

Anammox Treatment Performances Using Polyethylene Sponge as a Biomass Carrier

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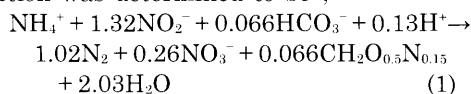
Abstract

Nitrogen removal using a polyethylene (PE) sponge biomass carrier was evaluated in a fixed-bed reactor for nitrogen removal by the anammox process. The fixed-bed reactor was operated continuously for 240 days. T-N removal efficiencies increased from 38 %, 67 %, 72 %, 74 % to 75 % with stepwise increases in volumetric T-N loading rates. A T-N removal rate of 2.8 kg N/m³/day was obtained after 240 days of operation. After 3 months, anammox biomass fully covered the surface of the PE sponge carrier and the color of the material changed from white to red. Following 5 months of operation, biomass proliferated on the surface of the material and a dark-red color was observed. With the obtained results, PE sponge is auspicious biomass carrier for growth of anammox bacteria.

Keywords: anammox, biomass carrier, polyethylene, PE sponge, fixed-bed reactor, NH₄-N, NO₂-N, T-N

INTRODUCTION

In 1995, the anammox process was discovered during experiments on a denitrifying wastewater treatment pilot plant at Gist-Brocades (Delft, Netherlands)¹⁾. It was demonstrated that anammox is a new process that ammonium and nitrite are transformed to dinitrogen gas as the main product under autotrophic anaerobic conditions. In the anammox pathway, hydroxylamine (NH₂OH) and hydrazine (N₂H₄) have been verified to be important intermediates²⁾. The anammox reaction is exergenic ($\Delta G^0 = -358$ kJ/mol NH₄⁺) and provides the energy for the fixation of CO₂³⁾. The stoichiometry of the anammox reaction was determined to be⁴⁾,



Anammox treatment performances for

nitrogen removal using polyvinyl alcohol (PVA) gel beads and malt ceramics (MC) as biomass carriers achieved good results. PVA gel beads were used in a fluidized-bed reactor⁵⁾ where a maximum T-N removal rate of 3.0 kg N/m³/day was obtained. In another study, two fixed-bed reactors using MC material with 3 to 5 mm and 10 to 15 mm diameter pieces as biomass carriers for anammox treatment were also carried out. These two fixed-bed reactors both achieved high T-N removal rates of 3.1 kg N/m³/day⁵⁾.

In this research, polyethylene (PE) sponge was used as a new biomass carrier for anammox sludge in a fixed-bed reactor with durable, steady and non-bloated characteristics. The objective of this study is to investigate nitrogen removal capability of anammox process with PE sponge as a biomass carrier.

MATERIALS AND METHODS

Biomass carrier Characteristics of PE sponge material are shown in Table 1 and unused PE sponge material is shown in Fig 1.

Batch experiments With a large pore diameter (1,060 μm) of PE sponge biomass carrier, batch experiments were carried out to investigate effect of oxygen trapped in the pores of PE sponge biomass carrier to the anammox process.

As preparation for batch experiments, anammox sludge taken from a nonwoven anammox reactor⁽⁶⁾ was used as seed sludge for attachment on the PE sponge materials which was cut into small pieces (1×1×1 cm). Three batch experiments were incubated carried out in flasks A, B and C with different conditions as shown in Table 2 and Fig.2. These experiments were shock at 35°C under 80rpm in rotary shaker (TAITEC ML-10F).

Sponge reactor set-up in laboratory-scale The reactor used in this study was made from acrylic material with a total volume of 2.9 l. The reaction zone was 2.6 l and had an inner diameter of 9.0 cm and height (to effluent port) of 41 cm. A schematic diagram of the PE sponge reactor is shown in Fig. 3a. The reaction zone contains 6 vertically strips of sponge material for a total one-sided sheet area of 960 cm². These 6 strips were folded as shown in Fig. 3b and fixed to a frame. The reactor had an airtight glass dome including vents for gas collection, gas emission and thermostatic sensing. Gas was collected by using gas a sampling bag for the first 110 days and a gas collector vessel for the next 130 days.

Seed sludge The continuous-flow fixed-bed reactor was seeded with 1.2 g of anammox sludge taken from an existing nonwoven anammox reactor⁽⁶⁾. Before start-up, the seed sludge was circulated in the reactor for attachment on the surface of the PE sponge material.

