Supporting Information

Gene expression of an *Arthrobacter* in surfactant-enhanced biodegradation of a hydrophobic organic compound

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1. Determination of the biomass of SA02 cells

Strain SA02 cells cultivated in the LB medium for 24 hours (300 mL) were centrifuged, washed twice with MSM medium and centrifuged again before the strain SA02 cell pellets were freeze-dried. Then, the mass of the freeze-dried SA02 cells was determined using the electronic scale with a weighing accuracy of 0.001 milligram (METTLER TOLEDO XP6). The biomass of the freeze-dried SA02 cells was 38.4 μg, 21.7 μg, 13.5 μg, 5.3 μg and 3 μg. The DNA from those SA02 cells were extracted by the microbial DNA isolation Kit (Dongsheng Biotech, China). The extracted DNA was diluted to 100 μL. The DNA contents were determined using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Delaware, USA). The absorbance ratio of 260 nm and 280 nm was restricted to the range of 1.7-1.9. The linear relationship between the biomass of strain SA02 cells (μg) and its DNA contents (ng) was established.

In the main experiments, 10 mL sample was centrifuged (12,000×*g*, 15min) every two days to obtain the SA02 cell pellets. The SA02 cell pellets were used to extract the DNA by the the microbial DNA isolation Kit as the method described by the manufacture. The DNA contents were determined using Nanodrop ND-1000 spectrophotometer. The SA02 biomass was calculated by the linear correlation established above.

2. Abiotic loss of phenanthrene

The results from the abiotic control experiments show that the loss of phenanthrene through volatilization and other abiotic processes was approximately 1.2% in the 12 days.

3. Phenanthrene extraction

The equilibrium concentration of phenanthrene in each supernatant was analyzed using our previous method,[4] where the method quantification limit value for phenanthrene was 3.21 μg L^{-1}, and the relative standard deviation (RSD) for the determination of phenanthrene was 0.36% (n=6). In brief, supernatants (0.5 mL) were diluted with methanol (0.5 mL) before filtration through Teflon filter units (Φ0.22 μm, Pallflex Products Corp., USA), and analyzed by high-performance liquid chromatography (HPLC, Agilent 1200 series) equipped a 4.6×150 mm reverse phase XDB-C18 column and a fluorescence detector using methanol and water (*V*:*V*, 90:10) as mobile phase at a flow rate of 1 mL min^{-1}. The excitation and emission wavelengths for phenanthrene were 244 and 360 nm, respectively. Methanol (0.5 mL) was added to avoid phenanthrene adsorption onto the Teflon filter units and the effect of surfactant sensitization. Then, the cell pellet was washed with 1.0 mL MSM for two times by the resuspension and then centrifugation, cleaning out the adsorbed surfactant on the cell surface of strain SA02. Next, the phenanthrene that was adsorbed in the surface and entered into the cells was ultrasonically extracted with 1.0 mL of methanol for 15 min at a stable temperature (less than 10 °C) by the cooling water circulation prior to analyze by HPLC. The recovery of methanol extraction covered the range of 97.6-99.8%.

4. Measurement of SDBS concentration

In every other day, 10 mL broth was centrifuged to collect the supernatant. The SDBS concentration in the supernatant was analyzed by HPLC (Agilent 1200 series) with a 4.6×150 mm reverse phase XDB-C18 column and a UV detector (224 nm) using methanol and water mixture (*V*:*V*, 70:30) as mobile phase at a flow rate of 1 mL/min.

5. Transmission electron microscopy analysis of SA02 cells

The effects of surfactants on the ultrastructural features of the cell surfaces were analyzed using transmission electron microscopy (TEM)[1,2]. Briefly, 100 mL broth was taken, centrifuged and resuspended in the mineral salt medium (MSM) twice. To fix strain SA02 cells, the samples were centrifuged and resuspended in 1 mL of 2.5% glutaraldehyde solution overnight at 4 °C in phosphate buffer (0.1 mol L^{-1}, pH 7.0). The fixed pellets were centrifuged, decanted and rinsed twice before embedding in 2% agar. Cells in agar were postfixed in phosphate buffer (pH 7.0) with 1% OsO_4 for 1 h, rinsed and serially ascending dehydrated (50%, 70%, 80%, 90%, 95% and 100% ethanol). Cells in agar were then transferred to acetone, infiltrated and finally embedded in Spurr resin (Ted Pella, Redding, California, USA) and left to polymerize in an oven at 60 ºC for 24 hours. The polymerized samples were sectioned into slices of 60 nm thick using a microtome. The slices were stained by uranyl acetate and alkaline lead citrate prior to examination in the TEM (Model JEM-1230, JEOL, Tokyo, Japan).

6. Exaction of total RNA of SA02 cells

SA02 cells (on the 2nd, 4th, 6th, 8th and 10th days) were harvested from the broth by centrifugation at 12,000×g at 4 °C for 3 min, after the addition of 1.0 mL frozen killing buffer (20 mmol L^{-1} Tris/HCl[pH7.5], 5 mmol/L $MgCl_2$, 20 mmol L^{-1} NaN_3) to the culture sample. After discarding the supernatant, 100 μL of the lysing solution was added to the cell pellets, along with 10 μL β-mercaptoethanol and 700 μL RD solution. The supernatant was added to a new tube after centrifugation at 12,000×g at 4 °C for 1 min, and 700 μL RB solution was added. The solution was subsequently transferred to the RNA purification column before centrifugation at 12,000×g at 4 °C for 1 min. RP solution (700 μL) was added to the above RNA purification column before centrifugation at 12,000×g at 4 °C for 1 min. RW solution (500 μL) was added to the above RNA

purification column, incubated for 2 min before centrifugation at 12,000×g at 4 °C for 1 min, and this process was repeated. After centrifugation at 12,000×g at 4 °C for 1 min, the above RNA purification column was transferred into a new RNase-free tube before reconstitution in 20 μL RNase-free water. [3]

7. Reverse transcription PCR (RT-PCR) setting

The random hexamers and RNA templates were mixed and denatured at 70 °C for 5 min followed by cooling for 2 minutes on ice. The 5×first-strand buffer (4 μL), dNTPs (1 μL, 10 mmol L^{-1} each), M-MLV (1 μL) and RNase inhibitor (0.6 μL) were added to the cooled template mix and incubated for 1 hour at 37 °C before enzyme inactivation at 70 °C for 5 minutes.

