American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)

ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Study on Sludge Characteristics and Nitrogen Removal Mechanism of Hybrid Partial Nitrification Anammox Reactor (HPNA)

MD. Asadur Rahoman[a*], Chai Tianyu[b], Zhu Tong[c]

[a,b]*Northeastern University, NO. 3-11, Wenhua Road, Heping District, Shenyang, 110819, P. R. China*

[a]*Email: asadkarat7@gmail.com*

[b]*Email: chai_tianyu@qq.com*

Abstract

Anaerobic ammonium oxidation (anammox) is a chemical process that involves the removal of inorganic nitrogen during the treatment of wastewater. Partial nitrogen-anammox process is the best alternative to the conventional nitrification-denitrification process in terms of cost-effectiveness. During the process, half of the ammonium is partly oxidized into nitrite. An Anammox bacterium is oxidized by ammonium to nitrogen gas with the use of an electron acceptor (nitrite) under anoxic conditions, and their growth occurs by carbon dioxide fixation. The process of partial nitritation-anammox demands no exogenous carbon source (e.g. methanol) and less oxygen as compared to the conventional nitrification-denitrification process. Another factor that lowers the operational costs is the low sludge production. Nevertheless, since there is low biomass production of anammox bacteria, the reactors that effectively retain biomass and can provide a long solids retention time are needed for the anammox process to be efficient and successful. A single reactor usage has a number of benefits to the process setup. Single–stage processes have easy reactor configuration and lower operational costs. *In this study, the hybrid anammox reactor has two types of reactors developed to be used, the fluidized and fixed beds.*

---

* Corresponding author.

The following had to undergo experimental evaluation which has vital theoretical and practical significance

- The rapid startup of the anammox systems
- Enrichment of anammox granular
- The best nitrogen removal performances

Keywords: Partial nitrification; anammox; hybrid reactor; sludge characteristics; nitrogen removal mechanism.

1. Introduction

Anammox is a microbial procedure for inorganic nitrogen removal from wastewater treatment [1, 2]. In the anammox reaction, ammonium (NH_4^+-N) is used aerobically as the electron donor for the reduction of nitrite (NO_2^--N), yielding dinitrogen gas (N_2) as the end product.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.066CH_20.5N_{0.15}$$

A partial nitrification- anammox process as compared to the conventional nitrification-anammox process is a cost-effective process. During the process, only half of the ammonium is oxidized partly to nitrite and then anammox bacteria oxidizes ammonium to nitrogen gas utilizing nitrite as an electron acceptor under anoxic conditions. Their growth occurs by carbon dioxide fixation. The partial nitrification-anammox process needs less oxygen and no exogenous carbon source (e.g., methanol). Anammox bacteria have tremendously low growth rates (a replication period of 11 days) and a very low biomass yield emanating from the anammox process, meaning there is the production of a small amount of excess sludge in the anammox treatment. The low sludge production contributes to lower operation costs. Nonetheless, since there is low biomass yield of anammox bacteria, reactors with effective biomass retention and capabilities of long solids retention period are required for the anammox process to be successful and efficient. Biofilm type reactors like the fluidized bed, fixed bed and gas-lift reactors were initially used in anammox treatment [3,4,5]. Also contributed to anammox sludge cultivation is enhanced by the sequencing batch reactor [5,6,7]. Fixed-bed reactors using various types of biocarriers, for example, polyester non-woven material [8], polyethylene (PE) sponge [9], polyethylene glycolgel [10], poly (vinyl alcohol)-gel beads [11]and novel acrylic resin material [12], were established for stable immobilization of anammox sludge. It has been a challenge to develop new types of anammox reactors and their application to actual wastewater treatment. In anaerobic wastewater treatment technologies, hybrid reactors combine the advantages of both fixed-bed and sludge blanket reactors in having both free and support matrix regions. The support matrix region retains suspended sludge within the reactor and treats the wastewater through the biofilm activity developed on the packing material. Even though the hybrid reactor design has been effectively applied to several wastewater treatments [13, 14, 15], studies of its application to the anammox process have not been carried out yet. In this section, a hybrid reactor design combining two parts, the fixed and fluidized beds, was developed. The fluidized bed was mechanically stirred continuously to escalate the mixing efficiency between anammox sludge and wastewater and then release the gaseous products from the sludge. The fixed bed with the use of a non-woven biomass carrier was designed to catch the suspended sludge and reduce its washout. Some of the measures investigated experimentally include anammox granular enrichment, the rapid

startup of hybrid anammox reactors, the hybrid anammox reactor's high nitrogen removal efficiency.

2. Material and Methods

2.1 Reactor structure parameters and operational strategy

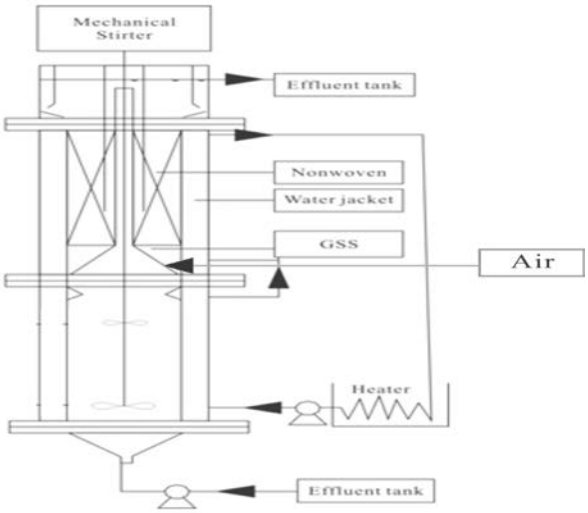


Figure 1: Schematic diagram of hybrid anammox reactor (HPNA).

The hybrid reactor was operated and evaluated to determine their treatment capabilities (as shown in Table 2.1). The reactor was in the upper part consisting of acrylic resin and had an effective volume (V_{eff}) of 30 L, an internal diameter of 280 mm and a length/diameter ratio of 5:2. The reactor comprised a fluidized bed in the lower region (0-450 mm from the bottom) and a fixed bed (400-700mm from the bottom). A mechanical stirrer (z-2100, Beijing, China) was fitted from the top to the lower region of the reactor. A turbine stirrer (60mm in diameter) with five blades made of stainless steel was utilized. The turbine stirrers were located at ca.60 from the bottom, to mechanically fluidize the lower section (fig 2.1). In the fluidized bed, there was no use of carrier particles for formation of granules. The upper region was filled with the porous polyester non-woven fabric carrier. Ten blades carrier were slot in the upper region with a superficial volume of 7.5L (21% of total volume of reactor). Two GSS were attached to the reactor, located at 280 mm (lower port) and 550 mm (higher port) from the bottom. The reactor was surrounded by a black vinyl sheet to prevent the growth of photosynthetic bacteria. The temperature was controlled by pH meter (IM-21p, TOA Electronis, (China) as shown in table 2.1. Nitrogen gas cleansed the influent storage tank to maintain an influent DO concentration below 1 mg/L in the reactors. The reactor operation was in an up-flow mode with the influent brought in from the bottom section by a peristaltic pump.

2.2 Inoculums and substrates

Seed anammox sludge containing strains and KSU-1 [8, 16] was collected from an anammox reactor operator in our laboratory. Using a polyethylene (PE) non-woven fabric as biomass carrier, the enriched granular sludge (accounting for 80% of the MLSS) was taken from a 30 L up-flow fixed-bed reactor and fed with synthetic

wastewater [17]. The reactors were seeded with 525g (MLSS) of anammox sludge, respectively, resulting in an initial concentration of about 17.55g-MLSS/L, respectively (as seen in Table 2.1).

In this study, synthetic wastewater was fed into the reactor with an NO_2 -$^-$N to NH_4^+-N molar ratio of 1.0-1.3. During the operational time, synthetic wastewater with the composition of $(NH_4)_2SO_4$ 283 g/L, KH_2PO_4 0.O68 g/L, KCL 0.4g/L, $MgSO_4.7H_2O$ 0.06 g/L, $FeSO_4.7H_2O$ 0.009 g/L, and EDTA-2NA 0.025 g/L was utilized.

Table 1: Operational strategy of hybrid HPN anammox reactors

Substrates	Concentration
Seed sludge	18 (g-MLSS/L)
$(NH_4)_2SO_4MgSO_4$	283 (g/L)
KH2PO4	0.068 (g/L)
$MgSO_4.7H_2O$	0.06(g/L)
$FeSO_4.7H_2O$	0.009(g/L)
KCL	0.04(g/L)
EDTA-2NA	0.025(g/L)

2.3 Feeding media of anammox sludge

The reactor were fed with synthetic media, with $Cacl_2$, $FeSo_4.7H_2O$, $MgSO_4.7H_20$, $MnSO_4.7H_2O$, Glucose, Starch, $NaHCO_3$, Urea(NH_2-NH_2), Inorganic salt, and KH_2PO4 respectively.

Table 2: Composition of synthetic medium

Compound	Concentration (g/L)
$FeSo_4.7H_2O$	1.375g/L
$MgSO_4.7H_2O$	15g/L
$MnSO_4.7H_2O$	15g/L
Glucose	30g/L
Starch	30g/L
$NaHCO_3$	12g/L
Urea (NH_2-NH_2),	5.4g/L
Inorganic salt	200mL
KH_2PO4	3.5g/L
$Cacl_2$	15g/L

2.4 Analytical method

A modified phenate method was used to measure NH_4^+-N by using ortho-phenyl phenol [18]. Concentration of NO_2-$^-$N, NO3-$^-$N, suspended solids (SS) were measured according to standard methods [19]. A DO meter (OM-52, Beijing, China) measured the DO, pH was measured by a digital P^H meter (IM-21p, TOA Electronis, China), respectively. A laser scattering particle size distribution analyzer (LA-922, Beijing, China) performed the particle size analysis. MLSS and MLVSS were measured by drying at 103℃ and 700℃ respectively on an evaporating dish. Extracellular polymeric substances (EPS) were obtained from sludge by formaldehyde plus NaOH and proteins were measured with the use of the method of Lowry and his colleagues [25] while carbohydrates were measured by the method of Dubois and his colleagues [26]. Microorganisms in the sludge were observed by an electron microscope (Nikon Eclipse E600, Japan) with a digital camera (Nikon 4500, Japan).

2.5 SEM observation

The anammox granules' outward and inner parts were observed using a scanning electron microscope (SEM). Samples were initially washed in a 0.1 phosphate buffer solution (pH 7.5) for 5 minutes. The samples were hardened for 90 minutes in a 2.5% glutaraldehyde solution prepared with 0.1M phosphate buffer solution (pH 7.5). The samples had to be washed thrice in buffer solution for 10 minutes each and fixed for 90 minutes in a 1.0% OsO_4 solution prepared using 0.1 M phosphate buffer solution (P^H 7.5). After washing the samples, they were dewatered for 10 minutes each in serially graded solution of ethanol with a concentration of 10, 30, 50, 70, 90 and 95%. SEM observations were conducted using a scanning electron microscope (JEISS, Germany).

