

**Table 1** Comparison of n-damo bacteria enrichments available in the literature

Source	Origin of inoculum	Scale (L)	CH <sub>4</sub> supply	Anaerobic control	T (°C)	pH	Stir	Medium supply	Headspace-CH <sub>4</sub>
Hu et al. (2009)	Mixture of sediments from freshwater lake, anaerobic digester sludge+return sludge from WWTP	2 (1.6 L liquid)	headspace was flushed periodically with mixed gas (N <sub>2</sub> , CH <sub>4</sub> and CO <sub>2</sub> )	.....	22 35 45 <sup>4</sup>	....	...	Nitrate added periodically	1.1–1.3 atm; He injected for confirming no gas leakage
Hu et al. (2011)	Parent culture from Hu et al. (2009) [Culture A (320 ml) + Culture B (160 ml) inocula]	2; (1.6 L liquid)	5%N <sub>2</sub> +90%CH <sub>4</sub> +5%CO <sub>2</sub> to flush headspace regularly with a P <sub>CH<sub>4</sub></sub> (0.5-1 atm)	N2 sparged into medium, then placed in an anaerobic chamber for 24 hours before it was used	35	7.0- 7.5	200 rpm	20 min settling; 400 ml fresh medium exchanged /4 weeks[NO <sub>3</sub> <sup>-</sup> : 1-10mM; NO <sub>2</sub> <sup>-</sup> : 1ml/72min-daily]	+80ml He into for increasing P > 1.2 atm after each flushing
Ettwig et al. (2008)	Biomass (1.5 liters) from an enrichment culture in Raghoebarsing et al. (2006) <sup>7</sup>	16; SBR (13 L liquid )	CH <sub>4</sub> -CO <sub>2</sub> 95:5%; 10 ml/min	Ar-CO <sub>2</sub> 95:5% (Continuously sparged in reactor and medium)	30	7.3- 7.6	200 rpm	Continuous supply; 0.2-0.8 L/day with nitrite conc. 3-15 mM(keep NO <sub>2</sub> <sup>+</sup> : 0-1 mM)	.....
Ettwig et al. (2009)	Biomass After 13 months enrichment from the above <sup>2</sup>	0.3 (0.22 liquid)	.....	Ar-CO <sub>2</sub> 95:5%	30	....	Stirre r	.....	6% (2.5% when measuring)
Ettwig et al. (2009)	Mixture sludge (2 liters) from four ditches draining agricultural land (upper 5 cm); Biomass After 230 days enrichment from the above	16; SBR (10-13 L liquid )	CH <sub>4</sub> -CO <sub>2</sub> 95:5%; 10 ml/min <sup>1</sup>	6 cycles of vacuuming and subsequent gassing with Ar-CO <sub>2</sub> 95:5, then 5 min of Ar-CO <sub>2</sub> , leaving 0.5 10 <sup>5</sup> overpressure (100 ml/min)	30	6.9- 7.5	100 rpm	0.3-1 L/day (pending on nitrite conc.: 0.2-1.5 mM)	Final conc.: 2.5 to 10%
Luesken et al. (2011b)	Enriched biomass from the bellow 15 L bioreactor WWTP in Lieshout	3; SBR 15; SBR	CH <sub>4</sub> -CO <sub>2</sub> 95:5%; 10 ml/min	Ar-CO <sub>2</sub> 95:5% (Continuously sparged in reactor and medium)	-----	6.8- 7.3	100 rpm	23.2 h/day; 0.3-1 L/day pending on nitrite conc.; (To maintain nitrite: 0.1-1 mM)	0.3 L; 4% (5.3%, 4.8% when measured on day 308,315)
Luesken et al. (2011a)	N-damo culture from Ettwig et al. (2009) (1 liter)	3; SBR	CH <sub>4</sub> -CO <sub>2</sub> 95:5%; 12 ml/min	Ar-CO <sub>2</sub> 95:5% (Continuously flushed)	30	7.3- 7.6	200 rpm	Synthetic medium from Ettwig et al. (2009)	.....
Zhu et al. (2011)	500 mL Anammox granules from WWTP (van der Star et al. 2007)	2 liquid; SBR	>60 days, CH <sub>4</sub> 10 ml/min <sup>1</sup> supplied with NO <sub>2</sub> <sup>+</sup>	SBR operated according to Luesken et al. (2011a)	Room temp.	7.2	...	Nitrite: ammonium=4:3	Always above 20% (255 μM)

