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Supplementary information

Cultivation of previously uncultured microorganisms with a continuous-flow down-flow hanging sponge (DHS) bioreactor, using a syntrophic archaeon culture obtained from deep marine sediment as a case study

In the format provided by the authors and unedited

Supplementary information

This file contains Supplementary Table 1, Supplementary Note 1, and Supplementary Figures 1–8.

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Supplementary Table 1 | Calculation of HRT and gas flow rate in DHS reactor.

<Sponge volume>

	Thickness (cm)	Length (cm)	Width (cm)	No. of sponges	Total sponge volume (cm^3)	Total sponge volume × void ratio (cm^3)	Total sponge volume × void ratio (L)
Sponge cubes	3	3	3	22	594	477.0	0.4770

<Medium HRT>

HRT (h)	Medium flow rate			1 min-on/9 min-off (mL/min)
	L/h	L/d	mL/min	
1	0.4770	11.45	7.95	79.50
2	0.2385	5.72	3.97	39.75
3	0.1590	3.82	2.65	26.50
4	0.1192	2.86	1.99	19.87
5	0.0954	2.29	1.59	15.90
6	0.0795	1.91	1.32	13.25
7	0.0681	1.64	1.14	11.36
8	0.0596	1.43	0.99	9.94
9	0.0530	1.27	0.88	8.83
10	0.0477	1.14	0.79	7.95
11	0.0434	1.04	0.72	7.23
12	0.0397	0.95	0.66	6.62
13	0.0367	0.88	0.61	6.12
14	0.0341	0.82	0.57	5.68
15	0.0318	0.76	0.53	5.30
16	0.0298	0.72	0.50	4.97
17	0.0281	0.67	0.47	4.68
18	0.0265	0.64	0.44	4.42
19	0.0251	0.60	0.42	4.18
20	0.0238	0.57	0.40	3.97 --->4 mL/min
21	0.0227	0.55	0.38	3.79
22	0.0217	0.52	0.36	3.61

<Methane gas flow rate in 20 h of medium HRT>

0.57 L medium is used at 1 day.

The medium contained 16.7 mM sulfate.----> 16.7 × 0.57 = 9.519 mmol/d (=9.519 mmol sulfate is provided to the reactor at 1 day).

CH_4 volume needed at one day is caluculated on the basis of the Ideal gas law and the chemical equation of sulfate-dependent anaerobic methane-oxidation (cultivation temperature is 10°C, and reactor operation is under atmospheric pressure).

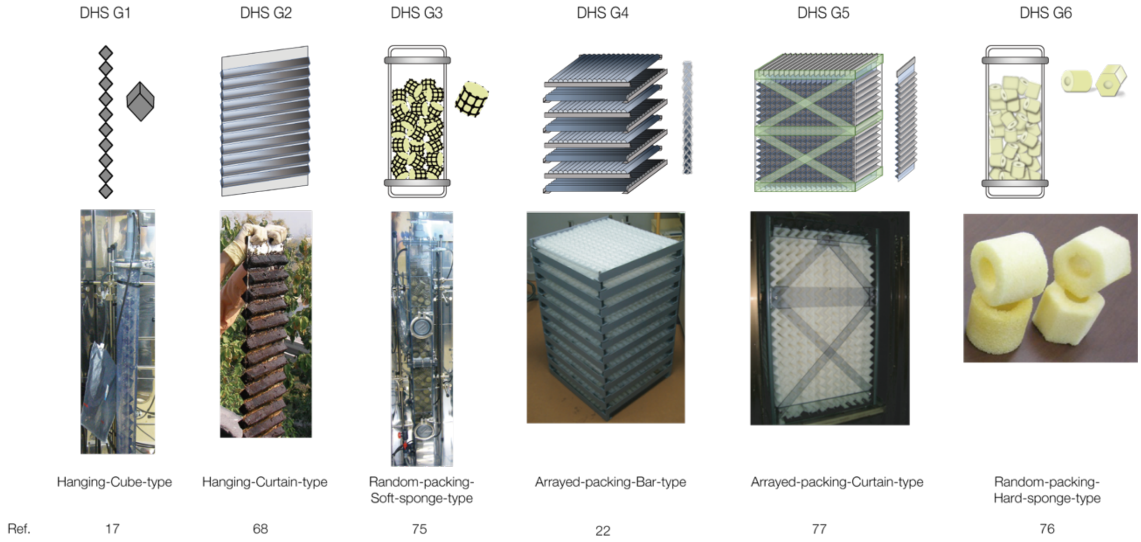
---->9.519 × 0.0821 × 283 = 220.90 -> 220.90 mL-CH_4 is needed in 1 day.

A mixture gas of CH_4 and CO_2 (95:5, v/v) was used in our study, thus 220 × 1.05 = 231.9 mL-CH_4/d is provided.

