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Supplementary Material

EXPERIMENTAL STUDY OF MICROBIAL HYDROGEN CONSUMPTION RATES BY *OLEIDESULFOVIBRIO ALASKENSIS* IN POROUS MEDIA

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1. METHODOLOGY AND RESULTS

1.1. Brines

Table S1: Summary of the composition of the brines (solutions) used in the study based on the modified DSMZ growth medium solution (GM)(5).

Brine (Solution)	Species	Amount	Unit
BM	Na_2SO_4	3	g/L
	NH_4Cl	0.3	g/L
	KH_2PO_4	0.2	g/L
	NaCl	21	g/L
	$MgCl_2.6H_2O$	3	g/L
	KCl	0.5	g/L
	$CaCl_2.2H_2O$	0.15	g/L
	SL-10 solution (Koblitz, n.d.-a)	1 0.25	g/L ml/L
	Na-resazurin (0.2% w/v)	1.5	g/L
	Na_2CO_3	0.4	g/L
	$Na_2S.9H_2O$	10	g/L
	Vitamin solution (Koblitz, n.d.-b)		
NS	BM	25	ml
	2M Na-lactate	300	µL
	2M Na-acetate	250	µL
BS	NS	25	ml
	Inoculum	2.5	ml

BM = base medium solution; NS = nutrient solution; BS = bacteria solution prepared from a mixture of NS and a concentrated BS inoculum. 2M = 2-molar solution. All solutions were prepared anoxically.

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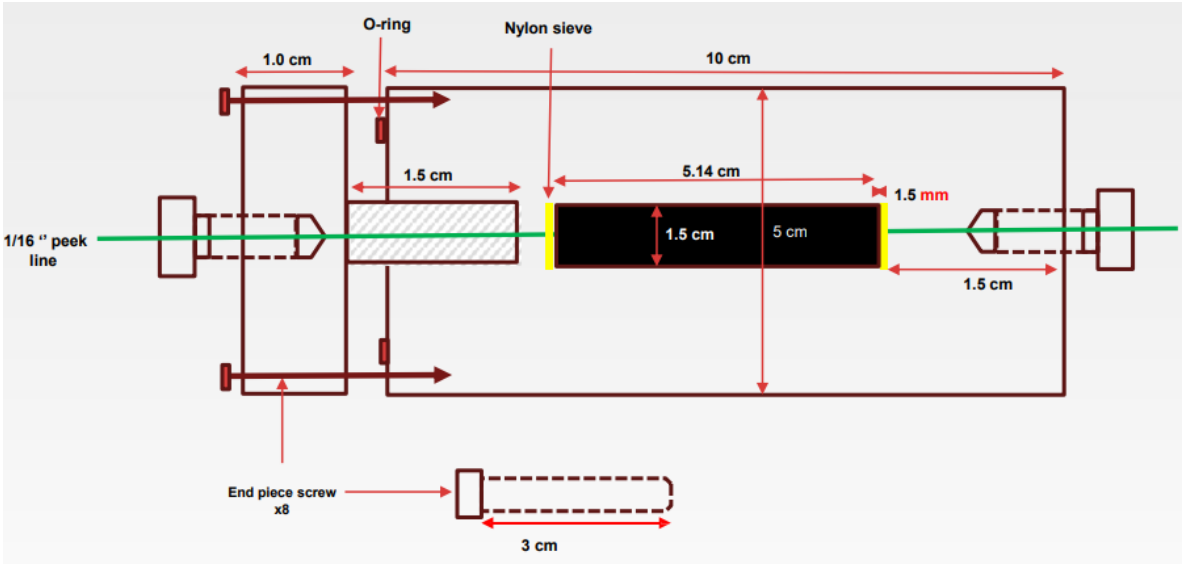

Figure S1: Details of the peek core holder used in the study.

