Supporting material accompanying the manuscript:

Effect of inoculum percentage and hydrogen supply on hydrogenotrophic denitrification driven by anaerobic granular sludge

Table S1 – Nitrate concentration values detected using different inoculum percentages, i.e., 10% (I10), 20% (I20) and 40% (I40) (v/v) and for control tests. All data are reported for the triplicate.

		NO_3^- concentration (mg NO_3^-/L)																	
Cycle	**Time** (d)	**C10.1**	**C10.2**	**C10.3**	**C20.1**	**C20.2**	**C20.3**	**C40.1**	**C40.2**	**C40.3**	**I10.1**	**I10.2**	**I10.3**	**I20.1**	**I20.2**	**I20.3**	**I40.1**	**I40.2**	**I40.3**
1st	0	223.4	200.3	218.4	199.7	211.3	224.4	238.7	199.8	216.9	199.4	195.8	219.6	198.5	200.7	206.9	214.9	207.3	208.2
	1	171.1	194.3	193.1	202.9	158.9	180.7	162.2	140.1	186.4	168.7	182.6	176.8	178.3	171.9	163.7	116.9	159.9	135.4
	3	166.7	156.5	201.7	109.4	105.4	163.5	96.6	25.0	107.0	103.5	129.5	109.9	38.4	12.1	21.6	50.4	13.7	21.0
	6	153.1	146.2	139.2	63.1	50.1	67.3	5.0	2.3	4.0	4.6	39.4	5.5	28.9	14.2	6.5	2.0	7.3	2.2
2nd	0	190.7	206.7		195.1	193.0	208.2	188.7		215.0	206.0	216.9		180.2	173.1	208.0	216.0	218.9	227.2
	1	211.5		209.1		183.7	192.4	156.3	140.6	198.4	188.7	129.2	217.3	187.1	123.7	174.5	179.2	188.5	168.5
	3	217.0	186.0	186.5	159.3	168.3	132.7	67.4	81.5	161.6	129.0	25.3	109.0	103.1	36.7	48.2	24.3		29.7
	6	160.7	183.6	181.7	88.7	93.5	59.4	34.7	15.4	30.8	10.2	9.5	6.7	4.7	9.8	6.9	15.8	23.1	22.5
3rd	0	223.4	211.0	183.2	198.2	211.7	228.5	200.7	213.4	223.0	202.5		181.4	206.7	229.4		225.4	198.5	229.8
	1	152.4		200.2	176.5	199.6	207.9	148.2	192.3	166.0	180.5	207.4		139.0	128.9		214.7	157.0	227.7
	3	198.5		158.6	156.6	130.0	164.7	84.0	93.0	89.1	92.8	172.2			112.5	140.5	12.2	89.7	99.9
	6	169.5		141.3	101.6	81.9	87.8	6.9	39.0	8.3	13.4	40.6		123.8	112.6	104.3	9.7	12.7	12.7

Table S2 – Nitrate concentration values detected for the different hydrogen supply, i.e., stoichiometric (Hst), 50% in excess (H50), and 100% in excess (H100) and in control test. All data are reported for the triplicate.

		NO_3^- concentration (mg NO_3^-/L)											
Cycle	**Time** (d)	**C.1**	**C.2**	**C.3**	**Hst.1**	**Hst.2**	**Hst.3**	**H50.1**	**H50.2**	**H50.3**	**H100.1**	**H100.2**	**H100.3**
1st	0	198.0	184.9	169.7	194.4	190.9	192.8	190.4	188.3	199.5	209.5	188.6	186.9
	1	151.3	166.4	133.2	143.2		163.3	168.6	164.4	144.9	186.8	145.1	184.0
	3	109.8	150.7	128.8	89.2	97.3	109.2	108.5	84.8	109.2	104.6	69.5	92.1
	6	93.8	88.1	71.9	42.6	19.3	9.7	20.3	22.4	13.6	11.4	9.0	25.0
2nd	0	187.5	202.8	161.7	187.8	199.7	200.0	203.8	210.6	193.4	203.4	198.0	
	1	187.0	114.6	178.8	191.8	155.3	137.9	133.9	129.2	142.3	118.8	178.7	128.1
	3	187.1	156.2	146.4	53.6	39.8	76.6	29.9	48.8	11.5	14.1	46.8	19.1
	6	87.1	111.7	67.0	11.3	7.8	32.2	5.9	5.6	19.0	6.6	8.5	12.9
3rd	0	201.3	229.3	192.9	194.0	202.2	197.3	198.2	179.3	201.2	194.1	202.5	206.0
	1	211.8	194.3		190.2	192.0		188.5		206.3	148.0	159.3	
	3	111.9	137.4	208.4	97.1	149.6	155.7	60.9	104.9	47.0	34.4	123.8	17.7
	6	84.7	70.4	117.9	38.4	60.1	48.5	24.5	35.5	49.4	27.2	33.3	16.2