Ref.	Nitrite reduction /Test time (Operating days)	Methane oxidation /Test time	Detection /Sample volume	FISH detection after X time	Observed stoichiometry
Hu et al. (2009)	0.065 mmol NO <sub>3</sub> <sup>-</sup> /day (day 220-260) 3.2 mmol NO <sub>3</sub> <sup>-</sup> /day (day 283-312) <sup>8</sup> .....(320 days in total)	0.038 mmol/day (day 220-260) 2.4 mmol/day (day 283-312) .....	GC/MS for 13C-SIP	270 day: no archaea 15% NC10 297 d: 40% archaea 50% NC10 ....	Consuming 16.7% more methane than predicted
Hu et al. (2011)	1.97 mmol/day for A (NO <sub>2</sub> <sup>-</sup> only, day 90-94) 0.062 mmol NO <sub>3</sub> <sup>-</sup> /day for B (NO <sub>3</sub> <sup>-</sup> only, day 50-105) (150 days in total)	A: close to calculated value B: 0.045 mmol/day	Test strip nitrite limit control daily FIA for NH <sub>4</sub> <sup>+</sup> NO <sub>2</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup> (1ml) GC-40 °C for CH <sub>4</sub> , N <sub>2</sub> ; 90 °C for He	Archaea disappeared after day 100 in A	B: 13.3% > stoichiometrical predicted: 0.039 mmol CH <sub>4</sub> /day
Ettwig et al. (2008)	Around 3.5(mmol/day) for 13 months Only increased (~9) till 450 ml new source added after 17, 19 months(22 months in total)	1.7 nmol /min/mg protein after 22 m; Test length: 36 h 3.7 nmol /min/mg protein after 22 m	Merckoquant test strip: NO <sub>2</sub> <sup>-</sup> , NO <sub>3</sub> <sup>-</sup> GC: 65 °C-- N <sub>2</sub> ; 120 °C--CH <sub>4</sub> ; BCA assay: total protein 100 µl gas; 1 ml liquid collected HPLC-UV: NO <sub>2</sub> <sup>-</sup> :NO <sub>3</sub> <sup>-</sup> ;10µl used GC: CH4;	Monthly, >70% NC10 all the time Archaea disappear from 10 to12 month	8:3.6=Consumption of NO <sub>2</sub> <sup>-</sup> :CH <sub>4</sub> (? 8:3 Stated by Ettwig et al, 2009) 5:1=NO <sub>2</sub> <sup>-</sup> :NO <sub>3</sub> <sup>-</sup> (21 h)
Ettwig et al. (2009)	Max=33.5 (mmol/day); 217(243 days in total) 4.7to5.1(nmol /min/mg protein <sup>-1</sup> ; red/ grey stopper) Day 136-142 3.4to5.6(nmol /min/mg protein); 2 months period with 4.75 protein	1.6to2.2(nmol /min/mg protein; red/grey stopper); Day 136-142 with bioreactor stop; tested in batches; Test length: 32 h	The same to the above except: 50 µl gas GC/MS-80 °C for N <sub>2</sub> (5 min)	5 m-can be used 6 m-be dominant <sup>6</sup> 7 m-70% n-damo	8:3.5=Consumption of NO <sub>2</sub> <sup>-</sup> :CH <sub>4</sub>
Luesken et al. (2011b)	After 70 d, activity increased (308 d in total) 0.4 mmol/day (day 112); 1.1 after day 175; 0.9 (nmol /min/mg protein) on day 308,315; 1.2 after day 315 2.1 (nmol NO <sub>2</sub> <sup>-</sup> /h/mg protein); 9.2 (nmol NO <sub>3</sub> <sup>-</sup> /h/mg protein); Denitrifying activity decreased	0.3 (nmol /min/mg protein) on day 308,315; 0.5 after day 315 Test length: 10 h	Routine nitrite analysis daily Batch exp. On day 308, 315 and Analysis according to Ettwig et al, 2008 1.7 (nmol /h/mg protein)	64 days-detected 2-3%; 308 days-60-70% n-damo Analysis according to Ettwig et al, 2008 No n-damo detected	9.8NO <sub>2</sub> <sup>-</sup> :3CH <sub>4</sub> (day 308,315) 10.1NO <sub>2</sub> <sup>-</sup> :3CH <sub>4</sub> (day 308,315) .....
Luesken et al. (2011a)	8.1 (nmol /min/mg protein) on 49 d; 17.2 mmol/day after day151 (?No data shown) 15.4 (nmol /min/mg protein) on day 142 (with anammox); 5.0 after 3 h when ammonium depleted (its rate was 9.0) [161 days in total]	3.1 (nmol /min/mg protein) on 49 d (then NH <sub>4</sub> <sup>+</sup> added); Coculture with anammox, (responsible for 77% NO <sub>2</sub> <sup>+</sup> ↓ and constituted 50%	Analysis according to Ettwig et al. (2008)	45 days-80% n-damo no anammox; 108 days- 5% anammox 151 days, 50% of each	8.1NO <sub>2</sub> <sup>-</sup> :3CH <sub>4</sub> (day 49)