Composition of synthetic wastewater Synthetic wastewater was prepared by adding ammonium and nitrite to a mineral medium. The various concentrations of ammonium and nitrite were adjusted in the forms of (NH₄)₂SO₄ and NaNO₂, respectively. The

Table 1 Characteristics of PE sponge materials

Pore diameter	1,060 μm
Thickness	10 mm
Total one-side area	960 cm ² (6 strips, 3.9 cm by 41 cm)
Packing ratio	37%
Porosity	96% (wet condition)
Specific gravity	0.995 g/ml
Material	polyethylene

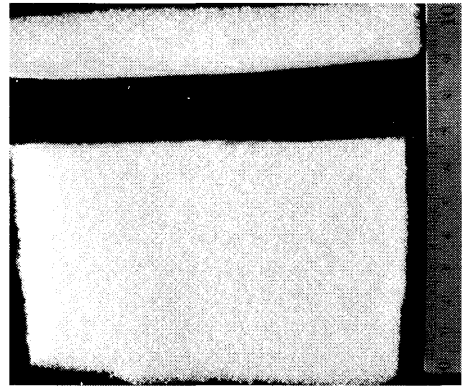


Fig. 1 Unused PE sponge material

Table 2 Parameters in 3 batch experiments

Batch experiments	A	B	C
Flask volume (l)	1.0	1.0	1.0
Synthetic wastewater volume (l)	0.5	0.5	0.5
PE sponge cube volumes (l)	0	0.3	0.3
Anammox sludge (MLSS)	1 g/l	1 g/l	1 g/l
Daily N ₂ gas purging	+	+	-

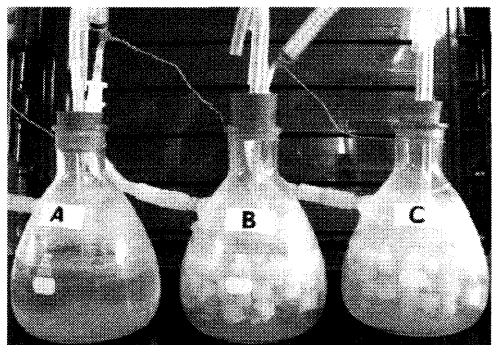


Fig. 2 Batch experiments

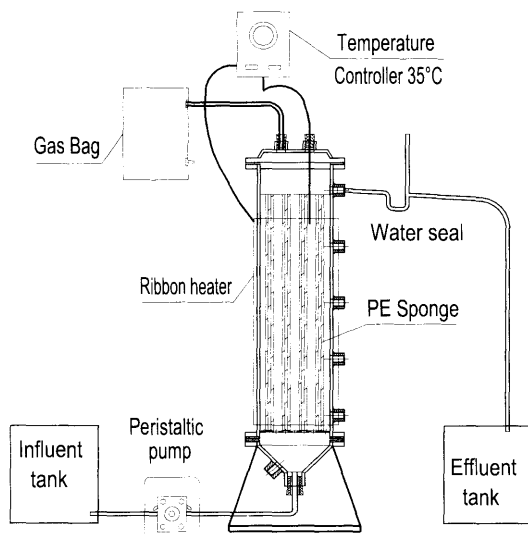


Fig. 3a Schematic diagram of PE sponge reactor

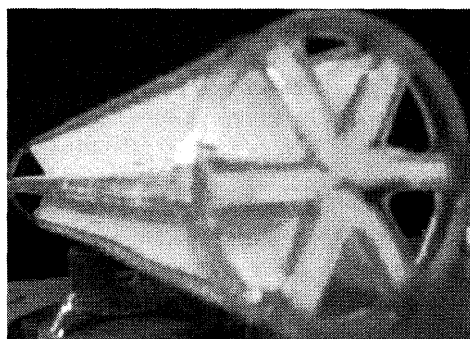


Fig. 3b PE sponge material frame

composition of the mineral medium was: KHCO_3 125 mg/l, KH_2PO_4 54 mg/l, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 9 mg/l, EDTA.Na 5 mg/l. Tap water of groundwater origins was used for the preparation of this synthetic wastewater.

Operational conditions The operational conditions of the reactor are shown in Table 3.

