Supplementary materials

Screen of microbial strains to reduce H_2S and CH_4 emission from wastewater

Zheng Qi [a], Tipei Jia [a], Wenjie Cong [a], Jinying Xi [a]*

[a] *Environmental Simulation and Pollution Control State Key Joint Laboratory, School of Environment, Tsinghua University, Beijing 100084, China*

∗Corresponding author: Jinying Xi

E-mail address: xijinying@tsinghua.edu.cn

Table S1. Characteristics of synthetic wastewater.

Water Quality Indicator	Concentration
COD	321 ± 10mg/L
TN	46.3 ± 3.6 mg/L
TP	7.1 ± 0.3 mg/L
NH_4^+-N	22.4 ± 1.1 mg/L
S^{2-}	0.008 ± 0.002 mg/L
SO_4^{2-}-S	145.6 ± 22 mg/L
DO	0.15 ± 0.05 mg DO/L
pH	7.2 ± 0.5

Table S2. Characteristics of sludge.

Sediment Indicator	Value	Unit
Density	1.4 ± 0.1	g/cm^3
Moisture Content	93.9 ± 0.4	%
TS (Total Solids)	64.4 ± 2.8	%（wet weight）
VS (Volatile Solids)	2.6 ± 0.2	%（wet weight）

Text S1

The extracellular polymeric substances (EPSs) from sludge samples were extracted using a modified ultrasonic centrifugation method. The extraction process is as follows: 10 mL of sludge was centrifuged using a high-speed refrigerated centrifuge at 4,000 rpm for 15 minutes at 4°C. The supernatant obtained was the slime-EPS. The precipitate was resuspended in 10 mL of 0.01 mol/L PBS solution, placed in a centrifuge tube with glass beads, and sonicated (40 kHz, 100 W) for 1 minute, followed by horizontal shaking for 10 minutes, and sonicated again for 1 minute. After a second centrifugation (9,000 rpm, 15 minutes, 4°C), LB-EPS was obtained. The precipitate was resuspended in 10 mL of 0.01 mol/L PBS solution again and sonicated (40 kHz, 100 W) for 2 minutes. After a third centrifugation (10,000 rpm, 15 minutes, 4°C), TB-EPS was obtained. All supernatants were filtered through a 0.45 μm membrane and characterized using three-dimensional fluorescence spectroscopy with excitation wavelengths (Ex) ranging from 200-400 nm and emission wavelengths (Em) ranging from 300-550 nm.

The biochemical components of EPSs were measured using sensitive colorimetric methods. Protein content was determined using a modified Bradford method with bovine serum albumin (BSA) as the standard. The procedure is as follows: dissolve 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% ethanol, add 100 mL of 85% (w/v) phosphoric acid solution, and dilute to 1,000 mL with distilled water to prepare the Coomassie Brilliant Blue reagent. Add 1 mL of the sample to each test tube (with physiological saline as a blank control) along with 5 mL of Coomassie Brilliant Blue reagent, vortex to mix, and let it stand for 5 minutes.

Measure the absorbance at 595 nm using a UV spectrophotometer, and calculate the protein content of the sample using the regression equation from the standard curve.

Polysaccharide content was determined using the phenol-sulfuric acid method with glucose as the standard. Add 1 mL of the sample to each test tube, then add 0.5 mL of 5% phenol solution and 2.5 mL of concentrated sulfuric acid to each tube, mix well, and quickly cool in cold water. Allow the tubes to stand at room temperature for 20 minutes. Measure the absorbance at 490 nm, and calculate the polysaccharide content of the sample using the regression equation from the standard curve.

