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Effect of COD:N ratio on biological nitrogen removal using full-scale step-feed in municipal wastewater treatment plants

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Abstract

This study investigated the effect of low and high chemical oxygen demand (COD):N ratios on biological nitrogen removal and microbial distributions in full-scale step-feed (SF) municipal wastewater treatment plants (WWTPs) in Thailand (SF_1) and Taiwan (SF_2). The SF_1 WWTP had a low COD:N (4:1) ratio, a long solids retention time (SRT) (> 60 d), and low dissolved oxygen (DO) conditions (0.2 mg L^{-1} in anoxic tank and 0.9 mg L^{-1} in aerobic tank). The total nitrogen (TN) removal efficiency was 48%. The SF_2 WWTP had a high COD:N (10:1) ratio, a short SRT (7 d), and high DO (0.6 mg L^{-1} in anoxic tank and 1.8 mg L^{-1} in aerobic tank). The TN removal efficiency was 61%. The nitrification and denitrification rates from these two plants were inadequate. Using a quantitative polymerase chain reaction (qPCR) technique, the populations of ammonium oxidizing bacteria (AOB) and ammonium oxidizing archaea were quantified. Measurement of ammonia monooxygenase (*amoA*) gene abundances identified these AOB: *Nitrosomonas* sp., *Nitrosospira* sp., *Nitrosoccus* sp. and *Zoogloea* sp. Higher amounts of the archaeal-*amoA* gene were found with long SRT, lower DO and COD:N ratios. Abundance of *Nitrobacter* sp. was slightly higher than *Nitrospira* sp. at the SF_1, while abundance of *Nitrobacter* sp. was two orders of magnitude greater than *Nitrospira* sp. at the SF_2. More denitrifying bacteria were of the *nirS*-type than the *nirK*-type, especially at higher COD:N ratio. Most bacteria belong to the phyla Acidobacteria, Actinobacteria Bacteroidetes, Chloroflexi, Proteobacteria. The results from this work showed that insufficient carbon sources at the SF_1 and high DO concentration in anoxic tank of SF_2 adversely affected nitrogen removal efficiencies. In further research work, advanced techniques on the next generation sequencing with different variable regions should be recommended in full-scale WWTPs.

Keywords: Biological nitrogen removal, COD:N, Full-scale, Step-feed

Introduction

Increases in water pollution are usually related to growing urban populations. Efficient removal of nitrogen in wastewater treatment plants (WWTPs) is essential to avoid downstream eutrophication which adversely affects not only animal but also human health globally. Nitrogen is removed from wastewaters with physical methods (air stripping), chemical methods (ion exchange), biological treatment (nitrification and denitrification processes), and/or combinations of these. Biological treatment processes are dominant over all other physical and chemical methods and are attractive because of relative low costs [1].

The most popular domestic wastewater treatment system for large communities is activated sludge process with plug flow configuration. However, with some site-specific conditions, existing processes or equipment and demand for high biological nitrogen removal efficiency, a modification of plug flow with step-feed is recommended. Dividing a reactor tank into anoxic and aerobic zones and/or using step-feed configuration are commonly recommended for improving nitrogen removal [2]. However, not all step-feed configurations require

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pre-anoxic process. The step-feed process has many advantages over conventional activated sludge processes, including more uniform distribution of oxygen demand, superior ability to handle peak wet–weather flows, and flexible operation. Step-feed systems can often achieve treatment objectives with smaller bioreactor volumes [3], and the process will often achieve low effluent total inorganic nitrogen concentrations [4].

The key factors affecting full-scale step-feed WWTPs are dissolved oxygen (DO), solids retention time (SRT), and hydraulic retention time (HRT). Operation with internal recycle reduced total nitrogen (TN) concentrations between 5 and 8 mg L^{-1} with SRT between 3 and 15 d, total HRT 3–5 h, anoxic zone time 0.5–1.5 h, aerobic zone time 2.5–3.5 h, aerobic tank DO 2–3 mg L^{-1} and anoxic tank DO ≤0.2 mg L^{-1} [5]. Wang and Chen [6] reported TN removal efficiency of > 64% in a full-scale step-feed system operated at DO of < 0.25 mg L^{-1} in anoxic tank and 2 mg L^{-1} in aerobic tank with temperature between 11.9–23.3 °C. Moreover, in a pilot-scale step-feed system investigated by Ge et al. [7], TN removal efficiencies varied from 75 to 86% with different SRTs at 10–15 d (75%), 10–12 d (82%) and 8–10 d (86%) under DO concentrations of 1.2–2.0 mg L^{-1} in aerobic zones.

Information on TN removal for full-scale step-feed municipal WWTP specifically for low and high chemical oxygen demand (COD):N ratios and various DO concentrations is rare in the literature. For this reason, our work was focused on two full-scale step-feed WWTPs in Bangkok, Thailand and Taipei, Taiwan. These two full-scale WWTPs with similar configurations were selected because of low and high COD:N ratios in influent. The definitions for high and low COD:N ratios of wastewaters are > 4.3:1 and ≤ 4.3:1, respectively. The study compared the efficiencies of nitrogen removal from these step-feed WWTPs, and different observations due to design parameters and operating conditions were explained. In addition, the abundance of microbial communities in these full-scale WWTPs were investigated and discussed. The results from this work could be applied to step-feed WWTPs in either country to solve carbon limitation when treating low COD:N wastewater and/or reduce aeration energy by using low-DO processes for improving biological nitrogen removal efficiencies.