Absolute permeability for each sand pack was measured with degassed deionized (DI) water and the base medium (BM) solution at 30 cm^3/hr and 45 cm^3/hr with the sand pack horizontally connected to the flow network (**Fig. S1**). The sand pack was kept submerged in a constant temperature water bath at 37°C using a water circulation pump. Flow lines before the sand pack were first flushed with degassed DI water through the bypass loop (**Fig. S1**). The bypass was used whenever there was a change of fluids to inject. DI water was injected with pump A, and a syringe pump (pump B) was used to inject the BM. The outlet was kept open to atmospheric pressure in the effluent collection flask. Permeability was calculated from the differential pressure measurements across the sand pack inlet and outlet measured with pressure transducers (± 0.1% FS, -1 to 2.5 barg). The viscosities of DI and BM used in the calculations were set to 0.797 cP (as pure water at 30°C and atmospheric pressure). The temperature effect on viscosity was corrected using tables accessed on the NIST Chemistry Webbook (1, 2). The injection of degassed and the BM solution created an anoxic environment conducive for inoculation of bacteria cells, adaptation and growth. Permeability was re-measured with the BM at 30 cm^3/hr after 5 days of bacteria cultivation in the bacterial sand packs following the procedure described in the next section.

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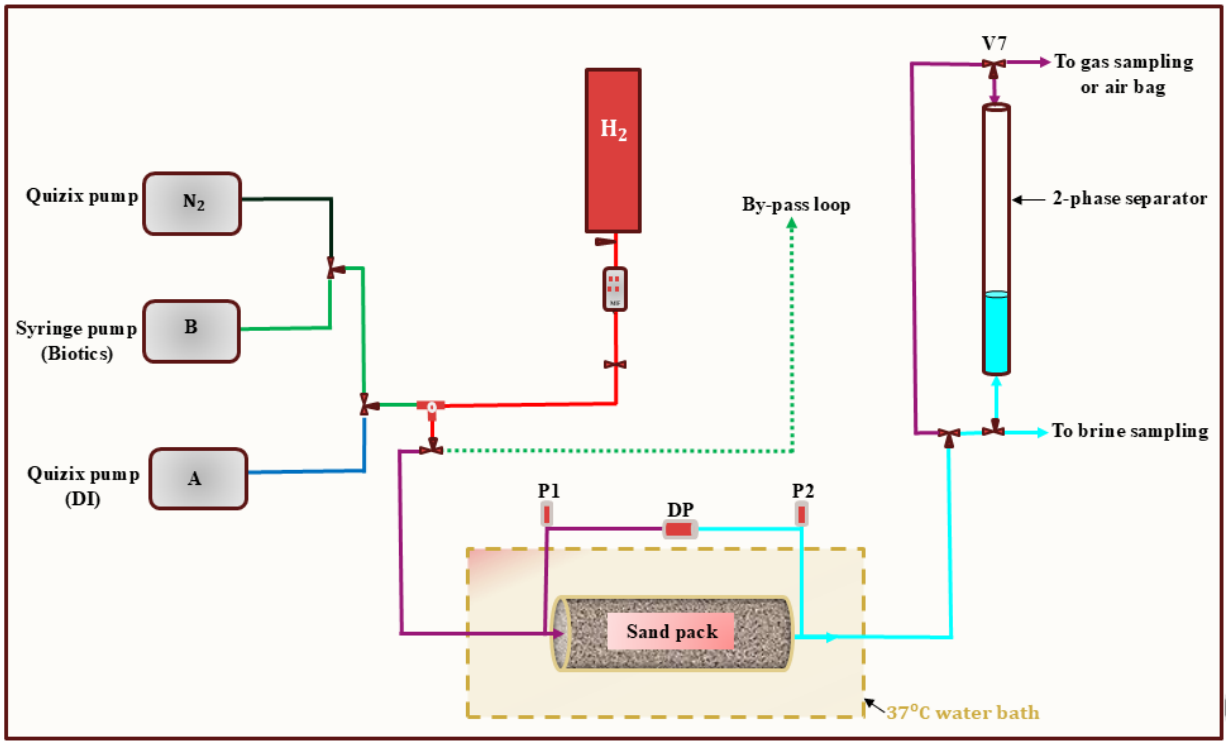

