Supporting Information

Exploit Carbon Materials to Accelerate Initiation and Enhance Process Stability of CO Anaerobic Open-Culture Fermentation

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This Supporting Information consists of twelves pages, three figures, and one table.

List of Texts, Figure and Table Captions

Text S1. Experiment description.

1) Basic nutrient solution per liter

The basic nutrient solution per liter was composed of: 0.1 g of magnesium chloride ($MgCl_2 \cdot 6H_2O$), 0.075 g calcium chloride ($CaCl_2 \cdot 2H_2O$), 0.02 g ferrous chloride ($FeCl_2 \cdot 4H_2O$), 0.53 g ammonium chloride (NH_4Cl), 0.1 g sodium sulfide ($Na_2S \cdot 9H_2O$), 2.77 g dipotassium phosphate (K_2HPO_4), 2.8 g monopotassium phosphate (KH_2PO4), 0.1 g yeast extract, 5 mL trace element solution, and 2 mL vitamin solution. The compositions of the trace element and the vitamin solution are described in Lin et al.[1].

2) The methods for detecting gas components, VFAs and alcohols

Gas components (H_2, CH_4, CO, CO_2, O_2, N_2) were measured using a Gas Chromatography (Trace 1300, Thermo Fisher Scientific, USA) equipped with a Thermal Conductivity Detector and a 5 m × 2.1 mm Carboxen-1000 column with argon as the carrier gas. The column temperature was held at 60 ºC for 7 min and increased to 225 ºC at a rate of 60 ºC per min. The concentration of volatile fatty acids (VFAs, including formate, acetate, propionate, n-butyrate, iso-butyrate, n-valerate, and iso-valerate) was measured by a high-performance liquid chromatography (LC-20AD, Shimadzu, Kyoto, Japan) with a COD-10AVP conductivity detector and a Shim-pack SPR-H analytical column. To measure alcohols (including methanol, ethanol, propanol, iso-butanol, and butanol), a gas chromatography (Focus GC, Thermo Scientific Co., Waltham, MA, USA) equipped with a Flame Ionization Detector and a 30 m × 0.53 mm HP-FFAP fused silica capillary column was used. The column operating temperature was held at 40 °C for 2.5 min, then increased by

12 °C/min to 100 °C and then held for 2 min.

3) The experiment to evaluate CO-liquid mass transfer efficiency

Six additional bottles were prepared in the same manner as described for the above reactors to determine the enhancement of carbon materials on CO-liquid mass transfer efficiency. Five grams of the five different carbon materials were added to the bottles separately. In order to rule out the effects of microbial utilization on CO dissolution, the six bottles without inoculum were purged with nitrogen to create an oxygen-free environment. Gas circulation was run for 6 hours which is equivalent to the ventilation cycle time. The amount of CO dissolved in the liquid was calculated by subtracting the vapor-phase gas concentration measured over the course of the experiment from the initial concentration of the gas in the experimental apparatus. At this point, it can be determined whether or not the addition of the carbon material can increase the amount of dissolved CO relative to the blank group.

Text S2 Principal component analysis.

Quadrant distribution and distribution distance can be used to assess similarities, if samples are in the same quadrant and the distribution distance of them is closer, they can be considered similar in nature. Therefore, microbiomes from different fractions at different p_{CO} are clustered into three groups according to the quadrant distribution and distribution distance in Figure S4. 1) The samples from the solid fractions, relating to higher abundance of *Methanosaeta*; 2) The samples from the liquid fractions in stage II or stage III (p_{CO} = 0.21 or 0.41 atm), relating to

WCHB1-69, Rhodocyclaceae, and Hydrogenophilaceae; 3) The samples from the liquid fractions in the final stage when p_{CO} was 0.69 atm, relating to Synergistaceae.

Reference:

[1] Lin Y, Lü F, Shao L, He P. Influence of bicarbonate buffer on the methanogenetic pathway during thermophilic anaerobic digestion. Bioresource Technology. 2013;137:245-53.

