Supplementary material

Synergy on Carbon and Nitrogen Removal of the Co-culture of Two Aerobic Denitrifying Bacterial Strains, *Acinetobacter* sp. GA and *Pseudomonas* sp. GP

Yan Guo,[ab] Ruili Yang,[ab] Zhaoji Zhang, [a]* Xiaojun Wang, [a] Xin Ye, [a] Shaohua Chen[a]*

a, Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, People's Republic of China;

b, University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

1. The detailed isolation procedure of strain GA and GP

In general, aerobic denitrifying bacterial strain was isolated by bromothymol blue (BTB) solid medium. The colour of fresh BTB medium was green, and the aerobic denitrifying bacterial colony would make the surrounding medium change to blue by changing the pH of the medium. Those blue bacterial colonies were picked and inoculated onto fresh BTB plates with the "streak palte method" to purify the bacterial strain. Repeat the last step until all bacterial colonies on the BTB plates were identical.

In present study, 10 strains of *Acinetobacter* and 10 strains of *Pseudomonas* were isolated from the bioreactor. These strains were cultured in medium M3 for 24 h at 25 ºC with the speed of 150 r/min to check their nitrogen removal efficiency. The total nitrogen (TN) and NO_3^--N before and after incubation was detected. At last, the strain 1-6 (GA) and strain 2-4 (GP) were choosen to conduct the following experiment.