Influent was fed continuously in up-flow mode by using a peristaltic pump (EYELA SMP-21). The process was operated under dark conditions in a black-vinyl sheet enclosure. The reactor temperature was maintained at 33°C to 35°C, controlled thermostatically with an external ribbon-

Table 3 Operational conditions for the PE sponge reactor

Period	Time (days)	Flow rate (l/d)	HRT (h)	Influent $\text{NH}_4\text{-N}/\text{NO}_2\text{-N}$ concentration (mg N/l)
1	1~54	2.6	24	30~125/30~125
	55~65	3.1	20	100/100
2	66~72	3.9	16	100~125/100~125
	73~83	5.2	12	125~150/125~150
	84~89	6.2	10	150/150
3	90~99	7.8	8	150/150
	100~106	10.4	6	150/150
	107~111	12.5	5	150/150
4	112~126	15.6	4	150/150
	127~130	15.6	4	175/175
	131~141	15.6	4	200/200
5	142~189	17.8	3.5	200/200
	190~240	20.8	3	200/200

heating element. In addition, daily purging with nitrogen gas was used to reduce dissolved oxygen (DO) levels in the influent to below 0.5 mg/l. Influent pH was 7.2 to 7.5 without adjustment.

Chemical analyses In accordance with Standard Methods⁷, nitrite concentrations were measured by the colorimetric method and nitrate concentrations were quantified by the UV spectrophotometric screening method with a correction for nitrite interference. Ammonium concentrations were measured by the phenate method using ortho-phenylphenol as a substitute for liquid phenol⁸. A Mettler Toledo-320 pH meter was used to measure pH levels. DO of the synthetic influent wastewater was measured with a DO meter (HORIBA D-55).

RESULTS AND DISCUSSION

Batch experiments Figs. 4 (a), (b) and (c) show the changes in $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$ and T-N concentrations and T-N removal efficiencies in the batch experiments that were conducted for almost 3 months and the results are summarized in Table 4. As shown in Table 4, influent T-N concentrations for the 3 flasks were the same. Average $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and T-N removal efficiencies and T-N removal rate were highest in flask B apparently because the PE sponge was used

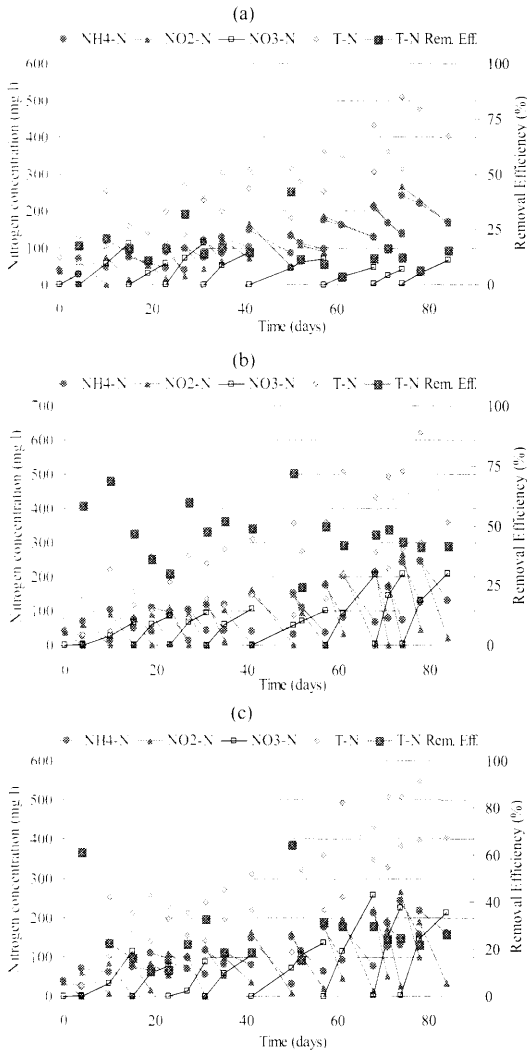


Fig. 4 Changes in concentrations of nitrogen compounds and T-N removal efficiencies for flask A (a), B (b), C (c).

in flask B and the flask was daily purged with N_2 gas. Therefore, the operational conditions used for flask B were preferred.