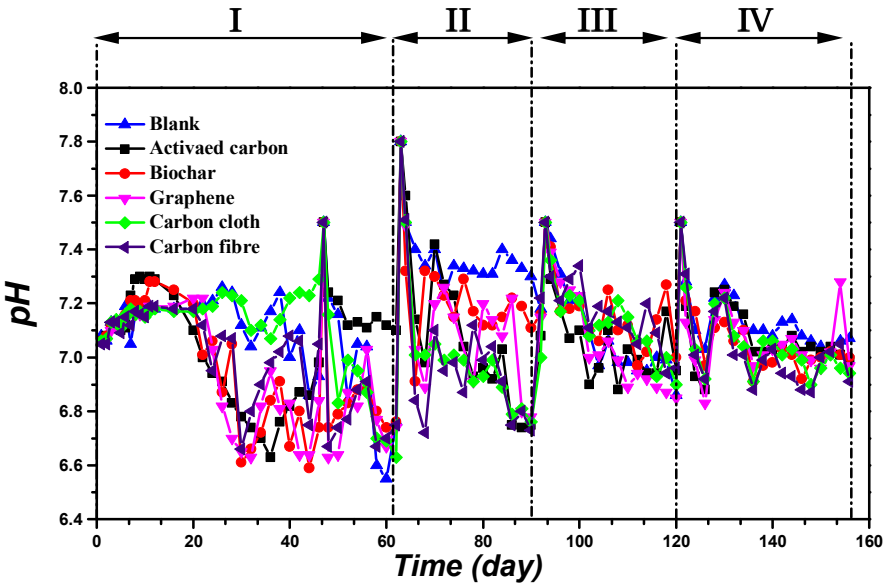

Figure S1 Changes in pH in four stages. According to the p_{CO}, the experiment was divided into four stages: I (0–62 d), II (63–92 d), III (93–120 d), and IV (121–156 d), corresponding to an initial p_{CO} of 0.07 atm, 0.21 atm, 0.41 atm and 0.69 atm, respectively.

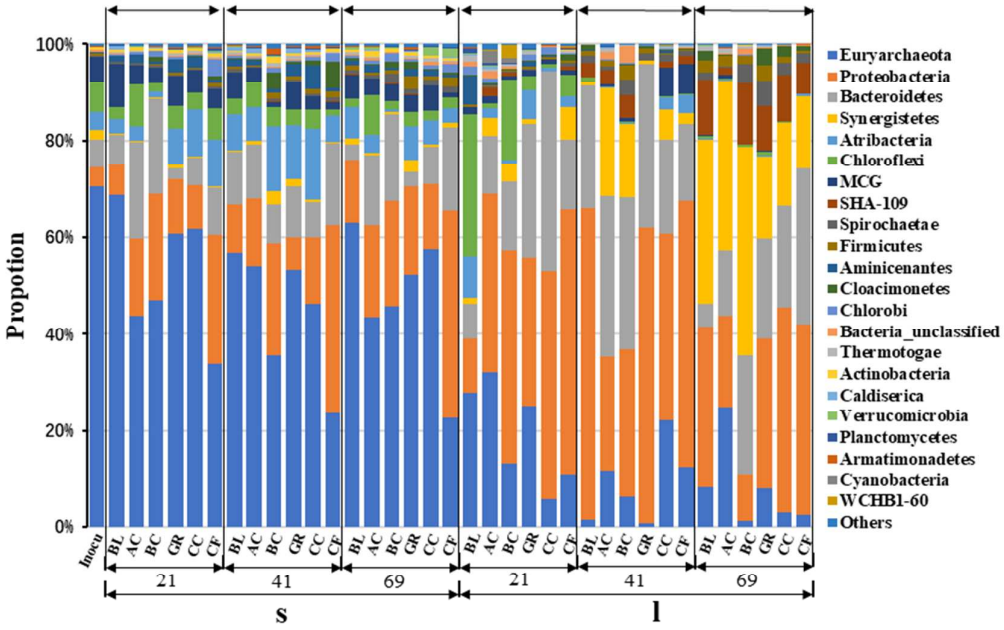

Figure S2 Taxonomic classification of the microbial communities in phyla level. "s" and "l": the fractions of sediment and liquid; "21" (0.21 atm p_{CO}), "41" (0.41 atm p_{CO}) and "69" (0.69 atm p_{CO}): different stages with p_{CO}; "BL": blank without additives, "AC": addition with carbon material activated carbon, "BC": biochar, "GR": graphene, "CC": carbon cloth, "CF": carbon fibre; "Inocu": the inoculum.

