Supporting Information

Matching different inorganic compounds as mixture electron donors to improve CO_2 fixing by non-photosynthetic microbial community without hydrogen

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Supporting Information summary

Non-photosynthetic CO_2 fixing micro-organism. Two NPMCs capable of fixing CO_2 without hydrogen were isolated from sea water and their sediments collected from the Yellow Sea, East China Sea, South China Sea and Antarctic waters. Two NPMCs were cultured continuously in two 3-L Applikon BioBundle fermentation systems (Cole-Parmer Instrument Co., USA) at 28°C and agitation at 120 rpm under aerobic and anaerobic conditions. The medium had a pH of 6.2 and contained (g/L): $(NH_4)_2SO_4$, 5.0; KH_2PO_4, 1.0; K_2HPO_4, 2.0; $MgSO_4•7H_2O$, 0.2; NaCl, 20; $CaCl_2$, 0.01; $FeSO_4•7H_2O$, 0.01. Two-mL of a trace element solution containing (mg/L) $Na_2MoO_4•2H_2O$, 1.68; H_3BO_3, 0.4; $ZnSO_4•7H_2O$, 1.0; $MnSO_4•5H_2O$, 1.0; $CuSO_4•5H_2O$, 7.0; $CoCl_2•6H_2O$, 1.0; and $NiSO_4•7H_2O$, 1.0 was also added. CO_2 as a sole carbon source and the N_2:O_2:CO_2 ratio was 60:20:20 under aerobic condition, the N_2:CO_2 ratio was 80:20 under anaerobic condition. Every 10 d, 2 L of medium was replaced by fresh medium. The mixture of these two NPMCs was then domesticated in the laboratory for more than 6 months under aerobic and anaerobic conditions.

Cultivation conditions. Forty mL of medium in a 150-mL serum bottle was prepared and the electron donor was added according to the experimental design. After autoclaving for 20 min at 121°C, the medium was inoculated with the microbial community. For aerobic culture, the serum bottle was sealed with a silicon stopper and filled with 20% (v/v) CO_2 with a syringe. For anaerobic culture, the air in the bottle was displaced by N_2 and the 20% CO_2 was then injected. For culture in the presence of hydrogen gas, the air in the bottle was displaced by H_2 and then 10% O_2 and 10% CO_2 were injected. The atmosphere ratio in the serum bottles under aerobic, anaerobic and hydrogen gas supply conditions were approximately 80:20 Air:CO_2, 80:20 N_2:CO_2 and 80:10:10 H_2:O_2:CO_2, respectively. All values were at 1 atm and were verified by analysis using a Shimadzu GC-14B gas chromatograph (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan). All cultures were maintained at 28°C and 120 rpm without light for 96 h (previous experimental results indicated that microbial CO_2 fixing efficiency in this period reached the highest). The atmosphere of the serum bottle was readjusted to the initial ratio after culturing for 48 h.

DNA extraction and PCR-DGGE. The bacterial DNA was extracted using a soil DNA kit (D5625-01, United States, Omega) according to the manufacturer's instructions. Primers 341f with a GC-clamp (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTAGGGGAGGCAGCAG-3') and 534r (5'-ATTACCGCGGCTGCTGG-3') were used to amplify the V3 region of the bacterial 16S rDNA. The PCR mixtures had a final volume of 25 μL and contained 2.5 μl 10×PCR buffer, 1 U Taq DNA Polymerase, 1.0 μl of each dNTP (2.5 mmol L-1), 0.5 μl of each primer (10 μM) and 50ng of

DNA as the template. The samples were amplified by subjecting the mixtures to the following conditions: 94 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, 72 °C for 50 s and a final extension at 72 °C for 10 min. DGGE was conducted using the DCodeTM Universal Mutation Detection System (BioRad, USA). The PCR products were electrophoresed directly in a 10% (v/v) polyacrylamide gel in 1×TAE buffer containing a linear gradient that ranged from 30% to 60% denaturant (100% denaturant was equivalent to 7 mol·L-1 urea and 40% (v/v) deionized formamide). Electrophoresis was performed at 60 °C under a constant voltage of 80 V. After 15 h of electrophoresis, the gel was stained with ethidium bromide (EB) for 15 min and then photographed. The contrast of the bands in different lanes was determined by Quantity One 4.6.2 (BioRad, USA), and the Dice coefficient (Cs) was defined as the following:

$Cs = (2L/L_0) \times 100\%$

where L was the amount of duplicate bands in both lanes and L_0 was the total amount of bands in both lanes.

