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APPENDIX A

Data of the performance of sequencing batch reactor (SBR) on Anammox system

Table A1 Characteristics of the experiment R1(100)-2 in reactor2

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
4/11/2010	70.46	0.02	73.1	6.2
5/11/2010	108.28	23.05	75.1	30.7
6/11/2010	106.74	0.01	84.1	7.9
7/11/2010	106.74	0.02	84.1	0.4
10/11/2010	106.74	5.76	84.1	15.9
11/11/2010	106.23	0.30	112.9	62.3
12/11/2010	108.11	0.44	105.0	39.1
13/11/2010	100.19	0.00	113.3	47.5
14/11/2010	127.18	0.01	135.4	50.8
15/11/2010	93.61	0.11	139.5	28.0
16/11/2010	94.28	0.01	128.5	46.9
17/11/2010	107.98	0.00	152.5	42.7
18/11/2010	109.99	0.03	110.2	38.9
19/11/2010	109.05	0.06	109.0	36.2
20/11/2010	106.23	0.02	112.8	36.3
21/11/2010	106.37	0.05	106.1	35.4
22/11/2010	106.10	0.00	110.9	30.7
23/11/2010	89.44	0.01	101.9	31.7
24/11/2010	91.46	0.03	106.1	28.1
25/11/2010	93.34	0.04	93.4	28.3
26/11/2010	101.62	0.01	101.7	38.9
27/11/2010	105.88	0.01	110.6	33.5
28/11/2010	101.91	0.01	104.0	34.7
29/11/2010	99.26	0.02	108.7	34.6
30/11/2010	101.91	0.03	103.5	33.4
1/12/2010	102.50	0.03	108.7	32.5
2/12/2010	101.91	0.03	102.8	29.7
3/12/2010	100.88	0.01	98.3	30.2
4/12/2010	104.26	21.49	99.3	33.4

Table A2 Characteristics of the experiment R0.75(75) in reactor2

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
7/12/2010	103.09	0.03	49.2	16.1
8/12/2010	96.62	0.03	59.1	17.1
9/12/2010	100.15	17.90	61.5	20.9
11/12/2010	98.82	0.10	60.4	0.8
12/12/2010	100.00	0.01	60.7	11.9
13/12/2010	87.50	0.00	56.3	11.5
14/12/2010	98.82	0.02	55.2	12.1
15/12/2010	105.59	0.01	57.3	11.3
16/12/2010	98.53	0.02	55.2	10.1
17/12/2010	110.88	0.02	48.6	9.3
18/12/2010	105.15	0.01	58.0	10.9
19/12/2010	103.68	0.01	64.6	14.9
20/12/2010	108.38	0.00	73.0	15.7
21/12/2010	104.12	0.01	71.5	15.5
22/12/2010	104.12	0.07	73.9	16.7
24/12/2010	104.12	0.01	73.9	16.9
25/12/2010	101.03	0.01	66.2	16.5
26/12/2010	103.38	0.03	73.0	15.1
28/12/2010	103.38	0.03	73.0	13.8
29/12/2010	105.88	0.03	63.0	13.0
31/12/2010	105.88	0.02	63.0	13.1
1/1/2011	103.82	0.03	72.1	22.1
2/1/2011	100.29	0.04	74.1	20.2
4/1/2011	100.29	0.04	74.1	23.7
5/1/2011	106.32	0.03	71.6	18.5
6/1/2011	105.44	1.11	73.6	19.4
7/1/2011	102.50	0.07	74.3	15.4
8/1/2011	106.18	0.07	75.4	15.6
9/1/2011	107.65	14.75	74.4	12.9
10/1/2011	106.18	21.16	74.4	12.2
11/1/2011	102.79	10.81	73.6	12.0
12/1/2011	100.59	29.26	73.6	11.1
13/1/2011	101.18	16.11	73.6	30.5
14/1/2011	101.32	52.21	74.6	41.3
15/1/2011	101.76	36.38	74.4	38.8
16/1/2011	102.35	62.23	74.6	37.7
17/1/2011	100.14	59.97	77.1	35.4
18/1/2011	100.14	73.31	73.6	55.2
19/1/2011	102.03	81.89	73.7	58.2
20/1/2011	99.73	82.53	74.3	53.6
21/1/2011	101.35	84.05	74.0	63.1
22/1/2011	102.03	94.32	74.9	58.3
23/1/2011	100.81	85.81	74.9	52.7
24/1/2011	98.92	90.61	76.7	49.0
25/1/2011	101.62	88.24	75.7	50.0

