Nitrite Accumulation at Low Copper Concentration in a Submerged Partial Bionitrification Reactor

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(Received March 3, 2021; Revised May 26, 2021; Accepted June 1, 2021)

Objectives: Effects of various Cu²⁺ concentrations in the synthetic wastewater on nitrite accumulation was investigated in a submerged partial biofilter reactor (SPBNR).

Methods: Experiments were carried out at the constant operational conditions (T=35°C; pH=9.0 and DO=2.0 mg O₂/L) by varying the concentrations between 5-50 mg Cu²⁺/L. The SPBNR, which was operated in an upward flow mode, set-up consisted of a cylindrical stainless steel. The support materials filling ratio was about 23% of the total reactor volume. The SPBNR was inoculated with microorganism drawn from a batch experimental biological reactor operated about one month by using the synthetic wastewater composition.

Results and Discussion: Before exposure to Cu²⁺, the highest loading rate of 1.3 g NH₄-N/(m².day) was determined under the operational conditions. Addition of 5 µg Cu²⁺/L into the waters promoted the activity of organisms and the loading rate achieved to 1.6 g NH₄-N/(m².day). At the control operational condition, the ratio of NO₂-N/NO₃-N was determined as 0.74, while the ratio increased to 0.78 at the Cu²⁺ concentration of 5 µg/L.

Conclusions: Results indicated that the ammonium oxidizing bacteria (AOB) is more stimulated than the nitrite oxidizing bacteria (NOB) at the concentration of 5 µg Cu²⁺/L. However, approximately equal NH₄-N removal rate (ANRR) and NO₂-N accumulation rate (NiAR) losses indicated that the AOB and NOB are approximately equally effected at the inlet concentrations of 35 and 50 µg Cu²⁺/L.

Keywords: Copper Inhibition, Nitrite Accumulation Rate, Partial Nitrification, Upflow Bioreactor

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1. Introduction

Nitrification process, which cause eutrophication in the surface water sources, greenhouse effect, acid rain, and plays significant role in the nitrogen cycle, has been widely studied in recent years.\(^2\) Nitrification is an autotrophic aerobic process and ammonium and nitrite are oxidized sequentially into nitrate by AOB and NOB. The rate limiting step of nitrification process is ammonia oxidation involve autotrophic AOB and ammonia-oxidizing archaea (AOA).\(^2\) Because of the growth constants of AOB and NOB are 0.9 and 0.5, respectively, the NOB is most sensitive organism in the nitrification process.\(^3\) Nitrate is converted to nitrogen gas under anoxic condition by heterotrophic denitrification organisms. In the treatment of industrial wastewater, which is poor in terms of organic substance, external carbon source such as methanol, ethanol, etc. should be provided in the denitrification process. Requirements of dissolved oxygen (DO) and external organic carbon source are the main disadvantages of conventional biological nitrogen removal (BNR) process.

The BNR process via nitrite (partial nitrification) is considered as a cost-effective alternative by the reduction of aeration about 25% and external carbon source requirement of 40%, compared to the conventional process.\(^4,5\) Moreover, Abeling and Seyfried\(^9\) reported that the denitrification rate with nitrite was 1.5-2 times greater than with nitrate. The partial nitrification process has been widely investigated in recent years due to the economical advantages.

The partial nitrification process can be carried out by controlling the nitrite oxidation without affecting the AOB. During this process, NOB must be adapted to high concentrations of nitrite. The factors affecting nitrite accumulation such as low DO concentration,\(^7,8\) higher operational temperature (≥35°C),\(^9\) control of sludge retention time (SRT),\(^10,11\) and hydraulic retention time (HRT)\(^4,12\) have been widely investigated. It is possible to provide NO\(_2\)-N accumulation at high pHs.\(^13\) However, there is a disagreement on the effect of free ammonia (FA) concentration and pH on the NO\(_2\)-N accumulation.\(^14\)

Although the influence of heavy metals on the organic carbon removal in a suspended growth process has been widely studied, few papers have been published about the nitrification process.\(^15\) Compared to the heterotrophic organisms, which use organic carbon source for growth, the autotrophic nitrification organisms are reported as more susceptible to heavy metal inhibition.\(^16,17\) Juliastuti et al.\(^18\) reported that the autotrophic nitrifying biomass was approximately ten times more sensitive than its aerobic heterotrophic counterpart.

