EVALUATION OF THE AMMONIUM REMOVAL CAPABILITIES OF ANAMMOX SLUDGE IN A FIXED BED REACTOR USING FELIBENDY CUBES AS BIOMASS CARRIER

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***Summary**: Nitrogen removal from ammonium-rich wastewater has been attracting increasing attention due to the serious water pollution consequences such as eutrophication of water bodies. The most widely used ammonium removal technology around the world was the conventional nitrification-denitrification process. However, the application of the conventional process was limited by high operational cost and addition of the external organic matters for the denitrification step [1]. Anaerobic ammonium oxidation (Anammox) is a biological reaction in which the anaerobic ammonium oxidation bacteria (AnAOB) combine nitrite and ammonium to form nitrogen gas using nitrite as electron acceptor under anoxic condition [1], [2]. The ANAMMOX process has some advantages compared to conventional nitrification-denitrification processes: it does not require the addition of external carbon, less sludge production and low energy consumption [3]. In the fixed-bed reactor using FELIBENDY cubes as biomass carrier, NH_4^+-N and NO_2^--N removal efficiencies were improved over the operational time from 40.87% and 31.56% to 86.35% and 82.96%, respectively. During operation period of 191 days, T-N removal efficiencies increased stepwisely from 22.8% to 71.47% while influent T-N concentrations gradually increased stepwise from 35 to 235 mg N/L. This showed the effectiveness of FELIBENDYcubes biomass carrier as the support material for extremely slow growing Anammox bacteria.*

***Keywords**: Anammox; rich Ammonium; waste water; NH_4^+-N; NO_2^-N; FELIBENDY cubes; biomass carrier; F-AX.*

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1. Introduction

In nature, at least two mechanisms of biological ammonium oxidation can occur under anoxic conditions. One of them can be carried out by nitrifiers like Nitrosomonas eutropha indicating ability to oxidize ammonium with nitrite as an electron acceptor [4, 5]. The other recently discovered and more effective group is autotrophic bacteria responsible for Anammox (Anaerobic ammonium oxidation) reaction. Bacteria belong to the order of the Planctomycetales [5]. Before they were identified by microbiologists, the existence of Anammox bacteria was preliminary predicted by Broda E. in 1977 [6]. He based his assumption on thermodynamic considerations and stated that if a chemical reaction yields energy, it is probable that there exist bacteria that are able to use it. Depending on electron acceptor, this reaction can yield enough energy to make it possible for chemolitho autotrophs to live.

For an ammonium-rich wastewater, combining with a nitritation step, this new process yields the formation of di-nitrogen gas under anoxic conditions from the concomitant oxidation of ammonium and reduction of nitrite. This combined process would require only 50% of the oxygen needed for the traditional nitrification-denitrification method and, being fully autotrophic, no addition of organic carbon is needed. The anammox process can be thus effective for nitrogen removal from wastewaters with low carbon content; in addition, sludge production is very limited amount thus making it an economically favourable treatment option. The stoichiometry of the anammox reaction has been determined as below [2]:

$$NH_4^+ + 1.32\,NO_2^- + 0.066\,HCO_3^- + 0.13H^+ \rightarrow$$
$$1.02\,N_2 + 0.26\,NO_3^- + 0.066\,CH_2O_{0.5}N_{0.15} + 2.03\,H_2O \quad (1)$$

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The application of anammox process for nitrogen removal from wastewater has been implemented such as reject water from more than 100 wastewater treatment plants [7] or mainstream wastewater in moving bed biofilm reactor (MBBR) [8].

Anammox treatment performances for nitrogen removal using polyvinyl alcohol (PVA) gel beads, malt ceramics (MC) and polyethylene (PE) sponge as biomass carriers achieved good results [9]. PVA gel beads were used in a fluidized-bed reactor where a maximum T-N removal rate of 3.0 kg N/m^3/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 achieved high T-N removal rates of 3.1 kg N/m^3/day. PE sponge reactor was operated continuously and T-N removal rate of 2.8 kg N/m^3/day was obtained after 240 days of operation.

Another study applied a fluidized bed reactor with small ring non-woven carriers using anammox process for nitrogen removal and obtained nitrogen removing rate (NRR) of 4.64×10^{-2} kg N/m^3/day on day 131 [10].

