Supplementary information to the article titled

Anaerobic digestion of pig manure supernatant at high ammonia concentrations characterized by high abundances of *Methanosaeta* and non-euryarchaeotal archaea

Anna Synnøve Røstad Nordgård[a], Wenche Hennie Bergland[b], Olav Vadstein[a], Vladimir Mironov[a], Rune Bakke[b], Kjetill Østgaard[a] and Ingrid Bakke[a]

a. Department of Biotechnology and Food Science, Norwegian University of Science and Technology (NTNU), Sem Sælands vei 6/8, 7491 Trondheim, Norway
b. Department of Process, Energy and Environmental Technology, University College of Southeast Norway (USN), Kjølnes ring 56, 3918 Porsgrunn, Norway.

anna.s.r.nordgard@ntnu.no, tel. + 47 73 59 16 47, + 47 472 44 895

wenche.bergland@hit.no, tel. +47 35 57 52 17, +47 928 27 207

olav.vadstein@ntnu.no, tel. +47 918 97 034

vladimir.mironov@ntnu.no, tel. +47 73 55 03 49

rune.bakke@hit.no, tel. +47 35 57 52 41, +47 986 24 908

kjetill.ostgaard@ntnu.no, tel. +47 73 59 40 68

Ingrid.bakke@ntnu.no, tel. +47 73 59 78 59, +47 932 47 872, Corresponding author

The reactors were operated at University College of Southeast Norway, Porsgrunn, Norway, while the microbial analyses were executed at Norwegian University of Science and Technology, Trondheim, Norway.

Table S1. Number of reads for each sample

Exp. Day	Bacteria				Archaea			
	HA1	**HA2**	**LA1**	**LA2**	**HA1**	**HA2**	**LA1**	**LA2**
D69	10498	9544	6697	6502	29021	27159	19375	29302
D114	13826	14097	19166	22362	33338	10454	33946	19725
D156	16931	20696	18045	16553	16378	26183	34419	14172
D230	10867	18075	13388	13514	35544	39937	59669	36248
D282	23593	24431	17596	18544	49475	26240	45905	28285
D316	27045	9909	8653	11454	28431	42880	30555	28233
D321	27553	21213	13641	15852	45634	38311	40409	37590
D341	17891	23001	26136	17361	35470	36607	37843	32789
D347	40816	29858	24563	34537	39812	37466	23900	45895

Table S2. Temperature, total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) concentrations in the reactor samples.

		Influent HA		Influent LA		HA1		HA2		LA1		LA2	
Day	Temp. [°C]	TAN [mg/L]	FAN [mg/L]	TAN [mg/L]	FAN [mg/L]	TAN [mg/L]	FAN [mg/L]	TAN [mg/L]	FAN [mg/L]	TAN [mg/L]	FAN [mg/L]	TAN [mg/L]	FAN [mg/L]
69	35	2030	153	2030	153	1910	186	1913	190	1845	187	1865	160
114	35	3490	1260	1935	58	3620	1086	3565	1104	1990	189	1935	169
156	25	3305	746	2015	53	3580	631	3450	637	2005	78	1980	84
230	25	3860	927	1950	60	4070	703	3990	690	2010	62	1980	65
282	25	3290	1237	1740	43	3470	600	3440	606	1700	101	1610	176
316	30	3500	990	1790	76	3690	850	3570	1078	1760	143	1920	166
321	30	3620	1315	1750	54	3690	1027	3670	1021	1770	200	1800	225
341	35	3680	1214	1780	76	3580	721	3700	732	1780	162	1800	145
347	35	3600	1557	1750	29	3860	980	3610	826	1810	162	1870	189

Table S3. Abundance of bacterial and archaeal OTUs in all reactor communities, found to be positively correlated to methane yield in HA samples (Spearman, $p<0.05$).