Wastewater effluents from the mining, smelting, metallurgy, electroplating etc. industries might contain high concentrations of heavy metals such as copper, zinc, nickel, and cadmium, which inhibit the biological treatment.\(^19,20\) Heavy metal ions distribution in a wastewater stream is a serious concern to our environment, which attributes to acute toxicity aquatic life.\(^21\)

Biological treatment of the industrial wastewater presents some challenges due to its toxic composition, such as heavy metals.\(^22,23\) While heavy metals in trace concentrations are essential for microbial growth, at high concentrations have some detrimental effects.\(^24\) Shock metal loads to the biological reactor inhibit the activity of organisms. Gradually increase of metal concentration up to certain limit in the reactor provides the adaptation of organism and limit the inhibition effects.\(^7\) On the other hand, at a low concentration of heavy metal, the biological activity might be promoted and results in the decrease of effluent pollutants concentration from the biological reactor.\(^7,22\)

The BNR needs longer HRT for the treatment of wastewaters contains heavy metal.\(^26\) Minimisation of heavy metals effect on the microorganism are usually overcome by adaptation of microorganism\(^22,25\), application of low pollutant loads, and physical-chemical units with an increase in treatment costs.\(^27\) Additionally, selection of appropriate reactor type minimize the inhibitory effect of heavy metals on the microorganisms.\(^28\) For example, it was reported that the biofilm system was 2-600 times higher capacity to resist heavy metal stress than the suspended growth process. Nitrification organisms in the biofilm are more tolerance than organisms in the suspended flocs when subjected to shock loads of heavy metals.\(^29\) Although heavy metals might inhibit both steps of nitrification reaction, the inhibition effects of metals are different.\(^30\)

Copper appears to have a unique mode of action involving rapid loss of membrane integrity.\(^31\) Madoni et al.\(^32\) reported that the tested concentrations of copper (in the range of 0.02 to 0.9 mg/L) was highly toxic to the heterotrophic microbial community while no effects was observed on the nitrification organisms. Vismara (1982) found that the nitrification process was inhibited at the concentrations of copper between 0.005-0.5 mg/L.\(^18\) Conversely, Tomlinson et al. concluded that the concentration of copper 10 mg/L had no effect on the proportion of ammonia oxidized, but a further increase to 20 mg/L started to decrease the degree of nitrification.\(^33\) Juliastuti
et al.\textsuperscript{18}) reported that the nitrite and nitrate production was inhibited when Cu\textsuperscript{2+} concentration was lower than 1.0 mg/L. The IC50 value was observed at the concentration of 0.09 mg Cu\textsuperscript{2+}/L\textsuperscript{18} and 0.5 mg Cu\textsuperscript{2+}/L.\textsuperscript{34)}

Biological wastewater treatment can be carried out in the attached and suspended growth systems. Since long sludge retention time is provided in the reactor, nitrifying bacteria in the attached growth processes is found abundant. In comparison to the other heavy metals that inhibit nitrification process, after the metal load was removed seemed to have the longest recovery time of the biological wastewater systems inhibited by copper.\textsuperscript{29)} Experimental results showed that nitrifiers in the biofilm were more tolerance than in the suspended flocs when subjected to shock loads of heavy metal.\textsuperscript{29)} The biofilm system is able to tolerate a higher total copper concentration, which is about more than 1.6 times higher, than the suspended growth system.\textsuperscript{29)} Because of the difference in exposure time, inhibition of Cu\textsuperscript{2+} on the nitrification organisms was ten times higher in the continuous flow reactor than what was observed in the batch reactors.\textsuperscript{35)} By the application of Cu\textsuperscript{2+} to the attached growth medium, some portion of Cu\textsuperscript{2+} could be sorbed to the medium and biofilm layer. Experimental results indicated that Cu\textsuperscript{2+} is released slower than it accumulated on the media.\textsuperscript{36)} Sustainable nitrification could be provided by the desorption of Cu\textsuperscript{2+} from the attached growth medium and the bacterial decay in the biofilm layer.\textsuperscript{36,37)}