Figure S2: A schematic of the anoxic experimental set up used in the study. The anoxic environment was achieved through injecting degassed deionized (DI) water and the base medium (BM) solution in combination with a network of valves. The dead volume in the network was minimized by using 1/16-inch peek flow tubing and connections. A hot water circulation pump ensured a constant temperature environment around the sand pack conducive to the growth of *Oleidesulfovibrio alaskensis* cells. A constant injection mass rate for hydrogen was possible with the use of the mass flow controller (MFC). Continuous pressure logging was limited to the sand pack inlet and outlet ends. Brine sampling was performed at the base of the 2-phase separator. Sampling was only performed at the end of the first drainage cycle or in-situ brine at the end of each sand pack study.

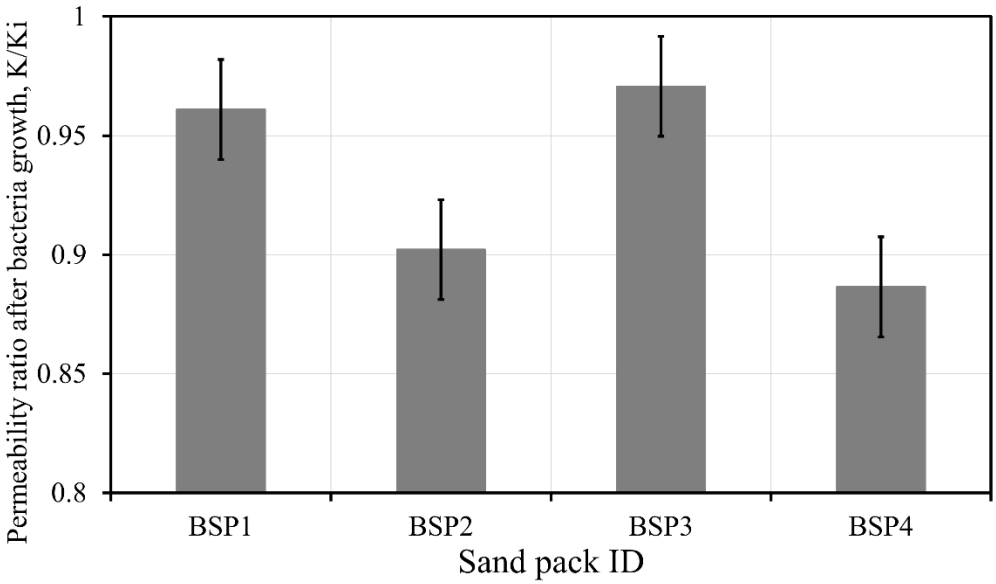

Figure S3: Permeability reduction after bacteria cultivation in non-sterile sand packs. Ki and K are the sand pack permeability before and after bacteria cultivation respectively.