However, the average NO_3 -N production rates and ratios of NO_3 -N production to influent T-N for flask A in compare with this results for flasks B and C show that NO_3 -N levels were very high in flasks B and C due to nitrification under aerobic condition and yielded high effluent nitrate. In addition, the ratio of NO_3 -N production to influent T-N for flasks B and C were a bit different as mentioned in Table 4. That it is thought that the oxygen trapped in the large pores of the PE biomass carrier could not be removed completely by N_2 purging. These results indicate that a more suitable deoxygenation method for PE biomass carrier is needed.

The lower NO_3 -N production rate and higher T-N removal efficiency in flask B as compared with flask C suggest that less oxygen was trapped in the PE carrier in flask B because of the daily purging with N_2 gas. Therefore, the daily purging with N_2 gas helps to reduce the oxygen trapped in the large pores of PE sponge material.

Continuous-flow fixed-bed reactor

Removal efficiencies of nitrogen compounds

Influent NH_4 -N and NO_2 -N levels were increased from 30 to 200 mg N/l over a period of 240 days (Fig. 5). During period 1 (the first 54 days), when the HRT was 24 h, effluent NH_4 -N levels were relatively high, ranging from 20 to 50 mg N/l, while effluent NO_2 -N levels were always very low with less than 10 mg N/l. NH_4 -N and NO_2 -N removal efficiencies were 44% and 81%, respectively.

Table 4 Summary of batch experiments (average values)

Parameters	A	B	C
Initial T-N concentration (mg N/l)	70-500	70-500	70-500
NH_4 -N removal efficiency (%)	24	56	34
NO_2 -N removal efficiency (%)	46	93	75
T-N removal efficiency (%)	16	48	27
NO_3 -N production rate (mg N/l/d)	15	28	32
Ratio of NO_3 -N production to Influent T-N (%)	16	19	21
T-N removal rate (mg N/l/d)	19	66	37

Furthermore, effluent $\text{NO}_3\text{-N}$ levels were also relatively high, from 20 to 60 mg N/l, with ratio of $\text{NO}_3\text{-N}$ production to influent T-N ranging from 25 to 38%, which was higher than the expected theoretical ratio of 10%. These results suggest the occurrence of nitrification, resulting in low effluent nitrite and high effluent nitrate. These conditions are thought to be due to oxygen persisting inside the pores of PE sponge material.

During period 2 (days 55-89), HRT was decreased stepwise from 24 h to 20 h to 16 h to 12 h to 10 h and effluent $\text{NH}_4\text{-N}$ levels progressively decreased to 20-30 mg N/l and $\text{NO}_2\text{-N}$ levels to 5-15 mg N/l. $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ removal efficiencies were about 78% and 93%. In addition effluent $\text{NO}_3\text{-N}$ levels were relatively high from 45 to 50 mg N/l and the ratio of $\text{NO}_3\text{-N}$ production to influent T-N was from 15 to 25%, which was lower than that of period 1.

During period 3 (days 90-111), the influent flow rate was increased from 6.2 l/d to 7.8 l/d to 10.4 l/d to 12.5 l/d for HRTs of 10 h, 8 h, 6 h and 5 h, respectively, and influent $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ levels were kept at 150 mg N/l. At day 92, the effluent $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ levels were at 46 mg N/l and 30 mg N/l, respectively, which were higher than the values of previous runs due to the trouble with the temperature control system. After some days, effluent $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations decreased to less than 40 mg N/l and 20 mg N/l, respectively. $\text{NH}_4\text{-N}$ and

$\text{NO}_2\text{-N}$ removal efficiencies of these 21 days of 78% and 92% were obtained, respectively. The ratio of $\text{NO}_3\text{-N}$ production to influent T-N was 12 - 13% lower than that of period 2, which was close to the theoretical ratio of 10%.

During period 4 (days 112-141), the influent flow rate was increased up from 12.5 l/d to 15.6 l/d for HRTs of 5 h to 4 h, respectively, and the influent $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations were increased stepwise from 150 mg N/l to 200 mg N/l. $\text{NH}_4\text{-N}$ removal efficiency was 75% and $\text{NO}_2\text{-N}$ removal efficiency was as high as 90%. The ratio of $\text{NO}_3\text{-N}$ production to influent T-N was 8 - 12%, which was in accordance with the theoretical ratio. Therefore, it appears the anammox process had become dominate in comparison with nitrification.