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
26/1/2011	100.41	92.36	74.8	56.2
27/1/2011	0.00	93.18	75.9	54.3
28/1/2011	0.00	47.26	75.9	35.2
29/1/2011	0.00	14.19	49.0	35.0
30/1/2011	0.00	0.56	62.7	9.5
31/1/2011	99.19	8.24	68.6	7.2
1/2/2011	102.03	9.96	68.2	5.0
2/2/2011	104.05	13.41	74.3	5.8
3/2/2011	100.68	13.12	73.2	36.1
4/2/2011	102.43	13.31	75.0	34.7
5/2/2011	100.68	11.05	76.0	43.3
6/2/2011	100.95	10.81	74.5	44.3
7/2/2011	103.51	12.23	74.4	40.6

Table A3 Characteristics of the experiment R0.5(75) in reactor2

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
23/3/2011	101.82	0.01	74.3	28.4
24/3/2011	123.24	0.01	75.1	24.2
25/3/2011	123.65	0.02	74.0	23.0
26/3/2011	123.10	0.01	75.0	27.7
27/3/2011	124.76	0.01	75.6	27.6
28/3/2011	150.32	0.02	75.5	24.2
29/3/2011	151.42	0.02	75.9	22.3
30/3/2011	150.59	0.02	75.8	22.8
31/3/2011	150.73	0.03	76.2	21.0
1/4/2011	150.87	0.01	76.3	21.7
2/4/2011	151.01	0.01	76.0	21.1
3/4/2011	150.32	0.01	75.8	21.1
4/4/2011	150.87	0.00	76.3	19.0
5/4/2011	150.18	0.00	75.9	19.5
6/4/2011	152.67	0.01	76.3	19.8
7/4/2011	151.70	0.00	76.0	18.8
8/4/2011	152.53	0.00	75.6	17.9
9/4/2011	152.25	0.01	75.1	16.6
10/4/2011	150.46	0.01	75.5	17.0
11/4/2011	151.84	0.01	74.3	17.1
12/4/2011	149.49	0.01	75.1	18.9
13/4/2011	151.56	0.01	78.8	19.1
14/4/2011	152.25	0.01	75.6	19.3
15/4/2011	150.73	0.01	74.5	19.3
16/4/2011	152.80	0.01	74.5	18.7
17/4/2011	152.53	0.01	74.6	19.9
18/4/2011	151.98	0.00	75.3	19.9
19/4/2011	150.87	0.00	74.9	19.3
20/4/2011	150.04	0.01	75.0	20.1
21/4/2011	150.32	0.01	75.6	18.8
22/4/2011	150.46	0.01	75.0	19.2
23/4/2011	151.28	0.01	74.9	19.7
24/4/2011	149.90	0.01	75.6	19.4
25/4/2011	150.18	0.02	76.0	20.1
26/4/2011	149.77	0.01	75.4	20.2
27/4/2011	150.70	0.02	75.8	19.8
28/4/2011	149.58	0.01	75.8	19.3
29/4/2011	149.44	0.08	74.9	20.6
30/4/2011	149.02	0.01	74.5	19.8
1/5/2011	152.67	0.01	74.4	20.1
2/5/2011	152.81	0.02	74.4	20.0
3/5/2011	152.67	0.02	74.4	19.4
4/5/2011	152.67	0.02	74.6	19.3
5/5/2011	152.25	0.02	74.5	18.1
6/5/2011	151.83	0.02	75.6	18.2

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
7/5/2011	151.54	0.02	76.9	18.8
8/5/2011	150.70	0.02	74.6	18.8
9/5/2011	150.98	0.02	74.9	18.4
10/5/2011	150.00	0.01	74.9	20.4
12/5/2011	151.40	0.05	75.6	22.6
13/5/2011	149.86	0.02	75.5	21.5
14/5/2011	149.58	0.02	75.6	21.5
15/5/2011	151.40	0.00	76.4	21.8
16/5/2011	150.70	0.01	74.1	21.7
17/5/2011	150.42	0.01	74.6	21.8
18/5/2011	149.58	0.01	75.2	20.9
19/5/2011	150.00	0.01	74.6	21.7
20/5/2011	150.84	0.01	75.6	21.6
21/5/2011	150.56	0.01	74.3	20.6