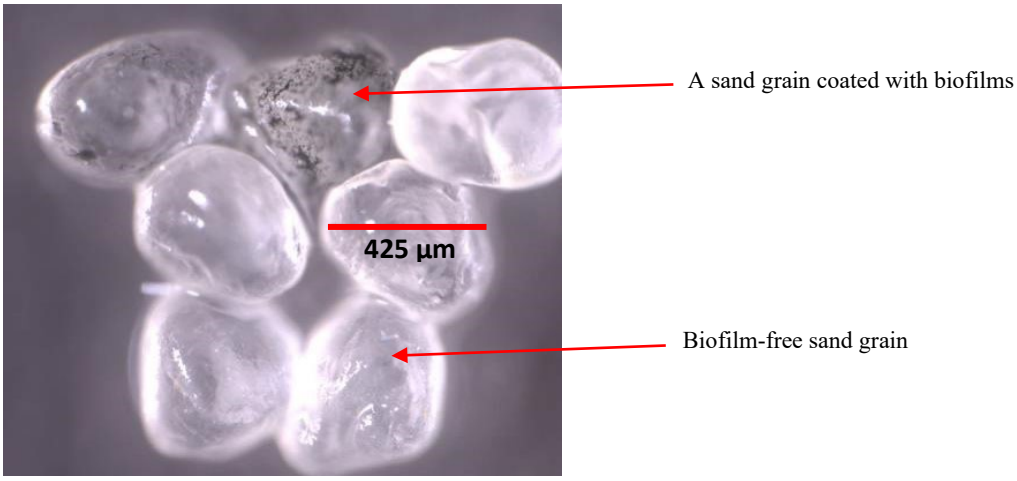

Figure S4: Sand grains (light gray) coated with organic matter (biofilms) after bacteria growth on hydrogen.

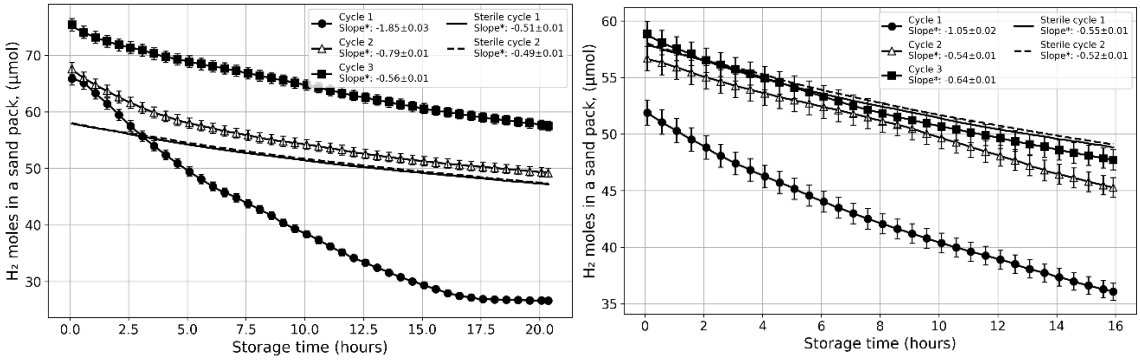

Figure S5: Evolution of the amount of hydrogen stored in each cycle for sand packs BSP1 and BSP4 against time. The solid and broken lines are moles in sterilized cycles.

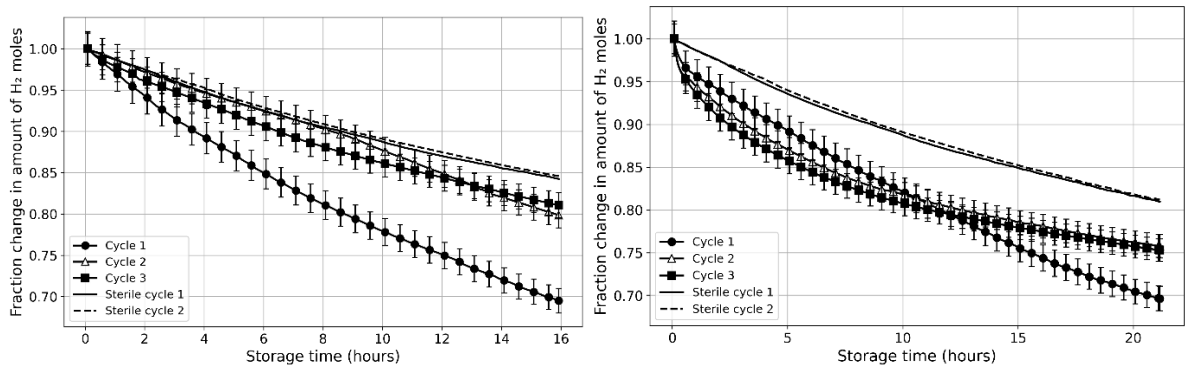

Figure S6: Relative moles in sand packs BSP1 and BSP4.