Table S3 – Inorganic and organic carbon concentration at the initial and final day of the hydrogenotrophic denitrification tests for the three different cycles. First set of experiments (fixed hydrogen (H_2) supply 50% in excess compared to the stoichiometry): I10 (10% (*v/v*) inoculum percentage), I20 (20% (*v/v*) inoculum percentage), and I40 (40% (*v/v*) inoculum percentage). Second set of experiments (fixed inoculum percentage of 10% (*v/v*)): Hst (stoichiometric H2 supply), H50 (H_2 supply 50% in excess compared to the stoichiometry), and H100 (H_2 supply 100% in excess compared to the stoichiometry).

ID Test	Cycle	Starting day		Final day	
		Inorganic Carbon (mg/L)	**Organic Carbon** (mg/L)	**Inorganic Carbon** (mg/L)	**Organic Carbon** (mg/L)
I10	1	59.7 ± 2.5	Not detected	82.2 ± 12.0	21.5 ± 10.2
	2	60.1 ± 5.5	3.6 ± 1.9	93.9 ± 24.9	15.4 ± 11.6
	3	47.6 ± 10.5	Not detected	73.9 ± 2.2	12.1 ± 1.2
I20	1	40.8 ± 3.4	3.3 ± 1.2	95.7 ± 1.6	36.7 ± 7.6
	2	42.7 ± 34.9	18.0 ± 16.1	97.3 ± 6.0	22.4 ± 2.1
	3	48.5 ± 8.7	Not detected	80.3 ± 7.3	15.4 ± 3.7
I40	1	53.3 ± 9.9	Not detected	21.4 ± 14.6	129.7 ± 21.1
	2	56.9 ± 6.6	11.1 ± 1.2	113.3 ± 8.7	33.1 ± 2.2
	3	71.8 ± 17.4	0.4 ± 0.6	62.1 ± 11.6	13.7 ± 1.2
Hst	1	19.8 ± 1.8	Not detected	46.1 ± 7.2	65.4 ± 21.3
	2	79.4 ± 8.6	10.5 ± 2.1	2.2 ± 1.4	14.2 ± 1.2
	3	75.2 ± 6.4	6.6 ± 3.3	73.4 ± 15.9	20.2 ± 5.2
H50	1	18.7 ± 1.2	Not detected	46.3 ± 2.0	66.4 ± 35.5
	2	70.1 ± 5.8	5.8 ± 3.6	8.1 ± 1.3	32.8 ± 1.3
	3	78.3 ± 12.6	2.2 ± 1.1	84.6 ± 8.3	34.8 ± 5.2
H100	1	20.7 ± 3.5	Not detected	48.4 ± 3.1	36.2 ± 10.6
	2	66.4 ± 4.9	Not detected	2.2 ± 0.0	23.4 ± 1.6
	3	76.8 ± 14.2	4.7 ± 1.7	93.2 ± 12.9	17.3 ± 5.7

