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Effect of COD/N ratio on the Feammox process in the treatment of fish processing wastewater

Danh Truong Trong, Chung Le Phuong*

Institute of Biotechnology and Environment, Nha Trang University, Nguyen Dinh Chieu 2, Nhatrang City, Khanhhoa Province, Vietnam, Phone: +84 (0) 918 780 795; emails: chungbiotech@gmail.com (C.L. Phuong), danhtt@ntu.edu.vn (D.T. Trong)

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ABSTRACT

Biological ammonium removal in wastewater treatment plants requires large quantities of energy input and the addition of organic carbon for denitrification. Anaerobic ammonium oxidation coupled to Fe(III) reduction (termed the Feammox process) has been considered a novel pathway to remove nitrogen from wastewater mediated by microorganisms in recent years. In this study, a 40 L anaerobic sequencing batch reactor with 14-d cycle was operated at different COD/N input ratios (24:1, 15:1, 9:1, 6:1 and 3:1). After 5 cycles of operation, the ammonia removal rate was 91.1%, 87%, 91%, 95%, and 82.9%, respectively. It was found that heterotrophic anaerobic microorganisms could coexist and co-consume carbon with Feammox bacteria at low COD/N conditions (<9). At high COD/N conditions (>9), Feammox bacteria had lower priority in carbon consumption. Nitrogen gas could be the final product of the Feammox process in this reactor. The mixed liquor volatile suspended solids/mixed liquor suspended solids ratio of Feammox sludge in this study (0.71–0.76) was equivalent to that of conventional activated sludge. This study provided a new possible solution to remove nitrogen from fish processing wastewater.

Keywords: Feammox process; COD/N ratio; Fish processing wastewater

1. Introduction

The commercial fish processing industry generates large quantities of solid waste and wastewater. Furthermore, fish processing wastewater is typically characterized by high suspended solids concentration, a high level of organic content as measured by either chemical oxygen demand (COD) (1.6–10 g/L) or biochemical oxygen demand (BOD_5) (0.7–6 g/L), and high protein concentration comes from the blood, entrails, fat and fish tissues [1]. The two most common techniques for biological nitrogen removal are denitrification and anammox. Although denitrification has a better effect, it is easily impacted by a lack of carbon sources [2]. Anammox is a popular study topic as well, although it still has drawbacks such as challenging biomass control, a long growth cycle, and environmental sensitivity [3–5]. Moreover, these two ways require aeration for nitrification or partial nitrification.

In 2005, a new process of ammonium oxidation coupled to ferric iron (Fe^{3+}) reduction in anaerobic conditions, called Feammox, was discovered [6]. Several studies have demonstrated that Feammox bacteria engage in the reduction of ferric ions (III) and anaerobic ammonium oxidation in soil environment such as: tropical rainforests [7,8], paddy fields [9,10], intertidal wetland [11,12], riparian wetland [6,13,14], marine sediments [15]. In addition, certain studies have revealed that the Feammox process was carried out in anaerobic digested sludge [16,17], Anammox sludge with Fe(III) added [18–20].

Feammox process is used in numerous studies to remove nitrogen from wastewater [21–26], so it could be applied to remove nitrogen in fish processing

* Corresponding author.

wastewater. Depending on the conditions, N_2, NO_2^-, or NO_3^- can be created during the Feammox process [8,16]:

$$3Fe(OH)_3 + 5H^+ + NH_4^+ \rightarrow 3Fe^{2+} + 9H_2O + 0.5N_2 \quad (\Delta G^\circ = -245 \text{ kJ/mol}) \tag{1}$$

$$6Fe(OH)_3 + 10H^+ + NH_4^+ \rightarrow 6Fe^{2+} + 16H_2O + NO_2^- \quad (\Delta G^\circ = -164 \text{ kJ/mol}) \tag{2}$$

$$8Fe(OH)_3 + 14H^+ + NH_4^+ \rightarrow 8Fe^{2+} + 21H_2O + NO_3^- \quad (\Delta G^\circ = -207 \text{ kJ/mol}) \tag{3}$$

Similar to other microorganisms, Feammox microorganisms consume carbon sources for growth and development. Both inorganic and organic carbon can be used as carbon sources in the Feammox process [21,27]. Autotrophic Feammox sludge do not require COD to oxidize NH_4^+ and it is inhibited by carbon sources [22]. However, in this research, heterotrophic Feammox sludge was taken from our previous study and it oxidized NH_4^+ extremely poorly if the input did not include COD [26]. Since the COD/N ratio affects heterotrophic denitrification bacteria [28], it is possible that it can influence heterotrophic Feammox bacteria. The aim of this study was to determine the effect of the COD/N ratio on the ammonia-removing ability of the Feammox bacteria in fish processing wastewater treatment.