2.6 PCR augmentation and DNA extraction

At day 160, a sludge sample was taken from the hybrid PN reactor. The granular sludge sample was first pounded with a pest under liquid nitrogen. Metagenomic DNA was extracted using an ISOIL kit (Wako, Osaka, Japan) according to the manufacturer's guidelines. The Phusion High-Fidelity performed the amplification of 16S rRNA gene. DNA polymerase (FINNZYMES Finland) utilizing preserved eubacterial primers at 6F (forward primer: 5'-GGAGAGTTAGATCTTGGCTCAG-3') and 149r(reverse primer: 5'-GGTTACCTTGTTACGACT-3'). PCR was undertaken in accordance with these thermocycling constraints: 30 seconds initial denaturation at 98℃, 25 cycles of 10 seconds at 98℃, 20 seconds at 51 C, 35 seconds at 72℃, and 5 minutes final elongation at 72℃. The amplified products were electrophoresed on a 1% agarose-gel and the excised fragments were purified using a PCR clean-Up System and Wizard SV Gel (Promega, USA).

2.7 Cloning and sequencing of 16S rRNA gene

The purified fragments were lighted into the EcoRV site of pBluescript II KS+ (Stratagene, USA) and E.coli

DH 5α was altered using the fabricated plasmids. White colonies (the insert included) were chosen randomly and the plasmids were extracted by the alkaline method. A 3130xl genetic analyzer and BigDye terminator v3.1 cycle sequencing kit (Applied Biosystem, USA) were used to determine the nucleotide sequences. The basic local alignment search tool (BLAST) program/application found on the NCBI website was used to compare the sequences determined in this study with the sequences in the nr-database.

3. Results and discussion

3.1 The treatment performance of the anammox reactor

160 days were used to monitor the reactor performance, during which the influent nitrogen concentrations and HRT were altered, as shown in Table 3.2. The treatment results for continuous operation of the reactor are shown in Fig (3.1; 3.2; 3.3) During the 160 days of operation, the influent total TN concentration was progressively increased from 100 to 800 mg/L and the HRT was decreased from 1.9 to 0.64 h with an average nitrite removal efficiency of 80% and an average total nitrogen removal efficiency of 58%. An NLR of 1.8kg-N/m^3/d and corresponding NRR of 0.95 kg-N/m^3/d were obtained after 160 days of operation.

Table 3: 180 days observation data of NLR; NRR; TN removal efficiency

Times (days)	NLR (kg-N/m^3/d)	NRR (kg-N/m^3/d)	TN removal efficiency (%)
0	1.2637	0.1356	10.73
1	1.1932	0.4038	33.84
2	1.1869	0.5893	49.65
3	1.1959	0.7435	62.17
4	1.2653	0.8117	64.15
5	1.1677	0.7884	67.52
6	1.2057	0.8365	69.38
7	1.3015	0.9423	72.40
8	1.2337	0.9278	75.21
9	1.269	0.7724	60.87
10	1.17	0.4992	42.66
11	1.1901	0.4362	36.65
12	1.2592	0.6126	48.65
13	1.1789	0.55755	48.81
14	1.1753	0.6148	52.31
15	1.2407	0.724	58.35

16	1.3168	0.8067	61.26
17	1.4408	0.811	56.29
18	1.4329	0.8996	62.78
19	1.4325	0.8491	59.28
20	1.4518	0.4204	28.95
21	1.4305	0.5822	40.70
22	1.358	0.5873	43.25
23	1.3737	0.8395	61.11
24	1.406	0.7995	56.86
25	1.4608	0.7676	52.55
26	1.4567	0.651	44.69
27	1.4538	0.6078	41.81
28	1.4251	0.7594	53.29
29	1.3949	0.8155	58.47
30	1.3908	0.9296	66.84
31	1.7415	1.0543	58.47
32	1.6961	0.8718	66.84
33	1.6447	0.727	60.54
34	1.7049	0.7952	46.64
35	1.6512	0.7203	43.62
36	1.7013	0.6641	39.03
37	1.7013	0.6609	38.85
38	1.6625	0.6647	39.98
39	1.6539	0.6092	36.83
40	1.7065	0.6199	36.33
41	1.7281	0.635	36.74
42	1.6433	0.54545	33.14
43	1.7132	0.5881	34.33
44	1.6136	0.6975	43.23
45	1.6555	1.0977	66.31
46	1.6856	1.1225	66.60
47	1.7116	1.0201	59.60

48	1.8866	1.1056	58.60
49	1.9242	0.722	37.52
50	2.0452	1.1272	55.11
51	2.0679	1.1875	57.43
52	2.0967	1.2230	58.33
53	1.9876	1.090440505	54.86
54	2.0355	1.232899984	60.57
55	1.9948	1.348424531	67.60
56	1.9294	1.301936058	67.48
57	1.8441	1.108087725	60.09
58	1.8643	1.177954395	63.18
59	1.8553	1.218772623	65.69
60	2.1097	1.370598723	64.97
61	1.9769	1.259501937	63.71
62	2.0421	1.347907061	66.01
63	2.0951	1.322639329	63.13
64	2.0605	1.186664174	57.59
65	2.0498	1.328969611	64.83
66	1.8606	1.08153826	58.13
67	2.0838	1.335334299	64.08
68	2.0088	1.317576294	65.59
69	1.9228	1.265638087	65.82
70	1.8731	1.286987396	68.71
71	1.9415	1.401169014	72.17
72	1.9928	1.406476984	70.58
73	1.9683	1.344978588	68.33
74	1.9457	1.358522872	69.82
75	2.0043	1.370472368	68.38
76	1.9554	1.382434044	70.70
77	1.91	1.197012029	62.67
78	2.4097	1.671095473	69.35
79	2.3318	1.364675277	58.52

80	2.335	1.554693551	66.58
81	2.4085	1.405885596	58.37
82	2.4356	1.284320488	52.73
83	2.4522	1.614293187	65.83
84	2.689	1.390410127	51.71
85	2.7802	1.788863854	64.34
86	2.6839	1.220217204	45.46
87	2.5515	1.101364198	43.17
88	2.6097	1.010565487	38.72
89	2.6871	1.017637628	37.87
90	2.5999	1.068050786	41.08
91	2.7904	1.085401713	38.90
92	2.394	1.01377595	42.35
93	2.1661	0.922170662	42.57
94	2.8835	1.013823112	43.18
95	2.9003	1.02346145	47.19
96	2.8218	1.4524	51.48
97	2.8725	1.1712	40.76
98	2.7389	1.5013	54.83
99	2.7292	1.5628	57.26
100	2.7487	1.7369	61.23
101	2.7883	1.9537	63.19
102	2.6463	1.8322	70.07
103	3.0758	1.9388	69.24
104	3.0068	1.8852	63.03
105	2.7896	1.8399	62.70
106	2.6835	1.8394	65.95
107	2.7156	1.9036	68.54
108	2.7058	1.955	70.10
109	2.6931	1.86	72.25
110	2.5572	1.7665	69.07
111	2.623	1.818	69.08

112	2.5923	1.8562	69.31
113	2.5817	1.8398	71.61
114	2.619	1.8699	71.26
115	2.3002	1.4735	71.40
116	2.6217	1.8586	64.06
117	2.6305	2.1117	70.89
118	2.698	0.704	80.38
119	2.666	0.6955594	26.09
120	2.7534	1.3313	44.63
121	2.6043	1.0053	41.23
122	2.6663	1.1972	42.34
123	2.6233	1.1923	40.76
124	2.6334	1.3462	54.19
125	2.7324	1.0054	61.28
126	2.79812	1.5325	54.19
127	2.73895	1.501814823	54.83
128	2.72918	1.562762546	57.26
129	2.7487	1.73687641	63.19
130	2.78835	1.953709217	70.07
131	2.64631	1.832243058	69.24
132	3.07583	1.938813224	63.03
133	3.00683	1.885169154	62.70
134	2.78961	1.83987996	65.95
135	2.68353	1.839400034	68.54
136	2.71558	1.903554449	70.10
137	2.70583	1.955000238	72.25
138	2.69309	1.86002332	69.07
139	2.55722	1.766459227	69.08
140	2.62296	1.817991396	69.31
141	2.59225	1.856193207	71.61
142	2.58171	1.839831636	71.26
143	2.61903	1.869912178	71.40

144	2.30021	1.47349	64.06
145	2.62172	1.858594842	70.89
146	2.6304	2.111704071	80.28
147	2.6783	2.134526	75.23
148	2.69795	0.70400072	26.09
149	2.65421	0.3563214	35.75
150	2.66596	1.189764701	44.63
151	2.75339	1.331345092	48.35
152	2.60426	1.005250063	38.60
153	2.66631	1.197175098	44.90
155	3.51017	1.502512429	42.80
156	3.91986	2.25348089	57.49
157	3.41765	2.16063833	63.22
158	3.74584	1.657444595	44.25
159	3.60265	2.30065229	63.86
160	3.53101	0.3074	8.71
162	1.26066	0.30032	23.82
165	1.20593	0.473332749	39.25
166	1.17209	0.583372552	49.77
167	1.19561	0.686575764	57.42
168	1.12044	0.620376239	55.37
169	1.17139	0.907056458	77.43
170	1.13508	0.649485356	57.22
171	1.10089	0.567106135	51.51
172	1.14074	0.801529095	70.26
173	1.10896	0.826151297	74.50
174	1.19031	0.891109739	74.86
175	2.30039	1.35312	58.82
176	2.30203	1.228797169	53.38
177	2.24592	1.393191027	62.03
178	2.26751	1.165099003	51.38
179	2.2591	1.428096384	63.22

