

Carbon Dioxide Micro-nano Bubbles Aeration Improves Carbon Fixation Efficiency for Succinic Acid Synthesis by *Escherichia coli*

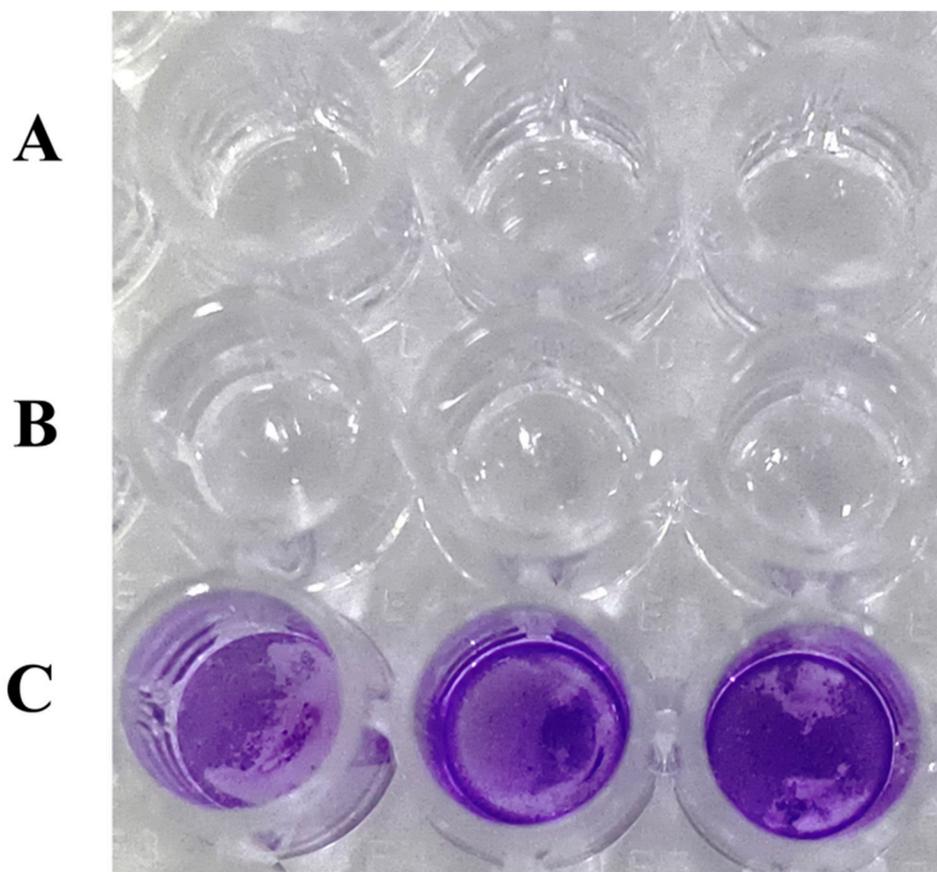


Figure S1. Crystalline violet staining of *E. coli* after formation of biofilm. (A) Negative control. (B) *E. coli* Suc260. (C) *E. coli* Suc260-CsgA.

Biofilm Formation

Static biofilms were grown in 96-well polystyrene microtiter plates (Sangon Biotech Co., Ltd., Shanghai, China). The overnight-culture *E. coli* suspension was diluted in fresh culture medium (1:100) and aliquoted into wells with 0.5% arabinose. The well plates were sealed and placed in an incubator at 30 °C for 72 h. At the end of the incubation, the planktonic bacteria were removed and the plates were washed with PBS solution three times and dried in an oven at 60 °C; then, 0.1% crystal violet solution was added to each well, and the plates were allowed to stand at room temperature for 30 min. The plates were washed with PBS solution three times to remove the excess crystal violet before being dried and photographed.



1: Switch valve. 2: Gas interface. 3: Gas guide metal pipe. 4: Metal membrane tube.

Figure S2. MNB generator device.

Table S1. Effect of inlet velocity on the number and mean diameter of MNBs under MNB aeration.

Inlet Velocity (mL/min)	Number of MNBs ($N \times 10^4 / \text{cm}^2$)	Mean Diameter of MNBs (μm)
313	315 ± 1	1.831 ± 0.09
375	455 ± 1	2.212 ± 0.15
500	319 ± 1	2.481 ± 0.20
625	180 ± 1	2.520 ± 0.16