Table A4 Characteristics of the experiment R1(100)-1 in reactor1

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
4/11/2010	70.46	0.01	73.1	3.6
5/11/2010	108.28	26.40	75.1	32.7
6/11/2010	106.74	0.09	84.1	5.0
7/11/2010	106.74	19.76	84.1	0.3
10/11/2010	106.74	12.56	84.1	14.0
11/11/2010	106.23	0.40	112.9	60.8
12/11/2010	108.11	0.00	105.0	39.5
13/11/2010	100.19	0.02	113.3	46.8
14/11/2010	127.18	0.00	135.4	51.6
15/11/2010	93.61	0.94	139.5	27.5
16/11/2010	94.28	0.03	128.5	48.7
17/11/2010	107.98	0.02	152.5	41.8
18/11/2010	109.99	0.03	110.2	39.6
19/11/2010	109.05	0.04	109.0	36.3
20/11/2010	106.23	0.03	112.8	35.8
21/11/2010	106.37	0.03	106.1	34.1
22/11/2010	106.10	0.01	110.9	31.1
23/11/2010	89.44	0.01	101.9	32.5
24/11/2010	91.46	0.03	106.1	27.0
25/11/2010	93.34	0.03	93.4	27.3
26/11/2010	101.62	0.03	101.7	37.5
27/11/2010	105.88	0.01	110.6	31.1
28/11/2010	101.91	0.01	104.0	32.4
29/11/2010	99.26	0.01	108.7	33.4
30/11/2010	101.91	0.01	103.5	32.4
1/12/2010	102.50	0.04	108.7	31.6
2/12/2010	101.91	0.01	102.8	29.0
3/12/2010	100.88	0.01	98.3	28.4
4/12/2010	104.26	23.05	99.3	31.1

Table A5 Characteristics of the experiment R0.5(50) in reactor1

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
7/12/2010	101.76	0.01	32.0	10.5
8/12/2010	100.74	0.01	38.5	9.0
9/12/2010	100.29	21.79	37.5	16.2
11/12/2010	100.51	0.04	37.8	7.9
12/12/2010	100.29	0.01	37.9	3.7
13/12/2010	90.74	0.00	35.2	2.2
14/12/2010	99.71	0.01	37.6	1.6
15/12/2010	103.01	0.00	38.5	0.9
16/12/2010	100.22	0.05	33.2	0.0
17/12/2010	99.26	0.01	33.8	0.0
18/12/2010	106.62	0.00	34.8	0.6
19/12/2010	106.10	0.01	39.6	0.4
20/12/2010	109.78	0.00	48.6	0.5
21/12/2010	103.97	0.00	49.3	0.6
22/12/2010	104.41	0.01	48.1	0.8
24/12/2010	104.41	0.00	48.1	0.6
25/12/2010	101.40	0.00	47.5	0.6
26/12/2010	103.68	0.01	48.4	0.6
28/12/2010	103.68	0.02	48.4	0.7
29/12/2010	106.25	0.00	43.5	0.8
31/12/2010	106.25	0.00	43.5	0.8
1/1/2011	102.79	0.01	50.5	0.8
2/1/2011	96.91	0.01	52.3	0.7
4/1/2011	96.91	0.01	52.3	0.9
5/1/2011	104.71	0.01	49.0	0.6
6/1/2011	105.81	0.01	51.3	0.9
7/1/2011	100.15	0.16	48.1	1.0
8/1/2011	102.87	0.01	50.5	0.9
9/1/2011	106.18	16.19	49.8	0.5
10/1/2011	102.87	14.16	48.7	0.6
11/1/2011	102.13	0.03	50.2	0.7
12/1/2011	103.38	0.02	51.5	1.3
13/1/2011	103.01	0.03	51.0	0.6
14/1/2011	101.54	0.05	51.8	1.3
15/1/2011	102.13	0.00	51.3	1.6
16/1/2011	101.18	0.00	51.7	1.3
17/1/2011	99.86	0.00	50.8	1.6
18/1/2011	100.81	1.31	53.5	2.2
19/1/2011	102.91	0.01	51.8	1.2
20/1/2011	100.54	0.02	51.4	2.3
21/1/2011	99.19	1.20	50.9	14.6
22/1/2011	99.66	1.35	48.7	12.5
23/1/2011	100.74	0.01	50.0	10.4
24/1/2011	100.20	0.01	49.7	9.1
25/1/2011	100.20	0.01	50.6	7.3