180	2.1561	1.37679255	63.86

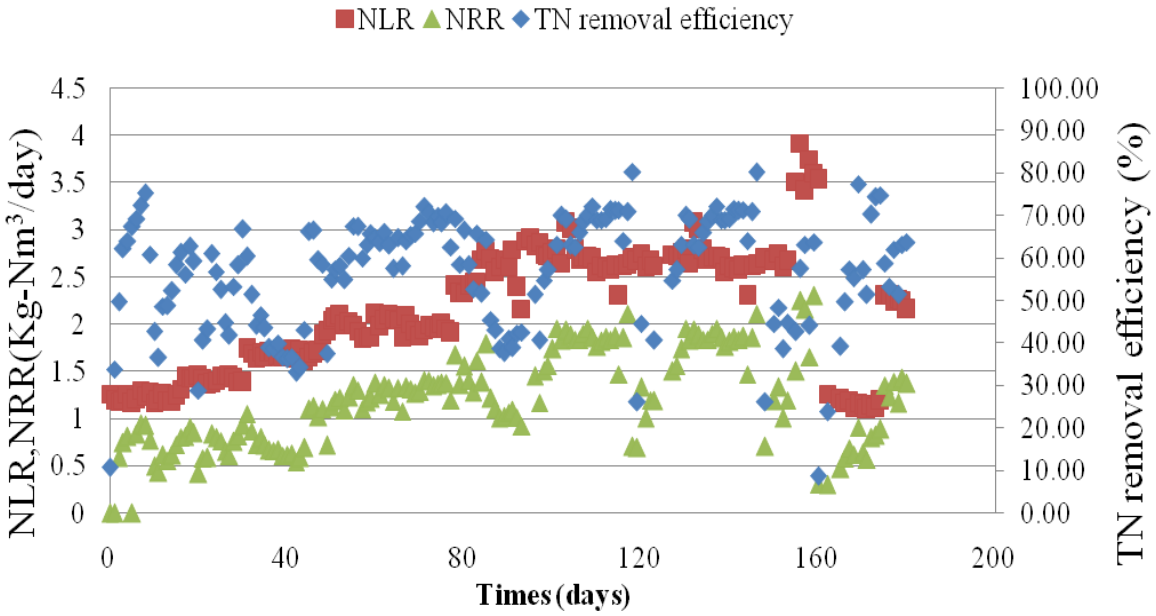


Figure 2: Time courses of total NLR, NRR, nitrogen removal efficiency

Table 4: 160 days observation data of inf. NH_4-N inf; NO_2-N eff; NO_3-N eff; NH_4-N eff

Time(days)	NH_4-N inf (mg/L)	NO_2-N eff (mg/L)	NO_3-N eff (mg/L)	NH_4-N eff (mg/L)
0	647.19	0.05	43.83	538.40
1	610.00	10.02	64.56	332.89
2	605.81	7.76	79.82	220.84
3	611.86	9.70	93.13	130.65
4	648.82	9.75	96.76	127.62
5	597.91	9.95	112.10	73.69
6	617.44	9.80	112.43	68.35
7	666.72	9.53	113.11	62.77
8	630.92	1.59	152.55	3.72
9	651.38	1.46	216.95	37.89
10	599.77	1.06	342.19	2.98
11	610.00	1.27	385.68	2.14

12	645.56	2.71	302.66	28.36
13	603.49	2.26	307.47	1.73
14	600.70	1.75	286.23	1.31
15	636.50	2.93	260.27	3.51
16	675.32	3.08	255.89	4.30
17	614.18	2.74	264.82	3.31
18	611.39	5.33	211.05	13.02
19	610.46	0.07	174.30	76.54
20	618.83	0.02	118.17	325.46
21	609.53	6.79	116.99	241.07
22	578.85	39.79	25.29	266.41
23	584.66	9.65	112.60	107.52
24	596.51	0.86	259.09	0.93
25	623.48	1.43	290.44	6.28
26	621.15	8.21	207.68	130.65
27	619.76	9.03	145.64	209.22
28	606.74	8.62	133.34	144.36
27	619.76	9.03	145.64	209.22
28	606.74	8.62	133.34	144.36
29	592.79	6.28	132.49	110.42
30	592.79	10.11	133.84	54.40
31	636.03	7.26	157.27	88.80
32	618.83	7.59	121.71	174.58
33	598.84	7.52	95.07	235.72
34	624.41	8.83	98.95	227.59
35	604.88	7.44	68.44	267.34
36	623.01	7.83	60.52	314.06
37	623.01	7.84	55.63	320.11
38	608.14	6.79	45.85	315.23
39	604.42	9.12	50.57	325.46
40	624.87	9.17	54.11	337.31
41	632.31	7.93	47.54	347.54

42	600.70	5.59	53.10	346.38
43	624.87	6.39	57.14	351.26
44	590.00	13.66	91.36	232.70
45	604.88	14.44	159.13	32.08
46	614.65	6.65	196.04	4.88
47	626.73	4.16	243.58	7.21
48	603.49	7.11	241.39	3.43
49	614.18	0.03	158.12	229.68
50	655.56	8.86	272.07	15.23
51	662.07	11.48	161.49	111.00
52	670.44	11.00	128.79	142.04
53	636.96	13.99	123.73	151.69
54	652.31	12.92	107.55	138.43
55	638.36	11.60	116.48	80.43
56	616.04	10.64	103.67	88.11
57	590.00	11.90	110.92	114.61
58	596.05	11.12	87.66	122.63
59	591.40	10.46	86.31	108.56
60	676.02	11.35	94.57	132.51
61	633.24	8.87	80.74	141.81
62	653.24	7.92	83.27	132.74
63	671.83	12.92	82.60	153.66
64	659.28	11.17	67.76	202.94
65	654.63	11.36	61.70	159.47
66	595.58	11.27	67.09	172.96
67	667.18	10.72	61.02	169.70
68	642.08	10.78	63.89	148.31
69	615.11	7.52	64.06	140.41
70	598.84	8.08	64.06	116.93
71	619.76	10.09	65.07	99.15
72	637.43	12.23	64.39	112.51
73	629.06	11.79	69.45	119.84

74	620.69	14.04	68.44	106.94
75	641.15	7.28	61.19	135.99
76	626.27	11.48	81.76	91.59
77	611.39	24.65	202.79	2.56
78	616.97	7.61	164.86	18.13
79	596.05	7.71	129.12	112.75
80	595.12	7.04	132.49	61.84
81	617.90	8.93	118.00	131.81
82	623.94	6.95	107.21	182.95
83	629.52	6.33	103.67	106.24
84	689.97	7.74	99.79	227.59
85	647.66	8.20	113.95	110.42
86	625.34	7.34	84.28	251.76
87	625.34	5.21	68.44	284.37
88	594.65	7.23	73.16	286.40
89	608.14	6.79	58.83	314.76
90	626.27	7.61	60.68	303.14
119	658.81	0.15	43.83	550.95
120	657.12	9.24	41.25	450.12
121	665.15	8.75	43.65	245.122
122	645.11	7.25	60.32	266.23
123	671.37	9.71	44.50	344.98
124	664.23	7.34	54.43	132.45
125	671.23	8.11	65.67	141.35
126	675.23	9.23	72.18	143.23
127	639.29	9.60	76.53	204.11
128	635.57	8.99	64.73	199.92
129	642.08	11.10	70.29	155.99
130	651.84	9.70	73.83	112.28
131	618.36	10.04	78.89	102.05
132	718.33	10.21	77.54	179.00
133	702.05	11.84	67.43	183.88

134	651.84	10.99	68.27	143.55
135	626.73	10.49	69.45	118.09
136	633.24	10.28	67.60	112.63
137	630.92	8.93	65.74	101.47
138	627.66	11.98	56.30	127.16
139	596.05	9.74	55.12	120.65
140	611.39	9.40	51.24	128.21
141	603.95	8.98	49.56	114.14
142	601.63	8.68	51.92	113.44
143	610.00	6.27	47.20	122.28
144	536.54	7.19	42.98	143.78
145	611.39	7.40	43.66	127.97
146	613.72	30.41	88.67	2.62
148	629.06	265.58	199.25	2.96
150	622.08	215.02	124.07	7.24
151	641.61	202.83	124.57	6.22
152	607.67	2.11	29.67	343.36
153	622.55	19.30	43.15	282.22
154	622.08	215.	124.07	7.24
155	641.61	202.83	124.57	6.11
156	607.57	202.11	29.57	343.36
157	622.55	11.30	43.15	282.22
158	623.55	12.25	41.25	275.11
159	601.16	6.11	13.49	325.8
160	671.83	8.61	13.49	255.57

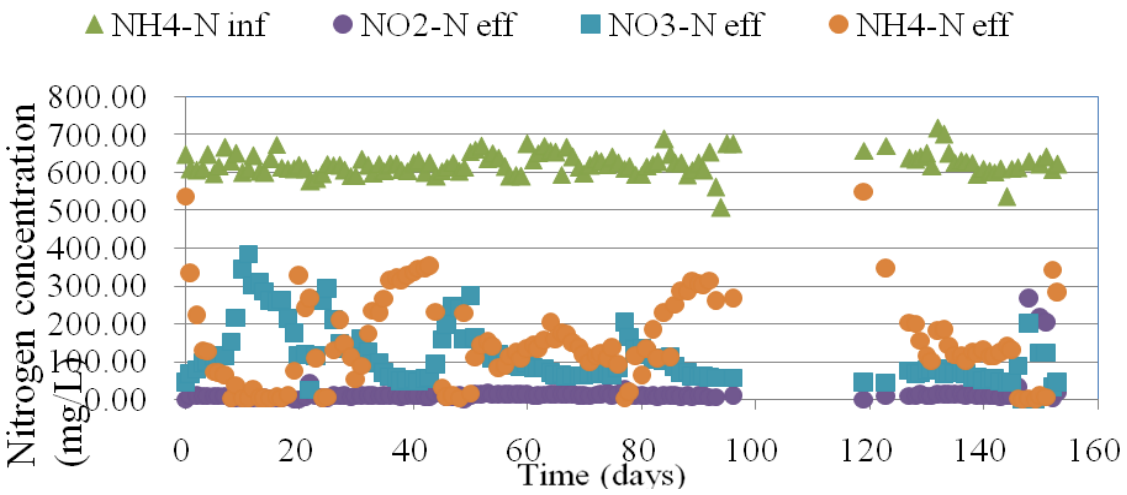


Figure 3: Time courses of total influent and effluent nitrogen concentrations

Table 5: 160 days observation data of HRT

Time (Days)	HRT (Hours)	Time (Days)	HRT (Hours)	HRT (Hours)	Time (Days)
1	1.9355	11	1.9355	21	1.6129
2	1.9355	12	1.9355	22	1.6129
3	1.9355	13	1.9355	23	1.6129
4	1.9355	14	1.9355	24	1.6129
5	1.9355	15	1.9355	25	1.6129
6	1.9355	16	1.9355	26	1.6129
7	1.9355	17	1.9355	27	1.6129
8	1.9355	18	1.6129	28	1.6129
9	1.9355	19	1.6129	29	1.6129
10	1.9355	20	1.6129	30	1.6129
31	1.6129	54	1.273514	78	0.9677
32	1.3825	55	1.265113	89	0.9677
33	1.3825	56	1.256713	80	0.9677
34	1.3825	57	1.248313	81	0.9677
35	1.3825	58	1.239913	82	0.9677
36	1.3825	59	1.231513	83	0.9677
37	1.3538	60	1.223112	84	0.9677
38	1.3825	62	1.2097	85	0.645161
39	1.3825	63	1.2097	86	0.645161
40	1.3825	64	1.2097	87	0.645161
41	1.3825	65	1.2097	88	0.645161
42	1.3825	66	1.2097	89	0.645161