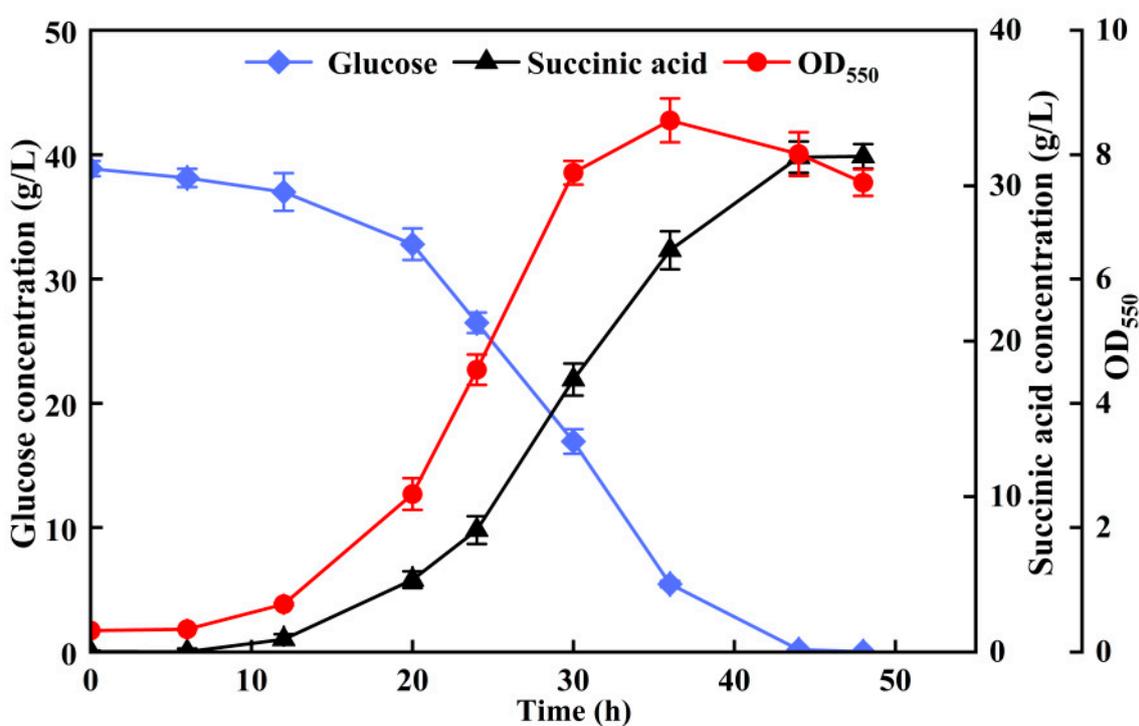


Figure S3. Succinic acid production during batch fermentation by biofilm-enhanced strain *E. coli* Suc260-CsgA in the AFB mode. (pH was kept at 6.8 by adding $2 \text{ mol}\cdot\text{L}^{-1}$ KOH and $2 \text{ mol}\cdot\text{L}^{-1}$ K_2CO_3 solution; CO_2 and K_2CO_3 were used as CO_2 donors in this process.).

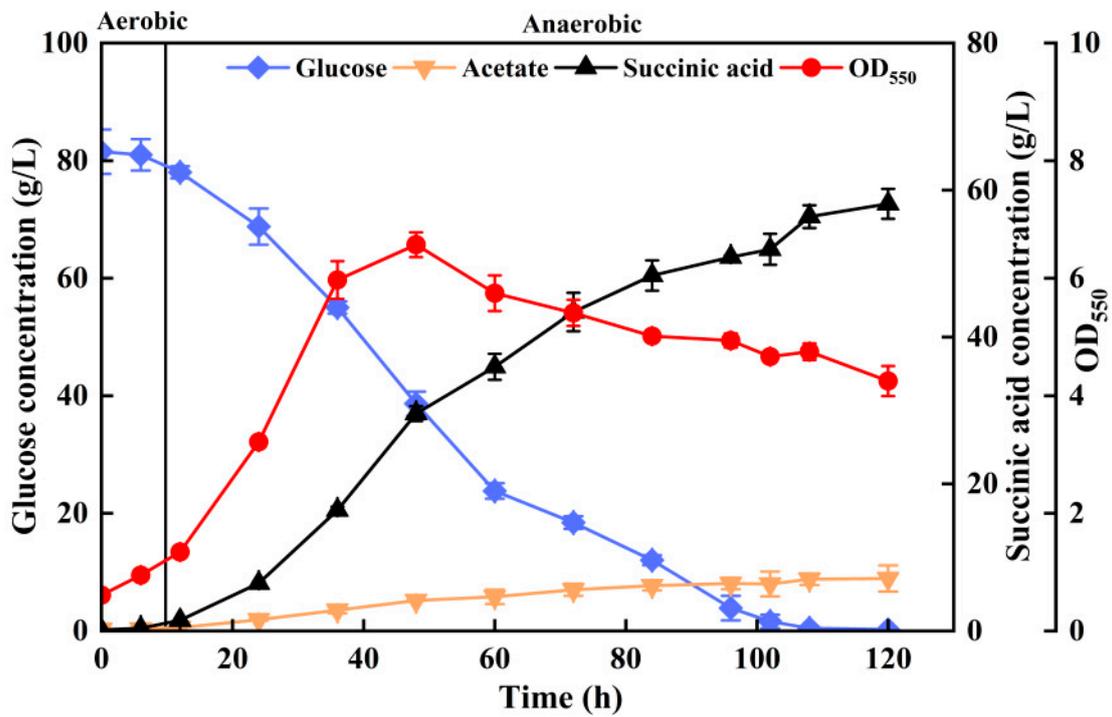


Figure S4. Succinic acid production during batch fermentation by biofilm-enhanced strain *E. coli* Suc260-CsgA in the PFM mode. (The initial glucose concentration was 80 g·L⁻¹, and 4 mol·L⁻¹ KOH was added to achieve an appropriate pH adjustment.).