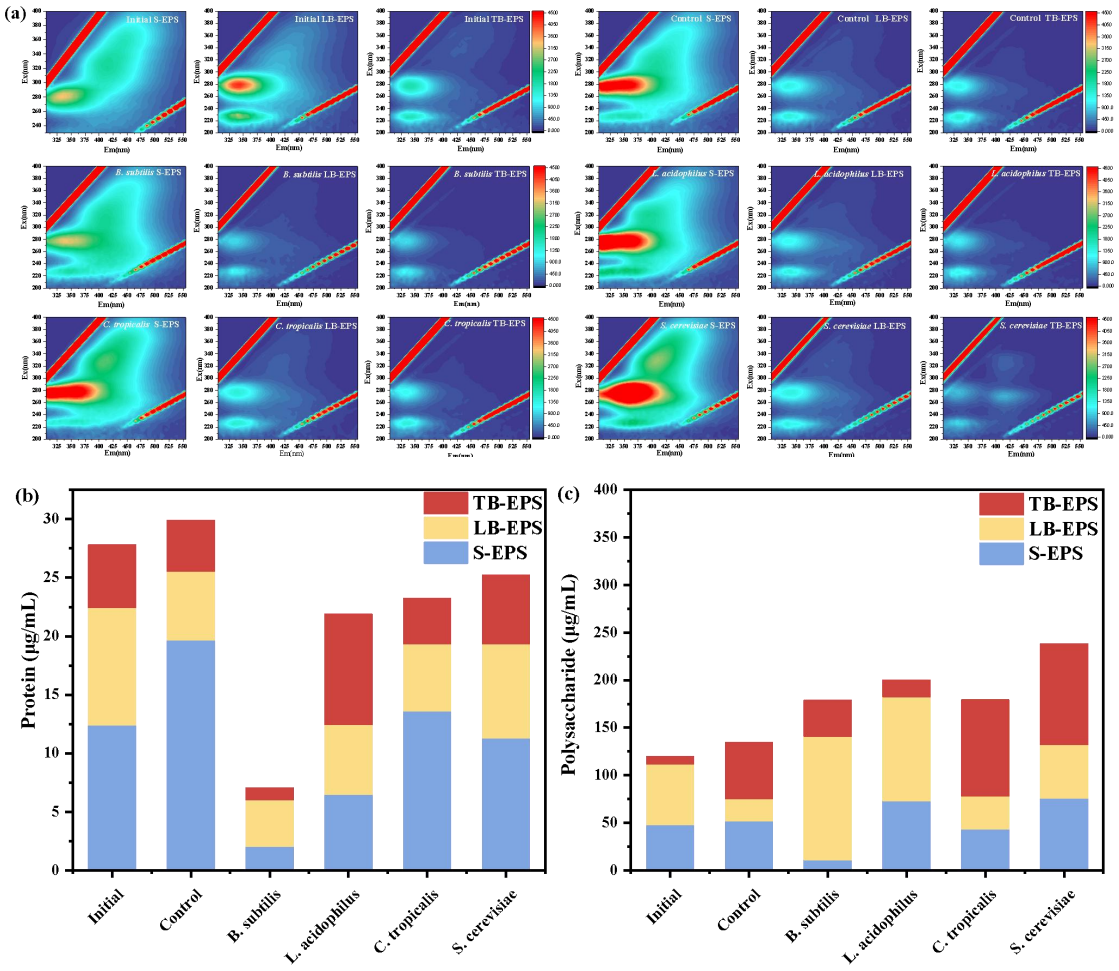

Fig. S1 3D-EEM spectra of S-EPS, LB-EPS, and TB-EPS (a) after adding yeast and bacterial strains and the extracellular protein (b) and polysaccharide (c) content.

Table. S3 Alpha diversity indices of microbial communities in wastewater after adding yeast and bacterial strains independently.

Microbiota	Samples	chao	Ace	Shannon	Simpson
Bacteria	Initial	385	409.4759	4.06	0.24
	Control	292	318.3851	3.06	0.34
	B. subtilis	132	174.3497	2.72	0.45
	L. acidophilus	281	311.7677	3.12	0.30
	C. tropicalis	264	300.6962	3.28	0.22
	S. cerevisiae	147	171.7192	2.72	0.35
Fungi	Initial	78	81.0548	2.24	0.435
	Control	87	85.4932	3.09	0.239
	B. subtilis	110	93.2114	2.83	0.303
	L. acidophilus	94	97.8117	2.87	0.282
	C. tropicalis	86	92.3330	1.95	0.549
	S. cerevisiae	80	83.9785	2.92	0.249