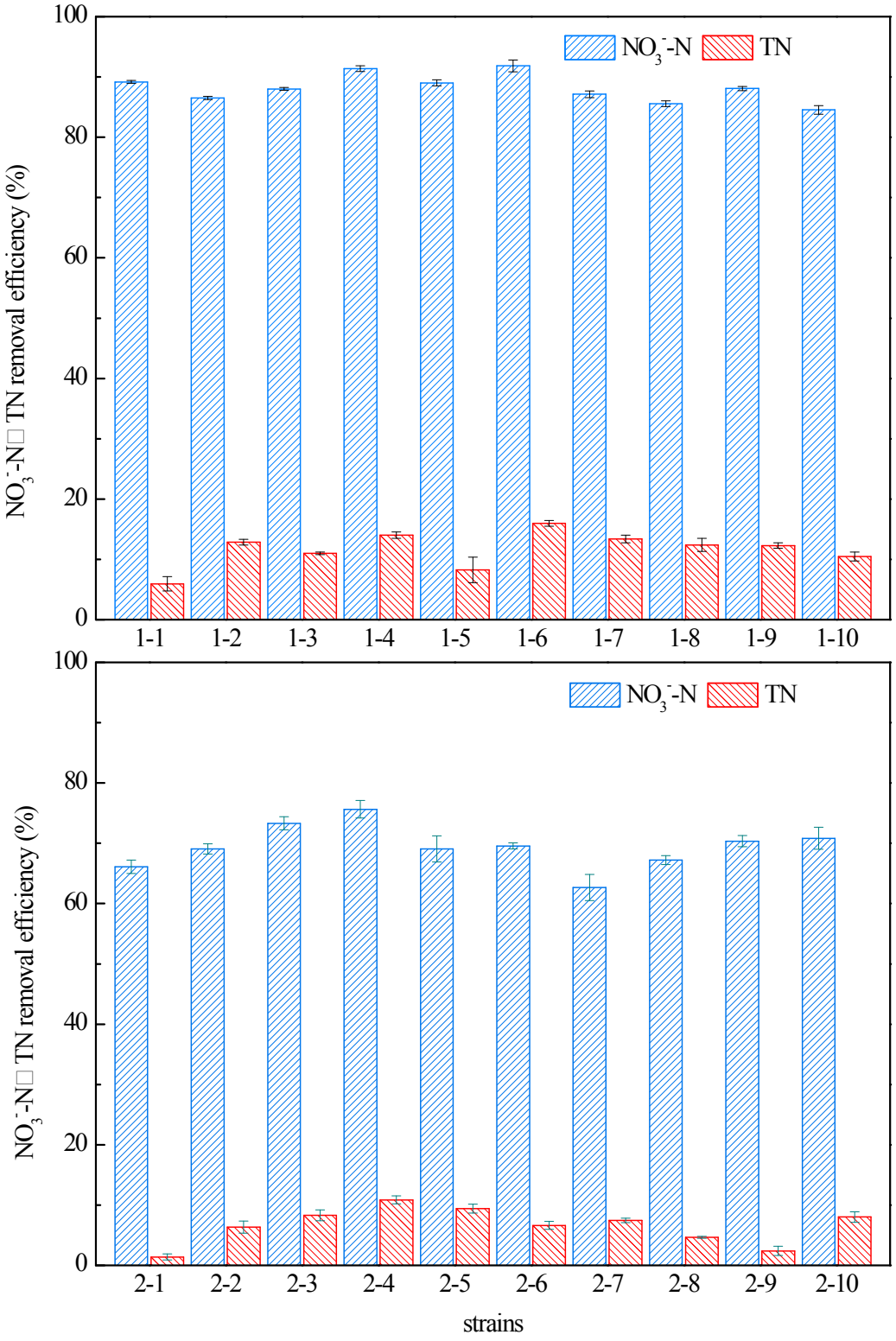

Fig. S1 the nitrogen removal efficiency of 20 isolated bacterial strains.

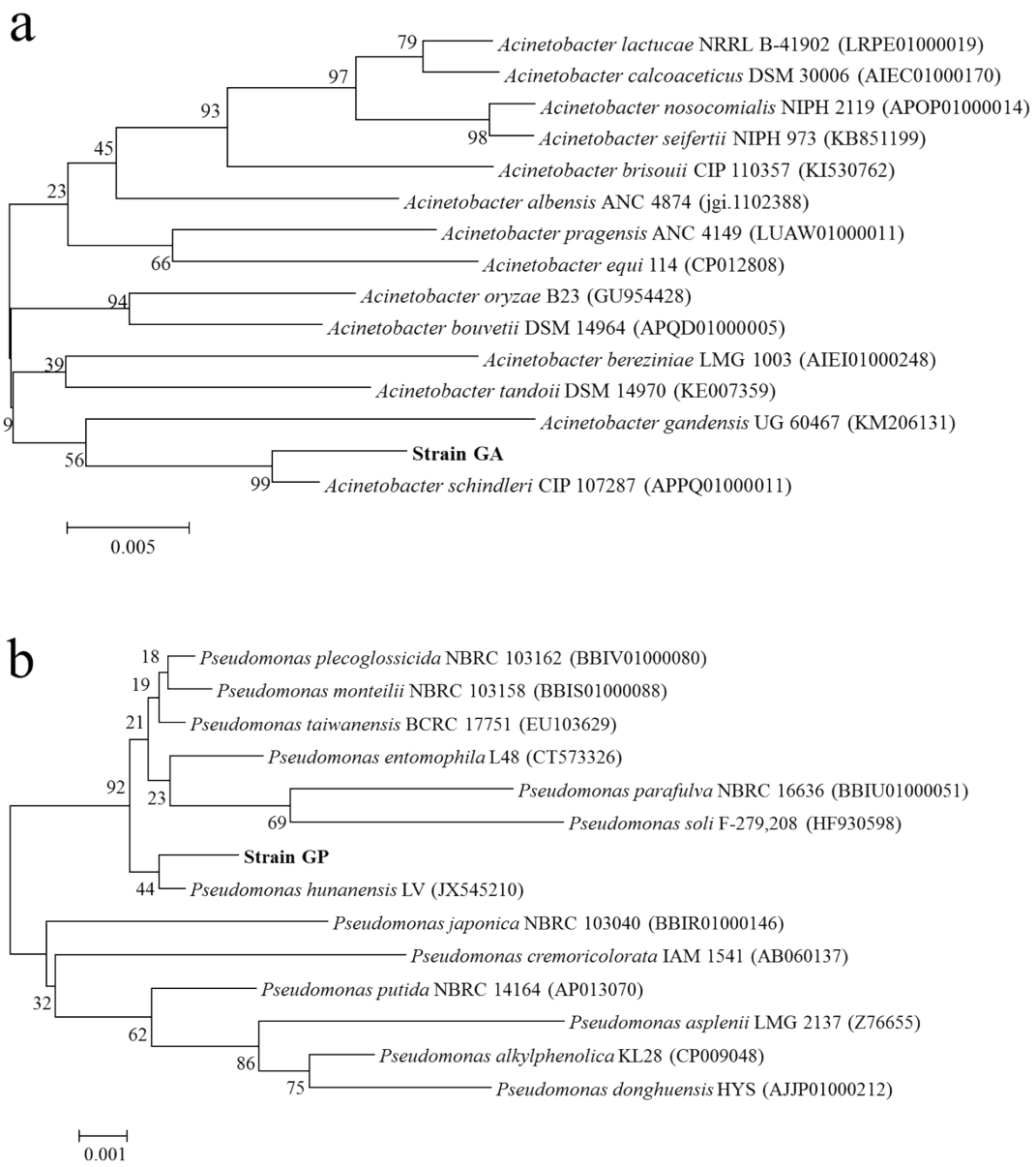

Fig. S2 Phylogenetic trees of bacterial strains GA (a) and GP (b).