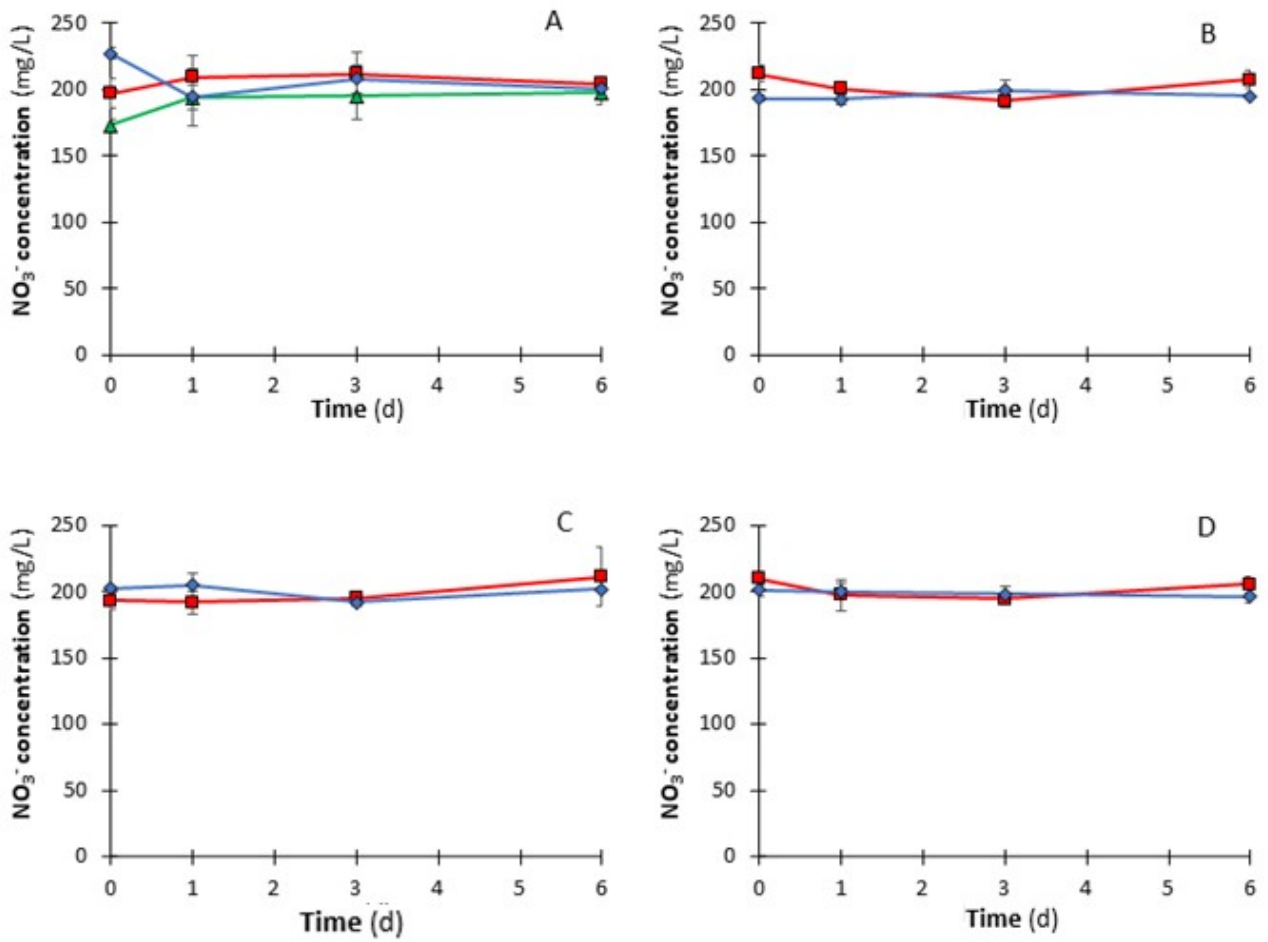

Figure S1 – Nitrate (NO_3^-) concentration trend in the abiotic controls carried out for the inoculum intake tests (A) and for the hydrogen supply tests, i.e., stoichiometry H_2 supply (B), 50% excess in H_2 supply (C), and 100% excess in H_2 supply (D). Cycles of denitrification tests: first cycle (■), second cycle (▲), third cycle (◆).