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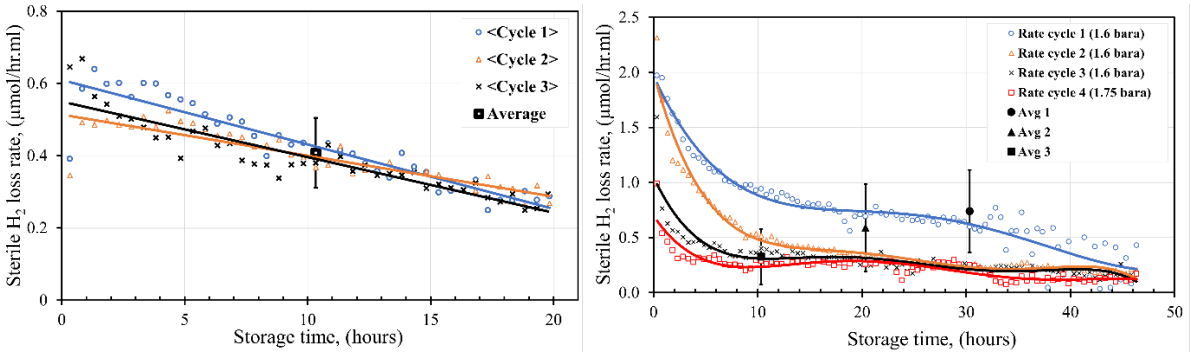

Figure S7: Total mechanical hydrogen loss rates in sterilized sand pack. **Left:** With initial pressure at 1.15 bara. **Right:** with initial storage pressure at 1.6 and 1.75 bara. The scatter points with error bars are the averages used in calculating the net microbial losses in non-sterile sand packs.

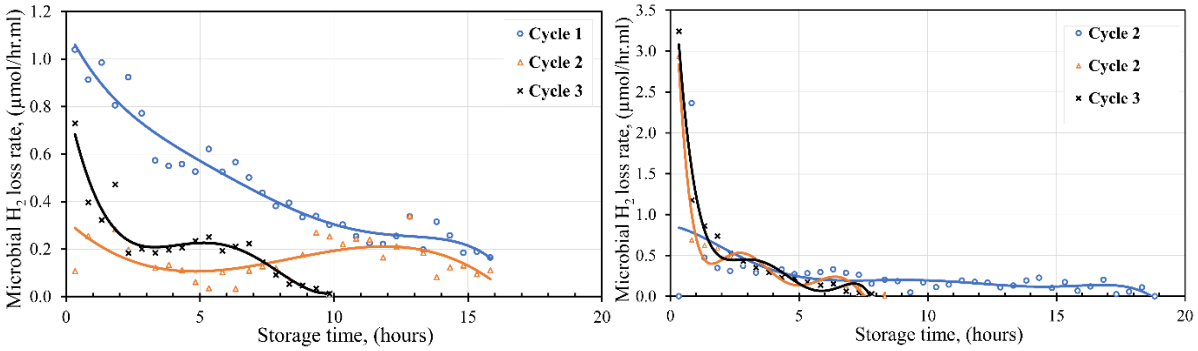

Figure S8: Microbial hydrogen loss rates in sand packs BSP1 and BSP4.

Table S2: Quantitative summary of the molar balance for the key reactants and products in **Equation 1** (from main paper). The calculations were based on the total number of hydrogen moles consumed by microbes in three storage cycles for each sand pack. BSP5 had only one storage cycle whereas BSP8 had two. The first three storage cycles in BSP7 were used in the calculations. The [ ] denotes concentration of the molecule. The relative change in sulphate ions is based on the initial sulphate concentration in each cycle.

