Supplementary information

High-efficiency CO_2 fixation and single-cell protein production in *Cupriavidus necator* via ARTP-driven metabolic rewiring

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Table S1 Primers for RT-qPCR.

Gene	Primer	Sequence（5' → 3'）	Gene	Primer	Sequence（5' → 3'）
glk	IF-glk	catcctcctgcgctgacttc	***cisY***	IF-cisY	ccatggcgtacaagtacaac
	IR-glk	catcctcctgcgctgacttc		IR-cisY	ctgcgtgcaggatgaagatg
gap	IF-gap	gccaagaaggtgatcatgtc	***odhA***	IF-odhA	cggtttcgaattcatgtacg
	IR-gap	cttgatgccccacttgtcgt		IR-odhA	cgacgtacttggtgtgcagg
fba	IF-fba	ctacaagttcacgcgcaagc	***sdhA***	IF-sdhA	tctatgcagcttccaccaac
	IR-fba	atcgcttcctggatttcctc		IR-sdhA	ggtgaaactgccagaactcc
eno	IF-eno	agaagatcctggccgacaag	***mdh***	IF-mdh	cctacatcgccatgaagtcg
	IR-eno	cttcgccttcgtggtagaac		IR-mdh	gttgatcaggtccttgacgc
pdhB	IF-pdhB	gaagtcaaggtcaaggtcgg	***soxY***	IF-soxY	aagagcgttgctgacgtgat
	IR-pdhB	cacgtcttcctgggtgatac		IR-soxY	gggttcttttccaccaggat
pdhA	IF-pdhL	ctacaagaaccaggtggtcg	***cysN***	IF-cysN	gacaacatcgtgcatgagag
	IR-pdhL	aagcggatcacggtcttctt		IR-cysN	gtcagcacttcagccaccac
cbbSp	IF-cbbSp	gacgattcacgatgaacgac	***hoxF***	IF-hoxF	ggacggatttgagatctttg
	IR-cbbSp	gcaatacctactgggagatg		IR-hoxF	cggcgttgcagattacatac
cbbP2	IF-cbbP2	ccgataccgatctgctgttc	***hoxH***	IF-hoxH	tcaacaacaacctgagcatc
	IR-cbbP2	ccgataccgatctgctgttc		IR-hoxH	ggtgtttctgatggaagtcg
			ppc	IF-ppc	ccagcgcaagagtattctcg
				IR-ppc	ggaaacaggtgcggtagtag

Table S2 Plasmids and primers used in the study.

