Support Information

CO_2 fixation in Anaerobic biological treatment: Amorphous carbon formation driven by electron bifurcation

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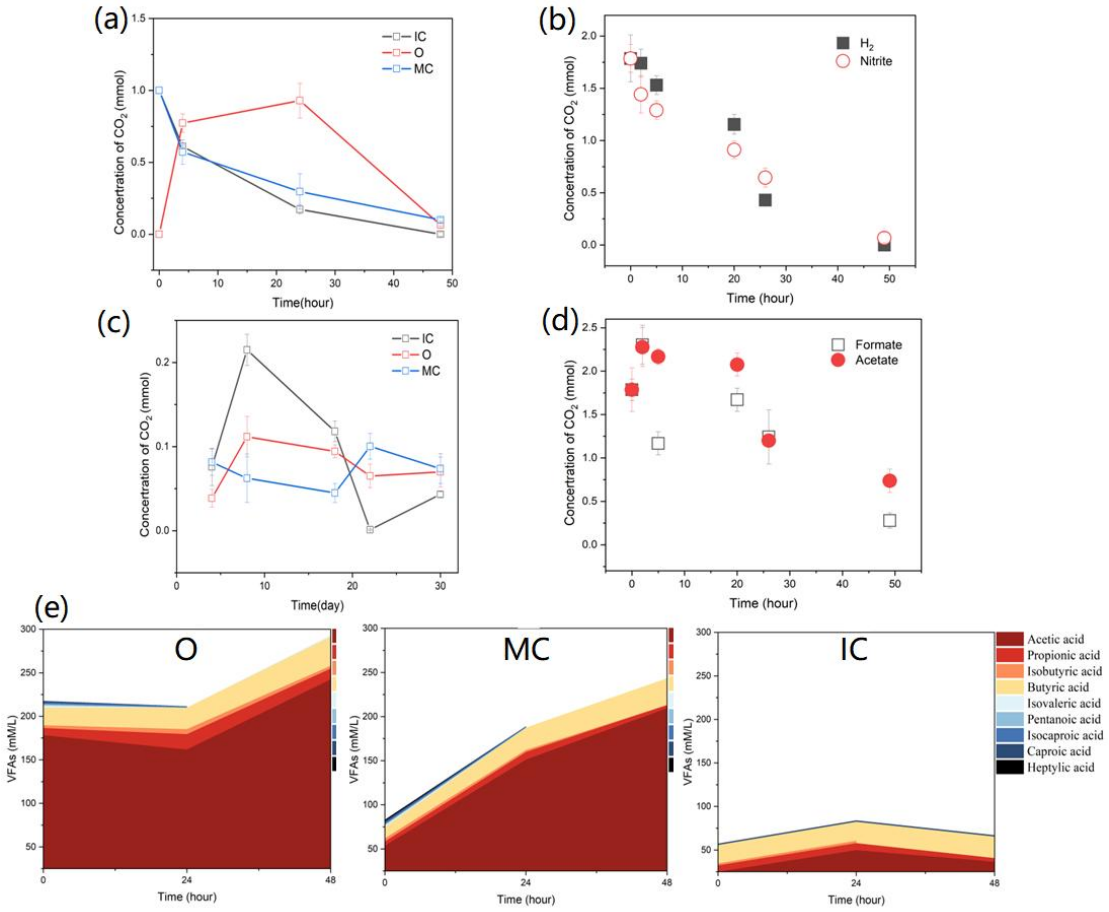

Fig. S1 Experimental 1: group with hydrogen as electron donor and different carbon sources, the concentration of CO_2 changed in one cycle and 30 days, (a and b). Experimental 2: group with carbon dioxide as carbon source and different electron donors (hydrogen, nitrite, formic acid, acetic acid), the changes in CO_2 concentrations in different groups over a 48-day period (c and d). Changes in VFAs concentrations in Experimental 1 reactor over a 48-day period (e). Organic carbon source (O), inorganic carbon source (IC) and mixed carbon source (MC).