8. Quantitative PCR setting

The qPCR settings of fatty acid desaturase gene, RHDase gene and 1H2Nase gene were: 2 minutes at 95 °C, followed by 40 cycles of 15 seconds at 95 °C, 30 seconds at 58 °C, 35 seconds at 72 °C and 2 minutes at 95 °C, followed by 40 cycles of 15 seconds at 95 °C, 30 seconds at 59 °C, 35 seconds at 72 °C, respectively.

9. Cultivation in the PUM medium

The liquid propionate uniquely modified (PUM) medium containing a single propionate as sole carbon and energy, was used to compare the changes of the CSH values for SA02 cells under different medium. As shown in Figure S6, the CSH values of SA02 cells grown in the PUM medium without addition of SDBS and phenanthrene were less than 15.5%, compared to 20.4-21.5% of the CSH values of SA02 cells grown in the LB medium. When strain SA02 cells grown in the propionate-free PUM medium with

addition of SDBS and phenanthrene, the CSH values were significantly higher than that of SA02 grown in MSM medium with SDBS and phenanthrene, and the CSH value even reached 95.5% at 30 mg L^{-1} of SDBS applied. These results indicated that PUM medium with a single carbon was more favorable to the CSH changes of SA02 cells, compared to the LB medium with a complex carbon, and also confirmed that SDBS in test concentrations could change the CSH of SA02 cells. Nevertheless, the changed trends of the CSH were similar under the LB or PUM medium cultured for strain SA02.

References

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Supporting Tables

Table S1. Oligonucleotide primers used in RT-qPCR.

Name	Sequence (5' to 3')
16S rRNA mF	GGTTGCGATAC TGTGAGGTGGA
16S rRNA mR	GCGTTGCTGATCTGCGATTACT
1H2Nase mF	AGCAGCGAAGGCATCGGAAC
1H2Nase mR	GCGTCGAGGTCGTAGAGATTGA
Desaturase mF	GCCATCTACGACCTTGAG
Desaturase mF	GTCCTTGGTGAACTGTGT
RHDase mF	CCGACAGCGTTCCTCAATGA
RHDase mR	GGCTTGGCGATCTCACTCTC

The suffixes F and R denote the forward and reverse primers, respectively.

The primer pair of 16S rRNA mF and 16S rRNA mR and the primer pair of 1H2Nase mF and 1H2Nase mR were designed by Primer 6.0, which were based on the partial 16S rRNA sequence (Genbank accession number: JQ796366.1), Δ9 fatty acid desaturase (Genbank accession number: CP002379.1 (3587630..3588817)) and the 1H2Nase gene sequence (Genbank accession number: KF527570) and RHDase gene sequence (Genbank accession number: KM279943), respectively.

Table S2 The changes of fatty acid content (%) of *Arthrobacter* sp. SA02 cells under different concentrations of SDBS.

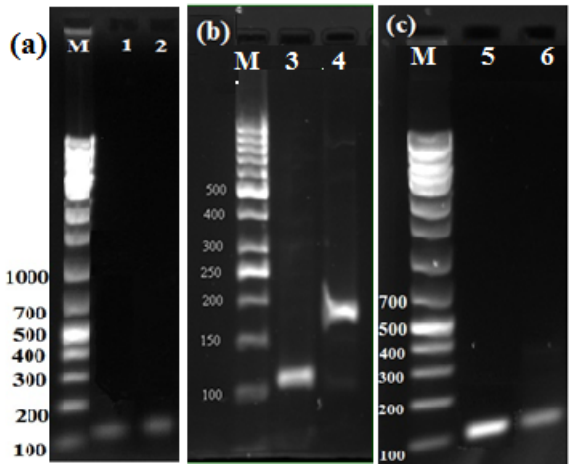
Fatty acids	10 mg L^{-1}			30 mg L^{-1}			50 mg L^{-1}			80 mg L^{-1}			0 mg L^{-1}		
	4h	6d	12d	4h	6d	12d	4h	6d	12d	4h	6d	12d	4h	6d	12d
Saturated															
C14:0	4.13±0.14	5.26±0.18	3.98±0.26	5.05±0.27	4.54±0.35	3.44±0.26	4.36±0.33	4.44±0.35	4.03±0.28	4.32±0.29	4.49±0.14	4.26±0.23	2.10±0.25	4.22±0.32	3.41±0.18
C14:0iso	4.81±0.25	3.67±0.37	4.56±0.39	3.42±0.48	3.18±0.17	4.02±0.15	3.98±0.20	4.19±0.24	3.62±0.19	4.45±0.29	5.30±0.37	4.22±0.23	3.21±0.24	5.66±0.33	2.82±0.29
C15:0	5.36±0.16	6.28±0.21	5.69±0.17	5.63±0.23	3.99±0.29	5.25±1.02	7.95±1.14	6.36±0.94	4.33±0.21	5.05±0.35	4.83±0.37	5.48±0.37	1.42±0.05	4.49±0.10	4.93±0.12
C15:0iso	31.2±0.19	31.3±0.53	34.1±0.44	33.2±0.29	33.3±0.36	33.6±0.43	33.7±0.25	34.5±0.43	34.3±0.57	32.8±0.26	31.5±0.28	33.3±0.39	39.4±1.18	33.9±0.75	34.8±0.81
C16:0	6.85±0.28	5.82±0.89	5.82±0.38	4.19±0.17	3.35±0.26	3.59±0.28	4.13±0.21	5.99±0.33	4.44±0.28	5.19±0.41	5.58±0.24	6.11±0.37	4.22±0.23	1.36±0.09	3.82±0.12
C16:0anteiso	25.8±0.24	27.5±0.75	33.7±0.28	25.6±0.37	31.6±0.14	28.1±0.19	24.3±0.22	24.5±0.32	26.8±0.15	25.8±0.29	25.4±0.36	25.9±0.28	31.9±0.25	32.1±0.33	30.5±0.20
C18:0	6.47±0.37	4.53±0.26	5.41±0.52	7.04±0.43	6.02±0.29	4.72±0.14	5.54±0.27	3.31±0.19	4.83±0.25	5.87±0.37	6.16±0.29	3.03±0.27	3.51±0.17	2.43±0.35	2.81±0.15
Unsaturated															
C16:1ω9	2.17±0.17	2.26±0.14	2.38±0.16	2.42±0.20	2.25±0.11	2.53±0.09	2.46±0.19	2.33±0.21	2.52±0.22	2.64±0.14	2.49±0.18	2.88±0.21	3.02±0.22	3.17±0.19	3.09±0.17
C18:1ω9	5.85±1.03	6.60±0.44	6.29±0.47	6.08±0.29	6.84±0.37	6.29±0.29	6.50±1.12	7.09±0.87	7.24±0.75	6.73±0.55	6.14±0.47	6.95±0.36	4.40±0.41	6.08±0.37	6.05±0.28
C18:2ω9,12	7.36±0.35	6.79±0.28	7.86±1.07	7.36±0.84	7.19±0.37	8.45±1.13	7.08±0.35	7.29±0.28	7.89±0.21	7.15±0.57	8.11±0.37	7.87±0.17	6.82±0.58	6.58±0.72	7.78±0.93
Sat./unsat. ratio	5.50±0.19	5.39±0.21	5.05±0.11	5.31±0.17	5.14±0.22	4.79±0.18	5.23±0.20	4.98±0.31	4.67±0.27	5.05±0.29	4.97±0.32	4.65±0.19	6.02±0.19	5.32±0.21	4.91±0.35