Ref.	Nitrite reduction /Test time (Operating days)	Methane oxidation /Test time	Detection /Sample volume	FISH detection after X time	Observed stoichiometry
Zhu et al. (2011)	21.8 mmol/day after 2 months [1 year in total] 13.9 mmol/l per day after 1 year; 8.4 for ammonium (Coculture with anammox,)	1.6 (nmol /min/mg protein)[0.7 mmol/l per day] after 1 year Test length: 100 min	.....	1 year is necessary	8NO <sub>2</sub> :3CH <sub>4</sub> (1 year later)

**Notes:**

1 3-7days supply+1-2 h settling+ 30-60 min pumping out liquid. As activity increased, after 6 months the cycle was shortened to 22.5h of supply of medium, 1 h of settling, and 30 min of drawing off of liquid.

2 After being made anoxic by thorough flushing with a mixture of Ar/CO<sub>2</sub> (95:5), culture liquid from the enrichment culture was being pumped through at a flow rate of 17.5 ml/min; After c. 24 h (accounting for at least 110 volume changes) it was disconnected from the main culture vessel by closing the influent and effluent tubes.

3 23.2 h supply+20 min settling+ 30 min pumping out liquid.

4 Stopped on day 90 as no denitrification was detected after day 40.

5 The use of black butyl rubber stoppers caused total inhibition of the activity of methane oxidation. Anoxic handling of the biomass, in contrast, was not crucial; exposure to oxygen during transfer to the bottles did not lead to lower activity.

6 Even though NC10 phylum bacteria already accounted for more than 50% of the population in the enrichment culture, they were not detected in a clone library (31 clones) obtained after 6 months with general 16S rRNA gene primers (primers 8F and 1545R). Instead, this library was dominated by uncultured *Acidobacteria* (11 clones) and *Chloroflexi* (10 clones) (data not shown).