Materials and methods

Wastewater treatment systems

Two underground full-scale municipal step-feed WWTPs were selected from the downtown area of two capital cities, Bangkok, Thailand, (SF_1) and Taipei, Taiwan, (SF_2). All wastewaters samples were collected and analyzed over an entire year (2018–2019). Both plants were designed for removal of both organic matter and nitrogen with reaction tanks consisting of anoxic-aerobic zones. The SF_1 (see Fig. 1a) had four feed points to four anoxic and four aerobic tanks. However, due to low flow conditions into the SF_1 system, only two feed points were operated and rotated with another two feed points. The SF_2 (see Fig. 1b) had three feed points to three anoxic tanks and three large aeration tanks (each large aeration tank was divided to four small aeration tanks) due to high flow of the system. These two WWTPs were built underground because of land limitations in these dense capital cities. The above ground areas of these two plants were used as recreation and education centers.

Analytical methods

Influent and effluent samples from each full-scale step-feed WWTP in this work were collected monthly during 2018–2019. Characteristics of these samples were measured by using the method described in Standard Methods for the Examination of Water and Wastewater (2005). Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) from each reactor tank were also analyzed by following the method described in Standard Methods. Temperature and pH were immediately measured in the field.

Nitrification and denitrification rates

To calculate the nitrification rates with various COD:N ratios, the concentration of ammonium-nitrogen (NH_4^+-N) in influent and effluent was determined. The nitrification rate was defined based on the NH_4^+-N removal as shown in Eq. (1) [8],

$$\gamma_{\text{nitrification}} = Q_{\text{in}} \times \frac{NH_4^+ - N_{\text{influent}} - NH_4^+ - N_{\text{effluent}}}{V_{\text{reactor}} \times VSS_{\text{nitrifying in reactor}}}, \quad (1)$$

where $\gamma_{\text{nitrification}}$ is the nitrification rate (d^{-1}), Q_{in} is flow rate (m^3 d^{-1}), V_{reactors} is volume of reactors (m^3), and $VSS_{\text{nitrifying in reactor}}$ is MLVSS of nitrifying organisms in reactor (mg L^{-1}).

The nitrifying organisms in the reactor is calculated based on MLVSS using Eqs. (2) and (3),

$$MLVSS_{\text{nitrifying in reactor}} = f_N \times VSS_{\text{reactors}}, \quad (2)$$

$$f_N = \frac{0.16 \times \left(NH_4^+ - N_{\text{influent}} - NH_4^+ - N_{\text{effluent}}\right)}{0.6 \times \left(BOD_{\text{influent}} - BOD_{\text{effluent}}\right) + 0.16 \times \left(NH_4^+ - N_{\text{influent}} - NH_4^+ - N_{\text{effluent}}\right)}, \quad (3)$$

where f_N is the fraction of nitrifying organisms presenting in the mixed liquor of a step-feed system. This fraction of nitrifying organisms can be estimated using Eq. (3). BOD_{influent} and BOD_{effluent} are the

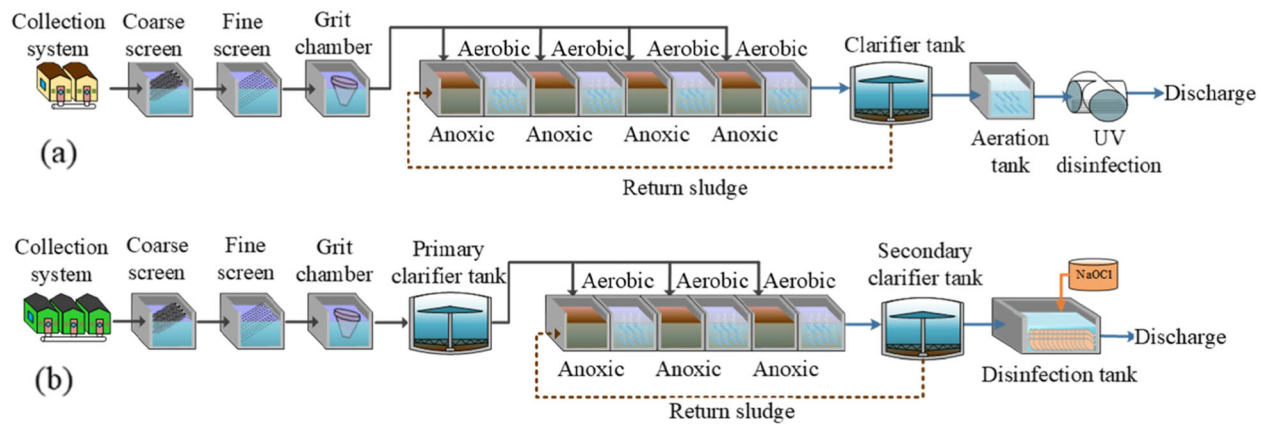

Fig. 1 Diagrams of the full-scale step feed systems in **a** Thailand and **b** Taiwan

concentrations of biochemical oxygen demand in influents and effluents (mg L^{-1}), respectively. The denitrification rate was defined as Eq. (4),

$$\gamma_{\text{denitrification}} = \frac{Q_{\text{in}} \times \left[\left(TKN_{\text{influent}} - NH_4^+ - N_{\text{effluent}}\right) - \left(NO_3^- - N_{\text{effluent}}\right)\right]}{\left(V_{\text{reactors}}\right) \times \left(VSS_{\text{reactors}}\right)}, \quad (4)$$

where $\gamma_{\text{denitrification}}$ is the denitrification rate (d^{-1}) and $[TKN_{\text{influent}} - (NH_4^+\text{-N})_{\text{influent}}]$ can be substituted with organic nitrogen in the influent. The TN removal (%) was calculated using Eq. (5).