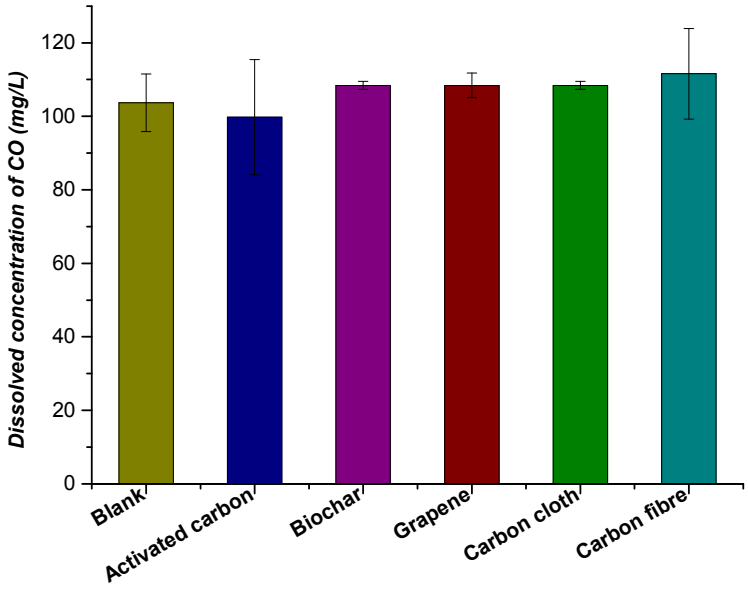

Figure S3 Concentrations of dissolved CO in water with existence of five different carbon materials.