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
26/1/2011	101.28	0.02	50.0	10.5
27/1/2011	100.61	0.95	46.7	11.6
28/1/2011	101.55	0.05	50.5	10.2
29/1/2011	103.24	0.02	46.5	3.9
30/1/2011	103.31	6.54	49.4	6.9
31/1/2011	103.11	13.28	50.6	6.0
1/2/2011	100.68	15.54	51.1	5.1
2/2/2011	102.57	21.32	51.0	4.9
3/2/2011	104.53	21.07	49.7	20.5
4/2/2011	103.45	22.15	53.6	24.9
5/2/2011	102.77	23.65	54.3	28.6
6/2/2011	103.58	25.30	54.3	29.3
7/2/2011	103.38	28.04	52.3	28.2
8/2/2011	102.91	30.91	50.2	30.6
9/2/2011	104.19	34.16	51.2	28.0
10/2/2011	104.73	34.97	50.6	27.6
11/2/2011	103.58	46.69	50.3	29.6
13/2/2011	102.91	64.70	51.1	33.7
14/2/2011	102.03	65.30	51.0	36.3
15/2/2011	104.12	65.57	51.0	37.0
16/2/2011	105.34	82.77	50.4	24.9
17/2/2011	104.80	83.72	49.4	52.7
18/2/2011	104.26	89.59	50.0	49.9
19/2/2011	104.66	91.55	51.5	50.9
20/2/2011	104.73	84.86	50.5	42.4
22/2/2011	104.12	83.38	50.6	43.5
23/2/2011	0.00	92.16	0.0	50.4
24/2/2011	0.00	82.84	0.0	48.5
25/2/2011	0.00	75.47	0.0	41.7
26/2/2011	0.00	75.95	0.0	46.1
27/2/2011	0.00	48.45	31.5	42.6
28/2/2011	0.00	30.81	22.7	31.5
1/3/2011	0.00	10.02	20.7	36.4
2/3/2011	0.00	0.35	11.2	22.5

Table A6 Characteristics of the experiment R1(100)-3 in reactor1

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
3/3/2011	102.38	14.99	103.0	31.3
4/3/2011	100.99	0.02	104.9	28.8
5/3/2011	103.07	0.00	102.2	31.9
6/3/2011	102.38	0.00	103.0	35.6
7/3/2011	101.55	0.00	102.9	36.7
8/3/2011	102.93	0.01	99.7	38.3
9/3/2011	101.55	0.01	99.7	38.3
10/3/2011	103.21	0.01	100.4	37.7
11/3/2011	101.27	0.01	99.7	42.2
12/3/2011	102.10	0.01	99.3	41.9
13/3/2011	102.24	0.01	100.4	43.2
14/3/2011	101.41	0.02	100.8	39.8
15/3/2011	102.24	0.01	99.3	41.9
16/3/2011	101.69	0.02	99.3	38.4
17/3/2011	102.10	0.02	100.1	41.5
18/3/2011	101.27	0.00	99.9	37.8
19/3/2011	102.10	0.00	100.4	39.3
20/3/2011	100.99	0.01	98.8	42.5
21/3/2011	101.55	0.01	98.8	44.7
22/3/2011	102.79	0.01	98.4	42.3