Sequencing of DGGE band.Prominent bands were excised from the DGGE gel for 16S rDNA fragment sequencing. The fragments were then re-amplified by PCR and purified using a B type Mini-DNA Rapid Purification Kit (BioDev, China), after which they were cloned using the pMD19-T plasmid vector system (TaKaRa, Japan). The DNA sequences were then determined by a commercial service (Shanghai Invitrogen Biotechnology Co., LTD., China). The vector sequence was cut off and the remaining nucleotides were compared to those available in GenBank using the BLAST program to identify the most similar 16S rDNA fragments.

Mergence of Aerobic and Anaerobic Models. The gas phase condition was set as the forth factor in the new experimental design. The minimum and maximum range of variables investigated and the full experimental plan with respect to the actual and coded forms are as the follows: $NaNO_2$ levels of -1 and +1 were 0.25% and 1.05% w/v, $Na_2S_2O_3$ levels of -1 and +1 were 0.50% and 1.10% w/v, Na_2S levels of -1 and +1 were 0.75% and 1.25% w/v and gas phase levels of -1 and +1 were anaerobic and aerobic. The data was Square Root transformed. The coefficients of the regression equation were calculated according to the results showed in Tables 1 and 2 using Design Expert and then used to evaluate experimental results, which enabled the TOC produced by CO_2 fixing micro-organisms to be expressed in terms of the following regression equations:

$$TOC = (7.84 + 1.43A + 1.23B + 2.29C - 0.34D + 2.68AB + 0.39AC - 0.28AD + 0.90BC - 0.047BD + 0.49CD - 1.12A^2 - 0.81B^2 - 0.53C^2 + 0.62ABC - 0.88ABD - 1.92ACD + 1.14BCD - 1.07A^2B - 1.92A^2C - 0.85A^2D + 0.97AB^2 - 1.12AC^2 + 1.28B^2D - 9.90ABCD - 0.63A^2B^2 - 12.61A^2BC + 0.90A^2BD - 1.34AB^2D)^2 \quad (3)$$

where A was the $NaNO_2$ concentration, B was the $Na_2S_2O_3$ concentration, C was the Na_2S concentration, and D was gas phase condition.

To evaluate the reliability of the model, the key elements of the regression equation were obtained by ANOVA. The R^2, adjusted R^2 and predicted R^2 values of this model were 0.9927, 0.9887 and 0.9858, respectively. In addition, the adequate precision, F-value and lack of fit P-value were 48.66, 247.85 and 0.8681, respectively. The key elements of the regression equation confirmed that the model was good and fitted the combined data well. The gas phase condition was changed from categoric factor to numeric factor. -1 level was changed from anaerobic to 0, and +1 level was changed from aerobic to 100. Then the relation between gas phase condition and other three factors could be expressed by 3-D response surface. The effects of electron donors under aerobic and anaerobic conditions were presented as 3-D response surface curves. The only difference in the curves generated under aerobic and anaerobic conditions was in the concentration of the oxygen gas, which was defined to be 100% (the actual value was about 21%) and 0%, respectively. Only one electron donor concentration changed with the oxygen gas concentration, while the other two electron donors concentrations were default values not equal to 0 (Figures S1a, S1b and S1c). Because there was an interaction of the electron donors, a combination effect was produced by the change in any one electron donor concentration. For example, the effect of $NaNO_2$ in Figure S1a led to a combination effect, that is, $\mathbf{[NaNO_2]} = [NaNO_2] + [NaNO_2\&Na_2S] + [NaNO_2\&Na_2S_2O_3] + [NaNO_2\&Na_2S_2O_3\&Na_2S]$. As shown in Figure S1a, the combination effect of $NaNO_2$ was enhanced as the level of oxygen gas decreased and the level of $NaNO_2$ increased. The combination effect of $Na_2S_2O_3$ appeared as a peak value under aerobic conditions, and there was a high value area when the concentration of $Na_2S_2O_3$ was 0.80%-0.90% under anaerobic conditions (see Figure S1b). When compared with the aerobic conditions, the combination effect of $Na_2S_2O_3$ increased to a steady and higher value, while the concentration of $Na_2S_2O_3$ was not highest under anaerobic conditions. Furthermore, as shown in Figure S1c, the combination effect of Na_2S at low concentrations under anaerobic conditions was greater than the effect observed under aerobic conditions. However, the combination effect of Na_2S was obviously enhanced with increasing concentrations of Na_2S under aerobic conditions, and even exceeded the effects observed under anaerobic conditions.