The following fatty acid nomenclature was applied: the number preceding the colon indicates the total number of carbon atoms, the number following the colon indicates the number of double bonds, and the suffix designates the position of the double bonds (from the methyl end of the molecule) or the position of hydroxyl groups (from the carboxyl end of the molecule), namely, the saturated fatty acids (Cn:0), the unsaturated fatty acids (Cn:0 m ω l). The standard deviation (SD) was calculated from analysis of triplicate samples. The values are given as means±SE.

Table S4. The expression levels of RHD gene, 1H2Nase gene andΔ9 fatty acid desaturase gene of strain SA02 grown in the LB medium containing SDBS (10, 30 and 50 mg L^{-1}).

medium	RHDase gene expression level			1H2Nase gene expression level			Δ9 fatty acid desaturase gene expression level		
	12 h	24 h	36 h	12 h	24 h	36 h	12 h	24 h	36 h
LB	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
LB+SDBS (10 mg L^{-1})	1.12±0.32	1.21±0.29	0.99±0.45	0.87±0.13	0.76±0.14	0.83±0.27	4.02±0.15	6.94±0.21	5.06±0.35
LB+SDBS (30 mg L^{-1})	0.97±0.26	1.08±0.12	1.13±0.41	0.91±0.27	0.65±0.31	1.07±0.35	6.92±0.53	7.14±0.39	6.99±0.25
LB+SDBS (50 mg L^{-1})	1.03±0.15	0.99±0.25	1.07±0.39	0.97±0.36	0.75±0.14	0.69±0.16	7.52±0.47	7.94±054	8.04±0.37

Supporting Figures



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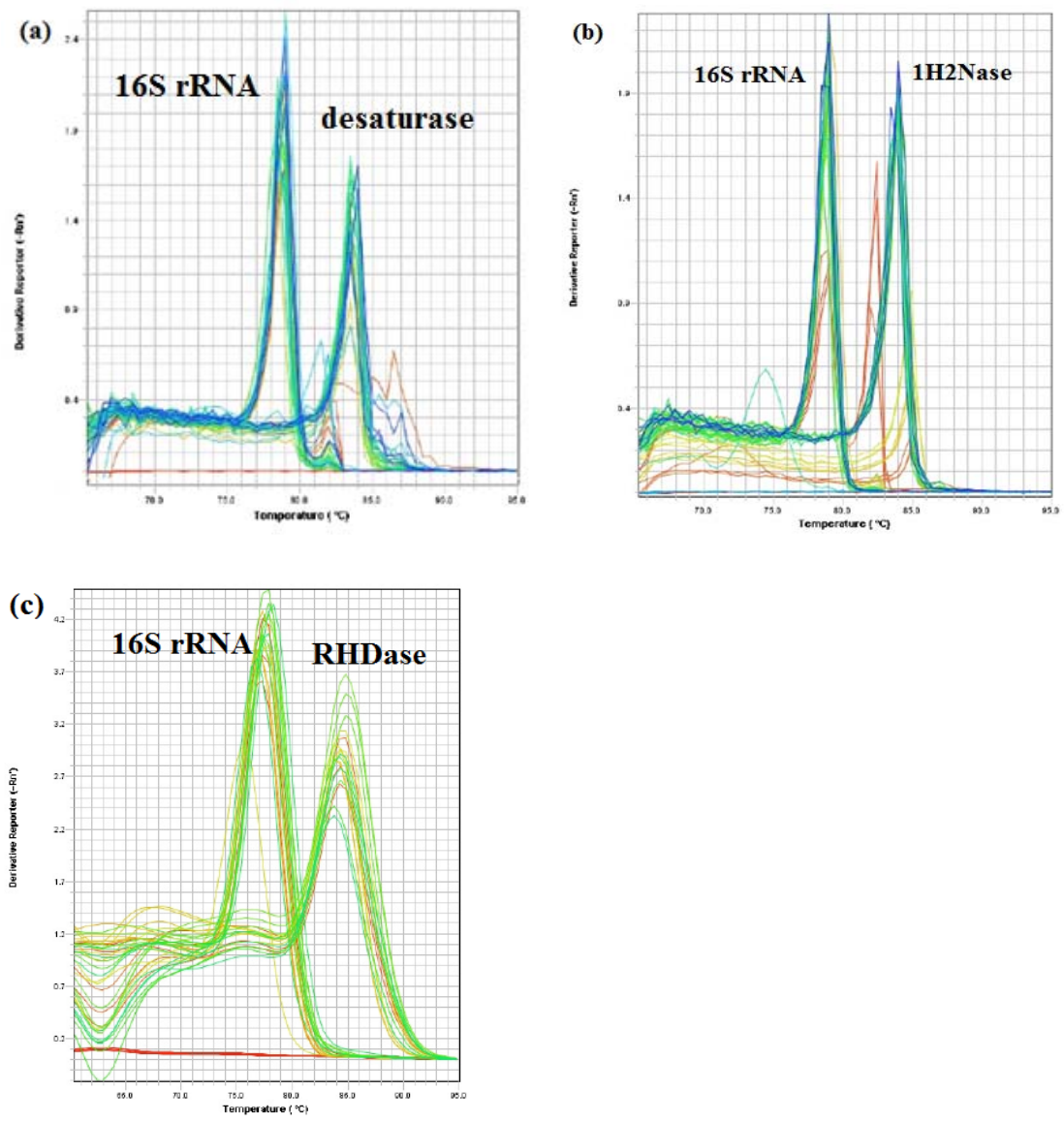