$$TN_{\text{removal}}(\%) = \frac{NH_4^+ - N_{\text{influent}} - NH_4^+ - N_{\text{effluent}}}{NH_4^+ - N_{\text{influent}}} \times 100, \quad (5)$$

Microbial communities analysis

Sludge samples from SF_1 and SF_2 and were taken from both anoxic and aerobic tanks for the analysis of microbial communities. The nitrifying bacterial communities were identified through analysis of ammonia monooxygenase (*amoA*) gene abundances of ammonium oxidizing bacteria (AOB) and ammonium oxidizing archaea (AOA). The 16S rDNA target gene of *Nitrospira* (NSR) and *Nitrobacter* (Nitro) was used to determine nitrite oxidizing bacteria (NOB) abundance. The functional targeted gene of *nirK* and *nirS* genes were used as molecular markers for denitrifying bacterial (DNB) abundances.

DNA extraction and polymerase chain reaction (PCR) amplification

1 mL of the samples were taken for DNA extraction following the manufacturer's method using FavorPrep™ soil DNA isolation mini kit (Favogen® Biotech Corp, Taiwan). The PCR protocol and oligonucleotide primers for quantitative PCR (qPCR) and denaturing gradient gel electrophoresis are shown in Table S1 in Supplemental Materials.

qPCR of functional and 16S rDNA genes

The qPCR mixture contained 10 μL of SYBR Green by Luna® Universal qPCR Master Mix (New England Biolabs, MA, USA), 10 pmol of each primer, 1 μL of DNA template (~ 10–20 ng μL^{-1}) and nuclease-free water up 20 μL per reaction. Each sample and the standard series (10^0–10^8) were performed in triplicate on CFX96 Touch™ Real-Time PCR detection systems (Bio-Rad Laboratories, CA, USA) and the results were active based on the correlation coefficient of the standard curve ($R^2 = 0.995$).

Denaturing gradient gel electrophoresis (DGGE) fingerprints

The PCR mixture contained 10X *Ex Taq*™ buffer, 5 units μL^{-1} *TaKaRa Ex Taq*™, 2.5 mM dNTP Mixture, 10 pmol of each primer, 1 μL of DNA template (~ 10–20 ng μL^{-1}) and nuclease–free water up to 25 μL per reaction. Each sample was completed on T100™ Thermal cycler (Bio-Rad Laboratories, CA and USA). 15 μL of each PCR product was loaded into individual lanes of a DGGE gel of 8% (W/V) acrylamide gel with 35–60% (EUB) and 35–50% (AOB), and 6% (W/V) acrylamide gel with 20–50% (AOA) denaturing gradients. Electrophoresis was performed for 16 h at 58 °C with a constant voltage at 80 V in 1X TAE buffer. Each DGGE band was excised with a scalpel, DNA fragment was eluted from the band by milli-Q water overnight in a refrigerator, followed by PCR with the same primer without attached CG-clamp. Representative sequences were aligned against the National Center for Biotechnology Information database using *Basic Local Alignment Search Tool*. In this work, *nirK* and *nirS* genes were used because they are typically contained in denitrifying bacteria, but are structurally different from nitrite reductase

(distinguish the copper-dependent nitrite reductase and cytochrome cd_1-containing nitrite reductase).

Results and discussion

Key operating conditions of the full-scale step-feed WWTPs and wastewater quality

The key average operating parameters of the SF_1 and SF_2 WWTPs are shown in Table 1. The characteristics of influent and effluent of each SF are shown in Table 2. The BOD:TN and COD:N ratios of SF_1 were 2:1 and 4:1, respectively. The BOD:TN and COD:N ratios of SF_2 were 4.6:1 and 10:1, respectively. The wastewater treatment loading rate, BOD:TN and COD:N ratios of SF_1 were significantly lower than those in SF_2. Both SF_1 and SF_2 were able to remove SS, COD, and BOD well, but not nitrogen and phosphorus. At SF_1, there was not enough carbon source (low BOD in the influent) for denitrifying bacteria as electron donor. For this reason, this insufficient carbon source would affect on denitrification process.

The process performances on COD, NH_4^+-N, NO_2^--N, and NO_3^-- N concentrations profile of SF_1 and SF_2 WWTPs are shown in Fig. 2. Although BOD:N and COD:N ratios of SF_1 were significantly lower than SF_2, the NH_4^+-N removal efficiency of SF_1 (> 88%) was higher than that of SF_2 (59%). Average temperature of wastewater at SF_1 was higher than average temperature of wastewater at SF_2 (Table 2). Higher average temperature and longer SRT for SF_1 could be significant factors promoting AOB activities. Although the DO concentration in the aerobic tank of SF_1 was quite low, the DO was sufficient for adequate nitrification. It is also noted that the concentration of nitrifier communities at SF_1 was significantly higher than that for SF_2 (see subsequent Section microbial communities AOB and NOB populations and communities).