7 FISH showed that upon inoculation the population was dominated by a bacterium affiliated with the phylum NC10 (approximately 80%) and an archaeon of the order *Methanosaecinales* (approximately 10%)

8 Decreased to 0.3 mmol NO<sub>3</sub>—N/day in 40 days; Increased after 220d to 2.0 on day 312 (35 °C); 0.14 without methane supply; 0.11 for day 50-260 (22 °C)

**Table 2** A summary of 16S rDNA PCR primers currently used for detecting n-damo bacteria from environmental samples

Primer	Froward/ Reverse	Position	Sequence (5'-3')	Speciality	References
202F	Froward	202-230	GAC CAA AGG GGG CGA GCG	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)
NC10-202Fdeg	Froward	202-230	RAC CAA AGG RGG CGA GCG	<i>M. oxyfera</i> 16S rDNA	Deutzmann and Schink (2011)
1492R	Reverse	1510-1529	GGT TAC CTT GTT ACG ACT T	Bacterial 16S rDNA	Lane (1991)
8F	Froward	8-27	AGA GTT TGA TYM TGG CTC AG	Bacterial 16S rDNA	Juretschko et al. (1998)
1043R	Reverse	1042-1060	TCT CCA CGT TCC CTT GCG	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)
1043Rb	Reverse	1042-1060	TCT CCA CGC TCC CTT GCG	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)
NC10-1043Rdeg	Reverse	1042-1060	TCT CCR CGY TCC CTT GCG	<i>M. oxyfera</i> 16S rDNA	Deutzmann and Schink (2011)
1545R	Reverse	1529-1545	CAK AAA GGA GGT GAT CC	Bacterial 16S rDNA	Juretschko et al. (1998)
qP1F	Froward	1001-1024	GGG CTT GAC ATC CCA CGA ACC TG	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)
qP1R	Reverse	1180-1201	CGC CTT CCT CCA GCT TGA CGC	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)
qP2F	Froward	1169-1189	GGG GAA CTG CCA GCG TCA AG	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)
qP2R	Reverse	1440-1460	CTC AGC GAC TTC GAG TAC AG	<i>M. oxyfera</i> 16S rDNA	Ettwig et al. (2009)

**Table 3** A summary of *pmoA* gene-based PCR primers specific for n-damo bacteria currently used

primer	Froward/ Reverse	Position	Sequence (5'-3')	GC%	Tm (°C)	Test 1	Test 2	Test 3	Test 4	References
A189_b	Forward	121-138	GGN GAC TGG GAC TT <sup>Y</sup> TGG	55.6	64.5-69.4	+*	+	+	+	Luesken et al. (2011c)
cmo682	Reverse	637-654	<b>AAA YCC GGC RAA GAA CGA</b>	44.4	61-65.8	-	-	+	+	Luesken et al. (2011c)
cmo182	Forward	167-184	TCA CGT TGA CGC CGA TCC	61.1	66.5	-	+	+	+	Luesken et al. (2011c)
cmo568	Reverse	537-556	GCA CAT <b>ACT CCA TCC CCA TC</b>	55.0	63.9	-	-	+	+	Luesken et al. (2011c)
NA638Rdeg	Reverse	593-612	RAA TGT TCG RAG CGT VCC BC	45.0	59.5-70.0	+	+	+	+	Deutzmann and Schink (2011)
NA720R	Reverse	675-694	TCC CCA TCC ACA CCC ACC AG	65.0	69.6	-	-	+	+	Deutzmann and Schink (2011)
682_NC10	Reverse	637-654	<b>AAA TCC GGC GAA GAA CGA</b>	50.0	63.0	-	-	+	+	Kojima et al. (2012)
HP3F1	Forward	209-233	CCC AGT ACT TCA TGT GGG ARA ARA T	40.0	59.3-61.8	+	+	+	+	Han and Gu (2013)
HP3R1	Reverse	463-484	GGG GGC CAG CCA NRY CCA RTT	57.1	68.1-78.0	+	+	+	+	Han and Gu (2013)

\* Test 1: Hairpins; Test 2: Self complementarity; Test 3: False priming in target group (FP565575 *M. oxyfera*); Test 4 False priming in excluded group (DQ119048 *Methylosinus spurius*); “+” means observed while “-” means not;

\*\* A189f (general *pmoA* primer; 5'-3'): GGN GAC TGG GAC TTC TGG (Holmes et al. 1995); key differences within the same priming site are highlighted in bold.