2. Materials and methods

2.1. Reactor design

An anaerobic sequencing batch reactor (anSBR) made of acrylic with a thickness of 8 mm was used in this study. This reactor was 20 cm × 20 cm × 120 cm (length × width × height) in size, with a total volume of 48 L and an effective volume of 40 L. Four valves (V1, V2, V3, V4) were located along the tank's height (Fig. 1).

This anSBR had a 14-d cycle. After a 10-min fill phase, the reactor was flushed with argon gas to remove dissolved oxygen. A small submersible pump was installed on the bottom of the reactor to stir and completely disturb the sludge during the react phase. The water settled for 30 min before being decanted at 50% volume from valve V3. Samples of water and sludge were collected from valves V1 and V4, respectively.

2.2. Wastewater and sludge

The wastewater samples utilized in this study were collected from the equalization tank of Hai San Ben Vung company's wastewater treatment plant in Nha Trang city, Khanh Hoa province, Vietnam. This company specializes in the initial processing of seafood (especially fish). The stages of the initial processing process are washing, sorting, packaging, and frozen storage. Wastewater mainly came from washing raw fish.

This experiment took place over 5 cycles with different input COD/N ratios: 24, 15, 9, 6 and 3. The raw wastewater had a base COD/N ratio of 25 (Table 1). NH_4Cl was added to the wastewater in order to decrease COD/N ratio.

The initial seed sludge was taken from a previous study [26]. The microbial community were most closely related to the *Aciclyphilus* sp., *Pseudomonas* sp., *Geoalkalibacter* sp., *Geobacter* sp. These strains shared several common physiological characteristics that are considered meaningful for the Feammox process, that is, (i) heteroptrophic ammonium oxidation, (ii) denitrification, and (iii) ferric iron reduction [29–31]. The experiment was operated for 40 d due to the enrichment of microorganism. Accordingly, 1.0 kg of seed sludge was resuspended in 4 L of anoxic distilled water in a tightly sealed glass bottle, shaken at 100 rpm for 5 min, and then added to the reactor. The reactor was then flushed with argon gas to remove oxygen and closed with gas-tight lids. The enrichment process was carried out at room temperatures (30°C ± 2°C).

Mixed liquor suspended solids (MLSS) of anaerobic sludge was initially 1,512 ± 40 mg/L. Ferrihydrite ($Fe_2O_3 \cdot 0.5H_2O$) was prepared by adding $FeCl_3 \cdot 6H_2O$ to NaOH

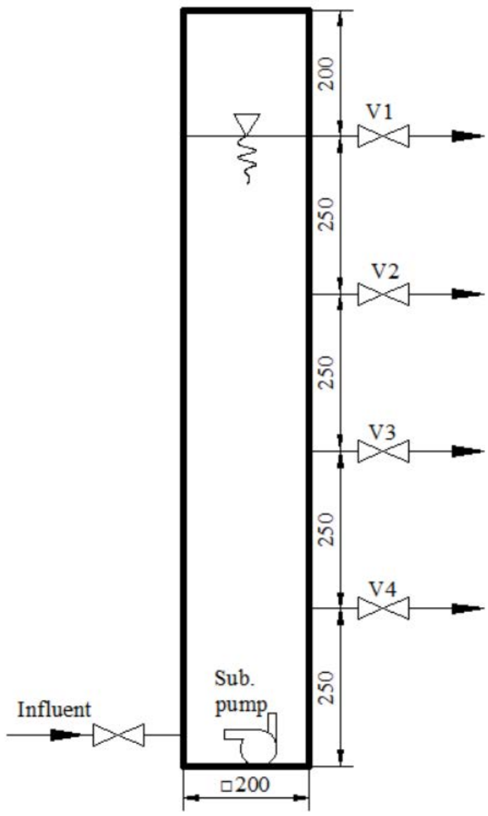

Fig. 1. Schematic diagram of the reactor.