Nitrification and denitrification rates

Overall biological nitrogen removal in SF_1 and SF_2 was determined by calculating nitrification and denitrification rates in the aerobic and anoxic tanks. These two rates should not be the same value [9]. In this work the nitrification and denitrification rates of SF_1 and SF_2 were significantly different (Table 3). There are several possible explanations for this inequality. First, Thai sewage piping combines wastewater and rainwater that occurs all seasons, diluting the Thai influent BOD and SS to very low levels ($< 50\ mg\ L^{-1}$ and $< 90\ mg\ L^{-1}$, respectively) [10]. Second, the MLVSS:MLSS ratio of SF_1 was only 0.45–0.55 compared with that of SF_2 (0.8–0.82) due to longer SRT in both anoxic and aerobic tanks in SF_1. For this reason, when MLVSS is used to calculate biomass, inaccurate higher estimations of microorganisms would result. The significant difference of MLVSS:MLSS ratio between SF_1 and SF_2 might also be due to the absence of primary clarifier in SF1. The main purpose of a primary clarifier is to remove solids and particulates. Third, other factors affecting the growth of nitrifiers and denitrifiers would include DO concentration, SRT duration and temperature. Especially important was maintenance of appropriate DO concentration ($< 0.2\ mg\ L^{-1}$) in the anoxic phase [6].

In this study, it was shown that the longer SRT (> 60 d) of SF_1 promoted TN removal efficiency (48%) although the COD:N at this plant was quite low. Davies et al. [11] reported that longer SRTs improved nitrification and denitrification, resulting in high TN removal efficiency. At SF_2, the operation was normal with sufficient carbon presence (high COD:N ratio) but TN removal efficiency (only 61%) was not much better than that of SF_1. Moreover, the nitrification and denitrification rates were only 1.23 and 0.12 g NH_4^+-N g^{-1} VSS d^{-1}, respectively. The main reason for the low nitrogen removal efficiency and differing microbial processes was excessive DO in anoxic tank ($0.6\ mg\ L^{-1}$). Other investigators [9, 12, 13] have stated that high DO concentration enhances nitrification rates in aerobic tank while not increasing denitrification rates in anoxic tank. Maintaining lower DO concentrations ($< 1.0\ mg\ L^{-1}$) throughout an entire year in aerobic tank adversely affected the nitrification process (TN removal efficiency only 55%). Meng et al. [14] reported that TN removal was increased to 78% by increasing DO concentrations to $> 1.0\ mg\ L^{-1}$. Wang and Chen [6] demonstrated that a simultaneous nitrification-denitrification (anoxic-aerobic) process could result in TN removal efficiency of 57%. They also suggested that DO concentration in anoxic tank should be $< 0.25\ mg\ L^{-1}$ and aeration should be reduced when the DO concentration exceeds $2\ mg\ L^{-1}$ in the aerobic tank. This current study showed that at both SF_1 and SF_2, DO levels between 0.2 and $0.6\ mg\ L^{-1}$ in anoxic tank could be postulated to impact on the denitrification rate.

Table 1 Key average parameters of two full-scale step feed (SF) WWTPs

Parameter	SF_1	SF_2
Avg. flow rate ($m^3\ d^{-1}$)	84,000 ± 5000	433,820 ± 8000
SRT (d)	> 60	7 ± 1
HRT (h)	4.1 (avg.)	4.5 (avg.)
Anoxic	1.8 ± 0.5	0.7 ± 0.5
Aerobic	2.3 ± 1.5	3.8 ± 1.2
DO ($mg\ L^{-1}$)		
Anoxic	0.2 ± 0.3	0.6 ± 0.5
Aerobic	0.9 ± 0.5	1.8 ± 0.5

Microbial communities by using qPCR and DGGE technique

AOB and archaea (AOA) populations and communities

The different COD:N ratios at SF_1 and SF_2 affected the sizes of archaeal-*amoA* (AOA) and bacterial-*amoA*