The above researches show that the anammox process has been applied for nitrogen removal in different wastewater with numerous material carriers and obtained positive results. Therefore, a material carrier of FELIBENDY cubes is a new choice for nitrogen removal using anammox process under fixed-bed condition in this research. The objective of research is to investigate the ammonium removal capabilities of anammox sludge in a fixed bed reactor (F-AX) using FELIBENDY cubes.

2. Materials and methods

Laboratory- scale Fixed Bed Reactor (F-AX)

The fixed-bed reactor was used with a total volume of 1.62 L. The reactor had an inner diameter of 7.1 cm and total height of 41 cm. The FELIBENDY cubes volume of 0.65 L was considered to be the reaction zone volume, which was used for determinations of hydraulic retention time (HRT). HRT was changed from 24 h to 6 h. The clarification zone (above the reaction zone) was 0.34 L. Schematic diagram of the fixed-bed reactors is shown in Fig. 1 and picture of the reactor shown in Fig. 2.

Influent wastewater was fed in up-flow mode using peristaltic pumps (Eyela Co., Ltd., Tokyo). Nitrogen gas was collected by using gas sampling bags. Airtight integrity was maintained in the capped reactors using effluent water traps. Reactor temperatures were maintained at 33°C to 35°C by using external ribbon heating elements. Black vinyl sheet enclosures were used to maintain dark conditions. Daily purging by nitrogen gas was used to reduce dissolved oxygen (DO) levels in the influent medium to below 0.5 mg/L.

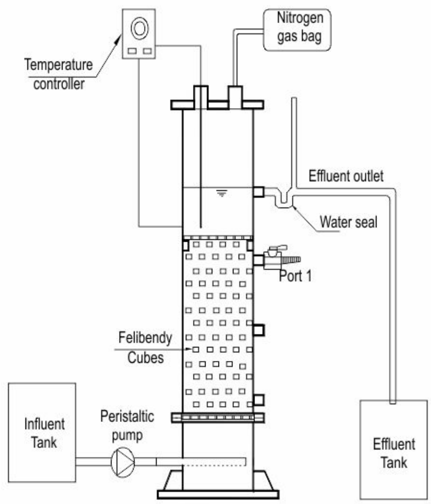

Figure 1. *Schematic diagram of F-AX using FELIBENDY cubes*

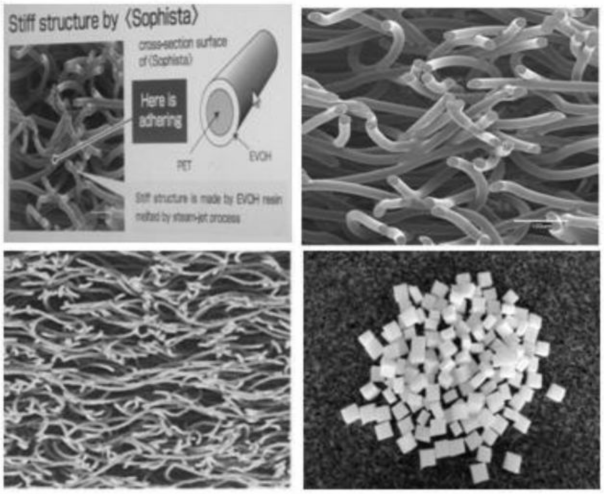

Figure 3. *FELIBENDY cubes as biomass carrier (Source: Kuraray Company) [11]*

Figure 2. *Picture of F-AX using FELIBENDY cubes*

Biomass carrier

FELIBENDY is the next generation material made with Steam-jet technology and special fibers of Kuraray. Steam-jet technology makes light and thick fiber-board with acoustic absorbent, air-permeable and shock absorbent properties easily.

Steam-jet process melts EVOH (ethylene vinyl alcohol) resin, with core of PET (polyethylene terephthalate) makes numbers of points of attachment and develops stiff structure with numbers of air space as shown in Fig. 3. FELIBENDY is a tough and unique fiber structure with air permeability and insulation characteristics. The physical properties of this biomass carrier are described in Table 1.