During period 5 (days 142-240), the influent flow rate was increased up from 17.8 l/d to 20.8 l/d for HRTs of 3.5 h and 3 h, respectively, and the influent $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations were kept at 206 mg N/l and 215 mg N/l, respectively. $\text{NH}_4\text{-N}$ removal efficiency was 77% and $\text{NO}_2\text{-N}$ removal efficiency was as high as 90%, demonstrating stable and efficient anammox treatment during period 5.

T-N removal efficiency Influent and effluent T-N and T-N removal efficiencies are shown in Fig. 6. Influent T-N was increased from 60 to 423 mg/l. During period 1 (the first 54 days), effluent T-N levels were

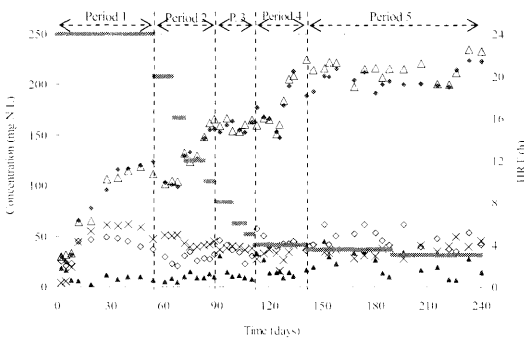


Fig. 5 Time courses of influent and effluent concentrations of nitrogen compounds

Symbols: ◆ Influent $\text{NH}_4\text{-N}$, ◇ Effluent $\text{NH}_4\text{-N}$,
 △ Influent $\text{NO}_2\text{-N}$, ▲ Effluent $\text{NO}_2\text{-N}$,
 × Effluent $\text{NO}_3\text{-N}$, ■ HRT

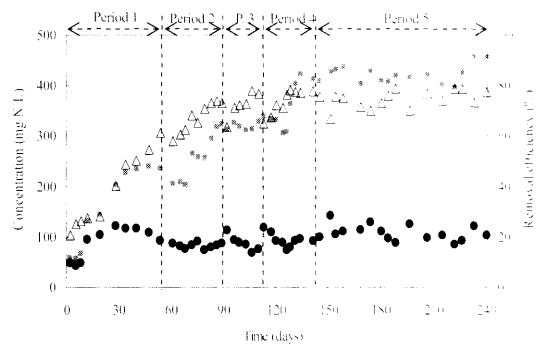


Fig. 6 Time courses of influent and effluent T-N concentrations and T-N removal efficiencies

Symbols: ■ Influent T-N, ● Effluent T-N,
 △ T-N Removal Efficiency

very high reaching a maximum of 120 mg/l and T-N removal efficiency was only 38%. During period 2 (days 55-89), effluent T-N levels were lower at 80-100 mg/l and T-N removal efficiency was higher at 67%. During period 3 (days 90-111), influent T-N was maintained at a level of 300 mg/l and effluent T-N decreased from 113 mg/l to 70 mg/l, while T-N removal efficiency was higher at 72%. During period 4 (days 112-141), influent T-N was increased from 300 mg/l to 400 mg/l and effluent T-N levels fluctuated from 117 mg/l to 73 mg/l. T-N removal efficiency was 74%, which was higher than period 3. During period 5 (days 142-240), the average influent T-N was 423 mg N/l and effluent T-N was about 108 mg N/l and T-N removal efficiency was stable at 75%. During an operational period of 240 days, T-N removal efficiencies improved stepwise from 38% to 75%.

T-N removal rates The reactor has been operated continuously for 8 months and T-N removal rate increased very slowly from 0.05 to 0.1 kg N/m³/day during period 1 (the first 54 days) as shown in Fig. 7. This was due to the co-existing nitrification and the anammox

processes in the reactor. However, T-N removal rates increased quickly from 0.1 to 0.6 kg N/m³/day during period 2 (days 55-89). Especially, T-N removal rate increased two times from 0.6 to 1.2 kg N/m³/day during period 3 (days 90-111). Consequently, the anammox bacteria were more active during this period than the previous period. In addition, T-N removal rates increased stepwise from 1.2 to 2 kg N/m³/day for a short time during period 4 (days 112-141). Subsequently, a T-N removal rate of 2.8 kg N/m³/day was obtained during period 5 (days 142-240), showing that the anammox bacteria were very active at this period.