As mentioned above, the effects of heavy metals on AOB and NOB are depending on the environmental and operational conditions. Although the effects of operational conditions on the partial nitrification has been well documented, there is few experimental study to date has specifically examined the susceptibility of nitrification organisms to metals. In this experimental study, the effects of Cu\textsuperscript{2+} on partial nitrification was investigated in the SPBNR that optimal experimental conditions were obtained considering the highest NO\textsubscript{2}-N/NO\textsubscript{x}-N ratio and NH\textsubscript{4}-N removal efficiency in our previous study. In order to determine the concentrations that promote or inhibit the AOB and NOB, experimental studies were carried out with the synthetic wastewater contains low concentrations Cu\textsuperscript{2+}.

### Table 1. Synthetic wastewater constituents\textsuperscript{10)}

| Chemicals | Concentrations (mg/L) | Chemicals | Concentrations (mg/L) |
|-----------|-----------------------|-----------|-----------------------|
| NH\textsubscript{4}Cl | 920-950 | CoCl\textsubscript{2}·6H\textsubscript{2}O | 0.0119 |
| Na\textsubscript{2}EDTA | 4.83 | Na\textsubscript{2}MoO\textsubscript{4}·2H\textsubscript{2}O | 0.066 |
| CuSO\textsubscript{4} | 0.0046 | MgSO\textsubscript{4}·7H\textsubscript{2}O | 36.97 |
| ZnSO\textsubscript{4}·7H\textsubscript{2}O | 0.023 | NaHCO\textsubscript{3} | 226 |
| CaCl\textsubscript{2}·2H\textsubscript{2}O | 36.74 | FeCl\textsubscript{3}·6H\textsubscript{2}O | 0.316 |
| H\textsubscript{3}BO\textsubscript{3} | 1.0 | KH\textsubscript{2}PO\textsubscript{4} | 1920 |

used for microbial growth (Table 1). Experiments were carried out at the constant operational conditions by varying the Cu\textsuperscript{2+} concentrations between 5-50 µg/L. The data for each Cu\textsuperscript{2+} concentration was obtained under the steady state conditions.

#### 2.2. The SPBNR set-up and operation

The SPBNR, which was operated in an upward flow mode, set-up consisted of a cylindrical stainless steel (Fig. 1). The specific properties and operational conditions are shown in Table 2.

### Table 2. Operational conditions of the SPBNR.

| Reactor properties | Active biological filter depth (cm) | 32.5 |
|--------------------|------------------------------------|------|
|                    | Diameter of the PNB reactor (cm)   | 10   |
|                    | Total Volume (L)                   | 2.6  |
|                    | Void Volume (L)                    | 2.0  |
|                    | Length of filling materials (m)    | 3.9  |
|                    | Total surface area of filling materials (m\textsuperscript{2}) | 0.49 |
|                    | Total surface area m\textsuperscript{2}/m\textsuperscript{3} | 188 |
|                    | MLSS (mg/L)                        | 11,633 |
|                    | MLVSS (mg/L)                       | 7,980 |

#### Fig. 1. Schematic diagram of the SPBNR.
In order to provide bacterial growth, the plastic coils used as support materials (diameter = 20 mm) was filled into the SPBNR. The filling ratio was about 23% of the total reactor volume. While the synthetic wastewater was inlet from the bottom of SPBNR by a peristaltic pump (Watson Marlow, 520 S), the treated water was withdrawn from the top. The temperature was about 35±1℃ in the SPBNR throughout the experimental study. The DO concentration, which was kept at a constant level of 2.0±0.2 mg/L, was measured twice in a day from the top of SPBNR by using a DO meter (YSI 5100). The influent pH of wastewater was adjusted to 9.0±0.01 by using stock NaOH solution.