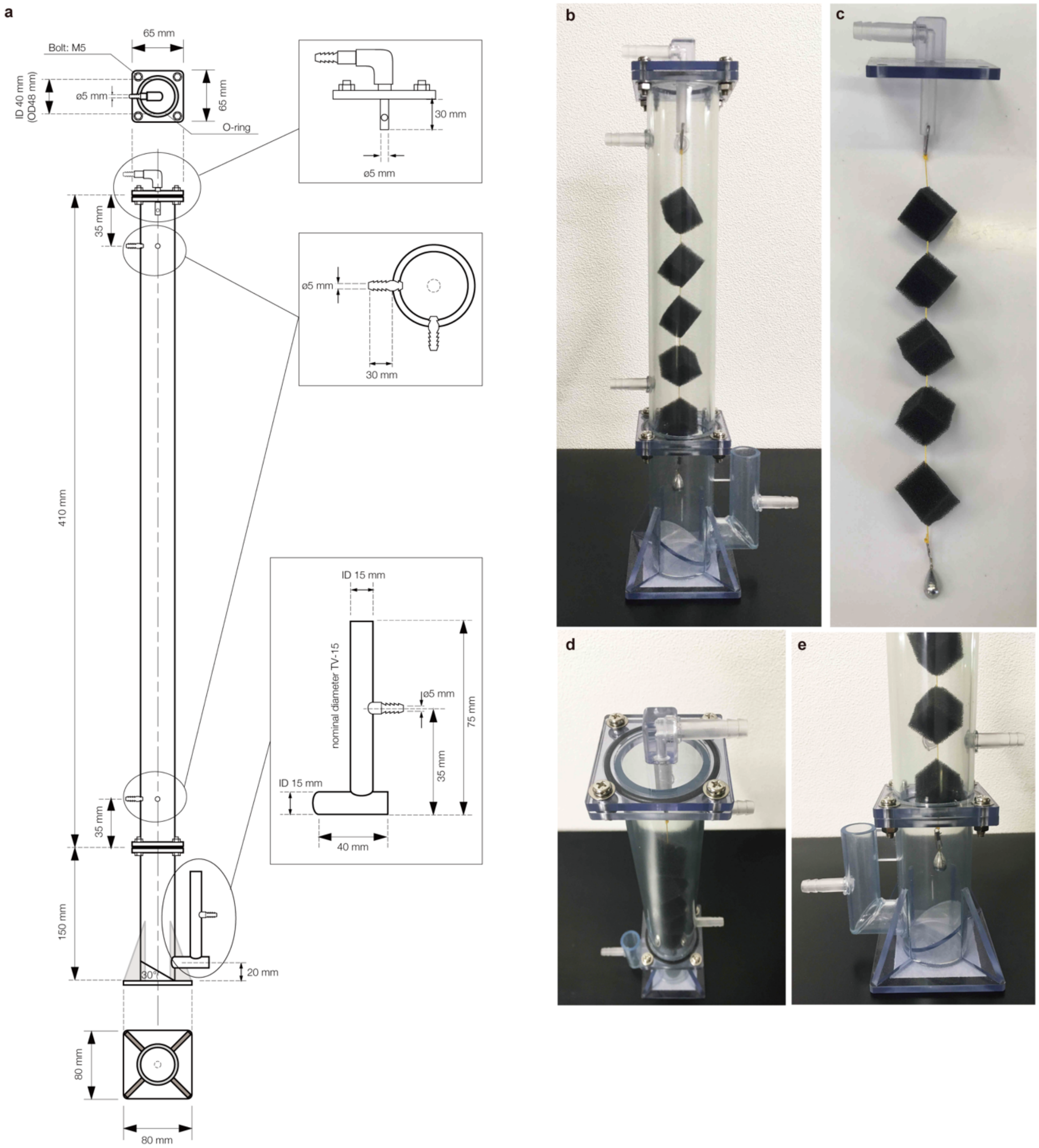
Consequently, the CH_4/CO_2 mix gas is provided at approximately 240 ml/d (=0.01 L/h). ---> 1.7 mL/min (1min-on/9 min-off)

Supplementary Note 1

How to determine the medium flow rate, and frequency of medium replacement. The flow rate of the medium (*i.e.*, HRT) was determined by how often the person running the reactor can make the medium. In the beginning of our experiment, 6 L of the anaerobic medium was made every 10 days, but now 10 L is made at approximately every 17 days. The frequency of medium preparation can be as often as once per a week, but a frequency of every 10 days or longer allowed a more feasible schedule around business trips or other unavoidable events. If the medium is prepared weekly, we recommend doing so on Thursday because preparing the medium on a weekday close to a weekend was a safer choice enabling us to avoid accidents (*e.g.*, we can remake the anaerobic medium on Friday if the medium made on Thursday is found to be unable maintain anaerobic conditions). It is possible to prepare the medium every three weeks or even once a month, but we do not recommend reducing the frequency as the anaerobic state must be maintained.