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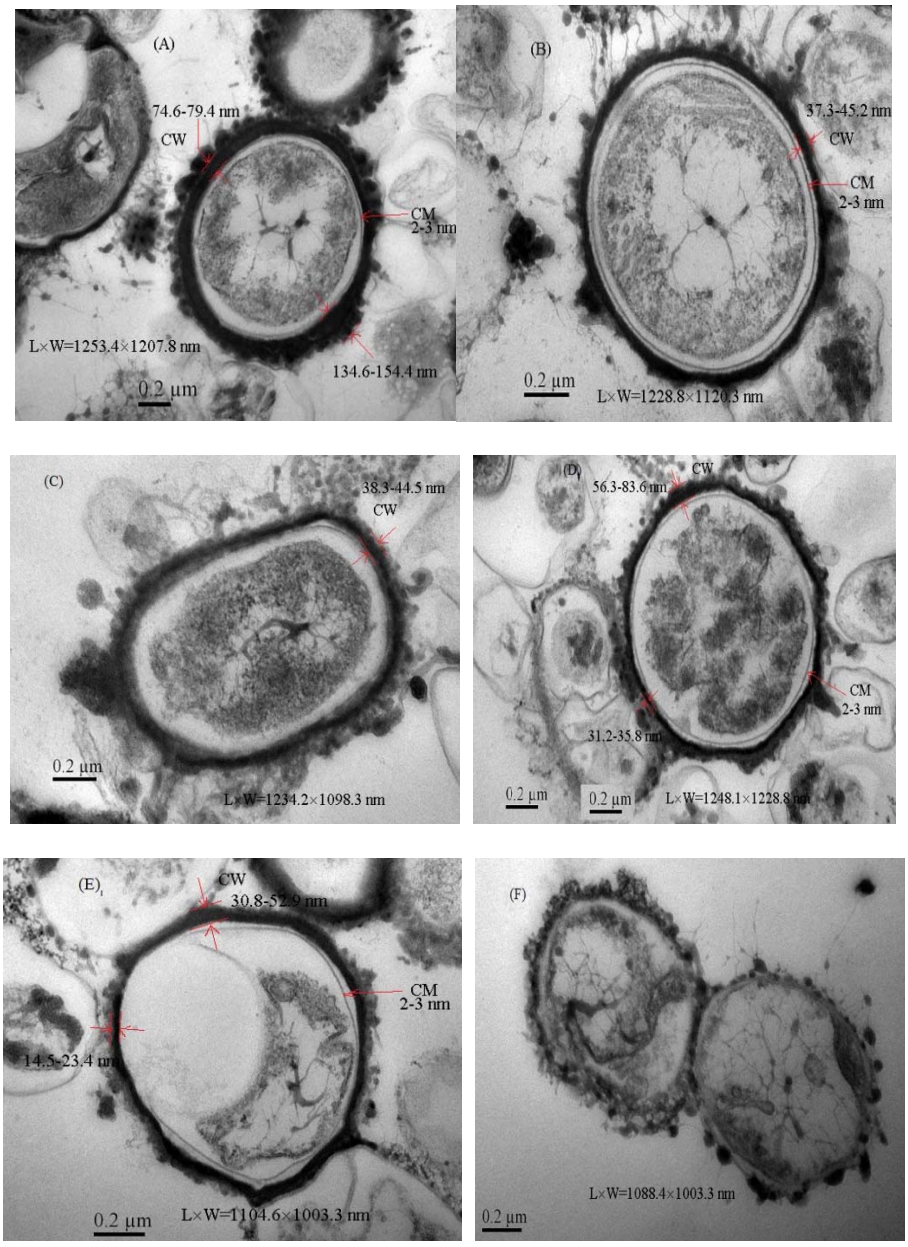