Table 1. *Physical properties of FELIBENDY [11]*

No	Parameter	Unit	Value
1	Weight	g/m^2	1000
2	Thickness	mm	10
3	Density	g/cm^3	0.1
4	Air permeability	$g/cm^3/cm^2/s$	26.2
5	Equivalent open area	cm^2/cm^2	0.005
6	Thermal coductivity	W/m.K	0.034

Figure 4. *Seed sludge*

Seed sludge

The anammox sludge (Planctomycetes) distributed by Meidensa company, Nagoya, Japan was used as seed sludge as shown in Fig. 4. Before start-up, 50 ml of the seed sludge was attached on the surface of the FELIBENDY cubes.

Synthetic wastewater

Synthetic wastewater was prepared by adding ammonium and nitrite in the forms of $(NH_4)_2SO_4$ and $NaNO_2$, respectively, to a mineral medium according to the composition given in Table 2.

Table 2. *Composition of wastewater*

Compositions	Units	Concentration
$(NH_4)_2SO_4$	(mgN/L)	Variable 10-120
$NaNO_2$	(mgN/L)	Variable 10-120
$KHCO_3$	(mg/L)	125.1
KH_2PO_4	(mg/L)	54.4
$FeSO_4.7H_2O$	(mg/L)	9.0
EDTA	(mg/L)	5.0

Operational conditions

Influent was fed in up-flow mode using a peristaltic pump (Eyela Co., Ltd., Tokyo). The reactor temperature was maintained at 33°C to 35°C, controlled by the thermal stability equipment of the aquarium. Light is known to have a negative effect (30-50% rate reduction) on ammonium removal rate; consequently, dark conditions were maintained using black vinyl sheet enclosures. Purging with nitrogen gas was used on a daily basis to keep dissolved oxygen levels in the influent below 0.5 mg/L.

Operational regime of this experiment is shown in the Table 3 with different HRT and variable influent concentration of ammonium and nitrite.

Table 3. *Operational parameters of Fixed Bed Reactor*

Period	Time (days)	Flow rate (L/d)	HRT (h)	Influent NH_4^+-N (mg N/L)	Influent NO_2^- N (mg N/L)
1	1- 43	1.62	24	10-40	10-40
2	44 - 87	2.16	18	40-60	40-60
3	88 - 143	3.24	12	50-120	50-120
4	144 - 191	6.48	6	60-120	60-120

Chemical analyses

The experiment was conducted in the laboratory of Water Supply and Sanitation Division, Faculty of Environmental Engineering, National University of Civil Engineering. Parameters of influent and effluent stream were analyzed 3 times per week. Ammonium concentrations were measured by colorimetric method with Nessler reagent at wavelength of 420nm. In accordance with Standard Methods [12], nitrite concentrations were estimated by the colorimetric method (4500-NO_2^- B) and nitrate by the UV spectrophotometric screening method (4500-NO_3^- B). Nitrite was determined to have an interfering response in the nitrate UV screening method of 25% of the nitrate response on a nitrogen weight basis, thus the results were corrected by calculation. Levels of pH were measured by using a Mettler Toledo-320 pH meter and DO was measured by using a DO meter (D-55, Horiba).

3. Results and discussion

Influent and effluent concentrations and removal efficiencies of nitrogen compounds