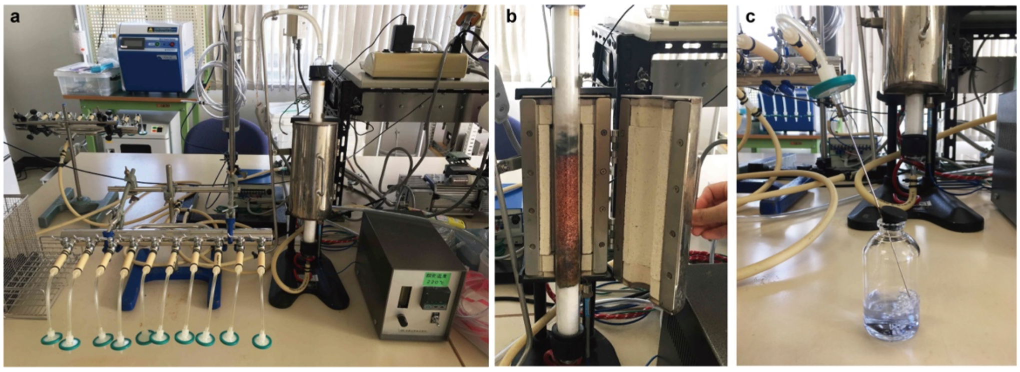


Supplementary Fig. 1| All types of DHS reactor developed so far. The first published papers for each reactor type are shown as references. The review papers[18,21] about the DHS reactor summarize the detail for each reactor type.



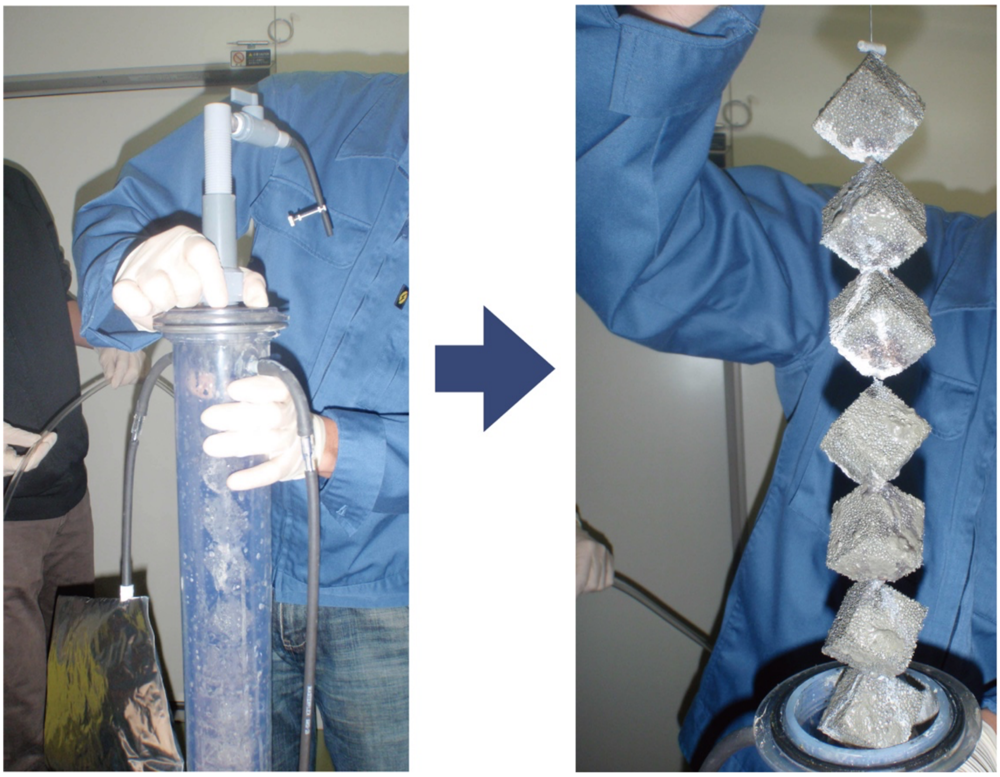
Supplementary Fig. 2| A small size DHS bioreactor. a, Schematic diagram. Note that the scale is not exact. **b–e**, Photomicrographs of a small size DHS reactor. View of the entire reactor (**b**), top part of the reactor and sponge line (**c**), upper view of the reactor (**d**), and bottom part of the reactor (**e**).

Supplementary Fig. 3| Aluminum bag and clamp. The image shows that a clamp is attached to the flow path between the influent gas bag and the reactor column.