Table A7 Characteristics of the experiment R0.75(100) in reactor1

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
23/3/2011	102.51	0.01	99.3	42.3
24/3/2011	131.39	0.02	99.2	40.3
25/3/2011	132.77	0.01	99.1	39.3
26/3/2011	133.19	0.01	98.6	43.5
27/3/2011	133.19	0.01	101.9	44.6
28/3/2011	132.91	0.01	101.6	42.8
29/3/2011	131.94	0.01	102.9	39.5
30/3/2011	132.63	0.01	102.8	43.4
31/3/2011	132.08	0.02	102.1	41.2
1/4/2011	133.32	0.01	102.1	42.4
2/4/2011	132.91	0.01	102.5	40.9
3/4/2011	132.22	0.01	102.5	36.3
4/4/2011	133.05	0.01	103.3	37.6
5/4/2011	132.36	0.01	102.7	39.1
6/4/2011	134.57	0.01	101.9	42.4
7/4/2011	131.53	0.01	102.5	41.0
8/4/2011	133.88	0.02	100.9	40.2
9/4/2011	134.71	0.03	100.5	40.4
10/4/2011	132.08	0.03	101.3	39.9
11/4/2011	132.91	0.04	101.6	39.6
12/4/2011	130.56	0.03	101.2	47.1
13/4/2011	133.05	0.03	105.7	45.9
14/4/2011	132.63	0.02	102.9	45.3
15/4/2011	133.05	0.02	101.9	42.5
16/4/2011	133.46	0.03	101.9	40.9
17/4/2011	133.74	0.02	101.4	43.4
18/4/2011	133.05	0.03	100.8	43.3
19/4/2011	133.05	0.02	101.1	41.6
20/4/2011	134.98	0.02	101.4	41.5
21/4/2011	133.32	0.03	101.0	38.6
22/4/2011	132.91	0.02	100.8	39.2
23/4/2011	132.91	0.02	101.7	42.3
24/4/2011	132.63	0.02	101.0	41.2
25/4/2011	132.08	0.04	101.5	41.4
26/4/2011	132.91	0.02	101.3	44.7
27/4/2011	133.29	0.03	101.1	43.9
28/4/2011	132.72	0.07	101.1	42.2
29/4/2011	133.15	0.08	100.7	45.1
30/4/2011	132.58	0.08	100.8	45.0
1/5/2011	133.15	0.08	102.5	43.4
2/5/2011	134.27	0.07	102.5	42.3
3/5/2011	133.57	0.08	102.0	43.1
4/5/2011	133.29	0.07	102.1	42.4
5/5/2011	133.29	0.08	101.8	41.2
6/5/2011	133.71	0.07	101.3	41.6

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
7/5/2011	133.71	0.08	101.0	41.8
8/5/2011	134.27	0.08	102.1	42.3
9/5/2011	133.71	0.08	101.5	41.9
10/5/2011	133.43	0.07	101.7	41.9
12/5/2011	134.55	0.19	102.9	44.6
13/5/2011	133.71	0.08	100.5	37.5
14/5/2011	133.15	0.09	100.8	37.4
15/5/2011	133.99	0.02	100.3	35.7

Table A8 Characteristics of the experiment R0.5(100) in reactor1

Date	Influent NO^-_2 (mg N/L)	Effluent NO^-_2 (mg N/L)	Influent NH^+_4 (mg N/L)	Effluent NH^+_4 (mg N/L)
16/5/2011	150.42	0.08	100.6	44.2
17/5/2011	203.09	0.34	100.5	40.6
18/5/2011	202.25	0.09	100.9	40.0
19/5/2011	203.23	0.08	99.8	39.8
20/5/2011	201.83	0.07	100.6	39.9
21/5/2011	200.98	0.08	98.8	39.5
22/5/2011	201.54	25.84	100.3	42.2
23/5/2011	201.40	49.37	100.5	44.4
24/5/2011	202.81	50.84	100.3	46.0
25/5/2011	202.25	60.67	101.2	49.7
26/5/2011	201.40	69.10	100.9	57.7
27/5/2011	200.70	69.73	101.5	60.9
28/5/2011	200.42	56.18	102.7	58.4
29/5/2011	201.26	45.29	102.4	57.2
30/5/2011	201.12	27.63	100.2	51.8
31/5/2011	200.56	27.95	100.6	49.2
1/6/2011	199.58	27.28	101.2	48.6

Table A9 N_2O concentration of the experiment R1(100) with 2.5 mL of sample and 100 ppm of standard