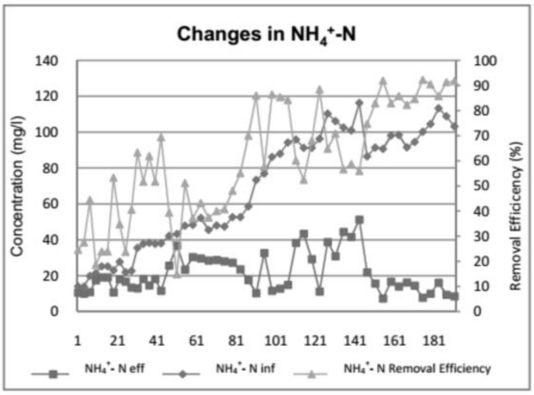

Figure 5. *Changes in Nh_4^+- N during 4 periods*

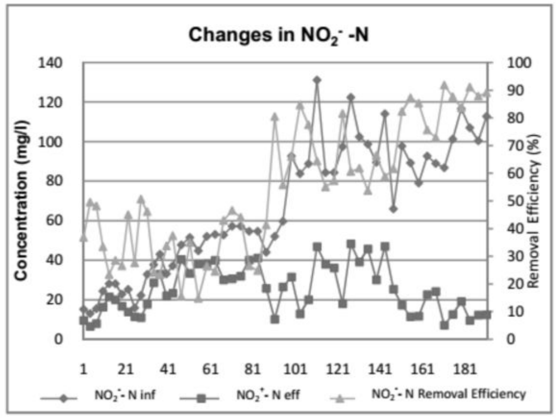

Figure 6. *Changes in NO_2^-- N during 4 periods*

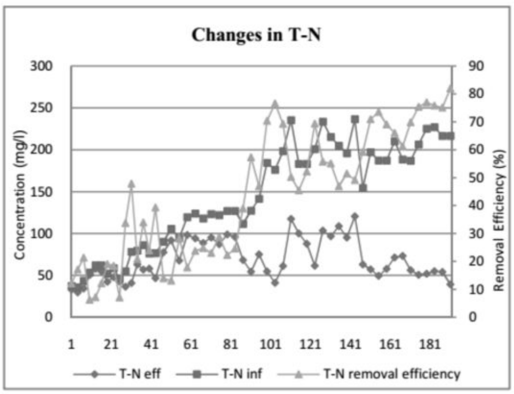

Figure 7. *Changes in T-N during 4 periods*

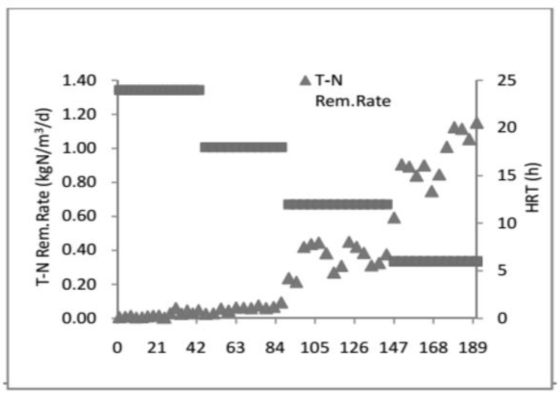

Figure 8. *Changes in T-N removal rate*

Influent NH_4^+-N and NO_2^--N levels were increased from 10 to 120mg N/L for operational time of 191 days (Table.3).

During period 1 (the first 43 days), when HRT was 24 h, effluent NH_4^+-N concentration was from 10 to 20 mg N/L, effluent NO_2^--N concentration was high from 6 to 32mg N/L. NH_4^+-N and NO_2^--N removal efficiencies were 40.87% and 35.56%, respectively. Furthermore, effluent NO_3^--N concentration was also very high from 14 to 23 mg N/L. The average ratio of NO_3^--N production to influent T-N was 11.99% as shown in Fig. 5 and Fig. 6.

During period 2 (days 44-87), HRT was decreased stepwise from 24 h to 18 h. Influent NH_4^+-N and NO_2^--N concentration increased from 40 to 60 mg/l. NH_4^+-N removal efficiency was 43.35% and NO_2^--N removal efficiencies were 31.09%.

During period 3 (days 88-143), the influent flow rate was raised up from 2.16 L/d to 3.24 L/d, for the HRTs of 18 h to 12 h and influent NH_4^+-N and NO_2^--N concentrations were increased from 50 to 120 mg N/L. NH_4^+-N and NO_2^--N removal efficiencies of this period were 69.66% and 65.94%, respectively.

During period 4 (days 144-191), the influent flow rate was raised up from 3.24 L/d to 6.48 L/d for the HRTs of 12 h to 6 h and the influent NH_4^+-N and NO_2^--N concentrations were kept 60 mg N/L to 120 mg N/L. NH_4^+-N removal efficiency was 86.35% and NO_2^--N removal efficiency was high of 82.92%. The average ratio of NO_3^--N production to influent T-N was 10.47% which was equal to the theoretical ratio. Therefore, anammox process has dominated in compare with nitrification process.

Influent and effluent T-N concentrations and T-N removal efficiencies

Influent and effluent T-N concentrations and T-N removal efficiencies were shown in Fig. 7. Influent T-N concentrations were increased from 35 to 230 mg/L.