Table 1
Characteristics of raw fish processing wastewater

pH	6.5–7.5
COD (mg/L)	884 ± 65
NH_4^+–N (mg/L)	35 ± 2
NO_2^-–N (mg/L)	ND
NO_3^-–N (mg/L)	ND

ND: Not detected

solution at pH 7.6 [32]. Then it was mixed with the influent wastewater for a final concentration of 30 mM. The pH in the system remained mostly steady between 6.8 and 7.2 during the enrichment and operating phases.

2.3. Analytical methods

Every 24 h samples of water were analyzed for their NH_4^+–N, NO_3^-–N, NO_2^-–N, COD and Fe^{2+} contents. The ammonium concentration was determined photometrically by using sodium nitroprusside reagent with a calibration curve obtained from a serial dilution of NH_4Cl solution (using previously dried NH_4Cl crystals) in the range of 0.018–1.8 mg/L [28,33]. Ferrous iron concentration was determined photometrically by using O-phenanthrolin reagent with a calibration curve obtained from a serial dilution of $FeSO_4$ solution in the range of 0.28–2.8 mg/L [29,34]. Nitrate concentration was determined by using NitraVer® 5 Nitrate Reagent Powder Pillows (Hach Instruments Inc., USA) with a calibration curve obtained from a serial dilution of $NaNO_3$ solution in the range of 0–30 mg/L. Using NitriVer® 3 Nitrite Reagent Powder Pillows (Hach Instruments Inc., USA) to determine nitrite concentration. The nitrite calibration curve obtained from a serial dilution of $NaNO_2$ solution in the range of 0–150 mg/L. COD determination was conducted according to USEPA 410.4 method (Hach Instruments Inc., USA). Sludge samples were collected at the end of every cycle. MLSS and mixed liquor volatile suspended solids (MLVSS) were determined based on the weighing method after the sludge was dried at 105°C and 550°C, respectively [30,35].

Unless otherwise stated, all measurements were performed in three replicate. The data were processed and graphed by Microsoft Excel 2013 software (average and standard deviation functions, the standard deviation was calculated using the "n–1" method).

3. Results and discussion

3.1. Influence of COD/N ratios

Fig. 2 shows the changing of COD and N–NH_4^+ contents over different cycles. Each 14-d cycle had a distinct COD/N influent ratio (24, 15, 9, 6 and 3). Ammonia removal rate of each cycle was 91.1%, 87%, 91%, 95%, and 82.9%, respectively. COD removal rate of each cycle was 94.5%, 94.7%, 96%, 95.3%, 96.4%, respectively (Table 2).

Generally, COD and N–NH_4^+ contents decreased over the period of treatment. However, the first two cycles were different from the others.

In first cycle (COD/N = 24), COD concentration dropped rapidly from 853 ± 52 mg/L to 76 ± 4 mg/L in 7 d and then it fluctuated until 14th day. Meanwhile, at the same time, N–NH_4^+ had a minor change (vary between 27–35 mg/L) in the first 7 d and just dropped to 4.85 ± 0.26 mg/L on 11th day. The 2nd cycle (COD/N = 15) was similar to 1st cycle: COD plunged over the first five days before varying for the following days while N–NH_4^+ had a small change before dropping. It could be seen that the low activity of the Feammox

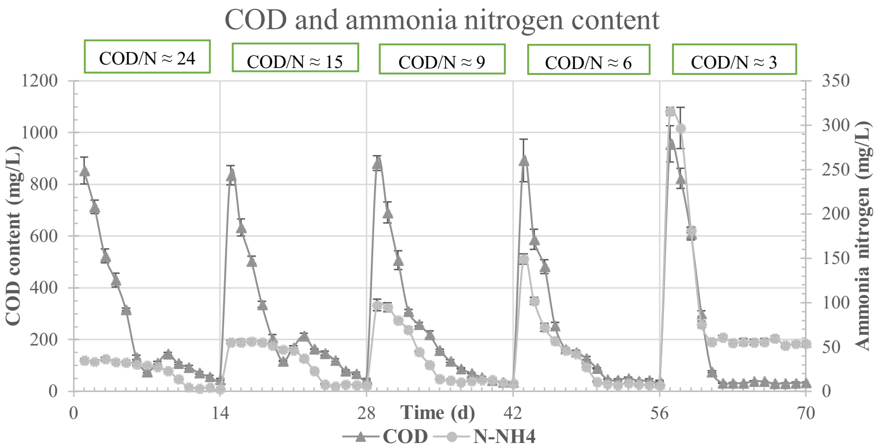

Fig. 2. Chemical oxygen demand and N–NH_4^+ content during different cycles.