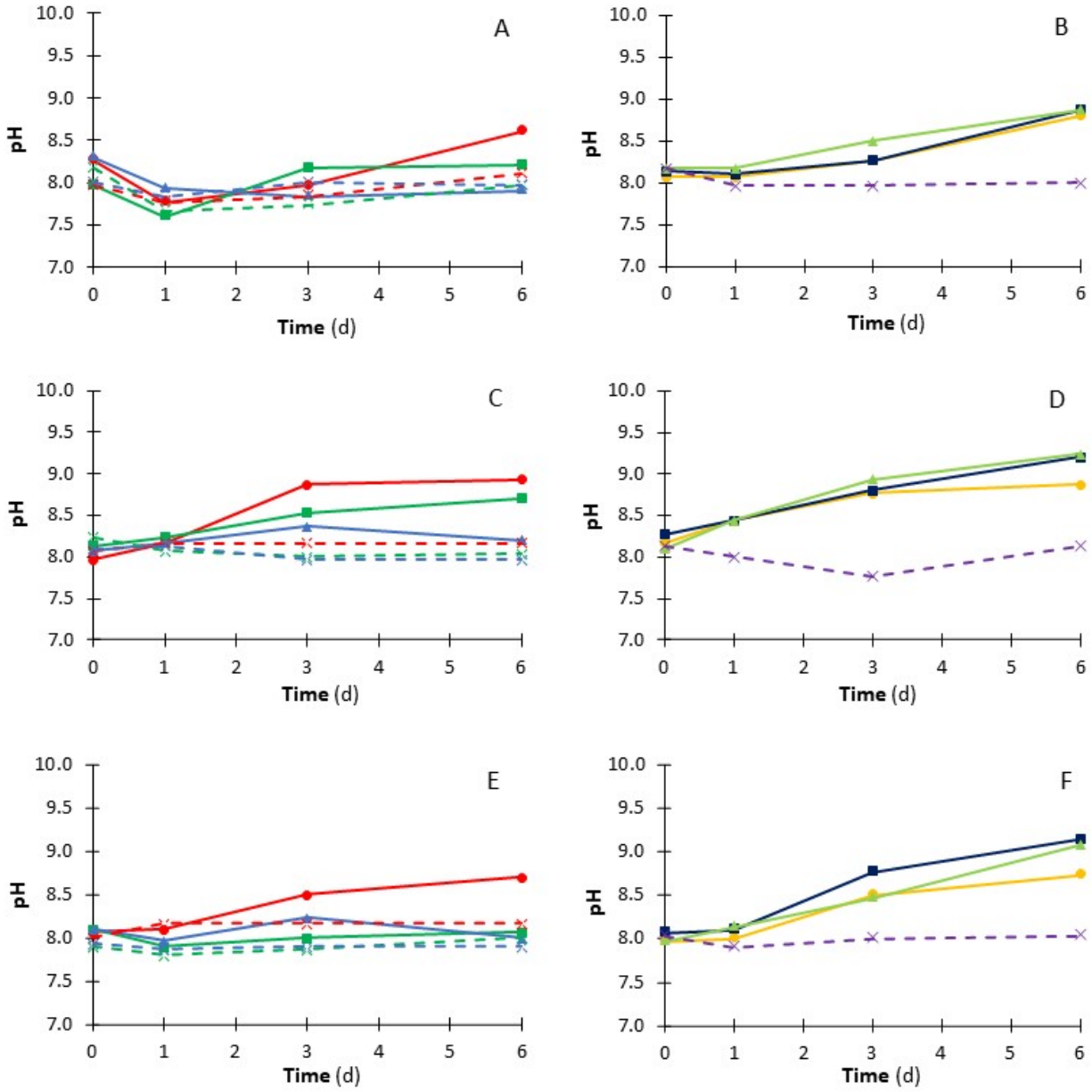

Figure S2 – pH trend during the hydrogenotrophic denitrification tests in the first (A and B), second (C and D), and third (E and F) cycle using different inoculum intakes, i.e., 10% (I10), 20% (I20) and 40% (I40) (*v/v*) and varying the hydrogen supply, i.e., stoichiometric (Hst), 50% in excess (H50), and 100% in excess (H100). Inoculum intake tests: I10 (●), I20 (■), I40 (▲). Controls of the inoculum intake tests: C10 (×), C20 (×), C40 (×). Hydrogen supply tests: Hst (●), H50 (■), H100 (▲). Control of the hydrogen supply test: C (×).

Table S4 – Nitrous oxide (N_2O) production expressed as percentage (%) in the headspace, with respect to the anaerobic granular sludge (AnGS) intake, and in proportion to the total nitrogen mass removal. First set of experiments (fixed hydrogen (H_2) supply 50% in excess compared to the stoichiometry): I10 (10% (*v/v*) inoculum percentage), I20 (20% (*v/v*) inoculum percentage), and I40 (40% (*v/v*) inoculum percentage). Second set of experiments (fixed inoculum percentage of 10% (*v/v*)): Hst (stoichiometric H_2 supply), H50 (H_2 supply 50% in excess compared to the stoichiometry), and H100 (H_2 supply 100% in excess compared to the stoichiometry).