During the first stage of period 1 (the first 43 days), influent T-N concentrations were small (from 35 to 85 mg/l) and effluent T-N concentrations were very high from 30 to 67 mg/L, T-N removal efficiency was 21.33% only.

During period 2 (days 44-87), influent T-N concentration were increased from 90 to 130 mg/l, effluent T-N concentrations were still high with 60-99 mg /L and T-N removal efficiency was 23.54%.

During period 3 (days 88-143), influent T-N concentrations was increased from 120 to 235 mg/L, T-N effluent concentrations fluctuated from 120 mg/L to 40 mg/L and T-N removal efficiency was higher of 56.85%.

During period 4 (days 144-191), influent T-N concentrations was kept from 150 mg/L to 235 mg/L, the effluent T-N was maximum at 70mg/l and T-N removal efficiency was higher of 71.27%.

The reactor has been operated continuously for 191 days of operation and T-N removal rate was increased very slowly from 0.005 to 0.006 kg N/m^3/day during period 1 as shown in Fig. 8. This is due to the co-existing of the nitrification and the anammox processes in the reactor.

T-N removal rates were increased slowly from 0.03 to 0.09 kg N/m^3/day during period 2 because of low loads of T-N. However, T-N removal rate increased quickly from 0.21 to 0.45 kg N/m^3/day for period 3.

Consequently, the anammox bacteria were more active at this period than the previous period. In addition, T-N removal rates were increased stepwise from 0.6 to 1.15 kg N/m^3/day for a short time of period 4. High T-N removal rates were obtained show that the anammox bacteria were very active at this period.

Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates

Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates during the operational time were summarized in Table 4.

Table 4.*Changes in Stoichiometric ratios of NO_2^-- N removal, NO_3^-- N production and T-N removal rates to NH_4^+-N removal rates during continuous treatment*

Periods	Times (days)	**NO_2-N/** NH_4^+-N	**NO_3-N/** NH_4^+-N	**T-N-N/** NH_4^+-N
	Theoretical ratios	**1.32**	**0.26**	**2.06**
1	1-43	0.61	0.43	1.18
2	44-87	0.64	0.45	1.20
3	88-143	0.92	0.24	1.68
4	144-191	0.94	0.24	1.69

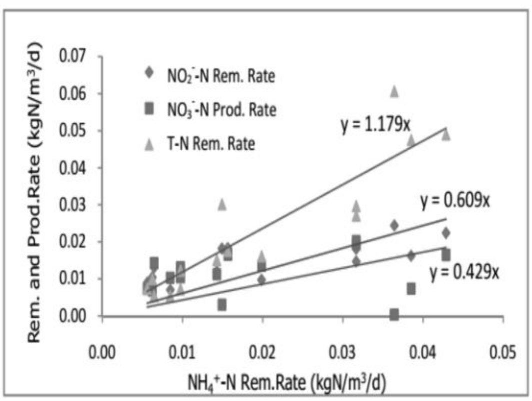

Figure 9a. *Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates during period 1*

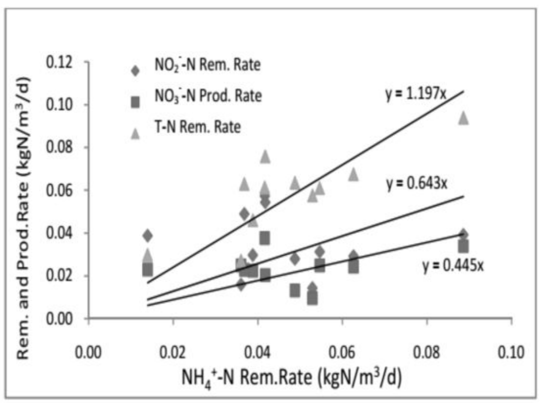

Figure 9b. *Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates during period 2*

NH_4^+-N removal rates during period 1 were very different in comparisons with the stoichiometry of the anammox reaction due to the nitrification phenomenon as discussed earlier.