Table 2
Chemical oxygen demand and NH_4^+–N removal rate of each cycle

Cycle	COD/N ratio	COD_{inf} (mg/L)	COD_{eff} (mg/L)	NH_4^+–N_{inf} (mg/L)	NH_4^+–N_{eff} (mg/L)	COD removal rate (%)	NH_4^+–N removal rate (%)
1	24	853 ± 52	45 ± 3	35 ± 1	3.1 ± 0.1	94.7	91.1
2	15	835 ± 37	48 ± 2	56 ± 2	7.2 ± 0.5	94.2	87
3	9	882 ± 28	36 ± 3	97 ± 6	8.9 ± 0.2	96	91
4	6	892 ± 82	42 ± 1	149 ± 6	7.2 ± 0.2	95.3	95.2
5	3	956 ± 70	34 ± 1	316 ± 4	54 ± 2.3	96.4	82.9

bacteria at the start of the first two cycles was what caused the minor change in the ammonium nitrogen level. The beginning of the first two cycles did not have any COD consumption by the Feammox bacteria because they were consuming COD while oxidizing $N–NH_4^+$ [26]. It was obvious that heterotrophic anaerobic bacteria may have been responsible for the decline in COD concentration at the start of the first two cycles, which had high COD/N ratios (>9). So, it indicated that under high COD/N ratio conditions, heterotrophic anaerobic bacteria had more priority than Feammox bacteria in carbon consuming, resulting a minor change in $N–NH_4^+$. This result was similar to that observed in a previous study, which showed that the lowest layer was dominated by heterotrophic bacteria while the upper and intermediate layers were enriched with iron-reducing and iron-oxidizing bacteria [36].

The input COD/N ratios of the 3rd, 4th, and 5th cycles were 9, 6, and 3, respectively. There was a consistent trend among the three cycles. COD and $N–NH_4^+$ contents declined in the beginning of the cycles and then remained stable for the following days. It proved that Feammox bacteria favored low COD/N conditions (<9), which resulted in ammonium nitrogen removal. Furthermore, the stable condition in each cycle was achieved when COD or $N–NH_4^+$ content was depleted. The 5th cycle's steady state lasted 9 d longer than the 4th cycle's (6 d). It demonstrated that under conditions with a lower COD/N ratio, COD and ammonium nitrogen removal rates might be faster. The COD elimination rate was 84.6% during the first six days of the 1st cycle, but the $N–NH_4^+$ concentration had a small change (30–35 mg/L). In contrast, the COD removal rate was 96.6% during the first six days of the 5th cycle, while the $N–NH_4^+$ concentration rapidly decreased from 315 ± 4 mg/L to 56 ± 2 mg/L. The difference between these 2 cycles could be explained by Feammox bacteria activities. Together with the heterotrophic anaerobic bacteria's carbon consumption, Feammox bacteria also consumed carbon while eliminating ammonium nitrogen. This result was similar to previous studies [25,26].

3.2. Ferrous ion

Fig. 3 illustrates the variations in Fe^{2+} and $N–NH_4^+$ concentrations across several cycles. Generally, the ferrous ion concentration varied between 2.1 ± 0.11 mg/L and 10.1 ± 0.55 mg/L. When NH_4^+ content changed slightly, Fe^{2+} concentration maintained a steady trend. Otherwise, while NH_4^+ level was reduced, ferrous ion concentration had a upward trend. Furthermore, Fe^{2+} concentration variations were proportional to NH_4^+ content variations when compared between cycles. These findings supported the hypothesis that Fe^{2+} is produced through reduction of Fe^{3+} and the oxidation of NH_4^+.

At the end of each cycle, Fe^{2+} concentration reduced. The reason could be that during the anSBR tank's withdrawal phase, around 50% of the tank's water was decanted, while the influent wastewater did not include Fe^{2+}. It indicated that the Feammox reactor discharged a tiny amount of Fe^{2+} and that the total amount of iron in the reactor would decrease over time.