Table 2 Characteristics of wastewaters from each full-scale step feed WWTP

Parameter	SF_1			SF_2		
	Influent	Effluent	% Removal	Influent	Effluent	% Removal
Temp (°C)	27.1 ± 0.1	27.4 ± 0.2	–	25.2 ± 2.0	25.1 ± 2.5	–
pH	7.4–7.6	7.2–7.4	–	7.0–7.2	6.9–7.1	–
SS (mg L^{-1})	36 ± 2	6 ± 1	83 ± 2	127 ± 16	14 ± 2	88 ± 0.5
BOD (mg L^{-1})	35 ± 2	6 ± 1	83 ± 2	126 ± 21	10 ± 2	91 ± 0.1
COD (mg L^{-1})	73 ± 5	18 ± 2	75 ± 1	275 ± 24	30 ± 2	89 ± 0.2
NH_4^+ (mg L^{-1})	13.3 ± 0.2	2.3 ± 0.4	83 ± 6	21 ± 2.5	8.6 ± 1.4	59 ± 5
NO_3^- (mg L^{-1})	0.4 ± 0.1	5.2 ± 0.5	–	0.20 ± 0.08	0.5 ± 0.04	–
NO_2^- (mg L^{-1})	0.1 ± 0.05	0.02 ± 0.01	78 ± 11	0.04 ± 0.02	0.02 ± 0.01	50 ± 1
Organic-N (mg L^{-1})	4.4 ± 0.1	1.9 ± 0.16	57 ± 3	4.2 ± 1.71	1.5 ± 0.9	67 ± 8
TKN (mg L^{-1})	17.7 ± 0.3	4.2 ± 0.56	76 ± 3	25.2 ± 4.2	10.1 ± 1.0	60 ± 3
TN (mg L^{-1})	18.2 ± 0.4	9.4 ± 2.1	48 ± 10	25.4 ± 4.3	10.6 ± 2.4	59 ± 2
TP (mg L^{-1})	3.0 ± 0.2	1.9 ± 0.1	37 ± 4	2.9 ± 0.8	1.5 ± 0.7	48 ± 12

Remark: All data values are averages ± S.D., $n = 12$ samples

(AOB) populations. Figure 3 shows that a large amount of AOA (1.0×10^5 copies mL^{-1} sludge) was found with the low COD:N ratio of SF_1. However, very low amounts of AOA (1.0×10^0 copies mL^{-1} sludge) were found with the higher COD:N ratio of SF_2. The very low amount of AOA found in SF_2, but not in SF_1, is due to the high DO concentration (> 1.8 mg L^{-1}) maintained in the aerobic tank. The quantitative results for AOA and AOB in this study were similar to that of Kayee et al. [15] who found an abundance of AOA in municipal full-scale anoxic and aerobic tanks at the Bangkok WWTP, which had low COD:N ratio (4.3:1), still higher than the COD:N ratio of SF_1 in this work. In Kayee's work, it was shown that the low DO concentration was maintained in

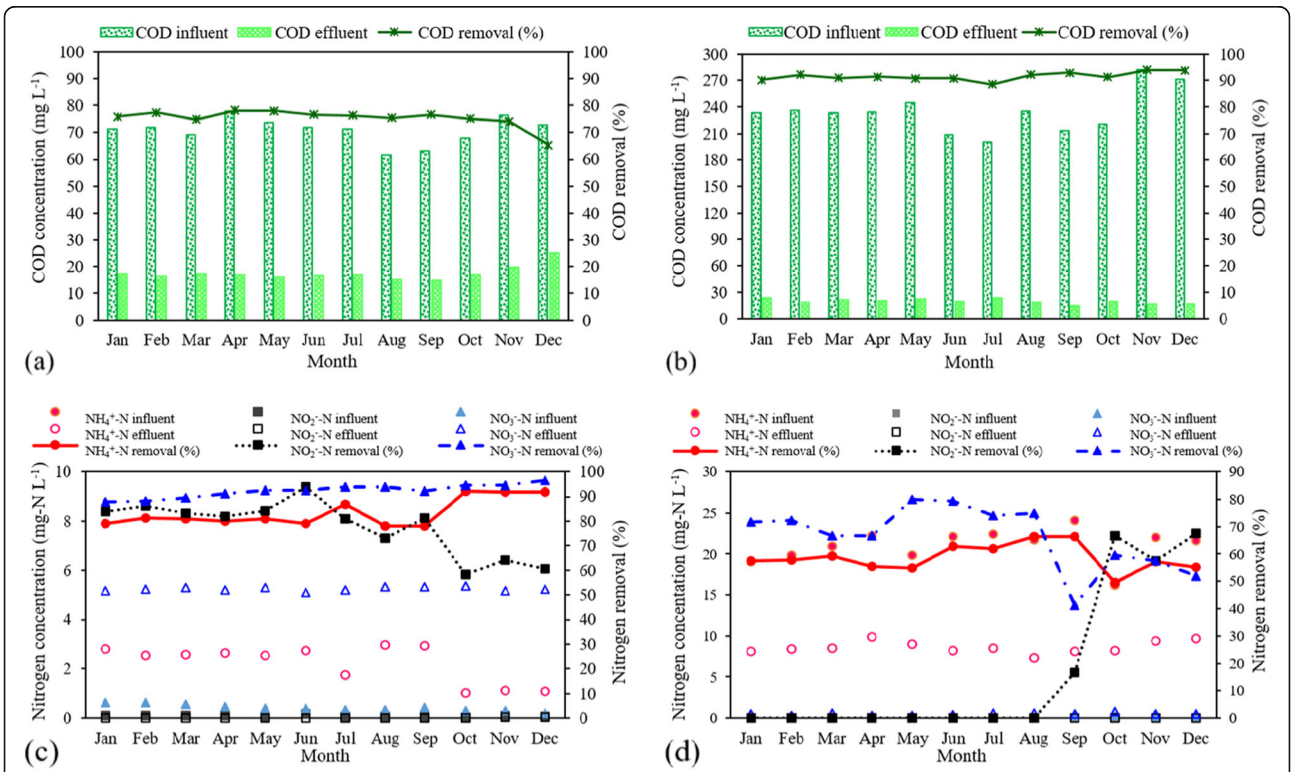

Fig. 2 Process performances on COD concentrations profile from **a** SF_1 and from **b** SF_2, and nitrogen (NH_4^+-N, NO_2^-- N, and NO_3^--N) concentrations profile from **c** SF_1 and from **d** SF_2