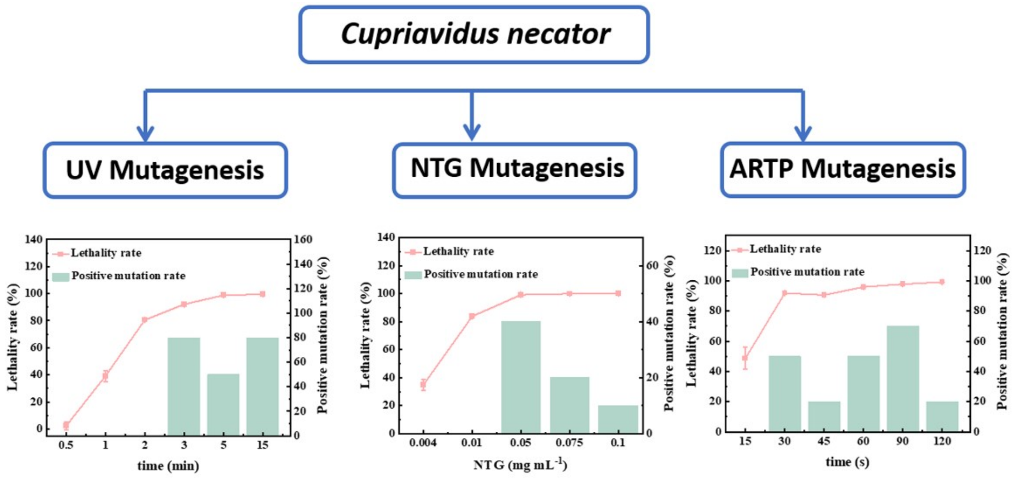
Strains	Primer	Sequence（5’→3’）
H16/R3-△*lysS*	IF-lys-C	atgaccatgattacgaattctggtgccgcacctgctga
	IR-lys-C	aagcgcttgcgatgtcttgtcggatcgcgc
	IF-lys-D	acaagacatcgcaagcgctttggcaaga
	IR-lys-D	cgacggccagtgccaagcttgccgggttgtgctgcccg
	F-PK18-lys	acaacccggcaagcttggcactggccgtcgtttta
	R-PK18-lys	cggcaccagaattcgtaatcatggtcatagctgtt
H16-P$_{mbh}$-A0443	A0443-Pmbh-up-F	aaacagctatgaccatgattacgaattcaagatcctgaccagctgcccgtcgt
	A0443-Pmbh-up-R	tccgagcaatgccacgcagctactcaggcgtcgccggcttcgagcagcgaaa
	A0443-mbh-F	tttcgctgctcgaagccggcgacgcctgagtagctgcgtggcattgctcgga
	A0443-mbh-R	gtgactgaggtcggagtggaggacataacctgtctcctaatttctgtattgg
	A0443-Pmbh-down-F	ccaatacagaaattaggagacaggttatgtcctccactccgacctcagtcac
	A0443-Pmbh-down-R	ttgtaaaacgacggccagtgccaagctttgccgcccagcgcaaagctgat
	A0443-Pmbh-V-F	atcagctttgcgctgggcggcaaagcttggcactggccgtcgttttacaa
	A0443-Pmbh-V-R	acgacgggcagctggtcaggatcttgaattcgtaatcatggtcatagctgttt
H16-P$_{chr}$-A0443	A0443-Pchr-up-F	aaacagctatgaccatgattacgaattcaagatcctgaccagctgcccgtcgt
	A0443-Pchr-up-R	ttgcagatcttcgtcaccgtggccaggcacgcctcaggcgtcgccggcttcgagca gcgaaa
	A0443-chr-F	tttcgctgctcgaagccggcgacgcctgaggcgtgcctggccacggtgacgaaga tctgcaa
	A0443-chr-R	gtgactgaggtcggagtggaggacatgcttgtctccttgcgtggttgagcgt
	A0443-Pchr-down-F	acgctcaaccacgcaaggagacaagcatgtcctccactccgacctcagtcac
	A0443-Pchr-down-R	ttgtaaaacgacggccagtgccaagctttgccgcccagcgcaaagctgat
	A0443-Pchr-V-F	atcagctttgcgctgggcggcaaagcttggcactggccgtcgttttacaa
	A0443-Pchr-V-R	acgacgggcagctggtcaggatcttgaattcgtaatcatggtcatagctgttt
H16-P$_{mbh}$-A2170	A2170-Pmbh-up-F	aaacagctatgaccatgattacgaattcgataacggcggtcatggttgcg
	A2170-Pmbh-up-R	tcgtccgagcaatgccacgcagctacatggaccgcttcgacgcgctgcaact
	A2170-mbh-F	agttgcagcgcgtcgaagcggtccatgtagctgcgtggcattgctcggacga
	A2170-mbh-R	tgacccgcgactcaggaacggcgctcataacctgtctcctaatttctgtatt
	A2170-Pmbh-down-F	aatacagaaattaggagacaggttatgagcgccgttcctgagtcgcgggtca
	A2170-Pmbh-down-R	taaaacgacggccagtgccaagcttagaagatggagacaaatgtaccggcaat
	A2170-Pmbh-V-F	attgccggtacatttgtctccatcttctaagcttggcactggccgtcgtttta
	A2170-Pmbh-V-R	cgcaaccatgaccgccgttatcgaattcgtaatcatggtcatagctgttt
H16-P$_{chr}$-A2170	A2170-Pchr-up-F	aaacagctatgaccatgattacgaattcgataacggcggtcatggttgcg
	A2170-Pchr-up-R	acttcgtcaccgtggccaggcacgccatggaccgcttcgacgcgctgcaact