Date	Average area of standard	Area of sample	ppm of sample
16/3/2011	31,646,200	483,212	1.53
	31,646,200	260,594	0.82
	35,859,044	483,212	1.35
	35,859,044	260,594	0.73
17/3/2011	31,646,200	374,443	1.18
	31,646,200	192,999	0.61
	31,646,200	458,180	1.45
	35,859,044	374,443	1.04
	35,859,044	192,999	0.54
	35,859,044	458,180	1.28
Average			1.05

Table A10 N_2O concentration of the experiment R0.75(75) with 2.5 mL of sample and 100 ppm of standard

Date	Average area of standard	Area of sample	ppm of sample
15/3/2011	31,646,200	249,098	1.57
	35,859,044	249,098	1.39
17/3/2011	31,646,200	222,580	0.70
	31,646,200	185,907	0.59
	31,646,200	204,464	0.65
	35,859,044	222,580	0.62
	35,859,044	185,907	0.52
	35,859,044	204,464	0.57
Average			0.83

Table A11 N_2O concentration of the experiment R0.75(100) with 2.5 mL of sample and 100 ppm of standard

Date	Average area of standard	Area of sample	ppm of sample
1/5/2011	4,256,020	76,327	1.79
2/5/2011	4,256,020	6,087	0.14
3/5/2011	4,256,020	70,921	1.67
4/5/2011	4,256,020	73,614	1.73
5/5/2011	4,256,020	85,111	2.00
6/5/2011	4,256,020	83,540	1.96
7/5/2011	4,256,020	123,877	2.91
8/5/2011	4,256,020	94,873	2.23
9/5/2011	4,256,020	166,603	3.91
Average			2.04

Table A12 N_2O concentration of the experiment R0.5(75) with 2.5 mL of sample and 100 ppm of standard

Date	Average area of standard	Area of sample	ppm of sample
1/5/2011	4,256,020	328,347	7.71
4/5/2011	4,256,020	380,176	8.93
6/5/2011	4,256,020	695,188	16.33
7/5/2011	4,256,020	947,617	22.27
8/5/2011	4,256,020	284,998	6.70
9/5/2011	4,256,020	738,898	17.36
17/5/2011	4,256,020	191,536	4.50
18/5/2011	4,256,020	733,722	17.24
Average			12.63

Table A13 N_2O concentration of the experiment R0.5(100) with 2.5 mL of sample and 100 ppm of standard

Date	Average area of standard	Area of sample	ppm of sample
24/5/2011	4,256,020	7,395,344	173.76
29/5/2011	4,256,020	8,556,383	201.04
30/5/2011	4,256,020	9,625,651	226.17
31/5/2011	4,256,020	8,564,454	201.23
Average			200.55

APPENDIX B

Analytical Method

Fluorescence *In Situ* Hybridization (FISH) Technique (Ahn *et al.*, 2002)

1. Reagents and Equipment

2.1 Fixative Reagent (Paraformaldehyde 4%)

Fixative reagent was used for fixation of bacterial cells to preserve ribosomal RNA. Fixative reagent was prepared by adding 2 g paraformaldehyde slowly into 33 mL of distilled water (add one drop of 10 M NaOH into distilled water). The mixture was heated gently to 60°C and stirred until dissolved. 3X PBS (16.5 mL) was added into paraformaldehyde solution and cooled down the solution on ice. The pH was adjusted to 7.2 with 1 M NaOH and filtered sterile through a disposable filter. The fixative reagent was kept on ice and used within 24 hours.

2.2 Acid Alcohol

Acid alcohol was used for cleaning slides before poly-L-lysine coating. Acid alcohol was prepared by adding 1% HCl (1 mL) into 70% ethanol (99 mL).

2.3 3xPBS Buffer

3X PBS buffer (390 mM of NaCl in 30 mM of $NaPO_4$ buffer) was prepared by adding 23.4 mL of 5 M NaCl to 18.0 mL of 0.5 M $NaPO_4$ buffer. The pH was adjusted to 7.2 and final volume was adjusted with distilled water to 300 mL.

0.5 M NaPO4 buffer was prepared by mixing 28 mL of 0.5 M NaH_2PO_4 with 0.5 M Na_2HPO_4.

2.4 Hybridization Buffer

Hybridization buffer contained 0.9 M NaCl, 0.01% Dodecyl sulfate sodium salt (SDS), 20 mM Tris-HCl (pH 7.2), and 5-40 % formamide.

