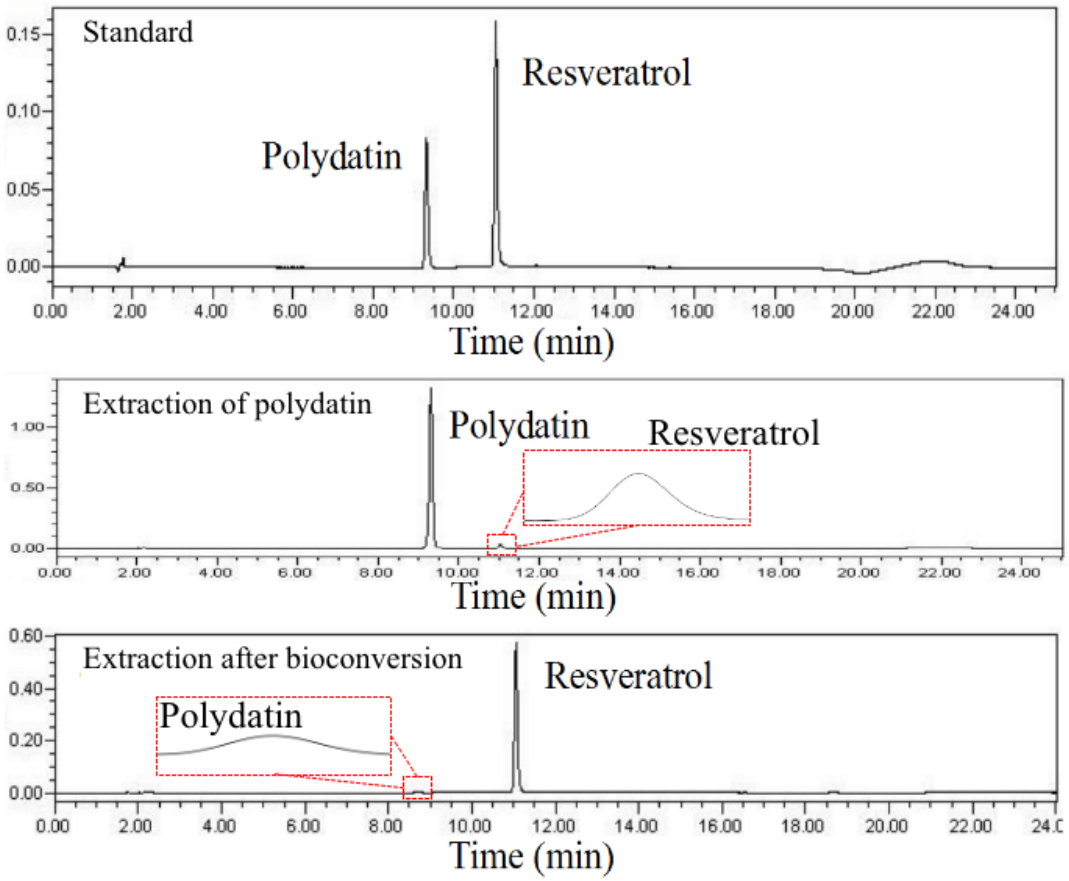

Fig. S16 The HPLC results before and after bio-transformation

Table S1. BET parameters of CE and CE-NiO

	S_{BET} (m^2/g)	Pore volume (cm^3/g)	Average pore diameters (nm)
CE	23.491	0.180	27.171
CE-NiO	11.643	0.094	26.528

Table S2 XPS data C 1s and O 1s

		CE		CE-NiO	
		Binding energy (eV)	**Component content (%)**	**Binding energy (eV)**	**Component content (%)**
C1s	**C-C**	287.93	54.72	286.51	32.82
	C-O	290.33	6.62	287.88	23.5
	O-C-O/C=O	286.30	38.66	284.8	43.68
O1s	**C-OH**	532.71	83.4	532.88	58.06
	C-O-C/C=O	531.58	16.6	531.45	41.94

References:

1. X. Tong, Z. Qi, D. Zheng, J. Pei, Q. Li and L. Zhao, Bioorg. Chem., 2021, 111, 104906.
2. B. Henrissat and G. Davies, Curr. Opin. Struct. Biol., 1997, 7, 637-644.
3. J. Zhou, M. Liang, Y. Lin, H. Pang, Y. Wei, R. Huang and L. Du, Molecules, 2022, 27.
4. M. L. Sinnott, Chem. Rev., 1990, 90, 1171-1202.