	A2170-chr-F	agttgcagcgcgtcgaagcggtccatggcgtgcctggccacggtgacgaagat
	A2170-chr-R	acccgcgactcaggaacggcgctcatgcttgtctccttgcgtggttgagcgtct
	A2170-Pchr-down-F	agacgctcaaccacgcaaggagacaagcatgagcgccgttcctgagtcgcgggt
	A2170-Pchr-down-R	taaaacgacggccagtgccaagcttagaagatggagacaaatgtaccggcaat
	A2170-Pchr-V-F	attgccggtacatttgtctccatcttctaagcttggcactggccgtcgtttta
	A2170-Pchr-V-R	cgcaaccatgaccgccgttatcgaattcgtaatcatggtcatagctgttt
	B1682-up-F	aaacagctatgaccatgattacgaattcaagcgtgtcgacggcattgtcgagt
	B1682-up-R	tgaactgactccctgtctgtgttggcgcgcgagggcctccagcaggtcgttca
H16-△B1682	B1682-down-F	tgaacgacctgctggaggccctcgcgcgccaacacagacagggagtcagttca
	B1682-down-R	acgttgtaaaacgacggccagtgccaagcttagcacgccaggtcggcatcgagat
	B1682-V-R	actcgacaatgccgtcgacacgcttgaattcgtaatcatggtcatagctgttt
	B1682-V-F	atctcgatgccgacctggcgtgctaagcttggcactggccgtcgttttacaacgt

Table S3 Strains、plasmids and primers used in the study.

Strains	Plasmids	Primer	Sequence（5'→3'）
HS01	pBBR1-blank	VF-blank	tttaagaaggagatatacatggatccaaactcgagtaaggatctccaggc
		VR-blank	atgtatatctccttcttaaaagatcttttgaattccaaaattatttctag
		MF-blank	aattgtccatattgcatcagacattgccgtcactgcgtcttttactggct
		MR-blank	tgagatgcgcaagaaggccgatcaagcccaagagacggcccgagatcttc
HS02	pBBR1-*pdh*	IF-pdh	gatcttttaagaaggagatatacatctacaagaaccaggtggtcg
		IR-pdh	gagatccttactcgagtttggatcctaagcggatcacggtcttctt
		VF-pdh	cacgtgtactgacgtgccgccgccgcgcaagcgccaccaccaccaccaccactgaggatccaaactcgagtaaggatctc
		VF-pdh	gcaccttgacttcgatcacactcatatgtatatctccttcttaaaagatc
HS03	pBBR1-*hoxF*	IF-hoxF	gatcttttaagaaggagatatacatatggatagtcgtatcacgacaatac
		IR-hoxF	gagatccttactcgagtttggatcctcagtggtggtggtggtggtgtcttgtcacctcttccagatccttcagcgccttc
		VF-hoxF	gaaggcgctgaaggatctggaagaggtgacaagacaccaccaccaccaccactgaggatccaaactcgagtaaggatctc
		VR-hoxF	gtattgtcgtgatacgactatccatatgtatatctccttcttaaaagatc
HS04	pBBR1-*gapdh*	IF-gapdh	gatcttttaagaaggagatatacatatgaccatcaagatcggcatcaacg
		IR-gapdh	gagatccttactcgagtttggatccttagtggtggtggtggtggtgcttggccgcgacgcggaccatttccagcaccttg
		VF-gapdh	caaggtgctggaaatggtccgcgtcgcggccaagcaccaccaccaccaccactaaggatccaaactcgagtaaggatctc
		VR-gapdh	cgttgatgccgatcttgatggtcatatgtatatctccttcttaaaagatc
HS05	pBBR1-*cisY*	IF-cisY	ggcaccctaccgcatggagataagcatgacgccgtccgatgtgaaagcca
		IR-cisY	tacgggttgctgcgcaacccaagtgtcagtggtggtggtggtggtggcgcttggcaacgtccggcacgtcgcgggtagct
		VF-cisY	agctacccgcgacgtgccggacgttgccaagcgccaccaccaccaccaccactgacacttgggttgcgcagcaacccgta
		VR-cisY	tggctttcacatcggacggcgtcatgcttatctccatgcggtagggtgcc
HS06	pBBR1-*ppc*	IF-ppc	ggcaccctaccgcatggagataagcatgacgcagcatgctgcgcgcccca
		IR-ppc	tacgggttgctgcgcaacccaagtgtcagtggtggtggtggtggtggcccgtattgcgcaggcccgccgcgaccccgttg
		VF-ppc	caacggggtcgcggcgggcctgcgcaatacgggccaccaccaccaccaccactgacacttgggttgcgcagcaacccgta
		VR-ppc	tggggcgcgcagcatgctgcgtcatgcttatctccatgcggtagggtgcc

a



b

4.648	3.728	4.902	5.15	4.368	5.34	6.75	4. 387
4.087	3.991	5.258	5.881	4.357	5.15	5.80	4. 084
5.91	5.12	5.17	4.795 H16	4.455	5.88	5.43	5. 304
4.42	4.56	4.06	6.16	4.12	4.80 H16	9.73	3. 595
5.54	5.99	5.867	6.745	7.14	6.16	7. 135	5. 39
10. 4	4	7. 2	4. 32	6. 402	10. 656	9. 20	8. 8
4. 12	4. 6	4. 71	5. 44	6. 204	6. 408	6. 316	6. 9