43	1.3825	67	1.2097	90	0.645161
44	1.3825	68	1.2097	91	0.645161
45	1.3825	69	1.2097	92	0.645161
46	1.3825	70	1.2097	93	0.645161
47	1.332315	71	1.2097	94	0.645161
48	1.323915	72	1.2097	95	0.645161
49	1.315515	73	1.2097	96	0.645161
50	1.307115	74	1.2097	97	0.645161
51	1.298714	75	1.2097	98	0.645161
52	1.290314	76	1.2097	99	0.645161
53	1.281914	77	1.2097	100	0.645161
101	0.645161	125	0.645161	149	0.645161
102	0.645161	126	0.645161	150	0.645161
103	0.645161	127	0.645161	151	0.645161
104	0.645161	128	0.645161	152	0.879765
105	0.645161	129	0.645161	153	0.645161
106	0.645161	130	0.645161	154	0.645161
107	0.645161	131	0.645161	156	0.645161
108	0.645161	132	0.645161	157	0.645161
109	0.645161	133	0.645161	158	0.645161
110	0.645161	134	0.645161	159	0.645161
111	0.645161	135	0.645161	160	0.645161
112	0.645161	136	0.645161		
113	0.645161	137	0.645161		
114	0.645161	138	0.645161		
115	0.645161	139	0.645161		
116	0.645161	140	0.879765		
117	0.645161	141	0.645161		
118	0.645161	142	0.645161		
119	0.645161	143	0.645161		
120	0.645161	144	0.645161		
121	0.645161	145	0.645161		
122	0.645161	146	0.645161		
123	0.645161	147	0.645161		
124	0.645161	148	0.645161		

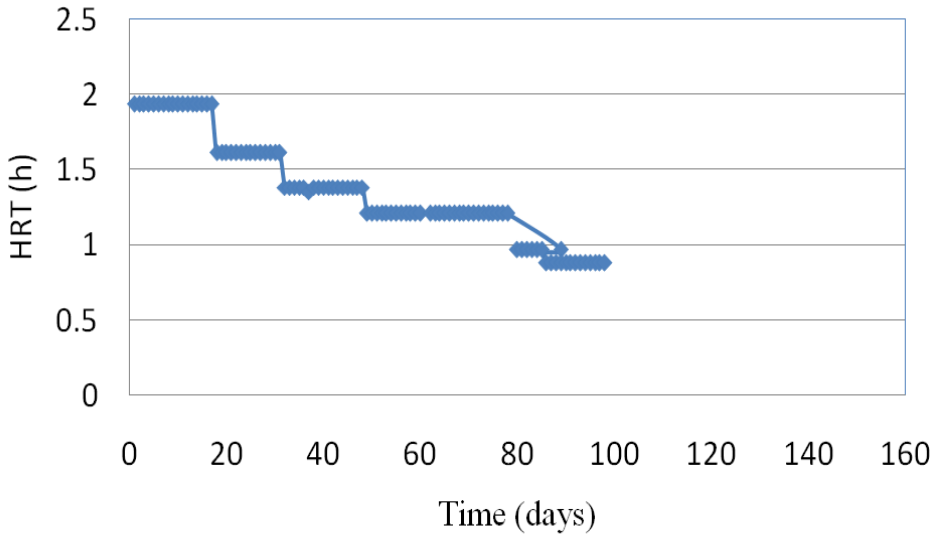


Figure 4: Time courses of concentrations of HRT in (HPNA) reactor

3.2 Nitrogen conversion

In the initial 16 days, the partial nitrification performance was unsatisfactory as a result of high pH level in the reactor average (8.16). Effluent nitrite levels increased gradually to 450 mg/L after adjusting reactor pH to a suitable range (pH 7.45) (effluent NO_2-N/NH_4-N ratio of 0.22±0.46). Consequently, the pH effects on the partial nitritation were investigated by regulating reactor pH from 7.4 to 7.45. It was noted that the effluent nitrite concentrations increased and the peak effluent NO_2-N/NH_4-N ratio of 4.72±44.80 was observed during this period (days 85 to 151). In the 17 days that followed (period 3), the reactor was run at a constant HRT without P^H, DO, and temperature control (the room temperature was about 25℃ due to continuous air-conditioning). Satisfactory performances were obtained and the average effluent NO_2-N/NH_4-N ratio was 0.17±0.67 in this period, which established that the partial nitritation reaction could be achieved successfully under a relatively NLR of 1.7116 kg-N/m^3/d even without control of running conditions. The effluent nitrite levels decreased slightly and the treatment capacity soon began to recover. On day 120, biomass detachment from BF materials resulted from SEM sampling, which caused the suspended solids level to rise more than before from (35 mg/L to 450 mg/L). The effluent NO_2-N/NH_4-N ratio on day 130 was 3.71±40.82 observed, shown in Fig.3.2. It was postulated that there existed a huge number of ammonium oxidizing bacteria (AOB) in the reactor and the attached state restricted the treatment potential of AOB. Additionally, it was considered that a suspended state could improve the contacting chances between nutrients and microorganisms, and therefore the performances of partial nitritation were greatly strengthened. Soon, NLRs were increased via1.3 kg-N/m^3/d to 2.6 kg-N/m^3/d, and the average effluent nitrite/ammonium ratio reached about 0.04±0.03 with temperature and P^H control.

From period 6 (from day 85 to day 151), the HPNA reactor was operated once again without P^H, DO, and temperature control in order to examine the partial nitritation capacity under variable conditions with a high NLR of 2.7 kg-N/m^3/d. Due to the FA and FNA beneficial effects, satisfactory partial nitritation performance

was achieved and the average effluent nitrite/ammonium ratios were 4.72±44.80 in period 6 respectively. From day 151, the effluent of the HPNA reactor was introduced into the anammox reactor and the resulting removal performance is showed in Fig. 3.1. Since there were different volumes of the two reactors, the NLRs of the anammox reactor were 1.8 kg-N/m^3/d (HRT of 1.3 h) even with the same rate of flow. However, there was an observation of high nitrite removal efficiencies (99.6%) and low nitrite production (effluent nitrates of 80mg/L) and an average T-N removal efficiency of 72% was obtained in this experimental period. But a relatively long HRT and high nitrite concentrations stimulated the growth of NOB, giving rise to increased rate of nitrite production in the system, with the maximum effluent nitrate concentration of the two reactors being 252.5 and 315.5 mg/L, respectively. Concurrently, the lower P^H value as a result of nitritation caused the FNA and FA concentration in the PN reactor varying, resulting in the reverse effects for AOB and NOB. Although there were high effluent nitrate concentrations, most ammonium and nitrite were eliminated by anammox reaction and an average T-N removal efficiency of 58% was achieved.

3.3 Start-up time

The anammox reactor was started up with an influent TN concentration of 100 mg/L and an HRT of 1.9 h. During days 1 to 16, intermittent stirring (run 5 minutes, stop 30 minutes) was originally applied and the NLR was about 1.31 kg-N/m^3/d but total nitrogen removal efficiency was only around 51%. During days 17-30, stirring continuously (30 rpm) and internal circulation were applied and the NLR was increased quickly from 1.31 to 1.39 kg-N/m^3/d by increasing the influent TN concentration and decreasing the HRT. During this moment, the influent NO_2^--N concentration stayed below 15 mg/L, with a TN removal efficiency of 54%. This shows that internal circulation and stirring continuously are important during the start-up moment.

Table6: Summary of different operational conditions and performances

Period (day)	Flow rate (L/h)	pH (reactor)	Temperature (℃)	HRT (h)	NLRs (kg-N/m^3/d)	NRRs	Effluent NO_2-N/NH_4-N
1 (0-16)	0.65	8.16∓0.32	28.5∓0.2	1.9355	1.3168	0.1356	0.37±0.67
2 (17-30)	0.775	7.40∓0.05	29∓0.5	1.61261	1.3908	0.9296	0.22±0.46
3 (31-47)	0.904	7.40∓0.05	28.5∓0.5	1.3825	1.7116	1.0201	0.17±0.67
4 (48-77)	1.033	7.40∓0.05	28.5∓0.5	1.2097	1.91	1.197	0.49±4.82
5 (78-84)	1.291	7.40∓0.05	28.5∓0.5	0.9677	2.689	1.3904	0.11±0.19

6 (85-151)	1.420	7.40∓0.05	28.5∓0.5	0.6452	2.7534	1.3313	4.72±44.80
7 (152-160)	1.420	7.40∓0.05	28.5∓0.5	0.6452	2.6663	1.1972	0.04±0.03

Note: Influent average ammonium concentration was checked as 600 mg-N/L to calculate NLRs.

Compared with previous anammox reactor studies, this hybrid anammox reactor showed a relatively high adaptability to the quick increase in NLR [9,20,21] reported that the NLR reached 2.1 kg-N/m^3/d on the 50^{th} day, with an original anammox VSS concentration of 0.8 g-VSS/L in a non-woven organic revolving contactor. As reported by [9], the NLR was increased to 1.3 kg-N/m^3/d on the 56^{th} day, with an inoculated 4g-MLSS/L of anammox biomass in a reactor that has an up-flow column. [10] Reported that the NLR was increased to 2.5 kg-N/m^3/d on the 24^{th} day, with an initial anammox sludge concentration of4g-MLSS/L in an up-flow column reactor containing a GSS in spiral-style. In addition to a high concentration of initial anammox sludge (about 4 g-MLSS/L), it was recommended that the operational tactic of applying continuous stirring (30 rpm) and internal circulation was beneficial for quick start-up of the hybrid anammox reactor.

3.4 The Rising NLR

During days 31-155, to increase the NLR, the hybrid reactor was operated under continuous stirring (30-100) with no internal circulating. With a step by step increase in influent NO_2^--N and NH_4^+ concentrations and reduction in the HRT, the NLR was increased from 1.31 to 2.7 kg-N/m^3/d. The influent TN concentration was increased from 100 to 600 mg/L and the HRT reduced from 1.9 to 0. 64h.A maximum NRR of 1.39 kg-N/m^3/L was obtained during this period with a nitrogen removal efficiency of 58%. When the TN concentration attained 600 mg/L, during days 157-160, the TN concentration was retained at 550 mg/L while the HRT was lessened from 0.87 to 0.64 h. As a result of this procedure, a maximum NLR of 3.7 kg-N/m^3/d was obtained with TN removal efficiency above 58%.

3.5 Operational glitches and The Recovery Process

On day 36, the electric power supply was eliminated, to ensure that influent could not be introduced and the temperature could not be controlled for approximately 24 hours. The temperature hence decreased to 18^0C and the NLR was dramatically decreased. During days 41-47, the NLR was reduced to 1.7 kg-N m^3/d by escalating the HRT to 1.39 hours. Through a step by step increase in influent NO_2^--N and NH_4^+-N concentrations and reduction of the HRT, the NRR was increased by day 65 to a similar level as that before the power loss .This result shows that the hybrid anammox reactor can recover from operational problems.