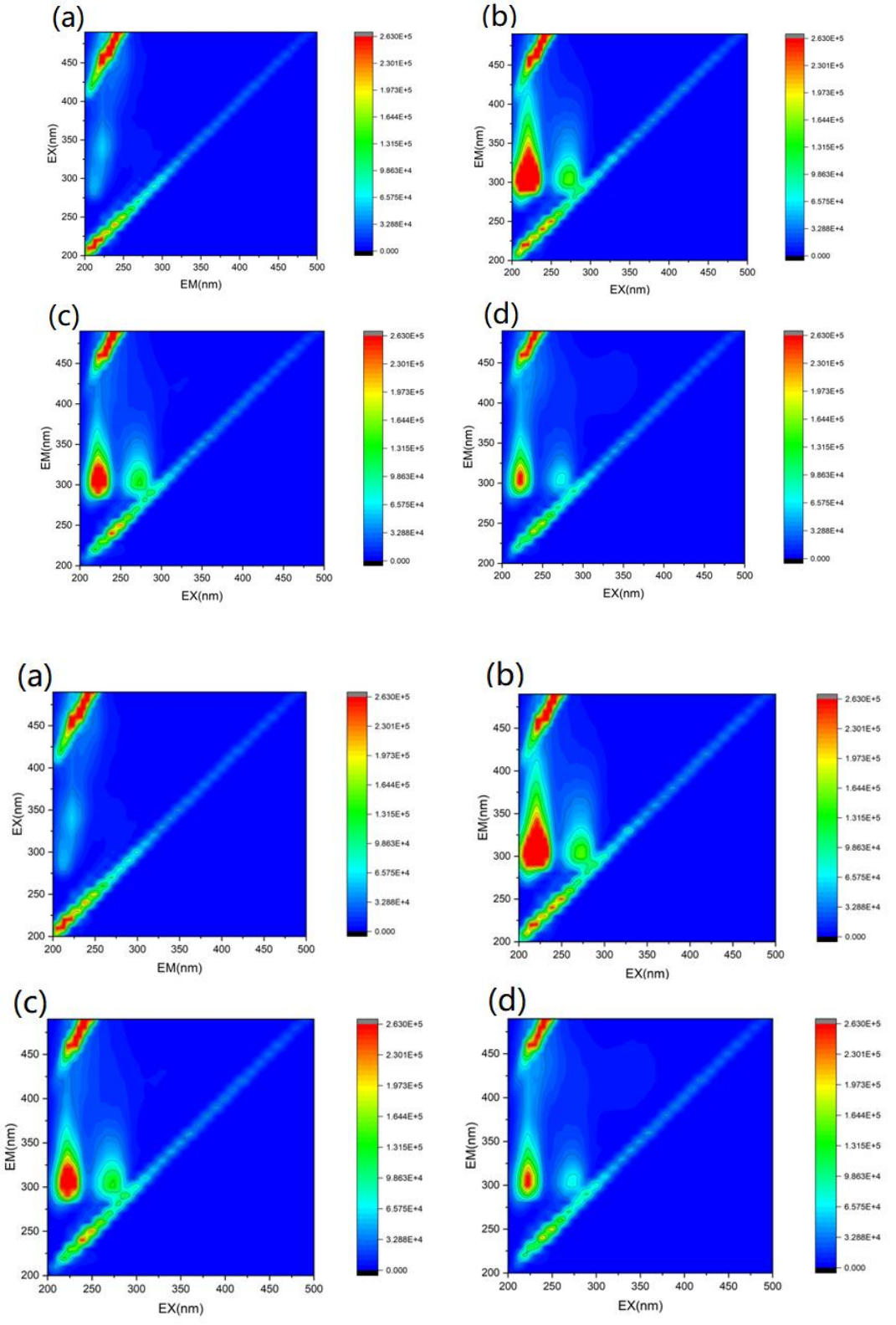

Fig. S2 Comparison of fluorescence intensity of microbial metabolites and tyrosine in each group, microbial initial state (a), group O: glucose only as substrate (b), group IC: CO_2 only as carbon source (c), group MC: glucose and CO_2 as mixed carbon source (d).