20 mM Tris-HCl was prepared by mixing Tris-(hydroxymethyl) aminometane $[NH_2C(CH_2OH)_3]$ 24.23 g in distilled water 150 mL and adjusted pH at 7.2 by HCl.

2.5 Washing Buffer

The washing buffer contained 0.9 M NaCl, 0.01% SDS and 20 mM Tris-HCl (pH 7.2).

2.6 DAPI Solution

4,6-diamidino-2-phenylindole dihydrochloride (DAPI) was used for counterstaining after whole cell hybridization. Stock solution was prepared by adding distilled water or dimethylformamide (DMF) to 100 μM. The stock solution was stored at 4°C. For counterstaining protocol, the DAPI stock solution was diluted to 300 nM in PBS. The working solution (300 nM) was added on the fixed cells and incubation at room temperature (5 min). After that, slides were rinsed by distilled water.

2. Procedure

2.1 Sample Fixation

Sample cells (1 mL) were harvested by centrifugation at 7,000 rpm for 5 min and removed 750 μL of supernatant. The cells were mixed thoroughly by 750 μL of fixative reagent and incubated cells at 4°C for 1-3 hours. The cell pellet was done by centrifugation (2 min, 700 rpm) and discarded supernatant, then washed twice with 1XPBS buffer by diluted 3XPBS buffer and discarded supernatant. The sample were stored in 1:1 mixture of 1XPBS buffer and 96% ice cold ethanol at -20°C.

2.2 Slide Preparation

Slide was cleaned in acid alcohol (1-2% HCl in 70% ethanol) for 5 min and placed slide into poly-L-lysin (0.01% v/v) at room temperature for 5 min. The coated slide was dried at 60°C for 1 hour or overnight at room temperature and was ready for use.

2.3 *In Situ* Hybridization

Fixed samples cells were spotted onto glass slides (3-5 μL/sample) and air dried at 37°C or room temperature for 10-15 min. Dried slides were dehydrated with ethanol series (50, 80, and 96%; 3 min/each), then air dried at room temperature. After that, samples were hybridized at 46°C for 1.5-2 hours with 9 μL of

hybridization buffer and 1 μL of probes (50 ng). Hybridization stringency was adjusted by adding formamide to hybridization buffer (5-40%). After hybridization, the slides were washed at 48°C for 15 min in washing buffer.

Washing buffer was removed with distilled water, then the slides were air dried. After that, the fixed samples were covered with 10-15 μL of DAPI for 15 min at room temperature and following with twice distilled water, then the slides were air dried and mounted with antifading (supplied by Molecular Probes). The slides were viewed on an Olympus microscope BX60 with appropriated filters.

3. Calculation

The fraction of microorganisms is calculated from the amount of cells after being hybridized with the target probe divided by the amount of cells after being hybridized with DAPI.

APPENDIX C

Analytical Method

Gas Chromatography (GC) Analysis of Nitrous Oxide (N_2O) Production

1. Gas Chromatography Instrument

GC-ECD Shimudsu 14B used the following conditions in analysis:

Column: 200×0.3 cm of porapack Q and porapack N

Column temp.: 65°C

Injection temp.: 150°C

Detector temp.: 300°C

Carrier gas: 99.99% of N_2 and 5% of CH_4 in Ar

2. Gas collection

2.1 Gas sampling

420 mL gas sample from the headspace of reactor sucked into gas bag at steady state by syringe of 60 mL. The gas bag of each gas sample was injected into GC-ECD Shimudsu 14B instrument for N_2O analysis. The 10% of N_2O gas standard (100,000 ppm of N_2O) was injected into GC-ECD before the gas sample injection then compared the gas sample concentration with 100 ppm of N_2O gas standard averaged.

2.2 Dilution N_2O gas standard

Gas-tight syringe of 2.5 mL was sucked 10% of N_2O gas standard (100,000 ppm of N_2O). Gas-tight syringe filled 2.5 mL with the gas standard and the gas in the gas-tight syringe was released to 0.25 mL to 10-fold of gas-tight syringe volume. After that, filled and released the gas standard in gas-tight syringe 3 times then get 100 ppm of N_2O gas standard in the final.