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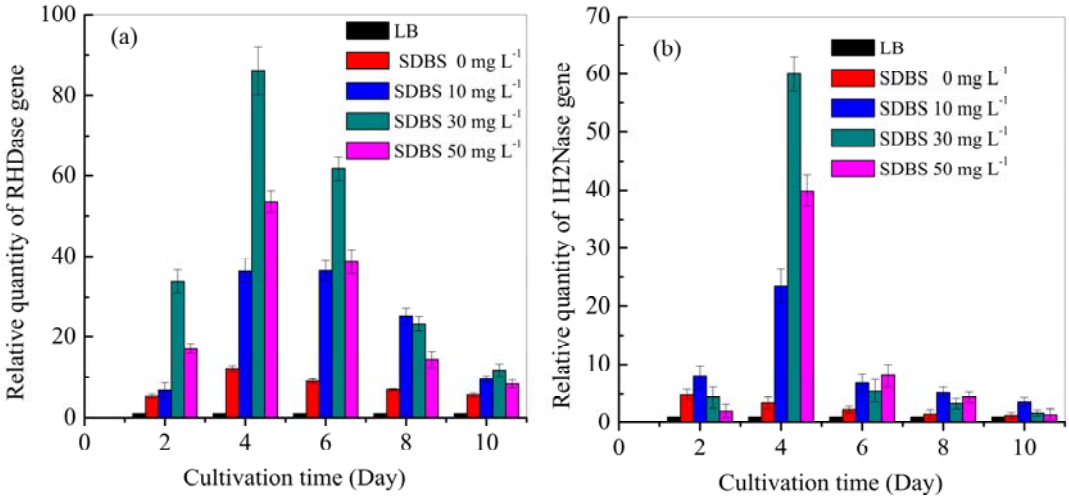

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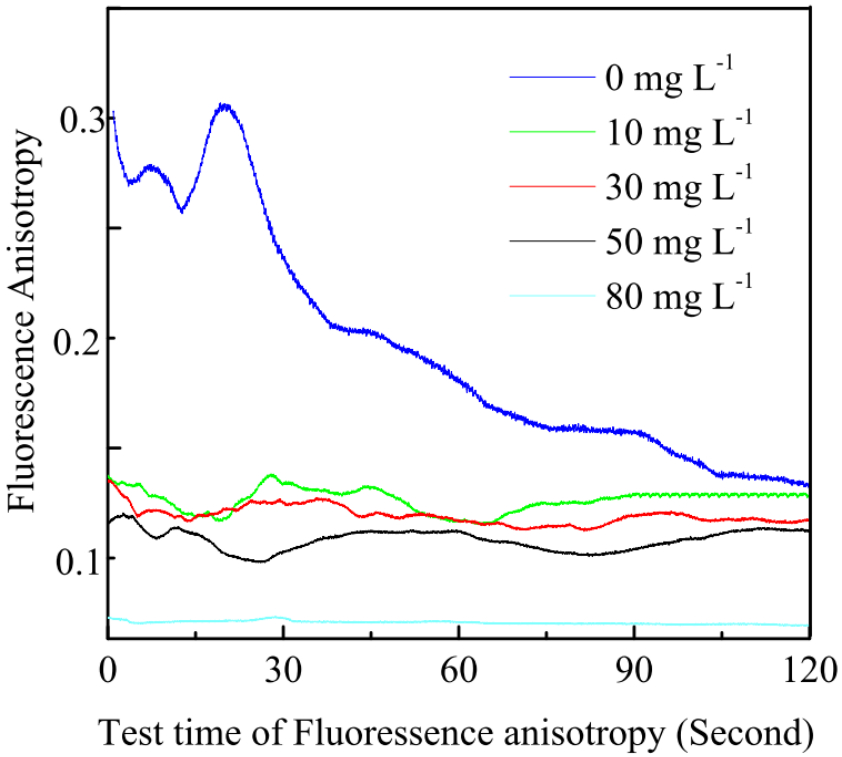

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314 **Figure S5.** The changes of DPH fluorescence anisotropy in the membranes of SA02 cells

315 under SDBS.

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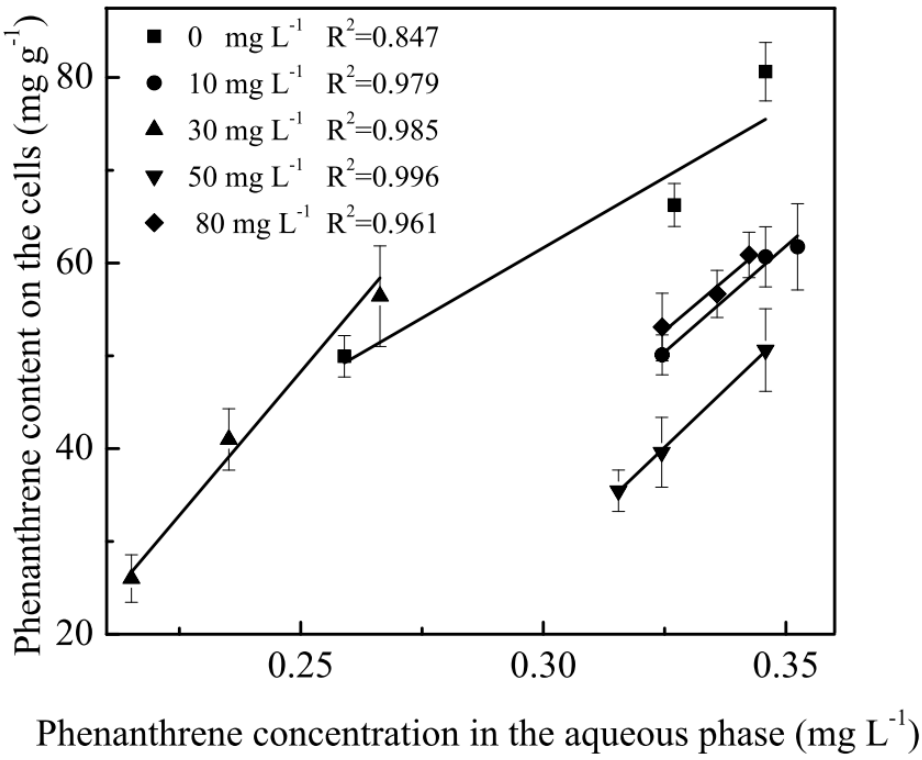