Sand pack ID	S_w (1st cycle) (%)	$[Na_2SO_4]$ (g/l)	$[SO_4^{2-}]_{start}$ $(\mu\ mol)$	$[H_2]_{total}$ $(\mu\ mol)$	$[H_2]_{used}$ $(\mu\ mol)$	$[SO_4^{2-}]_{used}$ $(\mu\ mol)$	$[SO_4^{2-}]_{used}$ (%)
BSP1	71	3	47	167	11	2.7	5.8
BSP2	56	3	39	208	21	5.3	13.3
BSP3	58	3	41	209	42	10.5	26
BSP4	70	3	47.	175	16	4.0	8.6
BSP5	89	3	56	32	9	2.3	4.0
BSP6	82	3	53	158	17	4.3	8.0
BSP7	66	3	44	201	42	10.4	23.4
BSP8	55	3	38	195	37	9.5	24.6

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Table S1: Quantitative summary of the molar balance for the key reactants and products in **Equation 1** (from the main paper). The calculations are only for the first storage cycle in test sand packs. The calculations are based on the moles of $\boldsymbol{H_2}$ consumed. The [ ] denotes concentration of the molecule, $\boldsymbol{V_{water}}$ volume of water produced, $\boldsymbol{\Delta S_w}$ change in water saturation.

Sand pack ID	S_w (%)	$[Na_2SO_4]$ (g/l)	$[SO_4^{2-}]_{start}$ $(\mu\, mol)$	$[H_2]_{start}$ $(\mu\, mol)$	$[H_2]_{used}$ $(\mu\, mol)$	$[SO_4^{2-}]_{used}$ $(\mu\, mol)$	$[SO_4^{2-}]_{used}$ (%)	V_{water} *(produced)* $(\mu\, l)$	ΔS_w (%)
SSP2	66	3	34	58	-	-	-	-	-
BSP1	71	3	37	52	12.6	3.1	8.5	0.23	0.013
BSP2	57	3	29	68	19.3	4.8	16.5	0.35	0.025
BSP5	59	3	30	66	18.4	4.6	15.3	0.33	0.023