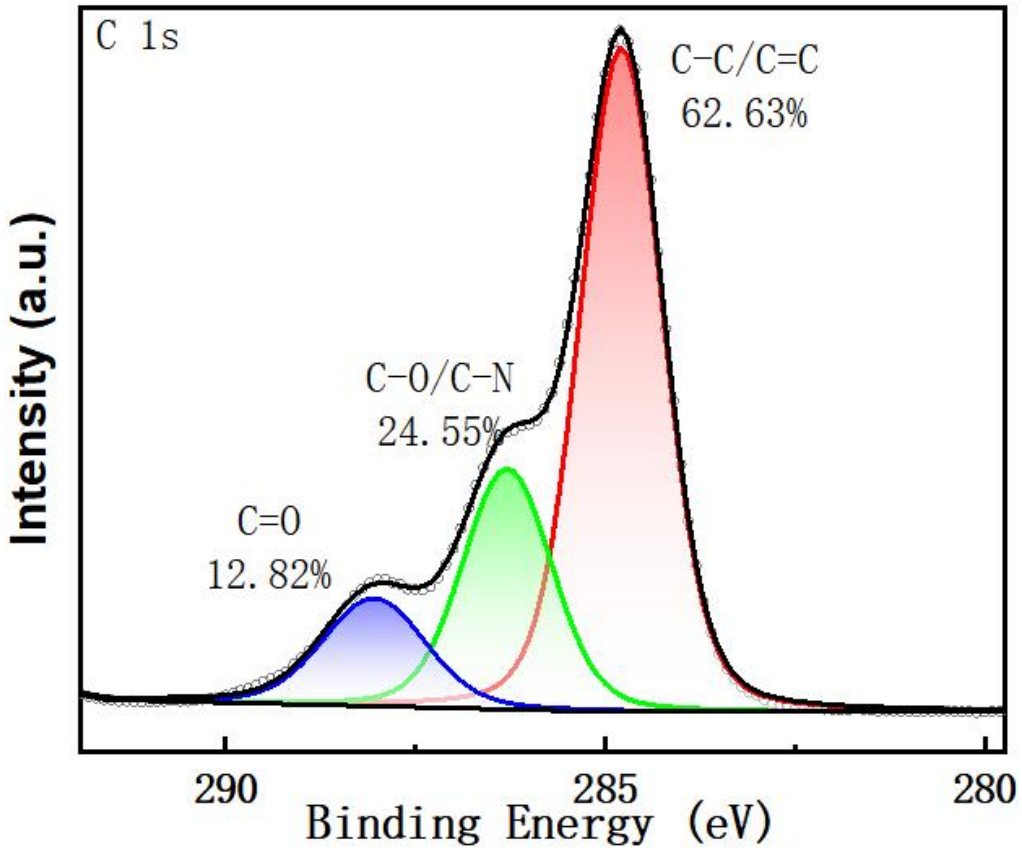

Fig. S3 XPS of C 1s for amorphous carbon.

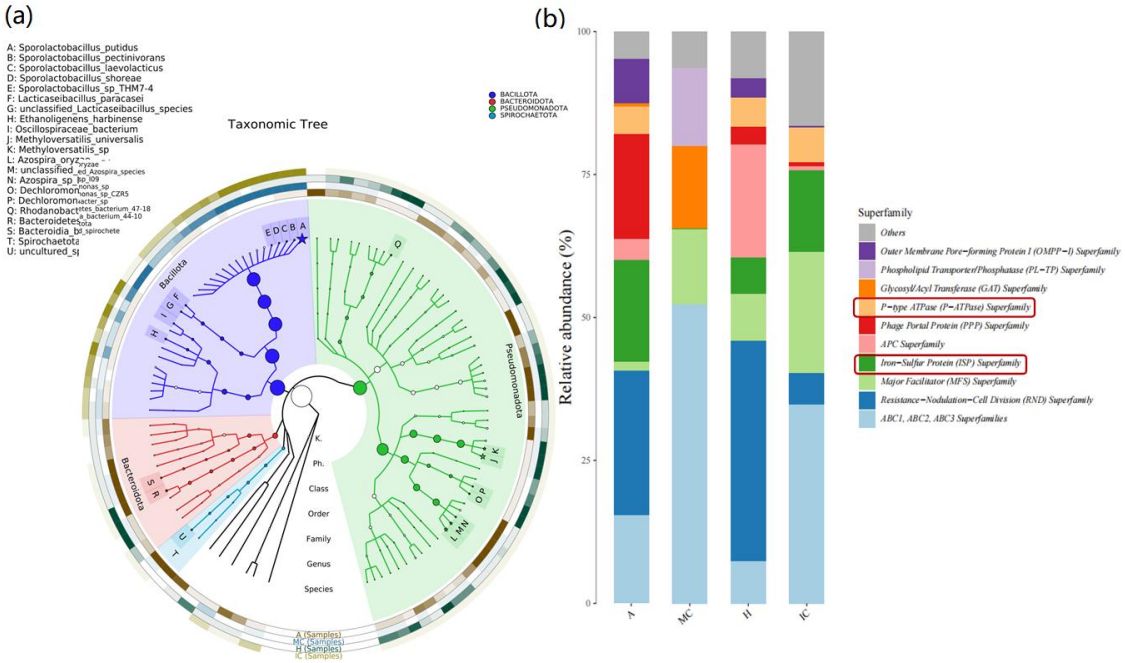

Fig. S4 Taxonomic Tree (a), Annotated proteins in TCDB database (b).