ID Test	**N_2O production** (%)	**AnGS intake** (mL)	**N_2O production** (µg/min/g AnGS)	**Nitrogen mass removed** (mg)	**N_2O production** (%) (mg N-N_2O produced/ mg nitrogen removed)
I10	0.180	12.5	$4.03 \cdot 10^{-3}$	5.2	2.65
I20	0.200	25.0	$2.29 \cdot 10^{-3}$	4.4	3.56
I40	0.260	50.0	$1.54 \cdot 10^{-3}$	5.8	3.66
C10	0.188	12.5	$4.61 \cdot 10^{-3}$	1.3	12.09
C20	0.183	25.0	$2.49 \cdot 10^{-3}$	3.7	4.64
C40	0.332	50.0	$2.22 \cdot 10^{-3}$	5.5	5.56
Hst	0.129	12.5	$2.80 \cdot 10^{-3}$	4.5	3.27
H50	0.019	12.5	$4.11 \cdot 10^{-4}$	4.6	0.30
H100	0.021	12.5	$4.88 \cdot 10^{-4}$	4.9	0.35
C	0.327	12.5	$8.34 \cdot 10^{-3}$	2.7	5.57

Table S5 – Headspace gas composition values measured for different inoculum percentages, i.e., 10% (I10), 20% (I20) and 40% (I40) (v/v) and for control tests. All data are reported for the triplicate.

Test	Gas composition (%)																			
	1st cycle							2nd cycle							3rd cycle					
	H_2	CH_4	$H_2O_{(v)}$	N_2	NO	N_2O	CO_2	H_2	CH_4	$H_2O_{(v)}$	N_2	NO	N_2O	CO_2	H_2	CH_4	$H_2O_{(v)}$	N_2	NO	N_2O	CO_2
I20.1	0.23	3.05	2.27	9.88	0.00	0.04	0.07	0.02	0.00	0.83	3.95	0.41	0.11	0.19	0.01	0.03	0.80	4.76	0.00	0.35	0.37
I20.2	0.05	3.66	1.58	9.24	0.00	0.10	0.11	0.37	2.72	0.79	10.27	0.00	0.03	0.07	0.01	0.04	0.79	10.08	0.00	0.05	0.00
I20.3	0.02	2.62	1.51	13.37	0.00	0.09	0.15	0.05	1.92	0.88	8.35	0.00	0.09	0.02	0.01	0.02	1.02	23.19	0.00	0.35	0.30
C20.1	0.01	0.19	1.58	4.62	0.00	0.17	0.21	0.02	0.02	0.89	5.02	0.15	0.16	0.23	0.01	0.03	0.85	4.68	0.00	0.33	0.38
C20.2	0.00	0.03	2.26	6.90	0.00	0.15	0.22	0.02	0.07	0.78	4.01	0.00	0.23	0.23	0.19	0.00	0.78	5.95	0.12	0.06	0.12
C20.3	0.00	0.01	2.64	9.68	0.02	0.21	0.28	0.01	0.09	1.03	11.93	0.00	0.10	0.07	0.01	0.00	0.82	3.06	0.00	0.24	0.20
I10.1	0.00	0.92	2.96	15.64	0.00	0.00	0.15	2.58	1.07	1.62	9.75	0.00	0.04	0.01	0.01	1.12	1.12	21.71	0.00	0.46	0.57
I10.2	0.00	0.56	2.88	12.08	0.00	0.02	0.06	0.12	0.25	0.80	10.89	0.00	0.04	0.00	0.01	0.43	1.16	15.78	0.00	0.57	0.77
I10.3	0.39	1.70	2.82	10.26	0.00	0.02	0.05	0.01	0.00	0.79	2.55	0.11	0.21	0.32	0.02	0.02	1.32	11.26	0.00	0.27	0.23
C10.1	0.00	0.00	1.74	3.85	0.16	0.14	0.18	0.02	0.00	0.67	1.69	0.00	0.00	0.09	0.01	0.02	1.40	13.97	0.00	0.28	0.32
C10.2	0.01	0.01	1.62	3.77	0.00	0.14	0.23	0.02	0.00	0.80	7.11	0.10	0.25	0.28	0.01	0.00	1.11	8.48	0.09	0.12	0.27
C10.3	0.02	6.60	1.73	8.10	0.00	0.28	0.48	0.03	5.88	0.70	6.71	0.00	0.13	0.24	0.01	0.03	1.06	13.19	0.00	0.36	0.16
I40.1	0.02	4.59	1.98	14.44	0.00	0.33	0.66	0.02	4.57	0.70	5.92	0.00	0.20	0.22	0.01	0.04	0.97	13.26	0.00	0.18	0.14
I40.2	0.02	0.49	0.95	11.58	0.00	0.00	1.13	0.02	3.63	0.74	8.23	0.00	0.18	0.48	0.01	0.07	1.09	14.44	0.00	0.32	0.38
I40.3	0.01	1.20	1.49	6.00	0.00	0.42	0.51	0.03	0.56	0.78	5.35	0.00	0.46	0.48	0.01	0.09	1.09	14.85	0.00	0.30	0.39
C40.1	0.01	0.88	1.31	3.74	0.00	0.58	1.01	0.03	0.82	0.79	6.51	0.00	0.44	0.59	0.01	0.05	0.91	13.15	0.00	0.16	0.16
C40.2	0.02	0.36	0.97	3.29	0.00	0.26	0.41	0.03	0.39	0.69	5.27	0.00	0.27	0.79	0.01	0.00	0.88	2.57	0.05	0.24	0.21
C40.3	0.01	1.00	1.64	4.27	0.00	0.70	1.06	0.02	0.40	0.72	4.68	0.00	0.34	0.64	0.02	0.53	1.04	3.53	0.00	0.00	0.45