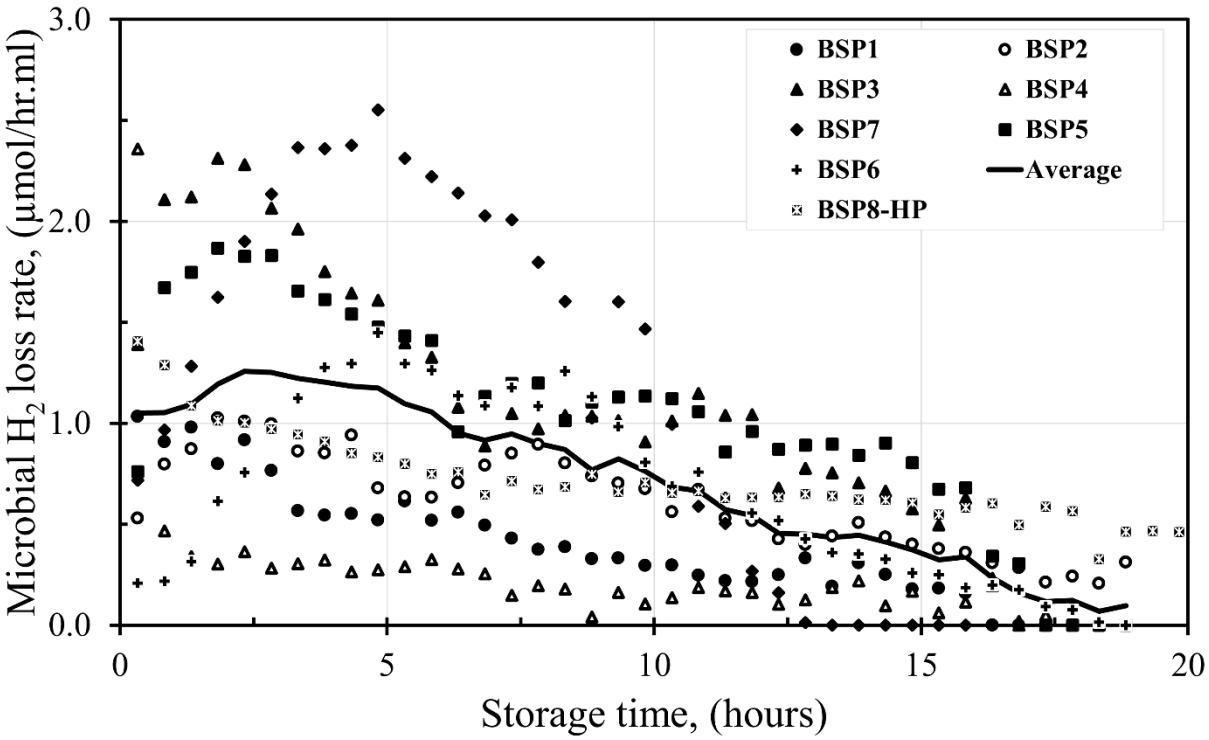

Figure S9: Microbial hydrogen consumption in the first storage cycle of all tests bacterial sand packs. The scatters are experimental measurements, and the solid line is the average of all cycles.