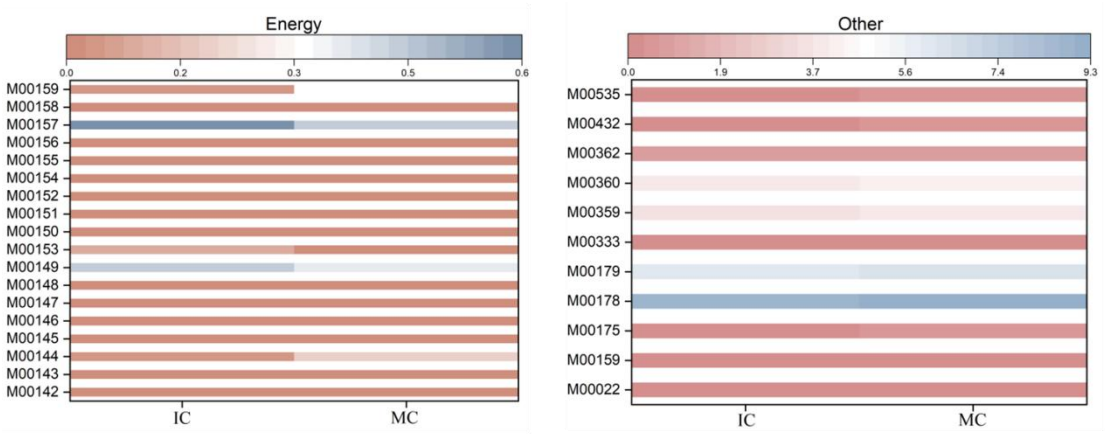

Fig. S5 abundance comparison of energy and other gene

Table S1 Elemental composition derived from XPS analysis of amorphous carbon.

Name	Peak BE	FWHM (eV)	Area (P) (CPS.eV)	Atomic (%)
O1s	532.06	4.05	1173134.16	27.48
C1s	285.22	4.37	1016192.39	59.74
N1s	399.95	3.71	128117.32	4.77
Si2p	102.13	3.82	72326.19	4.34
Na1s	1071.81	3.73	107750.72	1.13
Al2p	74.09	3.7	15975.13	1.52
Ca2p	348.58	7.42	55236.4	0.56
Zn2p3	1022.17	4.3	38098.7	0.19
Cl2p	198.69	4.18	12465.37	0.26

Table S2 Key genes in Ethanoligenens *harbinense*

Name	Kegg	Function	Abundance (‰)	
			MC	IC
Ethanoligenens *harbinense*	K00265	gltB, Glutamate synthase (NADPH) large chain 2 L-glutamate + NADP+ = L-glutamine + alpha ketoglutaric acid + NADPH + H+	0.24	3.86
Ethanoligenens *harbinense*	K00266	gltD, Glutamate synthase (NADPH) small chain	0.46	7.11
Ethanoligenens *harbinense*	K04072	Acetaldehyde dehydrogenase/alcohol dehydrogenase	0.22	3.47

Table S3 Abundance comparison of fixed carbon pathway.

KEGG pathway	Abundance (‰)			
	A	MC	H	IC
M00173:Reductive citrate cycle (Arnon-Buchanan cycle)	187.01	81.75	153.49	102.29
M00620:Incomplete reductive citrate cycle, acetyl-CoA = > oxoglutarate	63.27	2.67	35.53	20.68
M00377:Reductive acetyl-CoA pathway (Wood-Ljungdahl pathway)	24.07	29.97	20.29	32.46
M00579:Phosphate acetyltransferase-acetate kinase pathway, acetyl-CoA = > acetate	18.48	27.89	9.05	29.41
M00375:Hydroxypropionate-hydroxybutylate cycle	37.71	23.59	34.02	24.28
M00374:Dicarboxylate-hydroxybutyrate cycle	128.88	68.89	136.72	69.14
M00376:3-Hydroxypropionate bi-cycle	89.81	117.76	89.14	107.92
M00089:Triacylglycerol biosynthesis	25.59	7.50	24.11	9.21

Text 1

Experimental design

Take the fermentation solution form wastewater with 100 mmol/L Tris-HCl buffer pH7.4 (Sinopharm, China), wash twice, and suspend the microbial in the same buffer. The centrifuge tube containing bacterial suspension was placed in an ice bath with 80 kJ/L power ultrasound, and the microbial solution was filtered out with a 20 μm nylon filter membrane with a concentration of about 10^8. Under the strict hypoxia condition of 37℃, the gas phase was 100% N_2.