Moreover, in this study, nitrite and nitrate were not found in the reactor. The reason could be when nitrite and nitrate were generated by NH_4^+ oxidation, it could be consumed in the heterotrophic or autotrophic denitrification process [37]. Moreover, nitrite and nitrate could also interact with Fe^{2+} via chemodenitrification [38,39] or nitrate-dependant iron oxidation process [25,40,41]:

$$4Fe^{2+} + 2NO_2^- + 5H_2O \rightarrow 4FeO(OH) + N_2O + 6H^+ \quad (4)$$

$$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10FeO(OH)_3 + N_2 + 18H^+ \quad (5)$$

Because of the absence of nitrite and nitrate, it indicated that N_2 could be the final product of the Feammox process in this research. And this result was similar to our previous study [26].

3.3. MLSS and MLVSS

At the beginning and the end of each cycle, samples of the sludge were obtained from the reactor's lowest layer (valve V4) (Table 3). After 70-d operation, reactor's MLSS concentration varied from 1,512 ± 40 mg/L to 1,372 ± 24 mg/L. Little amount of sludge was decanted from the reactor after each cycle because the Feammox sludge settled poorly. In addition, daily water sampling also contributed to the loss

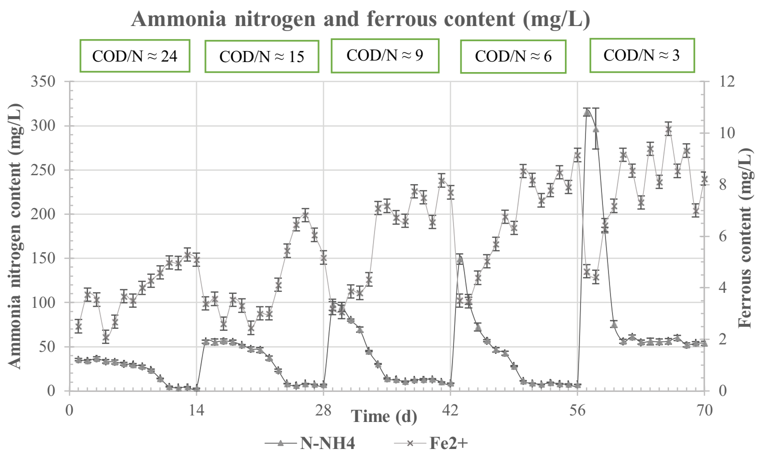

Fig. 3. Fe^{2+} and $N–NH_4^+$ content during different cycles.

Table 3
Mixed liquor suspended solids and mixed liquor volatile suspended solids in each cycle

Day	1	14	28	42	56	70
MLSS (mg/L)	1,512 ± 40	1,372 ± 24	1,473 ± 49	1,447 ± 29	1,379 ± 43	1,469 ± 32
MLVSS (mg/L)	1,107 ± 41	1,009 ± 35	1,076 ± 45	1,022 ± 20	1,045 ± 16	1,047 ± 33
MLVSS/MLSS	0.73	0.74	0.73	0.71	0.76	0.71

of sludge. MLVSS/MLSS ratios in this study varied from 0.71 to 0.76. These ratios were similar to the ratio of regular activated sludge (0.75–0.83 mg·VSS/mg·TSS) [36,42]. It shown that the amount of inorganic materials in Feammox sludge was not significantly higher than the amount in traditional activated sludge.

4. Conclusion

In this study, Feammox process occurred in the reactor treating fish processing wastewater. It proved that heterotrophic anaerobic microorganisms coexisted with Feammox bacteria and had a higher priority in carbon consumption at neutral pH and high COD/N conditions (>9). Feammox bacteria preferred low COD/N conditions (<9) and co-consumed carbon with heterotrophic anaerobic bacteria. The efficiency of COD removal could be improved by adding ammonium. The final product of the Feammox process could be nitrogen gas since nitrite and nitrate were absent. Variations in NH_4^+ content were proportionate to changes in Fe^{2+} concentration. Feammox sludge's MLVSS/MLSS ratio was similar to that of ordinary activated sludge.

In fact, wastewater from livestock farms, biogas storage tanks, and seafood processing plants, for example, often has high NH_4^+ and COD contents. These wastewater s are typically treated using a combination of expensive physico-chemical and biological processes to remove NH_4^+, COD, and other pollutants. Anaerobic processes are preferred over aerobic processes from the perspective of wastewater treatment because they use less energy and produce less secondary biomass. Feammox can be thought of as a potential process for treating anaerobic wastewater. Therefore, figuring out how influent wastewater's COD/N ratio affects COD and NH_4^+ treatment effectiveness, as presented in this study, helps put Feammox technology into practice.

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