Fig. S1 Comparative assessment of mutagenesis strategies for *C. necator*. (a) Lethality and positive mutation rate induced by UV, NTG and ARTP treatments. A positive mutant is defined as a strain whose autotrophic growth is ≥10 % higher than that of the wild-type H16 control. Data represent means ± SD of three independent experiments. (b) Numerical comparison of the screened mutant libraries. Each dot represents the relative autotrophic performance of an individual mutant. Green symbols: mutants performing worse than H16; red symbols: mutants outperforming H16.

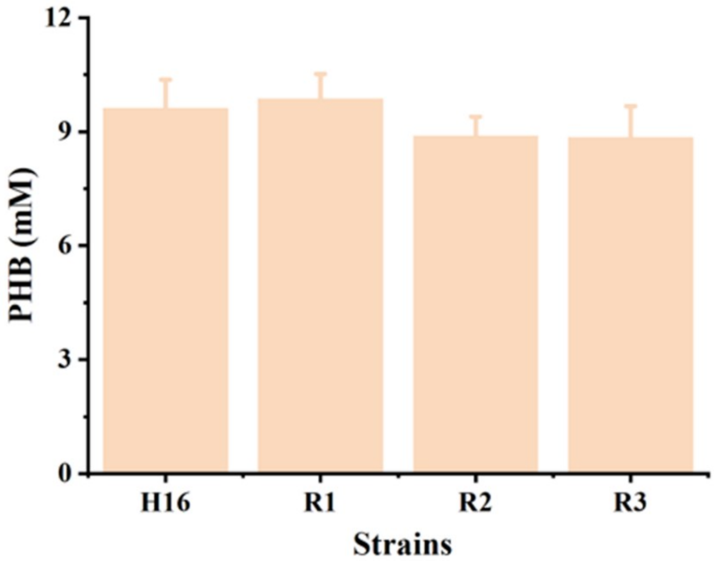


Fig. S2 PHB content of H16, R1, R2, R3.

Cupriavidus necator **[gbbct]: 74 CDS's (25789 codons)**

fields: [triplet] [frequency: **per thousand**] ([number])

UUU	4.7(	121)	UCU	1.9(	49)	UAU	6.9(	177)	UGU	0.7(	17)
UUC	28.2(	727)	UCC	10.1(	261)	UAC	19.0(	490)	UGC	8.5(	220)
UUA	0.7(	17)	UCA	1.9(	50)	UAA	0.5(	13)	UGA	2.2(	57)
UUG	6.4(	165)	UCG	19.9(	514)	UAG	0.2(	4)	UGG	13.2(	341)
CUU	4.2(	108)	CCU	2.9(	76)	CAU	7.5(	194)	CGU	7.0(	181)
CUC	16.2(	417)	CCC	14.7(	380)	CAC	17.6(	454)	CGC	45.1(	1164)
CUA	1.1(	28)	CCA	3.4(	87)	CAA	7.2(	185)	CGA	2.9(	75)
CUG	70.9(	1828)	CCG	33.1(	853)	CAG	30.1(	777)	CGG	10.1(	261)
AUU	5.3(	136)	ACU	2.6(	68)	AAU	5.9(	153)	AGU	1.5(	38)
AUC	42.5(	1095)	ACC	29.8(	769)	AAC	23.5(	605)	AGC	16.6(	427)
AUA	1.0(	27)	ACA	2.6(	68)	AAA	3.6(	94)	AGA	0.7(	19)
AUG	24.9(	643)	ACG	17.3(	445)	AAG	35.1(	905)	AGG	2.8(	71)
GUU	3.6(	93)	GCU	6.0(	155)	GAU	11.7(	301)	GGU	8.3(	215)
GUC	25.6(	659)	GCC	61.4(	1583)	GAC	41.1(	1060)	GGC	61.6(	1588)
GUA	1.8(	47)	GCA	11.8(	305)	GAA	25.2(	651)	GGA	3.4(	88)
GUG	41.4(	1068)	GCG	42.4(	1093)	GAG	31.9(	823)	GGG	8.0(	206)

Fig. S3 The codons of *C. necator*.