In the same laboratory, a 50-l upflow column anammox reactor using a polyester nonwoven biomass carrier⁵⁰ was being studied at the same time. Comparisons of the biomass carriers and T-N removal rates of the polyester nonwoven reactor and the PE sponge reactor (this study) are shown in Table 5.

The pore diameter of the PE sponge was larger than that of the polyester nonwoven. Therefore, a higher T-N removal rate (2.8 kg N/m³/day) was obtained after 240 days of continuous operation of the PE sponge reactor.

Ratios of T-N removal, NO₂-N removal and NO₃-N production to NH₄-N removal Ratios of T-N removal, NO₂-N removal and NO₃-N production rates to NH₄-N removal rates during the operational time are shown in Table 6. During period 1, NO₂-N/NH₄-N and NO₃-N/NH₄-N were higher than the theoretical ratios. In addition, T-N/NH₄-N was much lower than the value of the theoretical ratio. Therefore, ratios of T-N removal, NO₂-N removal and NO₃-N production rates to NH₄-N removal rates

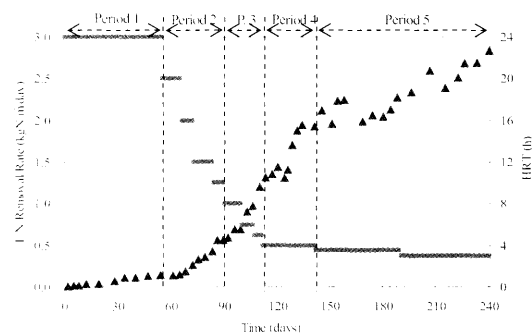


Fig. 7 Changes in T-N removal rate
Symbols: ▲ T-N Removal Rate, ■ HRT

Table 5 Comparisons of the characteristics of biomass carriers and T-N removal rates

Parameters	PE sponge reactor (this study)	Polyester nonwoven reactor ⁵⁰
Thickness	1 cm	0.7 cm
Porosity	96 % (wet condition)	99.5 %
Pore diameter	1,060 μm	510 μm
Packing ratio ($V_{\text{material}}/V_{\text{reactor}}$)	37 %	40 %
Operational time	240 days	260 days
T-N removal rate	2.8 kg N/m ³ /day	2.5 kg N/m ³ /day

Table 6 Changes in Stoichiometric ratios of $\text{NO}_2\text{-N}$ removal, $\text{NO}_3\text{-N}$ production and T-N removal to $\text{NH}_4\text{-N}$ removal during continuous treatment.

Time (days)	$\text{NO}_2\text{-N}/\text{NH}_4\text{-N}$	$\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$	T-N/ $\text{NH}_4\text{-N}$
Theoretical ratios	1.32	0.26	2.06
Period 1 (The first 54 days)	1.47	0.82	1.64
Period 2 (day 55~89)	1.2	0.38	1.82
Period 3 (day 90~111)	1.19	0.3	1.88
Period 4 (day 112~141)	1.23	0.24	1.99
Period 5 (day 142~240)	1.21	0.23	1.99

during period 1 were very different in comparisons with the stoichiometry of the anammox reaction due to the nitrification phenomenon as discussed earlier.

Ratios of T-N removal, $\text{NO}_2\text{-N}$ removal and $\text{NO}_3\text{-N}$ production rates to $\text{NH}_4\text{-N}$ removal rates during period 2 (days 55-89) were also different in comparisons with the stoichiometry of the anammox reaction. $\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$ was also higher in comparisons with the theoretical ratio. However, this differences became smaller compared with that of period 1.

Ratios of T-N removal, $\text{NO}_2\text{-N}$ removal and $\text{NO}_3\text{-N}$ production rates to $\text{NH}_4\text{-N}$ removal rates during period 3 (days 90-111) were close to the stoichiometric values of the anammox reaction. Though, $\text{NO}_3\text{-N}/\text{NH}_4\text{-N}$ was still little higher in comparisons with the theoretical ratio. These values were better than the values of period 2.

Finally, ratios of T-N removal, $\text{NO}_2\text{-N}$ removal and $\text{NO}_3\text{-N}$ production rates to $\text{NH}_4\text{-N}$ removal rates during periods 4 (days 112-141) and 5 (days 142-240) were improved clearly and almost equal to the theoretical ratios as shown in Table 6.