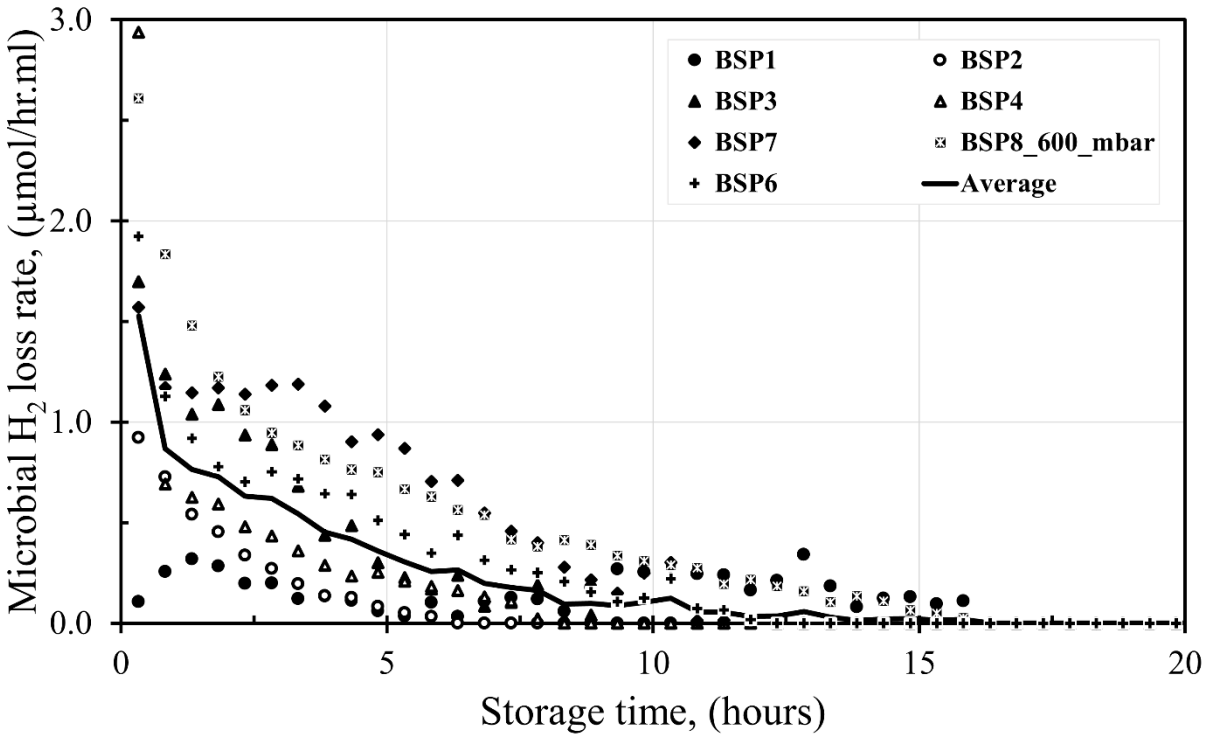

Figure S10: Microbial hydrogen consumption in the second storage cycle of all tests bacterial sand packs. The scatters are experimental measurements, and the solid line is the average of all cycles.

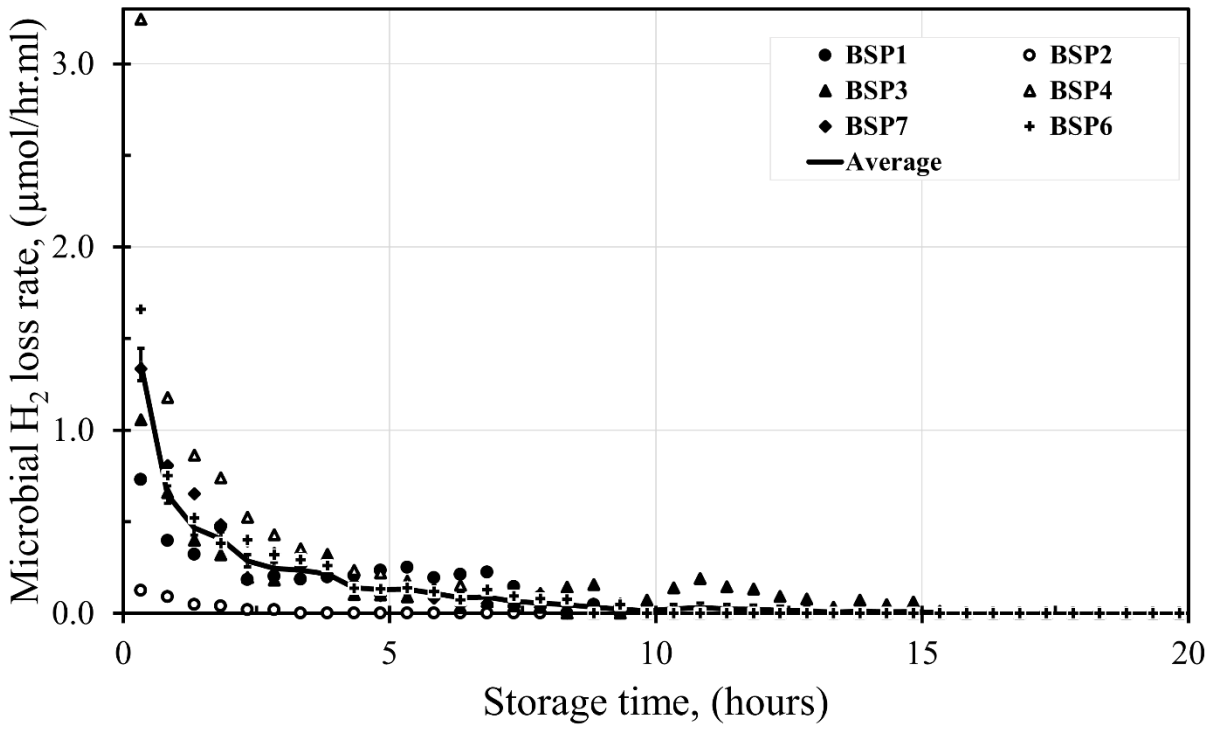

Figure S11: Microbial hydrogen consumption in the third storage cycle of all test bacterial sand packs. The scatters are experimental measurements, and the solid line is the average of all cycles.

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