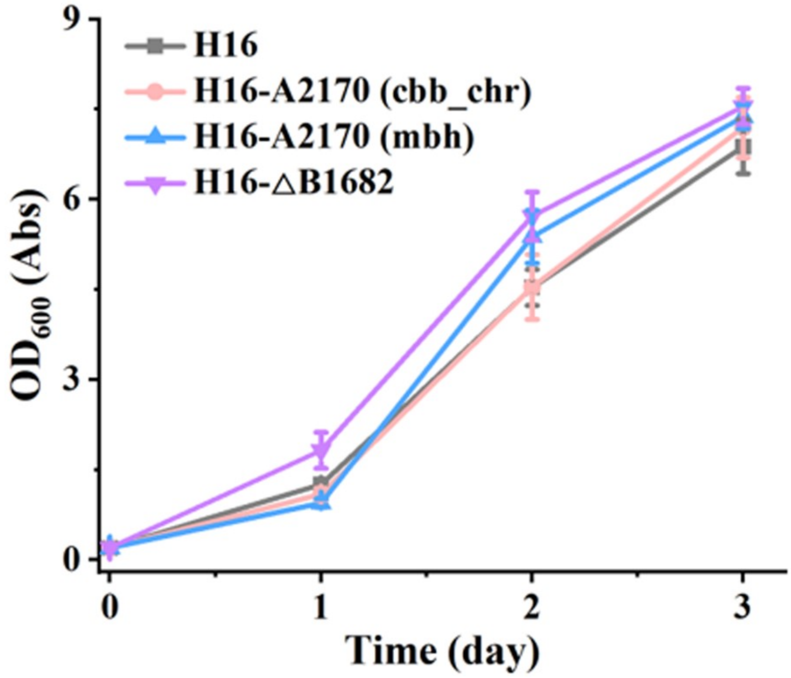


Fig. S4 The results of strengthening different promoter for A2170 gene or deleting B1682 gene in H16.