Table 3 Nitrification and denitrification rates and TN removal efficiencies of both SF_1 and SF_2

WWTP	Influent COD:N ratio (g COD g^{-1} N)	Nitrification rate (g NH_4^+-N g^{-1} VSS d^{-1})	Denitrification rate (g NO_3^--N g^{-1} VSS d^{-1})	TN removal (%)
SF_1	4:1	0.55 ± 0.02	0.047 ± 0.003	48 ± 10
SF_2	10:1	1.23 ± 0.15	0.12 ± 0.03	59 ± 2

aerobic tank of these WWTPs. Low DO concentration and longer SRT in full-scale WWTPs promoted large populations of AOA [16–18]. Gao et al. [16] found that a high ratio of COD:N (10.7:1) in full-scale activated sludge plants in Beijing, China, along with low DO concentration (0.5 mg L^{-1}) in the aerobic tank, led to large populations of AOA. It is noted that the relatively high temperature of wastewater in this work (only for SF_1) could be a contributing factor for the abundance of AOA and AOB populations. Several studies reported the effects of warm climate on AOA and nitrifying community. For example, Limpiyakorn et al. [17] found high AOA and other nitrifying communities in domestic and industrial WWTPs in Thailand. Sinthusith et al. [18] reported that long SRT with high temperature (30 °C) and pH > 7 at the WWTP in Thailand was associated with the dominance of AOA *amoA* genes over AOB *amoA* genes.

As indicated in Fig. 4, AOB species in the SF_1 were the same as in SF_2. AOB species included *Nitrosomonas europaea*, *Nitrosomonas halophile, Nitrosospira multiformis*, *Nitrosospira tenuis* and *Zoogloea caeni* via 16S rRNA of CTO primer pairs and *Nitrosoccus halophilus* via 16S rRNA of EUB primer pairs (see Table S2). Moreover, AOB communities present in this study were similar to those found by Shen et al. [19], who investigated the microbial community in a full-scale domestic WWTP (anoxic/oxic process). The main AOA communities at SF_1 were *Crenarchaeotal* sp. and uncultured *Thaumarchaeote*. *Thaumarchaeota* are autotrophic and capable of performing the oxidation of NH_4^+ to NO_2^- [20, 21]. Generally, *Crenarchaeotes* have been found in extreme environments, such as low oxygen concentrations in aquatic systems, hot springs, and full-scale anaerobic digester systems [22–24]. For full scale WWTP applications, it would be advantageous to maintain conditions which support AOA and AOB communities to improve biological nitrogen removal.

NOB populations and communities

In the second step of nitrification, *Nitrobacter* sp. and *Nitrospira* sp. are classically acknowledged as the most relevant NOB group in WWTPs. In Fig. 3, the copies number of *Nitrospira* via *NSR* gene were found 1.0×10^4 copies mL^{-1} sludge at SF_1 (low COD:N ratio), but they were present at less than 1.0×10^2 copies mL^{-1} sludge at SF_2 (high COD:N ratio). For *Nitrobacter* via *Nitro* gene 1.0×10^5 copies mL^{-1} sludge) no significant difference was found between SF_1 and SF_2. Yu et al. [25] reported on fluorescence in situ hybridization (FISH) technique results in their submerged membrane bioreactors under two SRTs (30 and 90 d). The fast-growing *Nitrobacter*

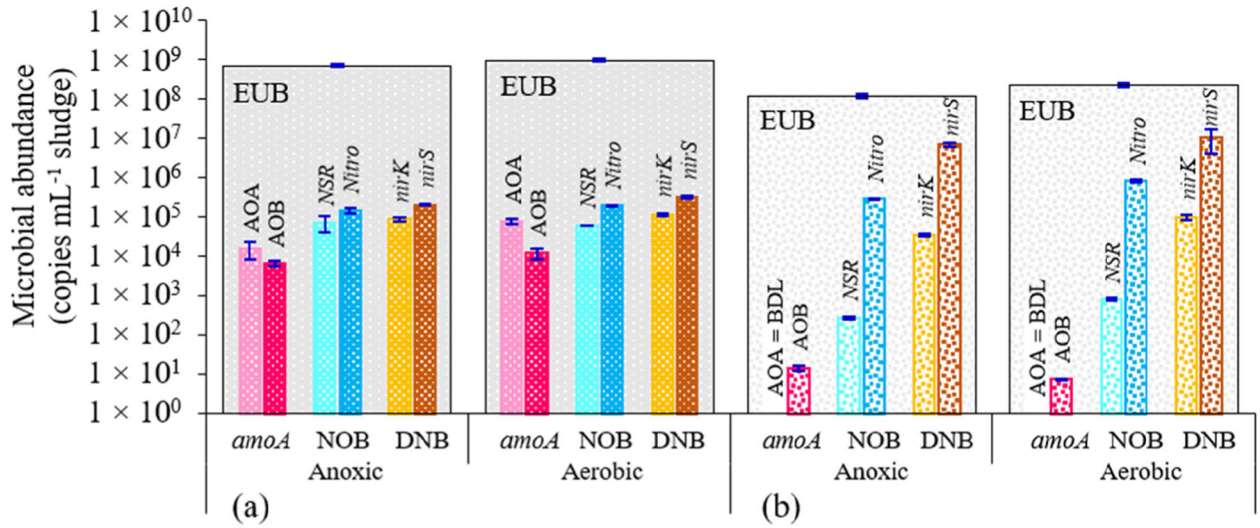

Fig. 3 Microbial abundance of AOA, AOB, NOB, DNB and total bacteria (EUB) **a** at the SF_1 with low COD:N and **b** the SF_2 with high COD:N. Remark: BDL = below method detection limit