	Bacteria			Archaea			
	OTU_9 Syntrophomonadaceae	OTU_38 Synergistia	OTU_95 Cloacimonetes	OTU_2 Methanosaeta	OTU_10 Methanoculleus	OTU_57 Methanobacteriaceae	OTU_1142 Methanobrevibacter
D69 HA1	1.34	0.10	0.00	11.53	0.01	0.00	0.00
D69 HA2	0.00	0.08	0.00	24.05	0.01	0.00	0.02
D69 LA1	0.78	0.07	0.00	11.54	0.00	0.00	0.02
D69 LA2	0.03	0.06	0.00	21.43	0.01	0.00	0.01
D114 HA1	0.71	1.01	0.00	5.37	0.83	0.00	0.00
D114 HA2	0.04	0.50	0.00	11.69	0.57	0.00	0.00
D114 LA1	2.68	0.38	0.00	5.91	0.05	0.00	0.03
D114 LA2	0.35	0.26	0.00	10.47	0.07	0.00	0.09
D156 HA1	0.02	0.71	0.04	9.80	0.28	0.00	0.00
D156 HA2	0.00	0.21	0.01	13.02	0.05	0.00	0.00
D156 LA1	2.48	1.15	0.00	4.24	0.12	0.00	0.00
D156 LA2	0.40	0.61	0.00	7.05	0.13	0.00	0.00
D230 HA1	0.64	0.22	0.01	16.45	0.71	0.00	0.13
D230 HA2	0.07	0.22	0.19	13.79	1.82	0.01	0.01
D230 LA1	1.37	0.78	0.00	2.74	0.26	0.00	0.02
D230 LA2	2.26	0.75	0.00	6.27	0.41	0.00	0.04
D282 HA1	1.14	0.45	0.06	13.62	4.03	0.03	0.05
D282 HA2	0.77	0.14	0.03	13.46	2.64	0.00	0.03
D282 LA1	6.85	0.73	0.00	6.08	0.21	0.00	0.05
D282 LA2	9.22	0.17	0.01	15.00	0.45	0.00	0.08
D316 HA1	4.71	0.46	0.59	17.76	4.09	0.52	0.03
D316 HA2	0.88	0.23	0.04	17.83	2.98	0.17	0.02
D316 LA1	5.14	0.25	0.02	4.94	0.67	0.00	0.03
D316 LA2	2.20	0.09	0.04	6.59	0.99	0.00	0.02
D321 HA1	7.59	0.24	0.14	16.47	4.35	0.85	0.10
D321 HA2	1.28	0.23	0.13	16.78	2.89	0.19	0.13
D321 LA1	13.46	0.41	0.00	5.23	0.22	0.00	0.04
D321 LA2	5.62	0.28	0.06	6.21	0.98	0.00	0.01
D341 HA1	7.26	0.31	0.20	19.82	6.26	1.18	0.19
D341 HA2	1.51	0.46	0.39	17.51	5.77	0.79	0.86
D341 LA1	22.98	0.29	0.02	10.53	0.16	0.00	2.35
D341 LA2	20.20	0.44	0.01	14.20	0.81	0.00	0.07
D347 HA1	2.61	1.06	0.28	26.27	11.55	0.61	0.06
D347 HA2	1.89	0.41	0.08	26.16	2.48	0.39	0.09
D347 LA1	32.27	0.20	0.02	16.43	0.06	0.00	0.35
D347 LA2	15.18	0.63	0.02	12.04	0.71	0.00	0.04