**Table 4** A summary and analysis of n-damo bacteria from different habitats based on *pmoA* gene sequences\*

Source/Environment	N-damo related clone	OTU	Shannon-Wiener	Chao1	Type	Accession number in GenBank	Reference**
CF5S	21	2	0.6365	1	M/R	KJ023443, KJ023450	Chen et al. (2014)
CF5B	4	1	0	1	M/R	KJ023444	Chen et al. (2014)
E201S	8	2	0.3768	1	M/R	KJ023445-KJ023446	Chen et al. (2014)
E407S	6	2	0.4506	1	M/R	KJ023447-KJ023449	Chen et al. (2014)
E407B	24	2	0.3768	1	M/R	KJ023451, KJ023455	Chen et al. (2014)
E510S	16	2	0.2338	1	M/R	KJ023452, KJ023456	Chen et al. (2014)
E525S	24	1	0	1	M/R	KJ023453	Chen et al. (2014)
E525B	1	1	--	--	M/R	KJ023454 KF528962-KF528968	Chen et al. (2014)
SCS E704S	24	3	1.0055	1	M	KF528973-KF528984 KF742451-KF742455 KF528961	Chen et al. (2015)
SCS E401	13	3	0.9110	1	M	KF528969-KF528972 KF742444-KF742450	Chen et al. (2015)
Lake Constance	15	6	1.4878	10.5	F	HQ906565-HQ906579	Deutzmann and Schink (2011)
Lake Biwa	21	1	0	1	F	AB661605-AB661625	Kojima et al. (2012)
Reed bed	12	1	0	1	F	JX898463-JX898474	Han and Gu (2013)
Reservoir	9	7	1.8892	13.2500	F	JX898486-JX898494	Han and Gu (2013)
WWTP^	10	7	1.8344	19.5	E	JX898495-JX898504	Han and Gu (2013)
Paddy soil	11	2	0.3046	1	F	JX898475-JX898485	Han and Gu (2013)
Paddy soil	14	1	0	1	F	JN704402-JN704415	Wang et al. (2012)
Paddy field	20	2	0.1985	1	F	KC341611-KC341630	Zhu et al. (2015)
Jingshan paddy field	27	1	0	1	F	KC905857-KC905883	Hu et al. (2014)

Source/Environment	N-damo related clone	OTU	Shannon-Wiener	Chao1	Type	Accession number in GenBank	Reference**
Xixi wetland	21	3	0.3805	1	F	KC905884-KC905904	Hu et al. (2014)
XZ wetland	4	1	0	1	F	KC905905-KC905908	Hu et al. (2014)
Freshwater Wetlands (Jingshan Paddy; Xixi wetland; XZ wetland)	52	5	1.0575	1	F	KC905857-KC905908	Hu et al. (2014)
Baiyangdian Lake	24	1	0	1	F	KC341248-KC341271	Zhu et al. (2015)
Bosten Lake	16	2	0.4826	1	F	KC341301-KC341316	Zhu et al. (2015)
Chaohu Lake	19	6	1.1513	1	F	KC341317-KC341335	Zhu et al. (2015)
Dongting Lake	21	2	0.6920	1	F	KC341336-KC341356	Zhu et al. (2015)
Jiaxing C. wetland-winter	13	8	1.8393	26	F	KC341375-KC341387	Zhu et al. (2015)
Jiaxing Constructed wetland-summer	11	8	2.0198	12.1667	F	KC341388-KC341398	Zhu et al. (2015)
Subsurface North canal	18	2	0.6870	1	U	KC341272-KC341289	Zhu et al. (2015)
North canal-12 m	3	1	0	1	U	KC341298-KC341300	Zhu et al. (2015)
North canal-15 m	8	1	0	1	U	KC341290-KC341297	Zhu et al. (2015)
Panjin swamp	21	1	0	1	F	KC341437-KC341457	Zhu et al. (2015)
Peat land China	23	6	1.3944	8	F	KC341414-KC341436	Zhu et al. (2015)
Poyang Lake	21	2	0.5983	1	F	KC341458-KC341478	Zhu et al. (2015)
Shahe River	18	5	1.2094	1	F	KC341479-KC341496	Zhu et al. (2015)
Songhuajiang River	18	7	1.6715	9.25	F	KC341497-KC341514	Zhu et al. (2015)
Tarim River	19	2	0.6918	1	F	KC341515-KC341533	Zhu et al. (2015)
Tiaoxi River	20	3	0.9973	1	F	KC341534-KC341553	Zhu et al. (2015)
Tibetan Lake	10	1	0	1	S/R	JQ429431-JQ429432	Yang et al. (2012)
Tulufan River	18	3	0.8487	3	F	KC341357-KC341374	Zhu et al. (2015)
Wuliangshuhai Lake	24	4	0.9366	4.5	F	KC341554-KC341577	Zhu et al. (2015)