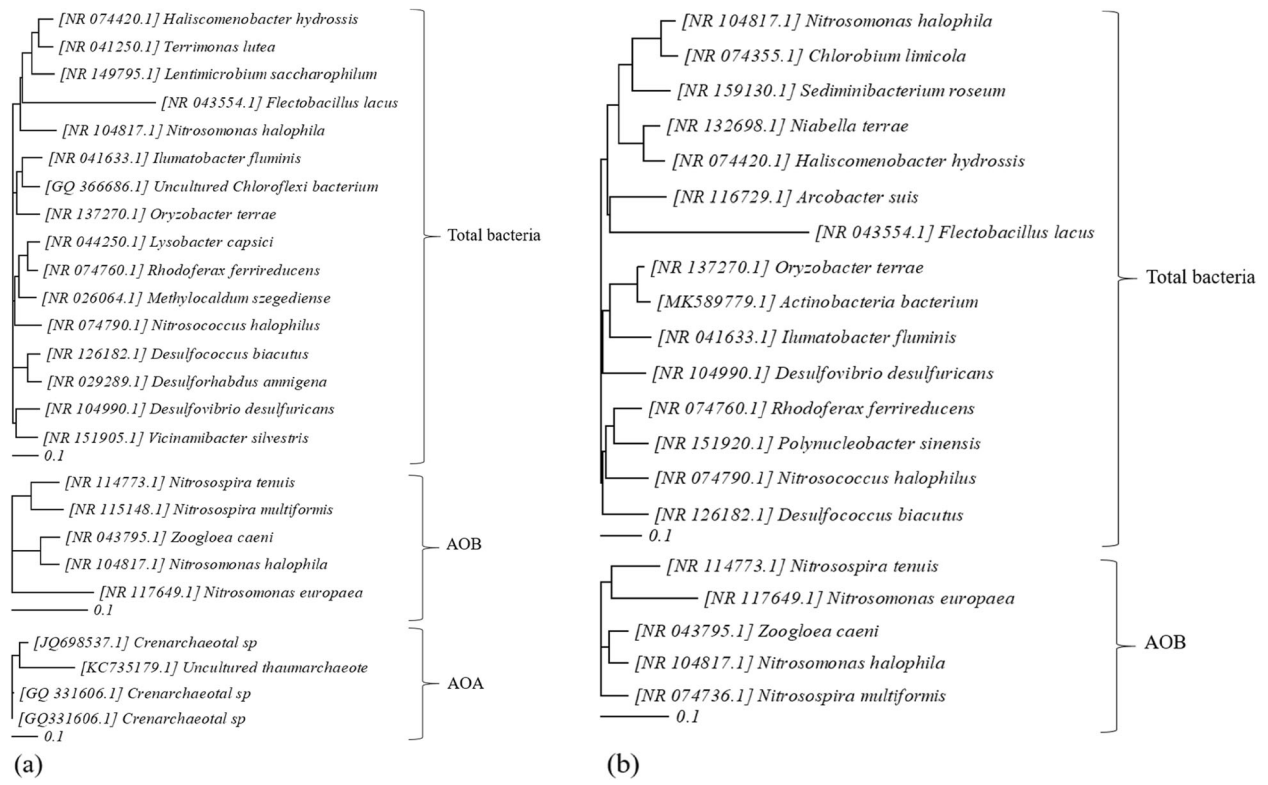

Fig. 4 Phylogenetic tree of microbial communities at **a** the SF_1 with low COD:N and **b** the SF_2 with high COD:N

sp. was the dominant species at SRT 30 d, while the slow-growing *Nitrospira* was dominant at SRT 90 d. The results from this work coincided with a study by Yu et al. The amount of *Nitrospira* that was found in SF_1 with long SRT (> 60 d) was significantly higher than the amount of *Nitrospira* in SF_2 with short SRT (7 d). Moreover, Huang et al. [26] studied distribution of NOB communities in the full-scale WWTP by controlling DO concentration. They found that *Nitrospira* was dominant when low DO (< 0.9 mg L^{-1}) concentration was controlled, while *Nitrobacter* increased when DO concentrations were increased at higher than 0.9 mg L^{-1}. This DO fact could explain why *Nitrospira* was dominant at SF_1 (operated with DO concentrations of 0.2–0.9 mg L^{-1}) and *Nitrobacter* was dominant at SF_2 (operated with DO concentrations of 0.6–1.8 mg L^{-1}. Consequently, low DO conditions and long SRT would be the major operating conditions that contributed to high *Nitrospira* population in WWTP. It should be noted that in this work other NOB communities were not analyzed because the qPCR technique for analysis of 16S RNA would only reveal *Nitrospira* and *Nitrobacter*.