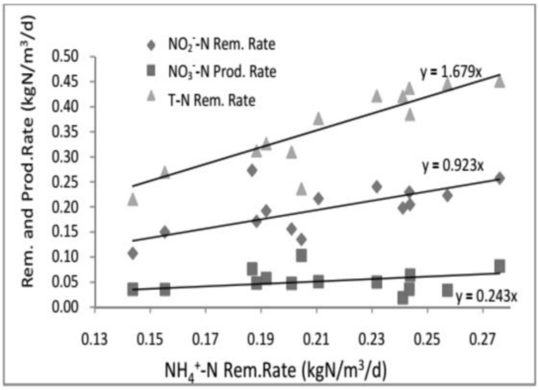

Figure 9c. *Ratios of T-N removal, NO_2-N removal and NO_3^--N production rates to NH_4^+-N removal rates during period 3*

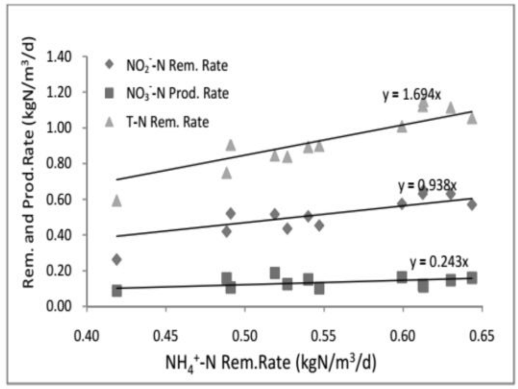

Figure 9d. *Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates during period 4*

Ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates during period 2 were also different in comparisons with the stoichiometry of the anammox reaction. NO_3^--N/ NH_4^+-N ratio was also higher in comparisons with the theoretical ratio as shown in Fig. 9b.

Ratios of T-N removal, NO_2-N removal and NO_3^--N production rates to NH_4^+-N removal rates during period 3 (were closer to the stoichiometric values of the anammox reaction. Though, T-N/ NH_4^+-N and NO_3^--N/ NH_4^+-N ratio was still little higher in comparisons with the theoretical ratio. These values were better than the values of period 2 as shown in Fig. 9c and Table 4.

After all, ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates during periods 4 were improved and almost equal to the stoichiometry of the anammox reaction as shown in Figs. 9d and Table 4.

Figure 10. *Attached biomass observation after 191 days*

During period 1, NO_3^--N/NH_4^+-N ratios were higher than the values of the theoretical ratios. In addition, T-N/NH_4^+-N ratio was lower much more than the value of the theoretical ratio. Therefore, ratios of T-N removal, NO_2^--N removal and NO_3^--N production rates to NH_4^+-N removal rates 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, NO_2^--N removal and NO_3^-- N production rates to NH_4^+-N removal rates during period 2were also different in comparisons with the stoichiometry of the anammox reaction. NO_3^--N/NH_4^+-N ratio was also slightly higher in comparisons with the theoretical ratio.

Ratios of T-N removal, NO_2^-- N removal and NO_3^- - N production rates to NH_4^+-N removal rates during period 3 and period 4were closer to the stoichoimetric values of the anammox reaction. Though, ratios of T-N removal, NO_2^-- N removal and NO_3^--N production rates to NH_4^+-N removal rates were little smaller in comparisons with the theoretical ratio.

Attached biomass observation

After 191 days of operation, anammox biomass was attached on the surface of FELIBENDY cubes material and the red color biomass was observed as shown in Fig. 10.

4. Conclusions

In the fixed-bed reactor using FELIBENDY cubes as biomass carrier, NH_4^+-N and NO_2^-N removal efficiencies were improved over the operational time from 40.87% and 31.56% to 86.35% and 82.96%, respectively. During operational time of 191 days, T-N removal efficiencies increased stepwise from 22.8% to 71.47% with influent T-N concentrations were also increased stepwise from 35 to 235 mg N/L. This showed the effectiveness of FELIBENDY cubes biomass carrier as the support material for extremely slow growing anammox bacteria.

Ratios of T-N removal, NO_2^--N removal, and NO_3^-N production to NH_4^+-N removal over191 days of 1.69:0.94:0.24 was nearly equal to the stoichiometry of the anammox reaction. After 191 days of operation, anammox biomass was attached strongly on the surface of FELIBENDY cubes material and the red color biomass was observed. It could be concluded that the FELIBENDY cubes are suitable biomass carrier because anammox bacteria grow quickly owe to the large number of air spaces.

Acknowledgement

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