Source/Environment	N-damo related clone	OTU	Shannon-Wiener	Chao1	Type	Accession number in GenBank	Reference**
Yellow River	15	1	0	1	F	KC341399-KC341413	Zhu et al. (2015)
Yuanmingyuan Lake	12	3	0.7215	3.5	F	KC341698-KC341709	Zhu et al. (2015)
Pearl River-winter	20	5	1.0098	6	F	KC341578-KC341597	Zhu et al. (2015)
Pearl River-summer	13	2	0.6172	1	F	KC341598-KC341610	Zhu et al. (2015)
Donghu Lake	15	3	0.8033	1	F	KC341631-KC341645	Zhu et al. (2015)
Honghaitan Tidal Land	17	4	1.1151	4.5	C	KC341646-KC341662	Zhu et al. (2015)
Shangqiu Reservoir	17	7	1.6100	8.5	F	KC341663-KC341679	Zhu et al. (2015)
Tianchi Lake	18	9	1.8121	18	F	KC341680-KC341697	Zhu et al. (2015)
WWTP^	128	3	0.8487	1	U	KC700842-KC700715	Gao et al. (2013)
Soils^	40	3	0.3813	1	U	KC700675-KC700714	Gao et al. (2013)
Qiantang River	50	16	2.1437	29.5	F	KC503613-KC503662	Shen et al. (2014)
West Lake	24	8	1.5359	12	F	JX531974-JX531997	Zhu et al. (2013)
Jiaojiang estuary	80	20	2.3787	30.1250	C	KC512302-KC512381	Li-Dong et al. (2014)
Freshwater habitats							
(Alpine peat bog							
WWTPs^	12	9	NA	NA	F/R	HQ698926-HQ698937	Luesken et al. (2011c)
Drainage ditch							
Contaminated aquifer^)							
WWTPs^	19	10	NA	NA	F/R	JF706214-JF706196	Luesken et al. (2011b)
SBR co-enrichment with anammox^	7	3	NA	NA	E/R	JN006731-JN006737	Luesken et al. (2011a)
Sewage treatment plants^	12	8	NA	NA	E/R	KC112371-KC112382	Ho et al. (2013)
Minerotrophic peatland^	3	3	NA	NA	E/R	JX262153-JX262155	Zhu et al. (2012)
Lab scale reactor^	13	3	1.0579	1	E/?	AB767281-AB767293	Masashi et al. (2013)

\*Abbreviations: NA, not applicable; M, marine; F, freshwater; S, saline lake; C, coastal; U, unknown; E, enrichment; R, representative sequences available in the GenBank database only;

\*\*Unpublished reference indicates that the related DNA sequences have already been released in the database without a published journal paper until 17-April-2014 when the information was accessed; OTU, Shannon-Wiener and Chao1 were recalculated using Fastgroup II (Yu et al. 2006) with the cutoff of 5%.

<sup>^</sup>Isolation sources/environments are not sediment

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