Effects of COD:N ratios on populations of DNB

The abundance of the denitrifying bacteria in this work is shown in Fig. 3. The denitrifiers are found in both anoxic and aerobic tanks of SF_1 and SF_2. Two gene types (*nirK* and *nirS*) were used to characterize denitrifiers. The *nirK*-type bacteria in both SF_1 and in SF_2 were found to be 10^4 copies mL^{-1} sludge in anoxic tanks and 10^5 copies mL^{-1} sludge in aerobic tanks. For the *nirS*-type denitrifiers, in SF_1 anoxic and aerobic tanks, the bacteria were present in the same order of magnitude (10^5). However, in SF_2 the *nirS*-type denitrifiers existed at two orders of magnitude higher (10^6 in anoxic tank and 10^7 in aerobic tank) than the *nirK*-type denitrifiers (10^4 in anoxic tank and 10^5 in aerobic tank). The higher population of *nirS*-type denitrifiers is attributed to the high COD:N ratio SF_2. This observation is similar to the results from Wang et al. [27]. They found the number of *nirS*-type denitrifiers (10^4 to 10^5) was higher than that of *nirK*-type denitrifiers (10^3 to 10^4) in two full-scale WWTPs (upflow anaerobic sludge blanket and anaerobic/aerobic). Geets et al. [28] reported that *nirK*-type denitrifiers (10^5) were lower than *nirS*-type denitrifiers (10^6) in the sludge of industrial influent of anaerobic digester. They also found that *nirK*-type denitrifiers (10^6) were lower than *nirS*-type denitrifiers (10^7) in the sludge of domestic wastewater influent from hospital wastewater. From the present work and the cited studies, it is recommended that the *nirS*-type denitrifier growth be encouraged in full-scale WWTPs.

The bacterial species of denitrifiers at SF_1 are shown to be similar to those at SF_2 by PCR-DGGE technique (Fig. 3). The identified denitrifiers belonged to phyla Acidobacteria, Actinobacteria Bacteroidetes, Chloroflexi, Proteobacteria (Table S2). These five main phyla from this study are consistent with the work of Shen et al. [19]. They found these same phyla in full-scale domestic WWTPs (step-feed and anoxic/aerobic process). Wang et al. [27] also investigated the denitrifier communities in full-scale anoxic/oxic reactors using different analytical technique (next generation sequencing (NGS) by pyrosequencing and Illumina high-throughput sequencing). For this reason, Wang et al. [27] were more specific than the results from this work, identifying Genus of *Thauera*, *Paracoccus*, *Hyphomicrobium*, *Comamonas* and *Azoarcus*. In further research work, new and/or advanced techniques on the NGS with different variable regions should be recommended in full-scale WWTPs in order to identify specific denitrifier groups. With additional information we should understand which denitrifier communities are most effective in overall biological nitrogen removal in WWTPs.

Conclusions

In earlier studies it has been shown that TN removal is improved with higher COD:N ratios, longer SRTs, and low DO concentrations in anoxic zones. This work demonstrated that each variable is important in order to achieve adequate treatment in full-scale step-feed WWTPs. SF_1 had longer SRT (> 60 d) and low anoxic DO (average 0.2 mg L^{-1}), but its low COD:N ratio (4:1) substantially impeded the denitrification portion of treatment. The COD:N ratio (10:1) at SF_2 would provide enough carbon for denitrification, but the average DO (0.6 mg L^{-1}) in the anoxic region was too high for complete TN removal.

The distributions of archaeal and bacterial communities are dependent on operating parameters of the WWTP. At SF_1 with low COD:N ratio, low DO, long SRT and high temperature the microbial abundance of AOA was greater than that of AOB. However, at SF_2 with opposite parameter values, the AOB was more abundant. The predominant AOB communities were *Nitrosomonas* sp., *Nitrosospira* sp., *Zoogloea* sp. and *Nitrosoccus* sp. Higher amounts of *Nitrospira* were present at lower COD:N ratios. Higher amounts of *Nitrobacter* were found with high DO concentrations and higher COD:N. This work shows that at high COD:N ratio, *nirS*-type denitrifiers are more prevalent than *nirS*-type denitrifiers. General microbial communities belonged to phyla Acidobacteria, Actinobacteria Bacteroidetes, Chloroflexi, Proteobacteria were identified.

The results from this work, longer SRT > 30 d could be suggested as possible practical solution on issue of solving carbon limitation when treating low COD:N wastewater. Another practical solution on issue of reducing aeration energy, DO concentration in aeration tank should be maintained from 0.9 to 1.4 mg L^{-1}.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42834-020-00064-6.

Additional file 1 : **Figure S1.** DGGE fingerprints of (a) total bacteria, (b) *amoA*-AOB and (c) Arch *amoA*-AOA genes. **Table S1.** Oligonucleotide primers for PCR amplification via quantitative polymerase chain reaction (qPCR) and denaturing gradient gel electrophoresis (DGGE) techniques. **Table S2.** AOA, AOB and total bacteria communities.

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Authors' contributions

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors declare they have no competing interests.

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