2. The nitrogen and carbon balance of strain GA and strain GP in different medium.

Pre-cultured strain GA or strain GP were cultured in medium M1~M6 for 24 h at 25 ºC with the speed of 150 r/min. The TN, total dissolved nitrogen (TDN), total chemical oxygen demand (TCOD), and dissolved chemical oxygen demand (DCOD) of every medium before and after incubation was detected.

Nitrogen removed through denitrification is calculated by formula $y(d1) = \frac{m_{0(TN)} - m_{24(TN)}}{m_{0(TN)}}$, where $m_{0(TN)}$ means the content of TN before incubation, and $m_{24(TN)}$ means the TN in medium after bacterial strain GA or GP cultured for 24 h. Nitrogen removal through assimilation is calculated by formula $y(a1) = \frac{m_{24(TN)} - m_{24(TDN)}}{m_{0(TN)}}$, where $m_{24(TDN)}$ means the TDN in medium after bacterial strain GA or GP cultured for 24 h.

Carbon removed through dissimilation is calculated by formula $y(d2) = \frac{m_{0(TCOD)} - m_{24(TCOD)}}{m_{0(TCOD)}}$, where $m_{0(TCOD)}$ means the content of TCOD before incubation, and $m_{24(TCOD)}$ means the TCOD in medium after bacterial strain GA or GP cultured for 24 h. Carbon removal through assimilation is calculated by formula $y(a2) = \frac{m_{24(TCOD)} - m_{24(DCOD)}}{m_{0(TCOD)}}$, where $m_{24(DCOD)}$ means the DCOD in medium after bacterial strain GA or GP cultured for 24 h.

Table S1 The nitrogen removal pathways of strain GA and strain GP

medium	strain GA		strain GP	
	denitrification (%)	assimilation (%)	denitrification (%)	assimilation (%)
M1	13.40 ± 1.71	79.39 ± 2.09	10.70 ± 1.04	81.45 ± 0.12
M2	13.95 ± 3.82	66.75 ± 1.65	12.94 ± 0.49	49.83 ± 4.05
M3	17.26 ± 0.71	74.97 ± 0.41	12.88 ± 3.03	58.14 ± 1.97
M4	16.72 ± 0.80	75.48 ± 0.91	1.78 ± 0.27	4.47 ± 1.06
M5	1.38 ± 0.46	2.00 ± 1.92	12.76 ± 0.19	56.24 ± 0.39
M6	2.90 ± 2.00	1.44 ± 1.04	12.42 ± 2.06	49.57 ± 1.75

Table S2 The carbon removal pathways of strain GA and strain GP

medium	strain GA		strain GP	
	dissimilation (%)	assimilation (%)	dissimilation (%)	assimilation (%)
M1	64.90 ± 0.88	32.45 ± 0.64	66.96 ± 0.85	31.65 ± 1.91
M2	70.04 ± 0.90	23.27 ± 1.89	67.58 ± 1.50	18.08 ± 0.69
M3	65.86 ± 1.46	31.18 ± 0.56	69.88 ±3.44	23.92 ± 2.50
M4	67.94 ± 1.32	27.99 ± 1.00	1.59 ± 0.91	1.21 ± 1.02
M5	16.70 ± 4.41	7.89 ± 0.61	71.73 ± 0.82	21.71 ± 1.75
M6	1.21 ± 1.05	2.18 ± 1.22	66.60 ±3.74	20.40 ± 5.29