On day156, the sludge concentration in the reactor reached 30 g-VSS/L and a low effluent was obtained. The HRT was shortened to 0.64 and the highest NLR of 3.9 kg-N m^3/d, with total nitrogen removal efficiency above 58%, was achieved .The highest NRR of 2.25 kg-N m^3/d was realized on same day.

From the studies in the recent past, only a few recorded an NLR above 20 kg-N m^3/d for the anammox process [22, 23, 24] reported that total NLR was increased to 26.0 kg-N m^3/d using up-flow fixed-bed glass biofilm column reactors having a liquid volume of 0.8 L. [23] reported record high total NLRs of 45.2 to76.7 kg-N m^3/d with HRTs of 0.22h and 0.16 h, respectively. Up-flow anaerobic sludge blanket reactors with a working volume of 1.1 were utilized in their researches and it was revealed that a great or rapid flow rate at concentrations with low substrate was more effective in achieving a high NRR compare to high substrate concentrations under low flow rates. In our study, the NLR was also increased, mainly due to a reduced HRT. In addition, our hybrid had an effective volume of 30 L, which is larger than that of earlier reported reactors having NLRs above 20 kg-N m^3/d. It was made known that the newly designed hybrid anammox reactor is appropriate to the anammox process, giving high NLRs and NRRs for wastewater treatment.

3.6 FA and FNA and their specific effects

$$\text{FA } (NH_3, \text{ mg/L}) = 1.214 \times \frac{\left[NH_4^+\right] \times 10^{PH}}{e^{(6344/(273+T))} + 10^{pH}} \quad (1)$$

$$\text{FNA}\left(\text{HNO}_{2,}\ \text{mg /L}\right) = \frac{46}{14} \times \frac{\left[NO_2^-\right] \times 10^{PH}}{e^{(-2300(273+t)) \times 10^{PH}}} \quad (2)$$

Free ammonia accelerates the rate of ammonium oxidation reactor and inhibits the activity of AOB and NOB. It was reported that AOB activity was inhibited at FA levels between 10 to 150 mg/L, whereas NOB activity was already greatly reduced at FA levels between 0.1 and 10 mg/L [27]. The inhibition of FNA to N eutropha was much weaker than to Nitrobacter; e.g.; the activity of Nitrobacter was reduced by 40% while there was no distinct influence on the activity of N.eutropha when the nitrite concentration was over 1400 mg/L. Additionally, the activity of Nitrobacter was totally inhibited at FNA level ranges between 0.22 and 2.60 mg/L [28].

Table 7: Time courses of 120 days observation data of FA

Time (days)	FNA (mg/L)	Time (days)	FNA (mg/L)	Time (days)	FNA (mg/L)
10	13.73	24	0.04	38	4.22
11	8.49	25	0.1	39	3.23
12	5.63	26	0.53	40	1.59
13	3.33	27	0.1	41	2.6
14	12.1	28	0.38	42	5.1
15	2.04	29	2.24	43	6.89
16	1.89	30	9.51	44	6.65
17	1.74	31	7.05	45	7.81
18	0.1	32	7.79	46	9.18
19	8.38	33	10.73	47	9.36

10	13.73	24	0.04	38	4.22
11	8.49	25	0.1	39	3.23
12	5.63	26	0.53	40	1.59
13	3.33	27	0.1	41	2.6
14	12.1	28	0.38	42	5.1
15	2.04	29	2.24	43	6.89
16	1.89	30	9.51	44	6.65
17	1.74	31	7.05	45	7.81
18	0.1	32	7.79	46	9.18
20	0.08	34	0.03	48	9.21
21	0.06	35	0.18	49	9.19
22	1.64	36	3.82	50	9.53
23	0.05	37	11.4	51	9.82
52	9.79	75	4.79	98	8.6
53	9.92	76	5.19	99	9.45
54	6.57	77	5.1	90	9.1
55	0.91	78	4.45	91	9.05
56	0.14	79	4.22	92	9.46
57	0.2	80	3.51	93	7.77
58	0.1	81	2.98	94	7.96
59	6.9	82	3.38	95	10.36
60	0.46	83	3.6	96	6.13
61	3.33	84	3.21	97	6
62	4.26	85	4.08	98	4.68
63	4.55	86	2.75	99	3.37
64	4.16	87	0.08	100	3.07
65	2.42	88	0.54	101	5.52
66	2.65	89	3.39	102	4.31
67	3.44	90	1.86	103	3.55
68	3.68	91	3.96	104	3.38
69	3.26	92	5.49	105	3.05
70	3.98	93	3.19	106	3.82
71	4.26	94	6.83	107	3.62
72	3.99	95	3.32	108	3.85
73	4.61	96	7.56	109	3.43
74	6.09	97	8.54	110	3.41

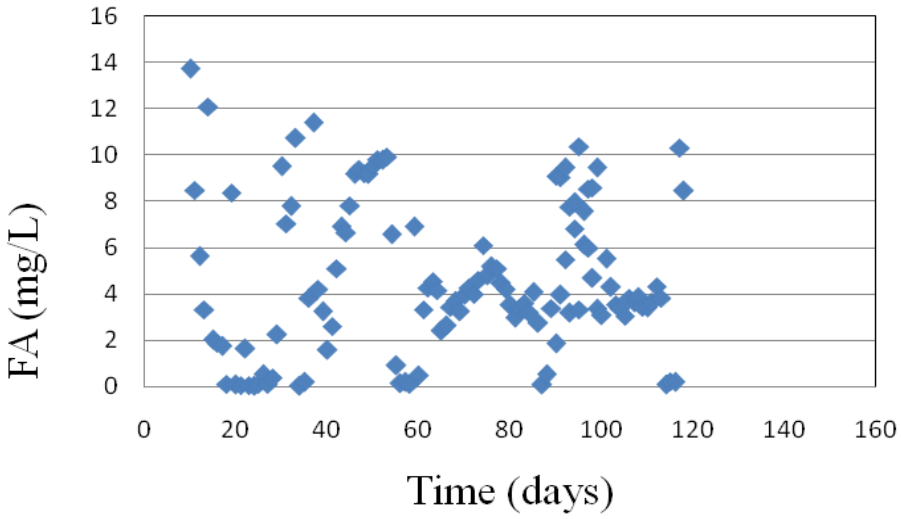


Figure 5: Time courses of FA concentrations in HPNA reactor

Table 7: Time courses of 120 days observation data of FNA

Times (days)	FNA(mg/L)	Times	FNA(mg/L)	Times	FNA(mg/L)	Times	FNA(mg/L)
34	0.03	58	0.1	82	3.38	96	2.13
35	0.18	59	1.9	83	3.6	97	0.06
36	3.82	60	0.46	84	3.21	98	1.68
37	0.07	61	3.33	85	2.08	99	3.37
38	4.22	62	3.26	86	2.75	100	3.07
39	3.23	63	2.55	87	0.08	101	1.52
40	1.59	64	1.16	88	0.54	102	2.31
41	2.6	65	2.42	89	3.39	103	3.55
42	1.22	66	2.65	90	1.86	104	3.38
43	0.04	67	3.44	91	3.96	105	3.05
44	0.01	68	3.68	92	1.49	106	3.82
45	2.11	69	3.26	93	3.19	107	3.62
46	3.11	70	3.98	94	2.83	108	3.85
47	0.02	71	1.26	95	3.32	109	3.43
48	0.03	72	3.99	96	1.56	110	3.41
49	0.06	73	2.61	97	2.54	111	3.67
50	0.08	74	1.09	98	8.6	112	1.32
51	0.02	75	2.79	99	1.45	113	3.82
52	2.34	76	2.19	90	9.1	114	0.08
53	1.92	77	1.1	91	1.05	115	0.22

54	2.57	78	2.45	92	1.46	116	0.19
55	0.91	79	1.22	93	1.77	117	0.31
56	0.14	80	3.51	94	1.96	118	0.47
57	0.2	81	2.98	95	1.36	119	0.23

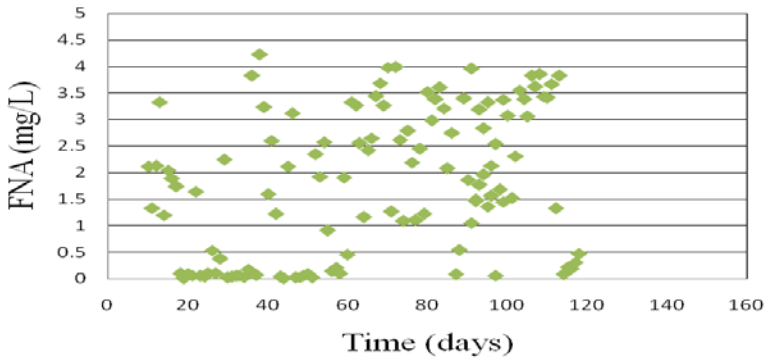


Figure 6: Time courses of FNA concentrations in HPNA reactor

Fig.(3.4;3.5) shows FA and FNA fluctuations over time as calculated based on Equations (1-2). In the initial 20 days, high FA concentrations suppressed the activity of both NOB and AOB therefore the effluent nitrite levels were very low. In the subsequent 115 days, FA concentrations fluctuated between 0.1 and 10 mg/L, which appeared to inhibit the NOB activity successfully and facilitate the amassing of nitrite even without pH temperature control, and DO. The detailed FA inhibition mechanisms to NOB activity are still unknown. [29]. deduced that it might be due to a direct FA inhibitory effect on nitrite oxidoreductase, or an enzyme involved in the proton translocation or electron transport. However, inhibition of FA on ATP production (with the usage of proton motive force) may result in a similar effect [29]. It was also assumed that the hydroxylamine formed by ammonia oxidizers might subdue the growth of nitrite oxidizers, though no formation of nitrate was observed [30].