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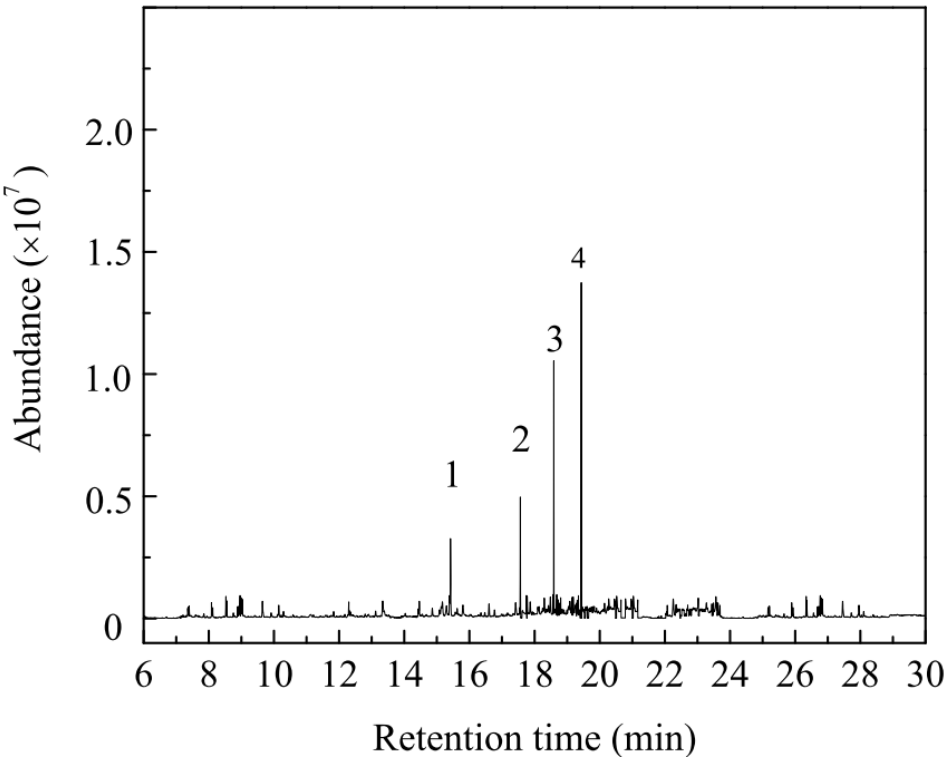

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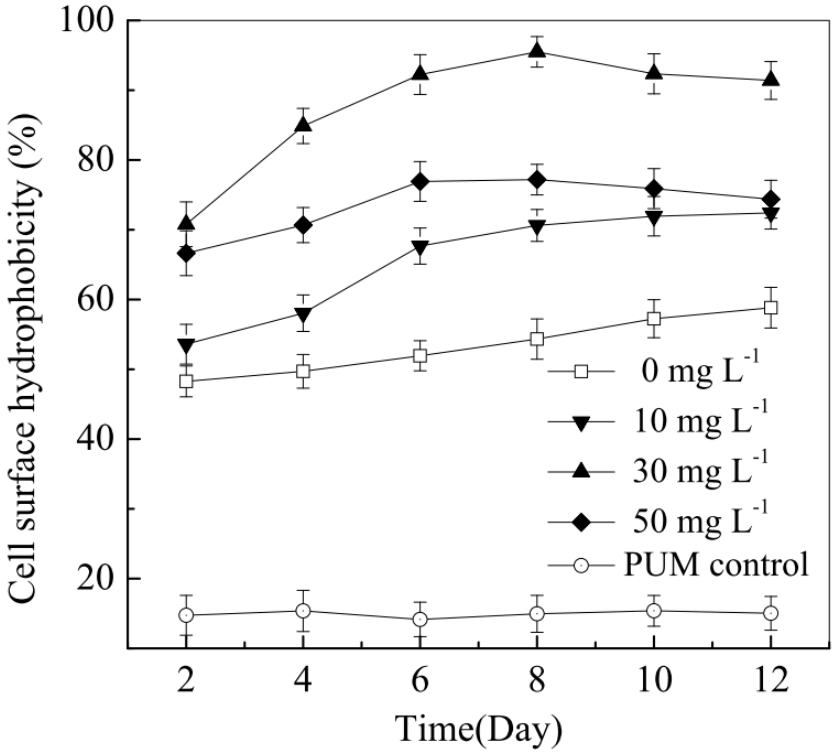

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347 **Figure S8.** Effect of SDBS on the cell surface hydrophobicity (mean ± sd) of strain SA02

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349 L^{-1}) plus SDBS (0, 10, 30 and 50 mg L^{-1}, respectively) as the treatments.

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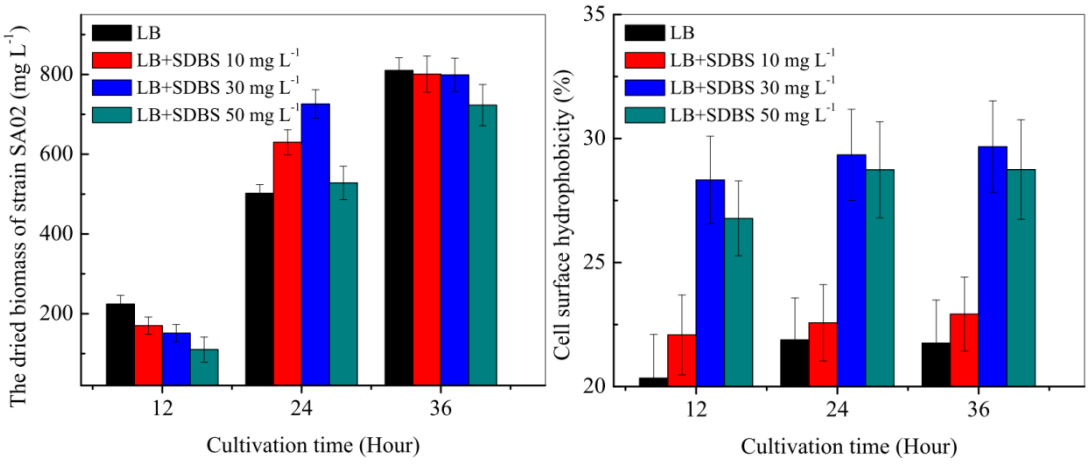