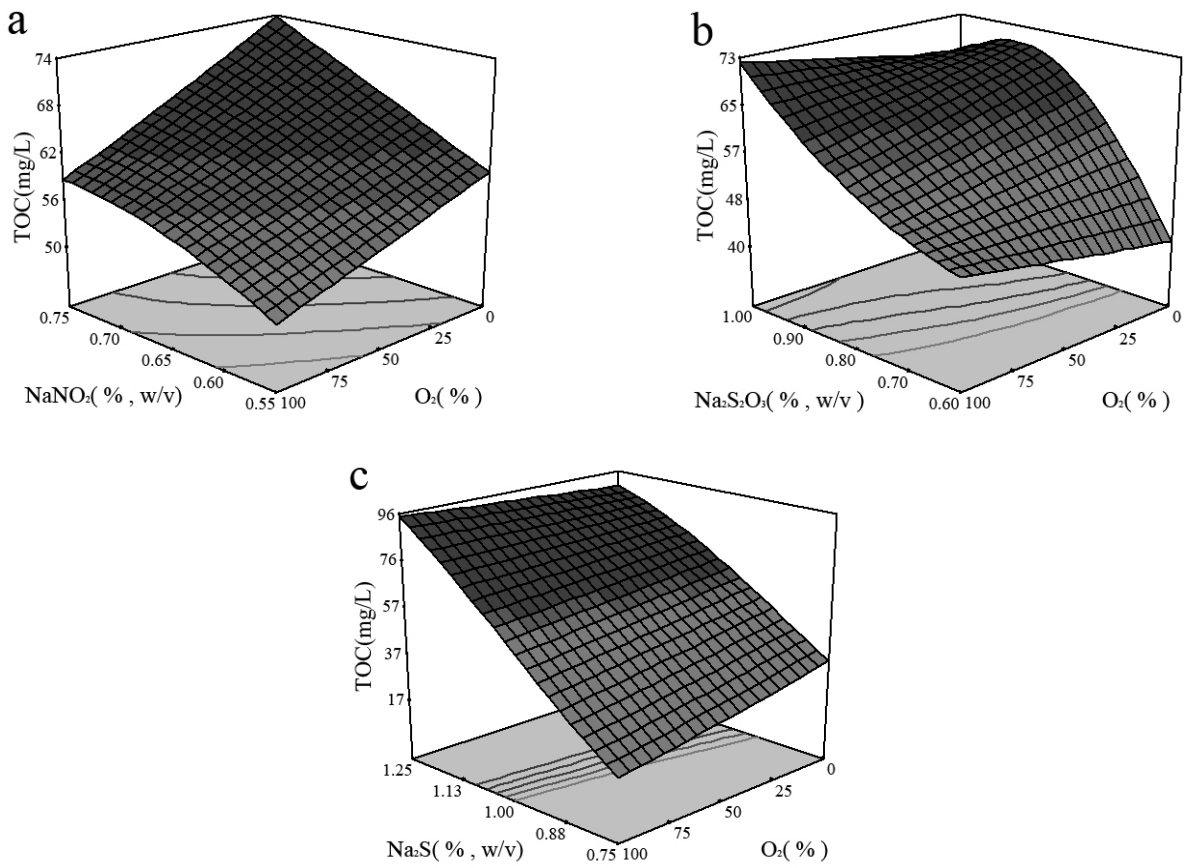

Fig. S1 (a) Relationship between $NaNO_2$ and oxygen gas. (b) Relationship between $Na_2S_2O_3$ and oxygen gas. (c) Relationship between Na_2S and oxygen gas. (a) - (c) Other two factors were set as the default level. $NaNO_2$ was 0.65%, $Na_2S_2O_3$ was 0.80% and Na_2S was 1.00%.

Table S1 16S rDNA V3 sequence similarities to closest relatives of DNA recovered from the prominent bands in DGGE gels

Bands	Similar name of bacterium of GenBank database (serial number)	Similarity (%)	lanes for the appearance of the prominent band (+)										
			a□	a□	a□	a□	a□	a□	b□	b□	b□	b□	b□
1	Uncultured bacterium clone(EU509144)	96		+									
2	Marine bacterium 'Isolate 5'(AY082665)	98		+		+	+			+	+	+	+
3	Uncultured bacterium(EU574677)	98						+				+	+
4	Marine bacterium HP22(AY239006)	99	+	+		+	+		+	+	+		
5	Uncultured bacterium(AY328485)	97	+	+				+	+	+	+		
6	Uncultured bacterium clone(EU857874)	90		+	+	+		+		+		+	+
7	Uncultured Pseudomonas sp.(AF467304)	100	+	+	+				+	+			
8	Uncultured bacterium(EU652656)	96		+									
9	Alpha proteobacterium DG1293(DQ486505)	99		+		+					+		+
10	Uncultured Thioclava sp.(EU167470)	98			+								
11	Uncultured gamma proteobacterium(AY711683)	99		+	+								
12	Uncultured Stenotrophomonas bacterium(EF562149)	98	+	+	+				+	+	+		
13	Uncultured bacterium (AB244004)	98		+	+								
14	Marine alpha proteobacterium RS.Sph.020(DQ097294)	99			+						+		
15	Uncultured bacterium(EF034329)	97	+			+			+				
16	Marine bacterium SE83(AY038922)	99						+			+		