Table 8: Time courses of 120 days observation data of nitrate concentration in HPNA reactor

Times (days)	Nitrate(mg/L)	Times (days)	Nitrate(mg/L)	Times (days)	Nitrate(mg/L)
1	43.83	22	25.29	43	57.14
2	64.56	23	112.6	44	91.36
3	79.82	24	259.09	45	159.13
4	93.13	25	290.44	46	196.04
5	96.76	26	207.68	47	243.58
6	112.43	27	145.64	48	241.39

7	113.11	28	133.34	49	158.12
8	152.55	29	132.49	50	272.07
9	216.95	30	133.84	51	161.49
10	342.19	31	157.27	52	128.79
11	385.68	32	121.71	53	123.73
12	302.66	33	95.07	54	107.55
13	307.47	34	98.95	55	116.48
14	286.23	35	68.44	56	103.67
15	260.27	36	60.52	57	110.92
16	255.89	37	55.63	58	87.66
17	264.82	38	45.85	59	86.31
18	211.05	39	50.57	60	94.57
19	174.3	40	54.11	62	80.74
20	118.17	41	47.54	63	83.27
21	116.99	42	53.1	64	82.6
65	67.76	86	113.95	107	67.6
66	61.7	87	84.28	108	65.74
67	67.09	88	68.44	109	56.3
68	61.02	89	73.16	110	55.12
69	63.89	90	58.83	111	51.24
70	64.06	91	60.68	112	49.56
71	64.06	92	63.72	113	51.92
72	65.07	93	56.3	114	47.2
73	64.39	94	57.48	115	42.98
74	69.45	95	57.31	116	43.66
75	68.44	96	43.83	117	88.67
76	61.19	97	44.5		
77	81.76	98	76.53	119	199.25
78	202.79	99	64.73		

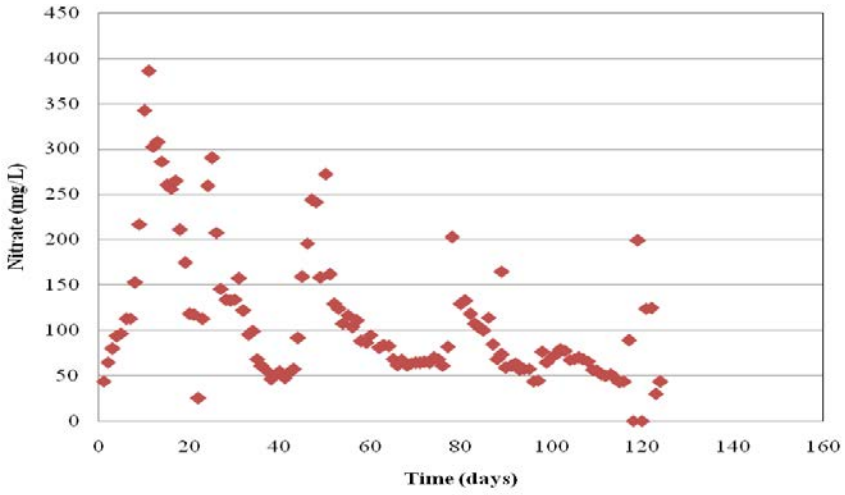
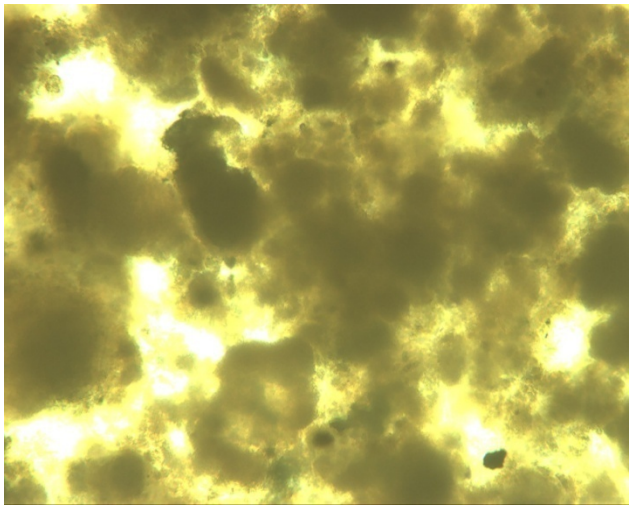


Figure 7: Time course of effluent nitrate concentrations in HPNA reactor

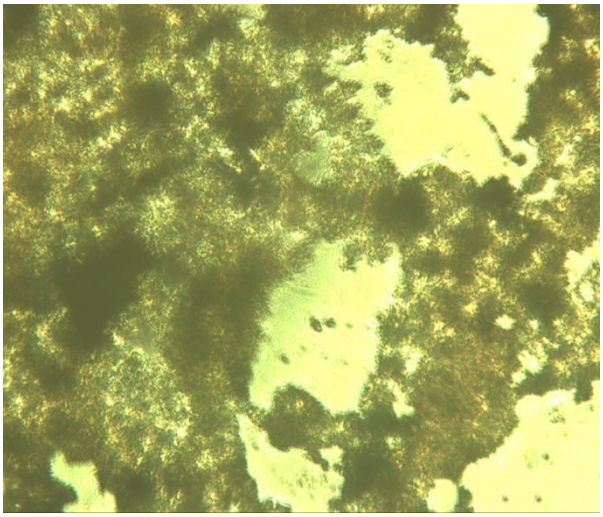
Fig.3.6 shows the course of the effluent nitrate concentrations in the HPNA reactor. From the beginning to day160, nitrate was at a low level with a high value of only 66 mg/L due to the inhibition effects of the NOB by FA and FNA. Later, a longer HRT (HRT of 1.9 h) supplied enough reaction time to the NOB and improved their growth. Concurrently, the nitrification reaction without P^H control resulted in lower pH values (the lowest of 7.4) in the HPNA reactor, which additionally caused levels to reduce below 0.1 mg/L and FNA to increase above 0.2 mg/L. AOB activity was gradually inhibited by the changes FA levels in the HPNA reactor. From day 150, the effluent nitrate levels increased gradually and reached the highest value of 80 mg/L on day 158. Later, pH control was applied (pH of 0.64) again in order to increase the FA concentration and further inhibit the AOB activity. Although the effluent nitrite levels increased,the nitrate upheld a relatively high level of over 100 mg/L.

4. Sludge characteristics

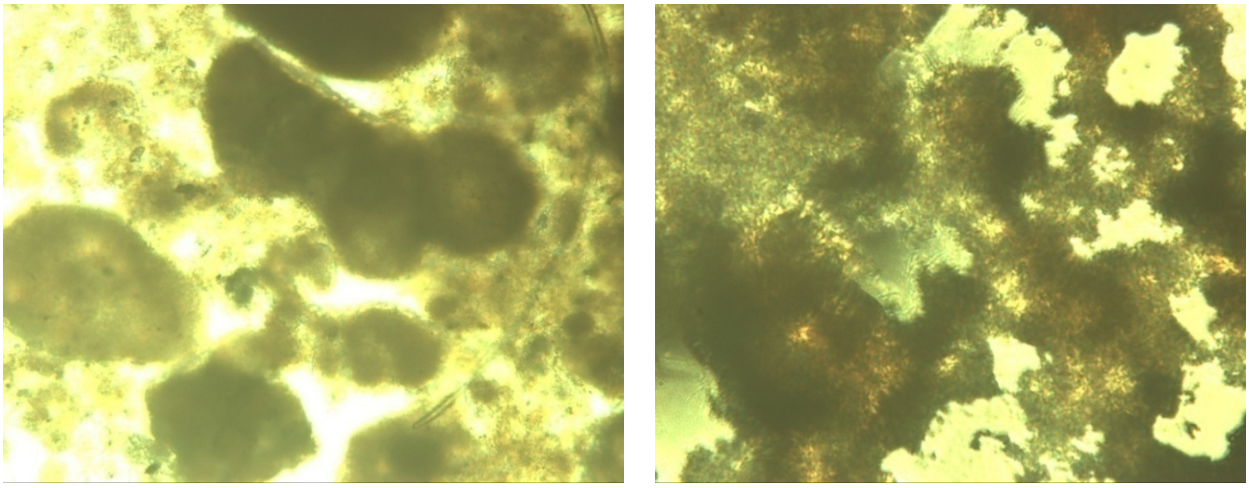
4.1 Microscopic observation



a



b



c **d**

Figure 8: Microscopic observation of anammox sludge.

(a) and (b) is anammox sludge on the upper part of the reactor;

(c) and (d) is anammox granule on the lower part of the reactor.

Figure-8 shows the different microscopic observation of anammox sludge in the reactor. These photos show that upper sludge had the law transmission compared with lower portion sludge.

The result supports that the compact anammox sludge was formed in the lower part.

4.2 SVI and EPS

Table 9: Diameter, MLSS, sludge volumetric index (SVI_5) EPS contents of the lower part sludge, upper part sludge and floating sludge in the reactor A

	Lower part	Upper part	Floating sludge
MLSS (g/L)	12	9	0.4 (g)
MLVSS/MLSS (%)	71	72	73
Diameter (mm)	0.62 ±0.5	0.52 ±0.6	>1
SVI_5 (Ml/g-MLSS)	47	55	ND
Proteins	65	52	103
Polysaccharides	54	36	47
PN/PS	1.1	1.3	2.2

Three samples of the lower part sludge, upper part sludge and floating sludge in the reactor A on day 158 were characterized by measuring mean granular diameter, MLSS, SVI_5 and EPS quantification. The lower part,

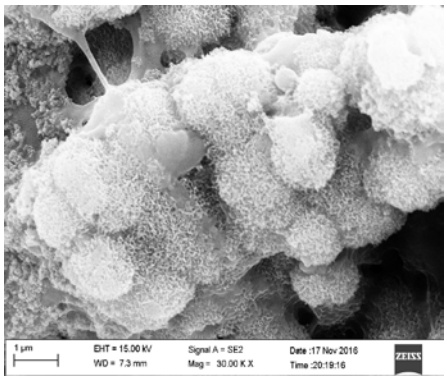
upper part and floating sludge diameter were 0.62±0.5mm, 0.52±0.5 mm and over 1mm respectively (as shown in Table- 9)

The granules showed a high settling property during the entire experiment. At the end of experiment of the SVI value, each of the sludge is measured (table 4.1). The SVI_5 for the lower part and upper part of sludge were 47 ml/g-MLVSS and 55 ml/g-MLVSS, respectively. [24] Also reported high setting velocities of 73-88m/h and SVI_5 of 24-25 ml/g-MLVSS. When these values are compared, it was noted that anammox granule were produced in the reactor A can be considered to have a good settling property.

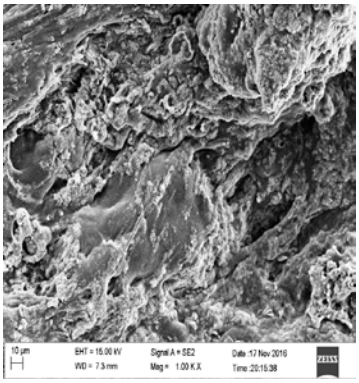
EPSs are supposed to play a vital role in microbial granules formation, regardless of whether the biomass is in suspended or biofilm states. EPS are considered to be a rich matrix of polymers, mainly including proteins and polysaccharides. In this study, the EPS concentrations were determined at different operational times. The proteins and the polysaccharide concentrations of the lower part, the upper part and floating sludges were 65 and 54 mg/g-MLSS, 52 and 36 mg/g-MLSS, 65 and 54 mg/g-MLSS, 103 and 47 mg/g-MLSS, respectively, on day 151 (Table -9).