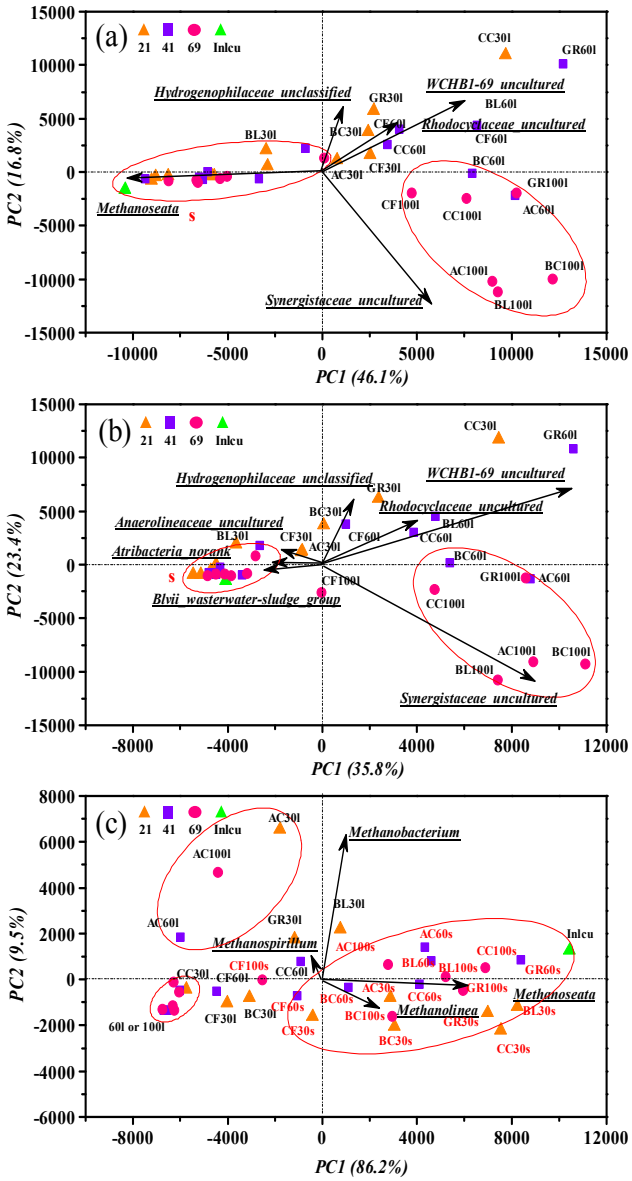

Figure S4 Principal component analysis. (a) total microbiomes, (b) bacteria, (c) archaea. "s" and "l": the fractions of sediment and liquid; "21" (0.21 atm p_{CO}), "41" (0.41 atm p_{CO}) and "69" (0.69 atm p_{CO}): different stages with p_{CO}; "BL": blank without additives, "AC": addition with carbon material activated carbon, "BC": biochar, "GR": graphene, "CC": carbon cloth, "CF": carbon fibre; "Inocu": the inoculum. According to the p_{CO}, the experiment was divided into four stages: I (0–62 d), II (63–92 d), III (93–120 d), and IV (121–156 d), corresponding to an initial p_{CO} of 0.07 atm, 0.21 atm, 0.41 atm and 0.69 atm, respectively.

Table S1 Parameters related to microbial community diversity.

Sample ID	Reads	Parameters			
		OTU	Chao*	Coverage	Simpson†
Inocu	35816	280	332	0.9982	0.2253
AC30s	31503	297	394	0.9970	0.1410
BC30s	29522	292	434	0.9965	0.1562
BL30s	32806	258	387	0.9971	0.2039
GR30s	32031	330	424	0.9968	0.1859
CC30s	35351	293	439	0.9971	0.1757
CF30s	30793	368	488	0.9965	0.0675
AC60s	33229	359	462	0.9969	0.1202
BC60s	32420	433	548	0.9966	0.0843
BL60s	31902	340	474	0.9964	0.1377
GR60s	42347	391	486	0.9975	0.1399
CC60s	37560	394	494	0.9970	0.1049
CF60s	38720	376	489	0.9972	0.0562
AC100s	34714	427	521	0.9971	0.0935
BC100s	29595	383	487	0.9962	0.1271
BL100s	29243	352	481	0.9961	0.156
GR100s	34256	407	582	0.9961	0.1421
CC100s	41028	376	552	0.9970	0.1195
CF100s	40365	391	492	0.9974	0.0557
AC30l	43074	385	474	0.9978	0.0630
BC30l	38427	252	343	0.9980	0.1002
BL30l	43869	294	419	0.9979	0.1321
GR30l	40633	302	379	0.9980	0.1128
CC30l	42724	236	298	0.9983	0.2447
CF30l	36592	243	355	0.9978	0.1015
AC60l	35186	248	338	0.9977	0.1542
BC60l	29907	215	313	0.9976	0.1134
BL60l	33640	175	235	0.9985	0.1353
GR60l	43355	184	290	0.9987	0.2642
CC60l	41843	253	375	0.9981	0.0762
CF60l	29933	200	303	0.9975	0.0922
AC100l	43830	215	278	0.9985	0.1476
BC100l	39727	228	415	0.9978	0.1963
BL100l	41748	213	279	0.9986	0.1322
GR100l	42970	243	307	0.9983	0.0950
CC100l	31482	224	313	0.9977	0.0900
CF100l	33999	203	243	0.9984	0.1460

* Chao: Chao1 algorithm is used to estimate the number of OTU samples contained in the index. Chao1 is commonly used in ecology to estimate the total number of species, first proposed by Chao (1984) (http://www.mothur.org/wiki/Chao).

† Simpson: Simpson is used to estimate one of the microbial diversity indices in the sample, proposed by Edward Hugh Simpson (1949), is commonly used in ecology to quantitatively describe a region's biodiversity. The larger the Simpson index, the lower the diversity of the community (http://www.mothur.org/wiki/Simpson).