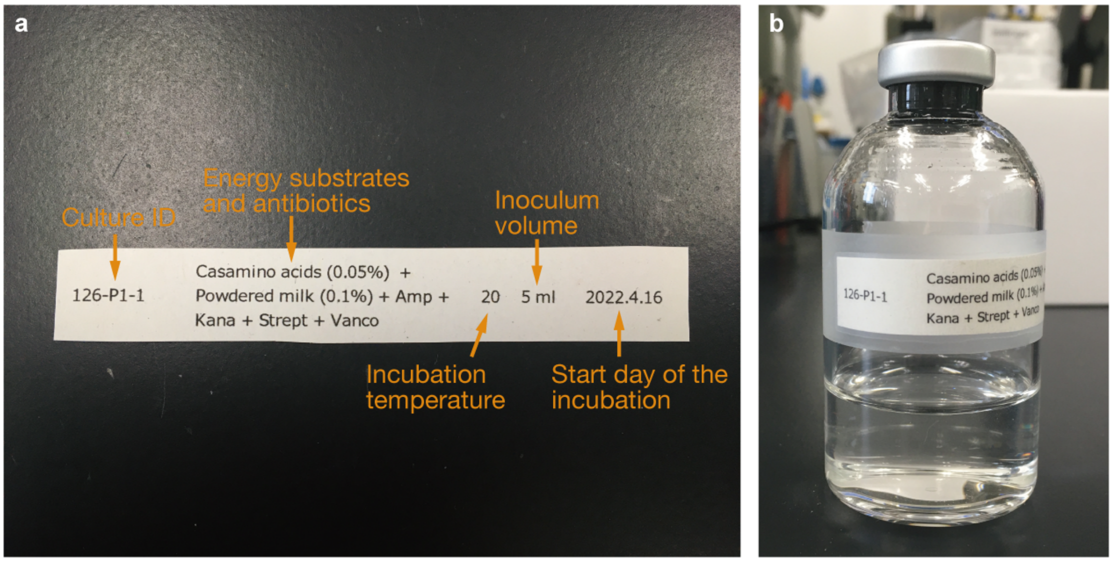


Supplementary Fig. 4| Oxygen-free gas supply system. a, whole view of the system. **b,** copper column, **c**, N_2 sparging while putting the long stainless needle into the medium

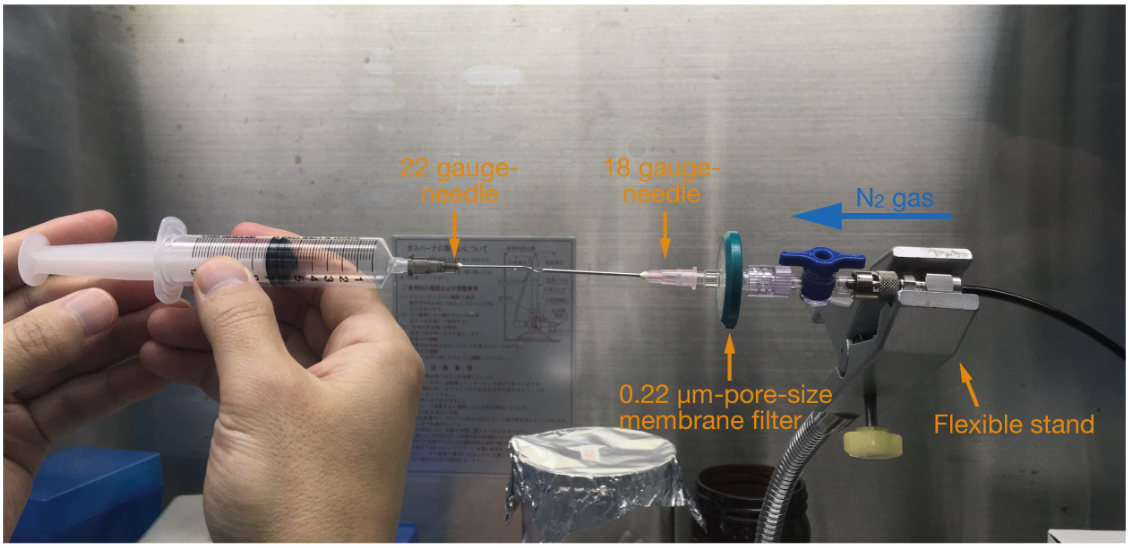
Supplementary Fig. 5| Effluent sampling using a 10-ml syringe attached with Viton tubing from the effluent pool in the bottom of the reactor column.



Supplementary Fig. 6| Opening the top part of the column and removing the sponge line.



Supplementary Fig. 7| The label for batch-type cultivation. a, An example of the label. b, A glass vial with the label attached with a mending tape.



Supplementary Fig. 8| Flushing a syringe with N_2 gas.