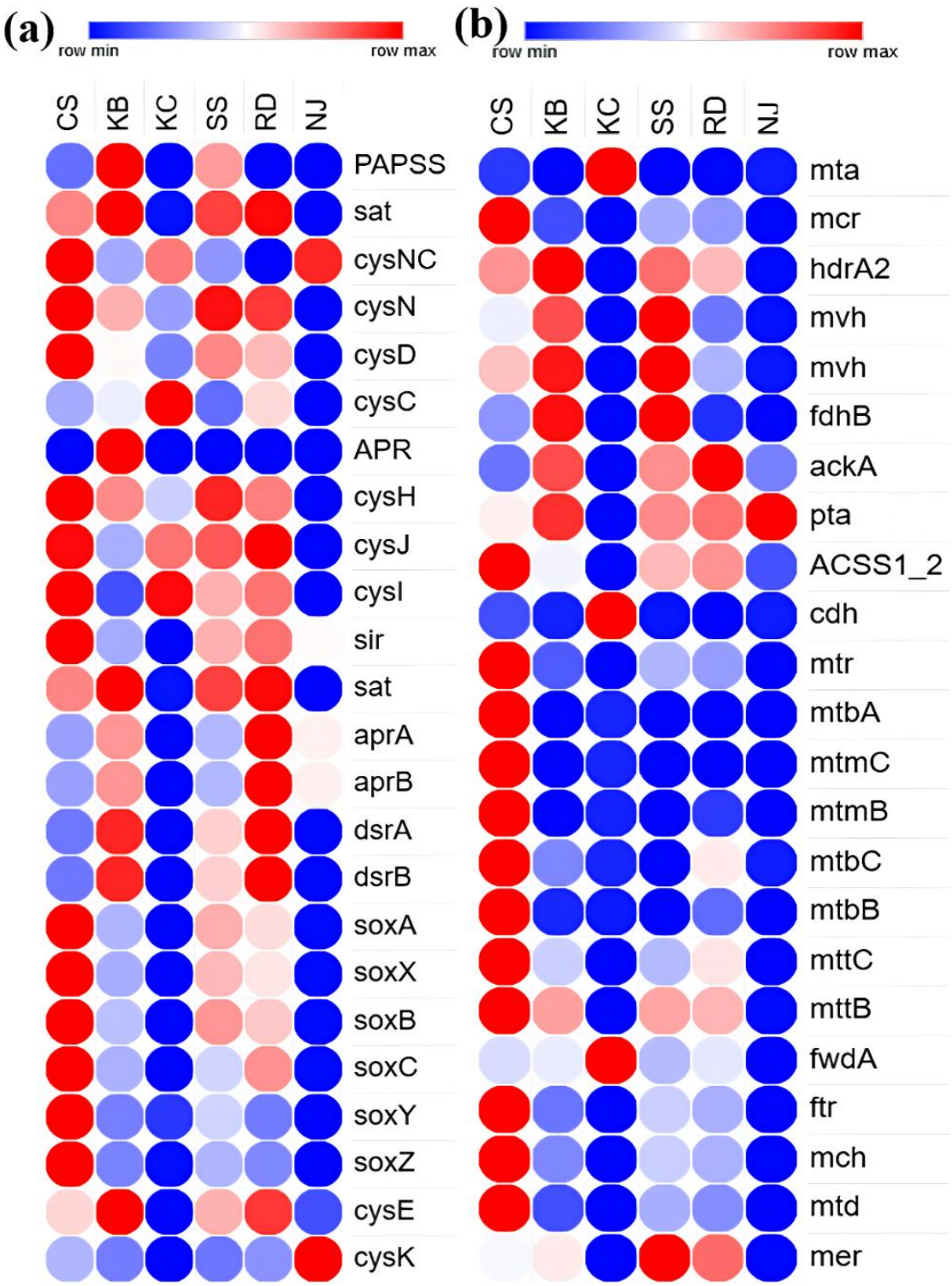

Fig. S2 Heatmap of relative abundances of functional genes involved in sulfur metabolism (a) and methanogenesis (b) predicted by PICRUSt (CS, KB, KC, SS, RD, and NJ represent the initial, control, *B. subtilis*, *L. acidophilus*, *C. tropicalis*, *S. cerevisiae* groups, respectively).