The proteins to polysaccharides ratio (PN/PS) is utilized to evaluate the granular strength and settle ability [31, 32, 33], where a higher PN/PS ratio of granules shows lower strength and weaker settle ability [31, 32, 33]. The low PN/PS ratio of the anammox granules suggests an excellent granular stability. Conversely, [34] reported thatheterotrophic anaerobic granules secreted extracellular protein which was highly propagated under high hydrodynamic shear forces in an internal circulation anaerobic reactor. Additionally, it was reported by [24] that the secretion of extracellular protein was enhanced at a rate higher than that of polysaccharides, resulting in an increase in PN/PS ratio of 2.29 when the hydrodynamic shear force increased HRT was short, May and his colleagues reported that PN/PS ration at an NLR of 27 kg-N/M^3/d was comparatively high value of 3.3, which would probably cause the high hydrodynamic shear and mechanically stirred forces in the hybrid reactor. In this study, the PN/PS ratio for the lower part, the upper part and floating sludge were 1.1, 1.3 and 2.2 respectively. The results are in support that the hydrodynamic shear is not high enough.

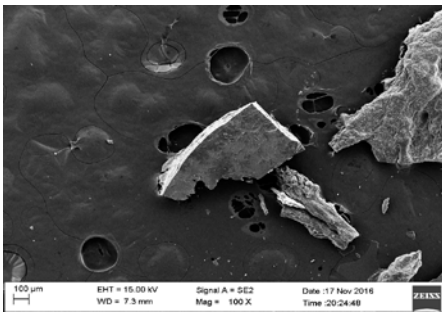
4.3 Strategic Observation through SEM



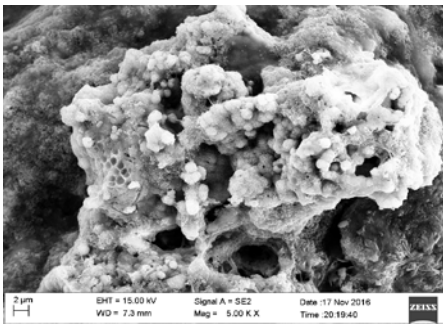
(a)



(b)



(c)



(d)

Figure 9: Outer appearance of anammox granule at the end of the experiment

The anammox biomass morphological features were observed using SEM. At the end of the experiment, sludge samples were obtained. It can be seen from the SEM photographs that the granule surface had an almost spherical shape with a smooth surface (Fig.4.2). Compared with our previous studies [12, 24], the micro-organization structure exhibited a higher degree of smoothness and sphericity. This structure would have been formed by the high hydrodynamics shear forces emanating from the mechanical stirring and relatively short HRT.

At the end of the process, the total biomass amount on the carrier (with a non-woven fabric material) in the upper part was to reach to 18 g-VSS. The fixed bed was deliberated to catch the biomass successfully. In view of an effective volume of the upper part of 4.0 L, the biomass concentration in the fixed bed was calculated to be 5.7 g-VSS/L which was four times smaller than that in the fluidized bed. Consequently, considering the biomass concentration, more than 72% of the total nitrogen removal was thought to be as a result of granular sludge contained in fluidized bed. The above sludge characteristics clearly show that the hybrid reactor is suitable for cultivation of granular anammox sludge possessing good settling properties. Future researches will emphasize on the impacts of the high hydrodynamic shear forces generated by the stirring on the granulation of anammox sludge

5. Discussion

The major aim of the study was to provide a clear clue on sludge morphology, reactor performance, and bacterial community composition of the nitritation-anammox HRT that is one-stage operated. It was noted that the capability for Nitrogen removal became steady at1.8 kg-N/m^3/d after 160 days of the operation of the reactor.

Coincidentally, distinctive granules (red in color) were observed among suspended flocs and they were gradually increased in number during the startup period. ZEISS analysis demonstrated these granules consisted of internal AnAOB sector protected by an AerAOB edge, while filamentous bacteria attached together in small flocs the scattered AerAOB. This unmistakable sludge morphology have been recounted in previous studies as well [35, 36], could be essential to optimize distribution of cumulative size in partial nitritation–anammox reactor that is one-stage process. Actually, Wett and his colleagues [37]have taken advantage of this fact by

developing a hydro-cyclone to wash out small flocs while retaining larger aggregates. Although HPN without this type of selective biomass retention can also operate successfully in the long term [38], this morphology selection basing on the settling velocity could give an extra opportunity to implement treatments PNA into mainstream wastewater.

It is established that three phyla (Planctomycetes, Chloroflexi, and Proteobacteria) were carefully chosen and recognized as a stable base in the community, although the inoculums had high diversity. Such selection was undoubtedly determined by the presence of a substrate within the influent. Park and his colleagues [39]also demonstrated that the compositions of the feed and existing substrate concentrations in the reactor were likely considered for the microbial community composition more than the configuration of the reactor and inoculums.

The main microbes in nitritation–anammox process are AerAOB and AnAOB and they perform nitrogen removal higher percentage from wastewater. The only AerAOB identified in the reactor was the genus Nitrosomonas, which is in accordance with previous studies [39, 40]. In the reactor, uncultured Planctomycetes-related Candidatus Jettenia was established as the dominant AnAOB (which accounted for about 16.8%), while anothertype of AnAOB, the Candidatus Brocadia was detected as well with the abundance of 3.3%.

Recently several different species of AnAOB have been found in nitrogen removal reactors. Furthermore, changes in the AnAOB population with time have been witnessed during the operation of anammox reactors [41, 42]. However, the ecological niches and physiological features for diverse AnAOB population, which are potentially responsible for the shifts, ought to be investigated in future researches.

Captivatingly, the species related to uncultured Planctomycetales bacterium clone SM1A02 takes a large portion of 17.8% in the system, but its function is still unknown. So it is possible that this species could be novel strains carrying out anammox, but further studies involving the system's metagenomes and metatranscriptomes need to be investigated to provide the species' functions.

In the reactor, high amounts of Chloroflexi (27%) make filamentous bacteria to be another dominant population besides AerAOB and AnAOB. However, apart from the reactor having no bulking problem, there is also the sludge that appeared like rigid granule .The filamentous Chloroflexi growth is considered a common phenomenon in activated sludge, and several similar observations in other anammox reactors investigating the proliferation of filamentous bacteria have also been reported [43, 44]. The Chloroflexi provide a strong backbone for the three-dimensional microbial aggregates (flocs) as suggested by Björnsson and his colleagues [45]. The Chloroflexi intrafloc position and their comparative abundance reported by Kragelund and his colleagues [46]supported this hypothesis which explains one vital role for these organisms in the activated sludge ecosystem. In addition, filamentous bacteria contribute to the aerobic granulation process because they have a capability of forming the primary framework for tiny sludge granules, which serve as cores or carriers [47]. Therefore, it could be inferred that the reactor performance in a one-stage partial nitritation–anammox systems are driven not only by AnAOB and AerAOB that remove nitrogen but by filamentous bacteria as well that could confer structural integrity to the aggregates.

The presence of heterotrophic bacteria belonging to the phylum Chloroflexi in the reactor makes us deduce that organic carbon sources reinforced the heterotrophs growth that were derived from the soluble microbial products (SMP) and extracellular polymeric substances (EPS), produced by autotrophs (AnAOB and AerAOB). Several publications have shown that the Chloroflexi, asa group, seem to be involved in the degradation of complex compounds such as polysaccharides and proteins [48, 49]. The uncultured Chloroflexi steadily used 14C-labeled products from an AnAOB culture as identified by Kindaichi and his colleagues [50], which meant that the bacteria favorably used the decaying anammox bacteria cell materials. Thus, it can be deduced that the pattern of Chloroflexi inferred substrate uptake in the reactor were plausible.

These results can assist in mathematical modeling of HPN process in future, that is, the existence of heterotrophic bacteria could not be neglected.

6. Conclusions

The hybrid anammox reactors merging the benefits of fixed and fluidized beds reactors were developed. Anammox granular enrichment, rapid startup, and the better nitrogen removal performances were presented experimentally. A hybrid anammox reactor for removal of nitrogen from synthetic wastewater has been applied successfully under relatively high NLR and short HRT. The reactor was applied for 160 days with an average NLR of 1.8 kg-N/m^3/d and an average total nitrogen removal efficiency of 75%. The anammox granular sludge formed through this procedure had a good settling performance and a relatively high PN ratio of 3.3. These outcomes clearly indicate that the newly designed hybrid anammox reactor is beneficial for attaining a high nitrogen removal rate and the stable maintenance of granular anammox sludge. Thirty-five clones had sequential structure identities of 99-100% to anammox bacterium strain KSU-1 (AB057453).The hybrid anammox reactor was also verified to be useful for anammox bacteria enrichment.

Acknowledgements

I would like first to express my deep gratitude to my advisor Dr. Zhu Tong, for giving me the opportunity for his invaluable guidance and inspiration throughout this work.

Mr. Tianyu has advised and encouraged me in every possible way. Thank you very much for being so nice to me and keeping door open every time. He has helped me develop my professional skills and I will emulate his drive and work ethic throughout my career. I would also like to thank all my friends who helped me get through the years of Graduate school especially Jumma,Abu Bokkar.

Finally, I owe special gratitude to my parents for continuous and unconditional help and support, sacrifice and consideration throughout my years of study here in Northeastern University. It’s their unselfish care that helps me finish this paper successfully. Thank you.

References

[1]. Mulder, A.,van de Graaf, A.A., Robertson, L.A; Kuenen, J.G., 1995, Anaerobic ammonium oxidation

discovered in a denitrifying fluidized bed reactor, FEMS.Microbiol. Ecol., **16**, 177-184.

[2]. Van de Graaf, A.A, De Bruijin, L.A.P., Jetten, M.S.M., Kuenen,J.G., Anaerobic oxidation of ammonium is a biologically mediated process, Appl. Microbiol., **61**, 1246-1251.

[3]. Van de Graaf, A.A, De Bruijin, P., Robertson, L.A., Jetten , M.S.M., Kuenen, J.G.,

1996, Autotrophic growth of anaerobic ammonium-oxidizing microorganinms in a fluidizing bed reactor,Appl.En.zviron.Microbiol., **142**,2187-2196.

[4]. Sliekers, A.O., Third,k; Abma,W., Kuenen, J.G., Jetten, M.S.M., 2003,canon and anammox in a gas-lifted reactor, FEMS. Microbiol.Lett., **218**,339-344.

[5]. Strous, M., van Gerven, E., Kuenen, J.G., Jetten, M., 1997, Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations, Water Res.,**31**,1955-1962.