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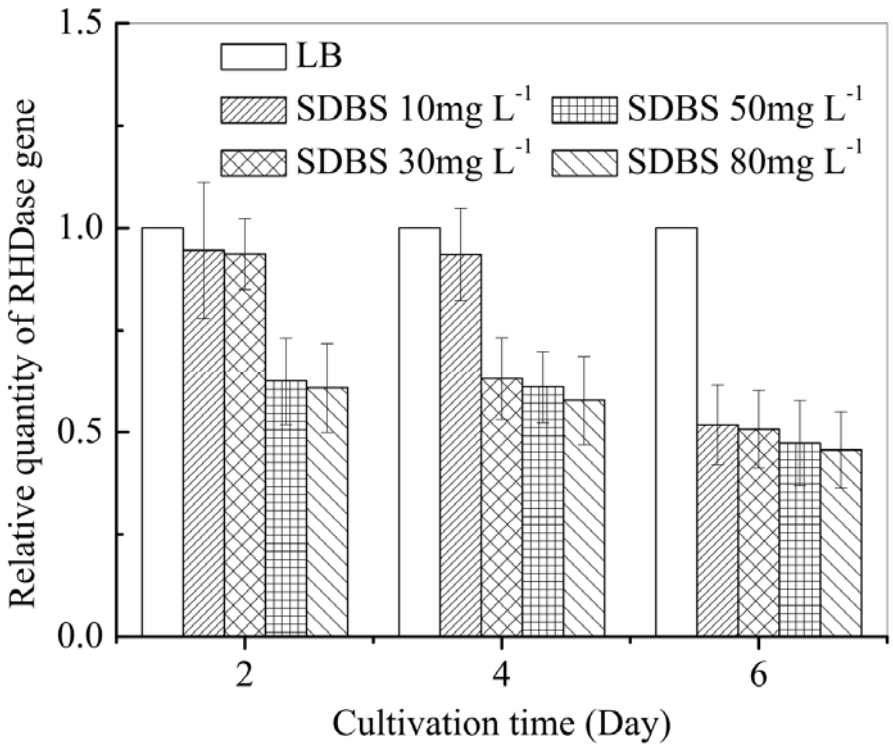

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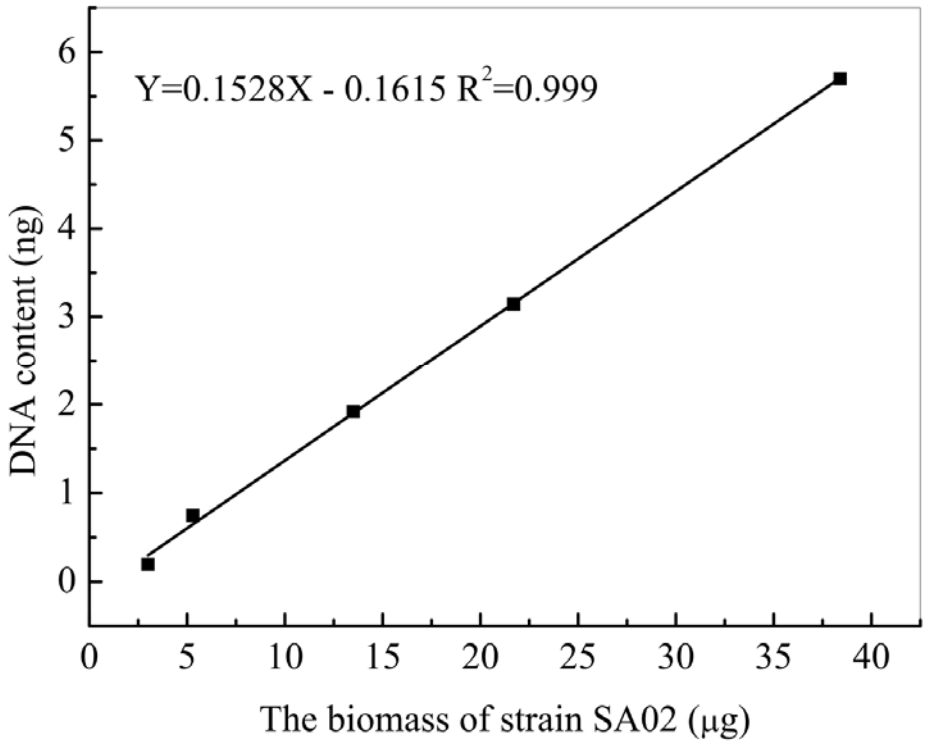

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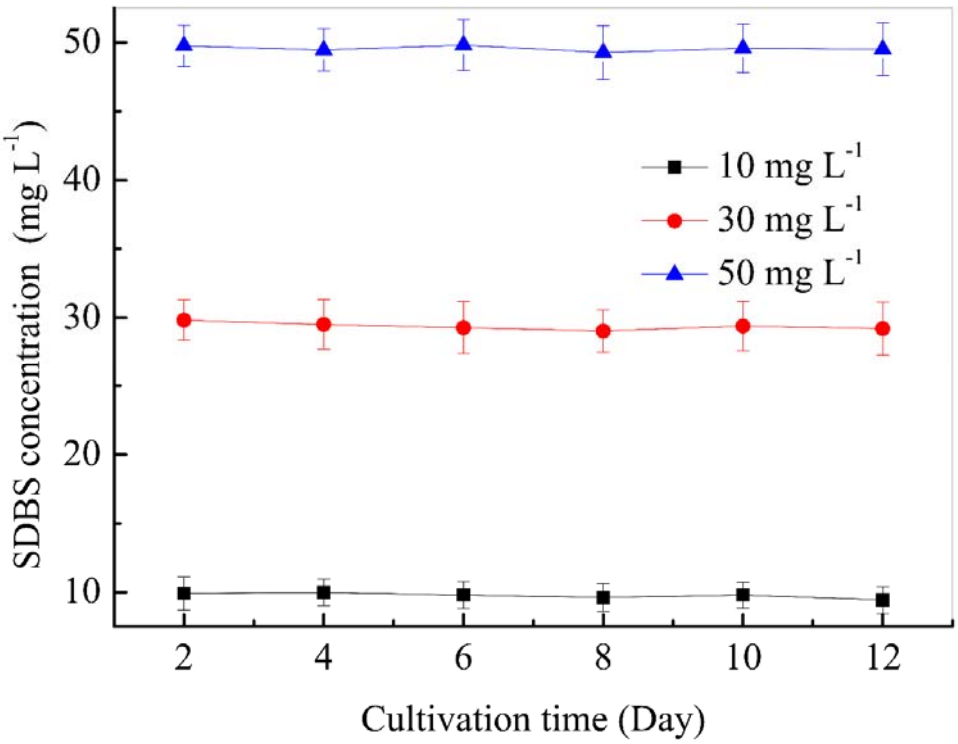