The complement to 100% is represented by the Argon gas used to flush the bottles at the beginning of the experiments.

Table S6 – Headspace gas composition values measured for the different hydrogen supply, i.e., stoichiometric (Hst), 50% in excess (H50), and 100% in excess (H100) and in control test. All data are reported for the triplicate.

Test	Gas composition (%)																			
	1st cycle							2nd cycle							3rd cycle					
	H_2	CH_4	$H_2O_{(v)}$	N_2	NO	CO_2	N_2O	H_2	CH_4	$H_2O_{(v)}$	N_2	NO	CO_2	N_2O	H_2	CH_4	$H_2O_{(v)}$	N_2	NO	CO_2	N_2O
C.1	0.03	0.064	1.12	3.61	0.00	0.08	0.07	0.01	0.02	1.31	2.24	0.00	0.24	0.28	0.02	0.00	1.57	2.14	0.02	0.14	0.57
C.2	0.01	0.03	1.12	2.80	0.42	0.08	0.08	0.01	0.02	1.27	1.49	0.00	0.18	0.35	0.02	0.02	1.70	2.08	0.00	0.22	0.61
C.3	0.01	0.05	1.07	2.78	0.00	0.08	0.04	0.01	0.01	1.41	2.69	0.00	0.20	0.26	0.02	0.02	1.83	5.04	0.00	0.28	0.68
Hst.1	1.96	0.24	1.33	6.05	0.00	0.05	0.00	0.04	0.65	2.99	7.12	0.00	0.00	0.09	0.01	0.07	1.80	6.17	0.00	0.03	0.04
Hst.2	1.49	0.22	1.25	6.46	0.00	0.03	0.01	0.14	0.75	1.54	6.87	0.00	0.06	0.04	0.01	0.11	1.85	5.18	0.00	0.03	0.14
Hst.3	0.96	0.14	1.29	9.23	0.00	0.01	0.03	0.01	0.02	1.68	28.89	0.00	0.10	0.41	0.02	0.02	1.88	8.42	0.00	0.10	0.39
H50.1	3.86	0.17	1.47	7.68	0.89	0.03	0.00	2.79	0.58	1.77	6.66	0.00	0.03	0.02	3.29	0.19	2.20	5.80	0.00	0.00	0.05
H50.2	3.83	0.56	1.47	7.81	2.46	0.07	0.00	0.78	1.02	1.62	10.35	0.00	0.03	0.04	3.30	0.21	2.55	6.98	0.23	0.00	0.05
H50.3	2.63	0.20	1.48	7.72	0.27	0.06	0.00	2.95	1.06	1.78	6.38	0.00	0.04	0.01	3.32	0.31	2.31	6.07	0.48	0.04	0.00
H100.1	1.21	0.18	1.21	20.76	0.00	0.01	0.04	4.09	0.93	1.94	7.38	2.17	0.03	0.01	6.52	0.17	1.99	5.13	0.67	0.03	0.02
H100.2	8.33	0.40	1.32	6.37	0.69	0.02	0.01	6.19	0.70	2.00	7.55	2.79	0.02	0.02	5.37	0.25	1.96	5.69	0.85	0.03	0.01
H100.3	2.56	0.59	2.31	20.43	0.00	0.04	0.04	3.76	0.96	2.00	8.55	1.99	0.03	0.01	6.12	0.29	2.38	7.05	0.72	0.01	0.03

The complement to 100% is represented by the Argon gas used to flush the bottles at the beginning of the experiments.