Text 2

Extraction and analysis of black materials containing amorphous carbon

The moist cell microspheres collected by centrifugation were washed with water, and the cell proteins were removed with 2 mL of 1 mol/L sodium hydroxide (AR, Sinopharm, China). Heat at 100℃ for 1 min and repeat 3 times. The resulting black particles are then treated with 1 mol/L hydrochloric acid (AR, Sinopharm, China) at 100℃ for 5 min to remove insoluble iron-bearing minerals from the sample. The samples with black particles still present after 1 mol/L hydrochloric acid (AR, Sinopharm, China) treatment were treated and further analyzed for the presence of elemental carbon. Therefore, the black particles are washed three times with water to remove the salt, then with methanol, and then dried at 100℃.

Text 3

Microbial calorimetry

The 1 mol/L (saturated concentration) ^{13}C-dissolved inorganic carbon (DIC) (MERYER, China) was configured with PBS and the concentrated sample was washed and suspended with PBS. The 500 μL sample was added to the reactor, the buffer solution was added to the other reactor, and the temperature of the detection chamber was set at a constant 35℃. The change of heat value in the process of reaction was measured, ATP and calorimetric respiration ratio were calculated.

Text 4

Isotope and polymer testing

^{13}C-labeled soluble inorganic carbon was added as the only carbon source to the reactor starved for 48 h, and atmospheric pressure H_2 was injected as the headspace. One week after the reaction, the amorphous carbon was extracted and the elemental carbon was determined by elemental analysis isotope mass spectrometry 7890B/Pegsus (BTLECO, USA).

The ^{13}C labeling results in isocitric acid, Alpha-ketoglutaric acid and other organic acids in roTCA cycle were measured by gas chromatography-time-of-flight mass spectrometer 7890B/Pegsus BT (LECO, USA). The polymers were tested using matter-assisted laser desorption

tandem time-of-flight mass spectrometry MALDI TOFTOF 7090 (Kratos Shimadzu, Shimadzu, Japan), the characteristic peaks were compared with TAGs configured with chloroform, and the species of biopolymers were predicted by combining the molecular weight of the repeating units and the potential monomers labeled with isotopes.

Text 5

Metagenomics

First, megahit was used to combine multiple books and obtain the initial contig sequence. The clean reads map was then used to concatenate the results using bowite2, unmapped reads were extracted and concatenated again using SPAdes to obtain low abundance contigs. Combined with the preliminary splicing results, the complete contigs assembly results were obtained. The Metawrap package was used to complete Bin sorting, Bin purification, Bin quantification, Bin reassembly and Bin identification in turn. After filtration, a single bacterial genome sketch with high integrity and low pollution was obtained. ORF prediction was performed on the splicing results by Prodigal. Genes with a length greater than or equal to 100 bp were selected and translated into amino acid sequences. The species annotation information and functional annotation information of genes were obtained by comparing gene sets with NR, KEGG, TCDB and other databases. Functional abundance and species abundance were obtained according to gene set abundance.

Text 6

Thermodynamic conditions support by electron bifurcation

The microorganisms from IC were concentrated 2.5 times to 10^4 cell/mL by PBS (Sinopharm, China). The reaction heat value was measured when the concentrated bacterial solution was added to saturated sodium bicarbonate solution. The microbes in the dissolved inorganic Carbon (DIC) group were inactivated by ultraciolet sterilization and gravity. Saturated sodium bicarbonate solution configured with PBS was the background value of M + DIC and 2M + DIC, and PBS was the background value of M and DIC. P0 is initial heat dissipation power, characterizes the basal metabolic activity and size of the microbial community. The basal metabolic activity of the microbial community is lower when carbon source free. The amplitude of the maximum signature peak (Pmax) and the time of registration of the maximum signature peak (Tmax), represents the maximum potential activity of the microbial community and the response speed of the microbial community. The larger Pmax is the higher the maximum potential activity of the microbial community. The smaller Tmax is the faster the response of the microbial community (Fredua-Agyeman and Gaisford, 2019). After exposure to inorganic carbon, the response rate of the microbial community decreased (1.58 h to 16.6 h) and the maximum potential activity increased (7.74 μW to 13.3 μW). QT, the total heat dissipation of the culture process, characterizing the total heat effect of the metabolic process, the larger QT indicates the more heat release of the metabolic process.

References

Fredua-Agyeman M, Gaisford S (2019). Assessing inhibitory activity of probiotic culture supernatants against Pseudomonas aeruginosa: A comparative methodology between agar diffusion, broth culture and microcalorimetry. World Journal of Micorbiology and Biotechnology, 35(3): 49