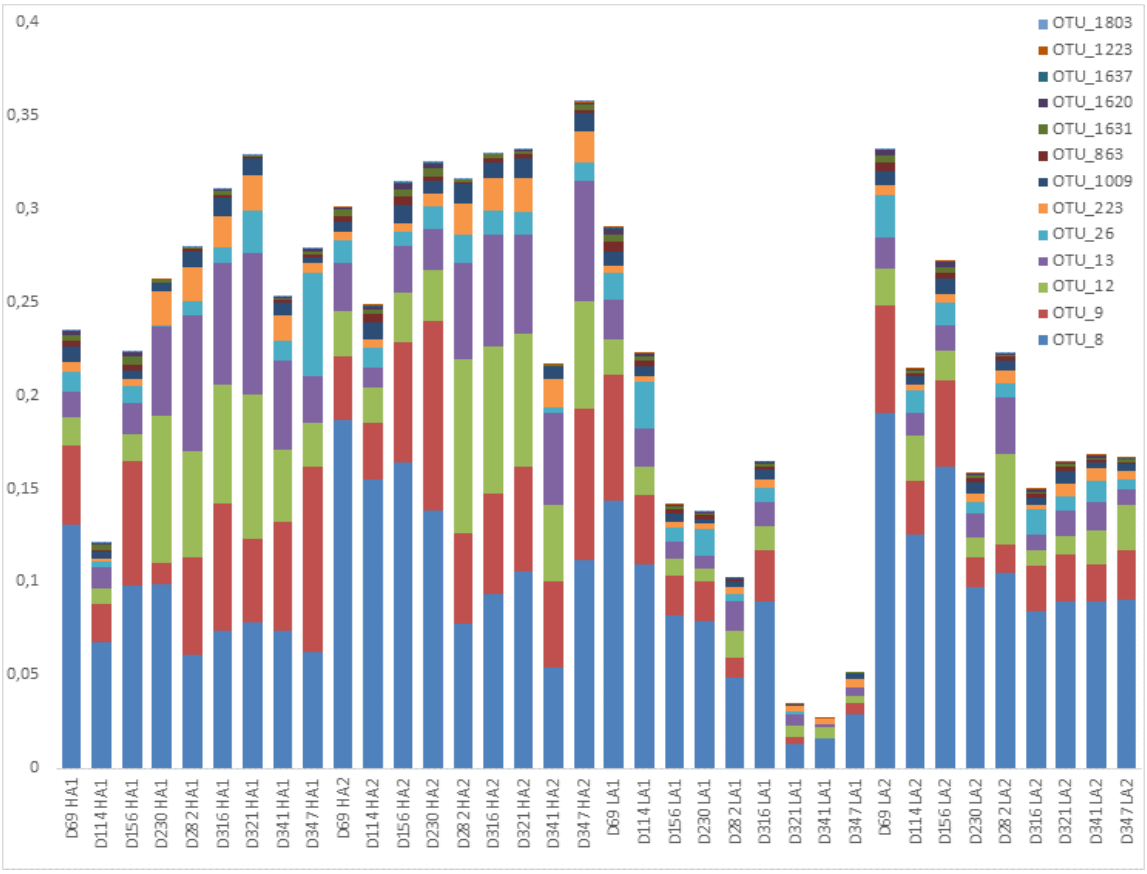

Figure S1. Relative abundances of 13 non-methanogen archaeal OTUs (see Fig. 4) in the reactor communities. D indicates experimental day.