3. The TOC removal performance of strain GA and GP in medium M1~M6

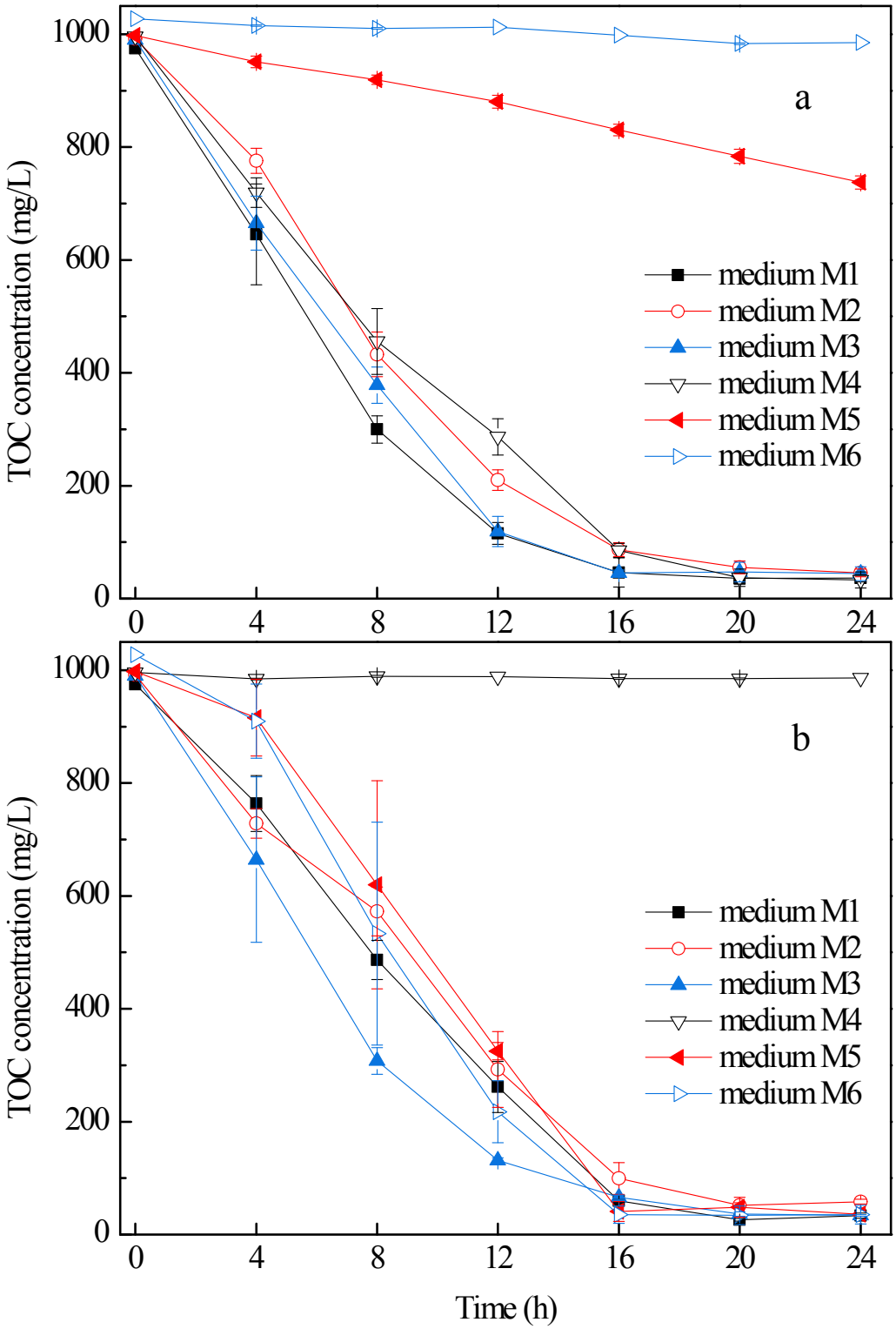

Fig. S3 The TOC removal performance of strain GA (a) and strain GP (b) in medium M1~M6

4. The carbon and nitrogen removal performance of strain GP with different C/N

The medium with citrate as carbon source, ammonium as nitrogen source was used to investigate the carbon and nitrogen removal performance of strain GP with different C/N. The inoculated media were incubated for 12 h at 25 ºC with the speed of 150 r/min. The content of TOC and NH_4^+-N before and after incubation was detected.

Table S3 The carbon and nitrogen removal performance of strain GP with different C/N

medium	TOC (mg/L)		NH_4^+-N (mg/L)	
	initial	final	initial	final
(1)	1024.75 ± 4.60	131.88 ± 46.68	19.79 ± 0.16	0 ± 0
(2)	1027.50 ± 1.41	73.82 ± 22.96	40.13 ± 0.27	0 ± 0
(3)	1006.75 ± 15.20	82.85 ± 18.74	60.20 ± 0.16	0 ± 0
(4)	1012.75 ± 3.18	59.52 ± 21.76	80.40 ± 0.27	0 ± 0
(5)	1010.50 ± 11.31	73.45 ± 15.03	99.78 ± 0.16	1.56± 0.42