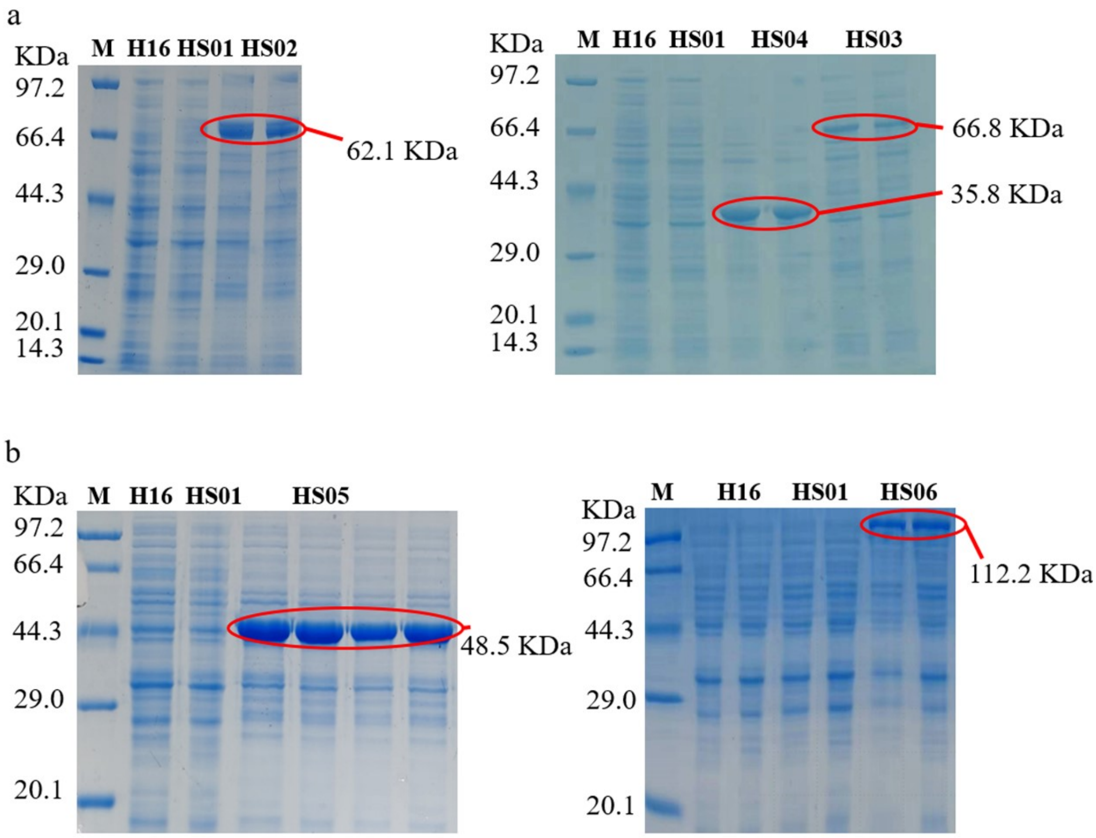


Fig. S5 The SDS-PAGE results of (a) the overexpression of engineered strains HS01, HS02, HS03, HS04, and (b) the overexpression of engineered strains HS01, HS06, HS05 and HS05 with oxalacetate or acetyl-CoA.

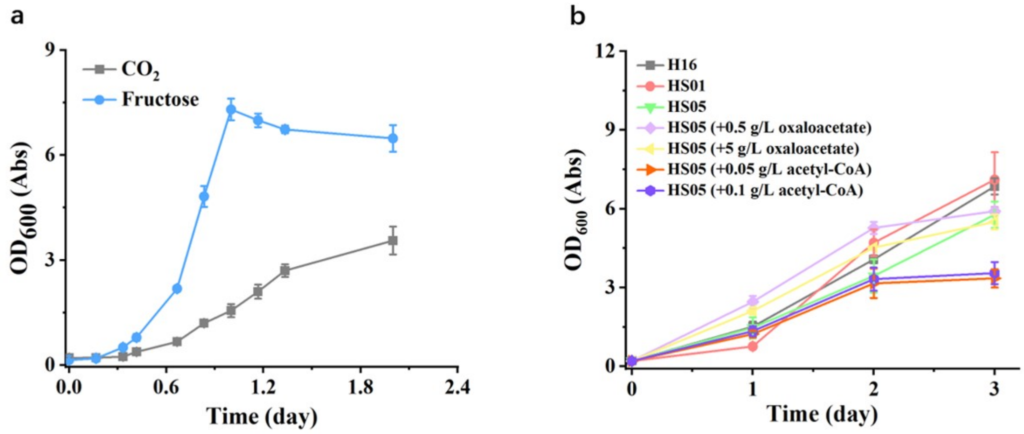


Fig. S6 The growth of *C. necator* H16 and engineered strain HS05. (a) The growth of *C. necator* H16 based on CO_2 or fructose. (b) The engineered strain HS05, and the comparison of the growth of strains HS05 with different concentrations of oxalacetate or acetyl-CoA added during its cultivation, versus strains H16 and HS01.

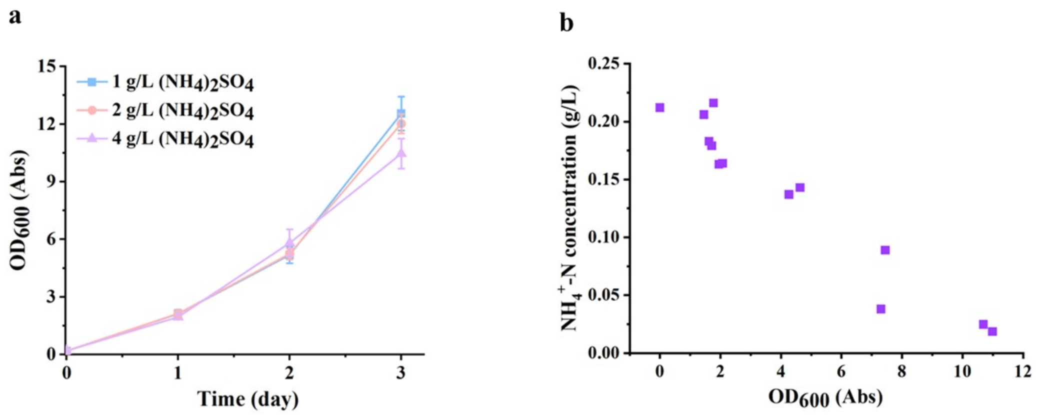


Fig. S7 The relationship between OD and nitrogen concentration. **(**a) Nitrogen source was verified by cultivating R3 with CO_2 as the carbon source. (b) The relationship between OD and NH_4^+-N concentration.