[6]. Strous,M., Kuenen, J.G., Jetten, M.S.M., 1999, Key physiological parameters of anaerobic ammonium oxidation , Appl. Environ. Microbiol., **65**, 3248-3250.

[7]. Dapena-Mora A., Arroja B., Campus J.L., Mosquera-Corral A. and Mendez R., 2004, Improvement of the settling properties of anammox sludge in an SBR,j. Chen.Technol. Biotechnol.,**79**,1417-1420.

[8]. Furukawa,K., Rouse, J.D., Imajo, U., Nakamura, K, Ishida, H., 2001, Anaerboic oxidation of ammonium confirmed in continuous flow treatment using a non-woven biomass carrier,Jpn.J.of water Treatment Biology,**38**(2), 87-94.

[9]. Zhang, L., Yang, J., Ma,Y., Li, Z., Fujii,T., Zhang, W., Nishiyama, T., and Furukawa, K., 2010, Treatment capacity of an up-flow anammox column reactor using polyethylene sponge strips as biomass carrier, J. Biosci. Bioeng., **110** (1), 72-78.

[10]. Isaka, K., Date, Y., Sumino,T., Tsuneda, S., 2007, Ammonium removal performance of anaerobic ammonium-oxidizing bacteria immobilized in polyethylene glycol gel carrier: anammox bacteria immobilized in gel carrier, App. Environ. Microbiol., **76** (6), 1457-65.

[11]. Hoa, T.T.H., Khanh, L.N., Liu, Z., Fujii,T., Rouse,J.D., Furukawa, K., 2006, Nitrogen removal by immobilized anammox sludge using PVA gel as biocarrer, Jpn. J. of Water Treatment Biology, **42** (3), 139-149.

[12]. Qiao, S., Kawakubo,Y., Chen, Y., Nishiyama, T., Fujii, T., Furukawa, k., 2009, Identification of bacteria coexisting with anammox bacteria in an uplow column type reactor, Biodegradation,**20** ,117-124

[13]. Colleran, E., Finnegan, S., Okeeffe,R.B., 1994, Anaerobic digestion of high sulphate containing wastewater from the industrial production of citric acid, Wat. Sci. Technol.,**30**,263-73.

[14]. Ozturk, I., Eroglu, V., Ubay,G., Demir,I., 1993, Hybrid upflow anaerobic sludge blanket reactor (HUASBR) treatment of dairy emuents, Wat. Sci.Technol., **28**, 77-85.

[15]. Boja, R., Banks,C.J., Wang, Z., 1995, performance of a hybrid anaerobic reactor, combining a sludge blanket and a filter, treating slaughterhouse wastewater, Appl. Microbiol. Biotechnol., **43**,351-357

[16]. Fujii,T., Sugino,H., Rouse, J .D., Furukawa,K., 2002, Characterization of the microbial community in an anaerobic ammonium-oxidizing bioflim cultured on a nonwoven biomass carrier, J. Biosci.Bioeng., **94**, 412-418.

[17]. Furukawa, k., Rouse, J.D., yoshida, N., H., 2003, Mass cultivation of anaerobic ammonium-oxidizing sludge using a novel nonwoven biomass carrier, J. Chen. Eng. Of Jpm., **36** (10),1163-1169.

[18]. Kanda, J., 1995,Determination of ammonium in seawater based on the indophenol reaction with o-phenylphenol(oop),Water res., 29, 2746-2750.

[19]. APHA,AWWA,WPCF: Standard methods for the examination of water and wastewater,19thedition,American public Health Association,Washington,D.C.(1995)

[20]. Yang, J., Zhang,L;Fukuzaki,Y., Hira,D., Fukuzaki,Y., Furukawa, K., inorganiccarbonsource2010,High-rate nitrogen removal by the anammox process with a sufficient,Bioresource.Technol., **101**,9471-1251.

[21]. Liu,S., Yang, F., Xue, Y., Gong, Z., Chen, H., Wang, T., Su, Z., 2008, Evaluation of oxygen adaptation and identification of functional bacteria composition for Anammox consortium in non-woven biological rotating contactor,Bioresour. Technol., **99**,8273-8279.

[22]. Tsushima, I., Ogasawara,Y., Kindaichi, T., Okabe, S., 2007,Development of high-rate anaerobic ammonium-oxidizing (Anammox) biofilm reactors, Water Res., **41**, 1623-1634.

[23]. Tang,C.J;Zheng,p; Hu,B.L; Chen,J.G; Wang.C.H;2010, Influence of substrates on nitrogen removal performance and microbiology of anaerobic ammonium oxidation by operation two USAB reactors fed with different substrate levels,J.Hazard. Mater;**181**,19-26.

[24]. Tang, C.J., Zheng, p., Wang, C.H., Mahmoo, Q., Zhang, J.Q., Chen, X.G., Zhang,L., Chen, J.W., 2011, Performance of high –loaded ANAMMOX UASB reactors containing granular sludge, Water Res., **45**,135-144.

[25]. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., 1951, Protein measurement with the foil phenol reagent Journal of Biological Chemistry, **193**, 265-275.

[26]. Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A., Smith F., 1956, Colorimetric method for determination of sugars and related substances, Analytical Chemistry**, 28 (3)**, 350-356.

[27]. Anthonism A.C., Loehr R.C., Prakasam T.B.S., Srinath E.G., 1976, Inhibition of nitrification by ammonia and nitrous acid, J. Water pollut. Control Fed., **48 (5)**, 835-852.

[28]. Sharma B., Ahler R.C., 1977, Nitrification and nitrogen removal, Water Research, **11**, 897-925.

[29]. Vadivelu V.M., Keller J., Yuan Z.G., 2007, Effect of free ammonia on the respiration and growth processes of an enriched Nitrobacter culture, Water Research, **41 (1),** 826-834.

[30]. Stuven R., Bock E., 2001, Nitrification and denitrification as a source for NO and NO_2 production in high-strength wastewater, Water Research, **35(8),** 1905-1914.

[31]. Quarmby, J., forster, C.F., 1995, An examination of the structure of UASB granules, Water Res**., 29**, 2449-2454.

[32]. Batstone, D.J., Keller, J., 2001, Variation of bulk properties of anaerobic granules with wastewater type, Water Res., **35**.1723-1729.

[33]. Franco, A., Roca, E., Lema, J.M., 2006. Granulation in high-load denitrifying upflow sludge bed pulsed reactors, Water Res. **40**, 871-880.

[34]. Wu, J., Zhou, H.M., Li, H.Z., Zhang, P.C., Jiang, J., 2009, Impacts of hydeodynamic shear force on nucleation of flocculent sludge in anaerobic reactor, Water Res., 3029-3036.

[35]. S.E. Vlaeminck,A. Terada, B.F. Smets, H.D.Clippeleir, T. Schaubroeck,S.Bolca, L. Demeestere,J. Mast, N. Boon, M. Carballa,W. Verstraete, Aggregate size and architecture determine microbial activity balance for one-stage partial nitritation and anammox ,Appl.Environ.Microbiol.76 (2010)900-909.

[36]. E.M. Gilbert, E. Muller, H. Horn, S. Lackner, Microbial activity of suspended biomass from a nitritation-anammox in SBR in dependence of operational condition and size fraction,Appl. Microbial.Biotechnol.97 (2013) 8795-8804.

[37]. B. Wett, M.Hell, G. Nyhuis, T. Puempel, I. Takacs, S. Murthy, Syntrophy of aerobic and anaerobic ammonia oxidizers, Water Sci. Technol. 61 (2010) 1915-1922.

[38]. A. Joss, D. Salzgeber, J. Eugster, R .Kong, K.Rottermann, S. Leumann, J. Mohn, H. Siegrist, Full-scale nitrogen removal from digester liquid with partial nitritation and anammox in one SBR, Environ. Sci. Technol. 43 (2009) 5301-5306.

[39]. H. Park ,A, Rosenthal, R. Jezek, K. Ramalingam,J. Fillos, K. Chandran, Impact of inocula and growth

mode on the molecular microbiology ecology of anaerobic ammonia oxidation (anammox) bioreactor communities, Water Res. 44 (2010) 5005-5306.

[40]. J.Vazquez-padin, A. Mosquera-Corral, J.L. Campos, R. Mendez, N.P. Revsbech, Microbial community distribution and activity dynamics of granular biomass in a CANON reactor, water Res.44 (2010) 4359-4370.

[41]. M. Oshiki, M. Shimokawa, N. Fujii, H. Satoh, S.Okabe, Physiological characteristics of the anaerobic ammonium-oxidizing bacterium 'Candidatus Brocadia sinica', Microbiology 157(2011) 1706-1713.

[42]. M.K . Winkler, R. Kleerebezem, M.C.M. van Loosdrecht, Integration of anammox into the aerobic granular sludge process for main stream wastewater treatment at ambient temperatures, Water Res. 46 (2012) 136-144.

[43]. H. Park, A. Rosenthal, K. Ramalingam, J. Fillos, K. Chandran, Linking community profiles, gene expression and N-removal in anammox bioreactors treating municipal anaerobic digestion reject water, Environ. Sci. Technol. 44 (2010) 6110-6116.

[44]. J. Li, B.Hu, P. Zheng, M. Qaisar, L. Mei, Filamentous granular sludge bulking in a laboratory scale UASB reactor, Bioresour. Technol. 99 (2008) 3431-3438.

[45]. L. Bjornsson, P. Hugenholtz, G.W. Tyson, LL blackall, Filamentous Chloroflexi (green non-sulfur bacteria) are abundant in wastewater treatment processes with biological nutrient removal, Microbiology 148 (2002) 2309-2318.

[46]. C. Kragelund, C. Levantesi, A. Borger, K. Thelen, D. Eikelboom, V.Tandoi, Y. Kong, J. van der Waarde, J. Krooneman, S. Rssetti, T.R.Thomsen, P. H. Nielsen, Identity, abundance and ecophysiology of filamentus Chloroflexi species present in activated sludge treatment plants, FEMS Microbiol. Ecol. 59 (2007) 671-682.

[47]. D. Gao, L. Liu, H. Liang, W. Wu, Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment, C rit. Rev. Biotechnol. 31 (2011) 137- 152.

[48]. Y.Xia, Y. Kong, P.H, Nielsen, In situ detection of starch-hydrolyzing microorganism in activated sludge, FEMS Microbiol. Eco. 60 (2008) 156-165.

[49]. C. Kragelud, T.R. Thomsen, A.T. Mielczarek, P.H. Nielsen, Eikelboom's morphotype 0803 in activated sludge belongs to the genus Caldilinea in the phylum chloroflexi, FEMS Microbiol. Ecol. 76 (2006) 451-462.

[50]. T. Kindaichi, S.Yuri, N. Ozaki, A. Ohashi, Ecophysiological role and function of uncultured chloroflexi in an anammox reactor, Water Sci. Technol. 66(2012) 2556-2561