Changes of pH levels Table 7 shows the pH changes during the operational period as

Table 7 Changes of pH levels during continuous treatment

Period, days	Influent pH	Effluent pH
Period 1, 1-54 (n=10)	7.4 ± 0.1	7.6 ± 0.1
Period 2, 55-89 (n=9)	7.3 ± 0.1	7.9 ± 0.3
Period 3, 90-111 (n=6)	7.3 ± 0.1	8.1 ± 0.2
Period 4, 112-141 (n=9)	7.2 ± 0.1	8.0 ± 0.1
Period 5, 142-240 (n=16)	7.2 ± 0.1	8.0 ± 0.1

shown in equation 1, the effluent pH value shifted to the alkaline range with the progression of the anammox reactor. Effluent pH levels of period 1 (the first 54 days) did not increase more than 7.6, which might be due to the co-existence of nitrification and anammox reactions in the PE sponge reactor. This shows the decrease in nitrification activity owing to the depletion of oxygen.

Effluent pH levels were higher than influent pH levels during period 2 (days 55-89). Effluent pH values of during periods 3 (days 90-111), 4 (days 112-141) and 5 (days 142-240) were significantly higher (0.8 pH units) than influent values, this reflect the chemistry of the anammox reaction, i.e., consumption of acidity. Therefore, it could be confirmed that the anammox process dominated at this time.

Attached biomass observation Fig. 8 shows the variations in biomass color of the PE sponge reactor at start-up and after 3 months, 4 months, 5 months and 8 months of operation. After 3 months, biomass was

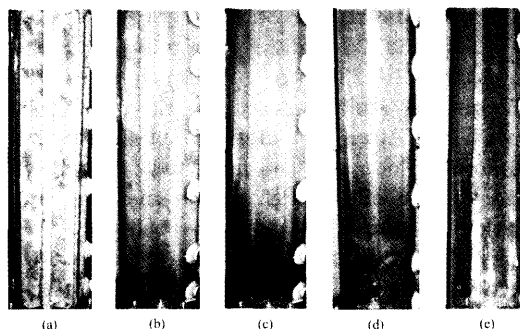


Fig. 8 Changes in biomass color (a) Start-up time; (b) after 3 months; (c) after 4 months; (d) after 5 months; (e) after 8 months

attached strongly on the surface of the PE sponge material and its white color had changed to a reddish color. After 5 months, anammox biomass had proliferated on the surface of the PE sponge material and the color of biomass carrier changed to dark-red color. This observation shows that anammox bacteria grew quickly on the large porous PE sponge biomass carrier. Influent wastewater was supplied from bottom of the reactor so that substrate concentration at bottom of the reactor is highest. Consequently, biomass grew better at the bottom of the reactor than at the upper part of the reactor.

CONCLUSIONS

In the batch experiments, nitrification occurred in flask B that had oxygen trapped in the large pores of the biomass carrier. However, the oxygen penetration in flask B was less than in flask C because of the daily N_2 gas purging in flask B. In the continuous fixed-bed reactor using the PE sponge sheet as a biomass carrier, NH_4 -N and NO_2 -N removal efficiencies improved over the period of testing from 44% to 77% and 81% to 90%, respectively. During the 240 days of operation, T-N removal efficiencies increased stepwise from 38% to 75% as influent T-N was also increased stepwise from 60 to 423 mg N/l. The reactor was operated continuously for 8 months and the T-N removal rate reached 2.8 kg N/m²/day after 240 days of operation. This showed the effectiveness of the PE sponge sheet biomass carrier as a support material for the extremely slowly growing anammox bacteria. Ratios of T-N removal, NO_2 -N removal, and NO_3 -N production to NH_4 -N removal over 30 days (days 112-141) of 1.99:1.23:0.24 and 98 days (days 142-240) of 1.99:1.21:0.23 were almost equal to the theoretical stoichiometry of the anammox reaction. After 5 months of operation, anammox biomass was attached strongly on the surface of the PE sponge material and dark-red color biomass was observed. This observation confirmed that anammox bacteria grew quickly within the large, 1060 μ m pores of the PE sponge biomass carrier, thus demonstrating its suitability as a biomass carrier for anammox bacteria.

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