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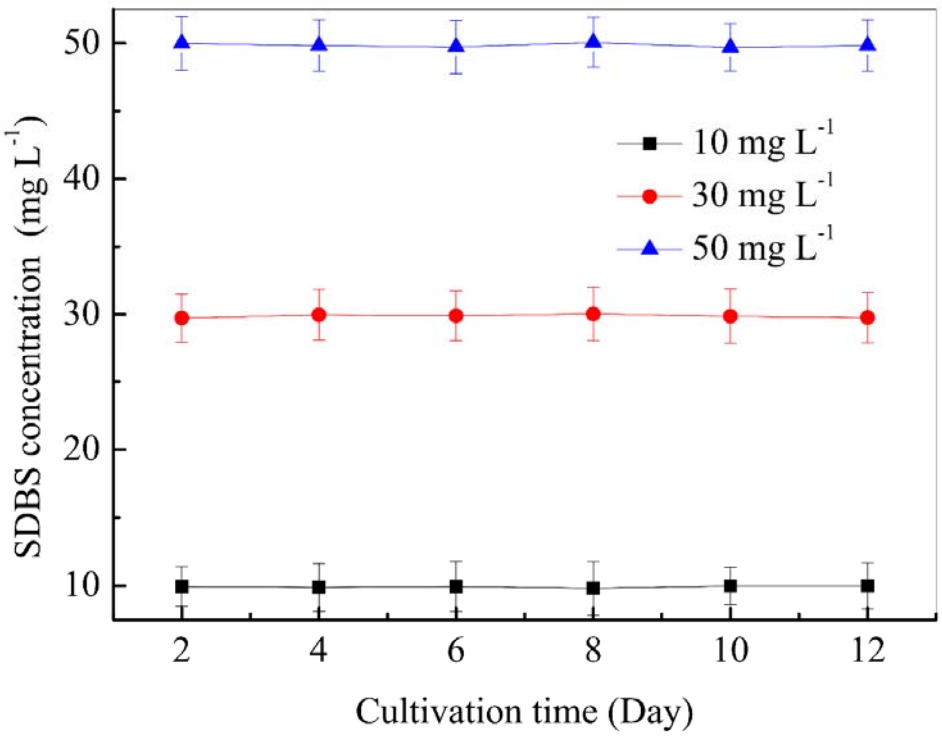

Figure S13. The concentration changes of SDBS when strain SA02 grew in the MSM medium with SDBS (10, 30 and 50 mg L^{-1}) and in the presence of phenanthrene (1.0 mg L^{-1}).

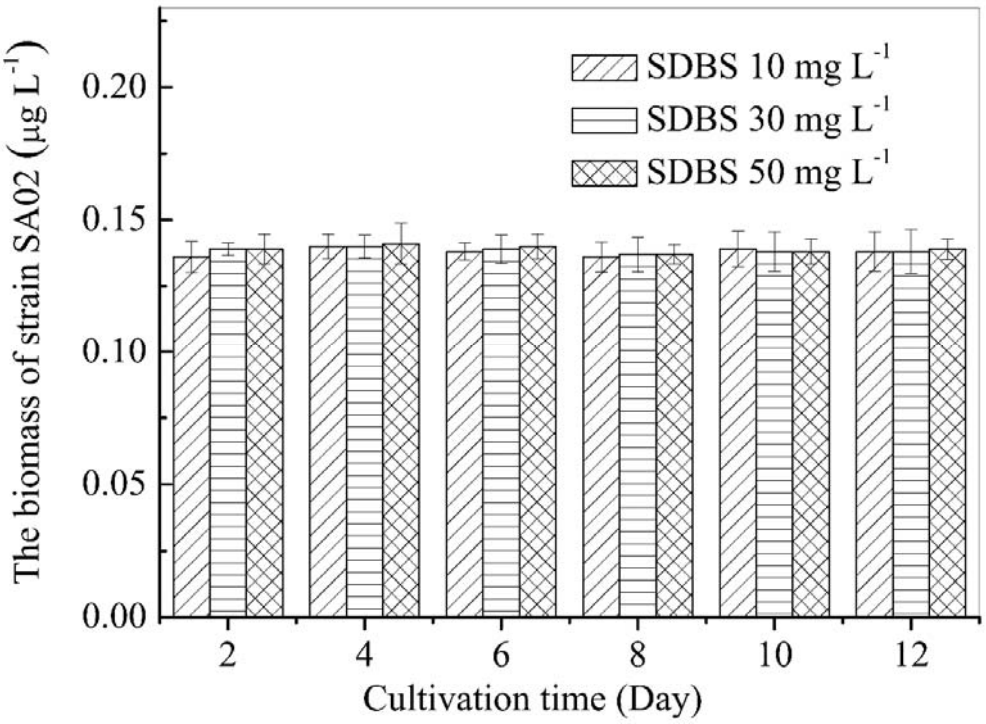

Figure S14. The biomass changes of strain SA02 grown in the MSM medium containing SDBS (10, 30 and 50 mg L^{-1}) as the sole carbon source in the absence of phenanthrene.