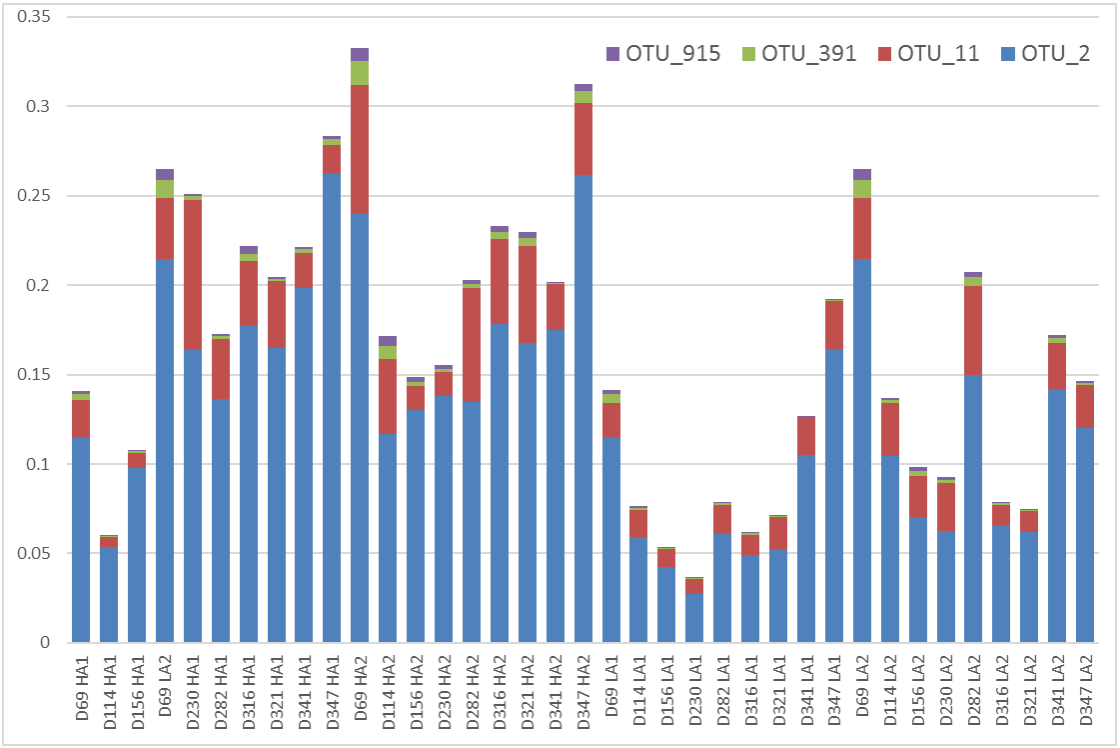

Figure S2. Relative abundance of the four most abundant *Methanosaeta* OTUs in the archaeal reactor communities. D indicate experimental day.

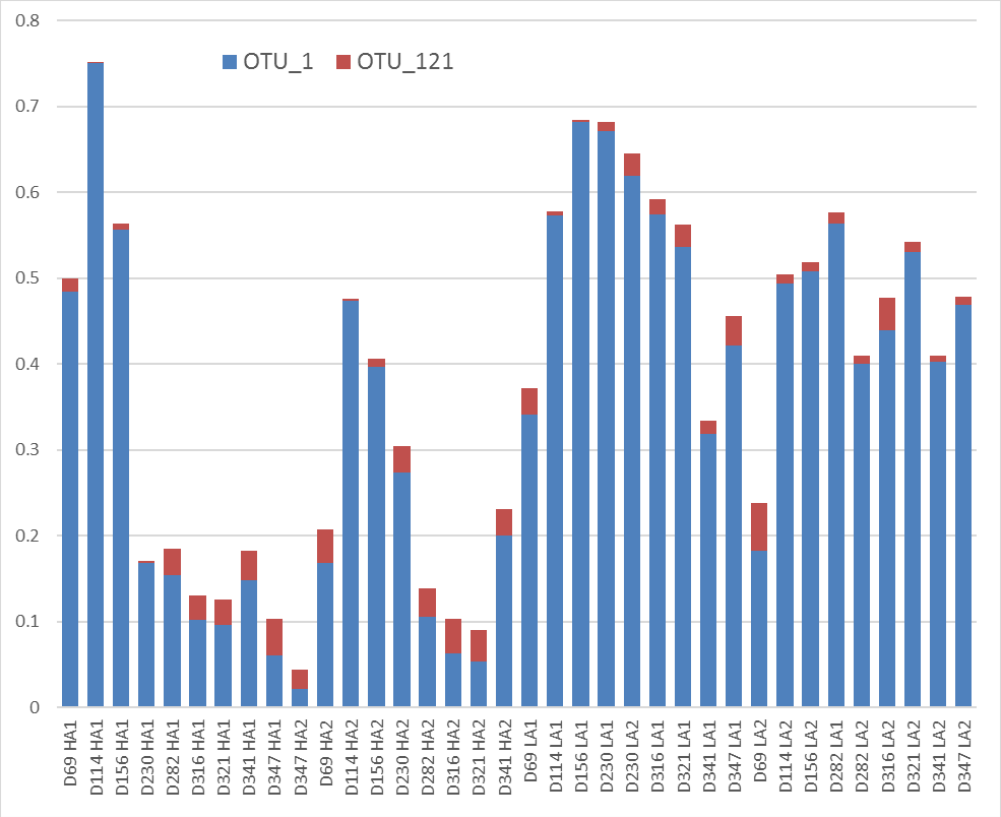

Figure S3. Relative abundance of the two most abundant *Methonosarcina* OTUs in the archaeal reactor communities. D indicate experimental day.

FAN was calculated from day 114 using equations S1 and S2.

$$K_a = \frac{[NH_3][H^+]}{[NH_4^+]} \quad \text{(S1)}$$

$$FAN = \frac{TAN}{1+\frac{10^{-pH}}{K_a}} \quad \text{(S2)}$$