The SPBNR was inoculated with microorganism drawn from a batch experimental biological reactor operated about one month by using the same synthetic wastewater composition. The removal efficiency (E) and inhibition (I) of NH₄⁺-N oxidation were calculated by using Eq. (1) and (2), respectively.

\[
E(\%) = \left(\frac{(NH₄)ᵢ - (NH₄)ₑ}{(NH₄)ᵢ - (NH₄)ₑ}\right) \times 100
\]

\[
I(\%) = \left(\frac{E_{NH₄} - Nₑ}{E_{NH₄} - Nᵢ}\right) \times 100
\]

Where, NH₄⁺-Nᵢ: influent NH₄⁺-N concentration; NH₄⁺-Nₑ: effluent NH₄⁺-N concentration; NH₄⁺-Nᵢₑ: removal efficiencies of control operation; NH₄⁺-Nₑₑ: removal efficiencies of SPBNR operation with Cu²⁺.

In order to evaluate NH₄⁺-N removal efficiency, optimum experimental conditions for the NLR, temperature, DO concentration, and pH were determined for the Cu²⁺ free synthetic wastewater. The highest NH₄⁺-N removal efficiency (58%) and NO₂⁻-N/NO₃⁻-N ratio (0.74) were observed at the temperature of 35°C, the nitrogen loading rate (NLR) of about 2.0 g NH₄-N/(m².day). Before feeding with Cu²⁺ containing waters, the reactor operating conditions were adjusted according to the conditions, which the highest ammonium removal efficiency and NO₂⁻-N/NO₃⁻-N ratio were observed (T = 35℃; pH=9.0 and DO=2.0 mg O₂/L). In order to achieve a steady-state condition, the SPBNR was operated about two months and then the Cu²⁺ contaminated synthetic wastewater was used for the growth of nitrification organisms.

3. Results and discussion

Since the activity of enzyme ammonia monooxygenase, which catalyzes the first step in the oxidation of ammonia to nitrite, was greatly increased by addition of copper³⁰, the effect of copper on partial nitrification process was investigated. The data which are presented in figures are the mean value (standard deviation ≤ 5%).

3.1. Optimal operating conditions of SPBNR

The optimal operating conditions of SPBNR were determined in our previous study. Considering the highest NO₂⁻-N/NO₃⁻-N ratio and NH₄⁺-N removal efficiency, optimum experimental conditions for the NLR, temperature, DO concentration, and pH were determined for the Cu²⁺ free synthetic wastewater.⁷ The highest NH₄⁺-N removal efficiency (58%) and NO₂⁻-N/NO₃⁻-N ratio (0.74) were observed at the nitrogen loading rate (NLR) of about 2.0 g NH₄-N/(m².day).³⁷ Before feeding with Cu²⁺ containing waters, the reactor operating conditions were adjusted according to the conditions, which the highest ammonium removal efficiency and NO₂⁻-N/NO₃⁻-N ratio were observed (T = 35℃; pH=9.0 and DO=2.0 mg O₂/L). In order to achieve a steady-state condition, the SPBNR was operated about two months and then the Cu²⁺ contaminated synthetic wastewater was used for the growth of nitrification organisms.

3.2. The activity of AOB and NOB at low Cu²⁺ concentrations

Changes in the concentrations of inorganic nitrogen compounds in the treated waters as a result of Cu²⁺ addition are shown in Fig. 2. Improvement of the nitrification organisms activity at the concentration of 5 mg Cu²⁺/L was determined by ICP-AES.

After completing the experimental studies, the attached biofilm onto filling materials from different heights of SPNR and water samples in the void volume were taken to determine the total biomass amount in the SPNR. The filling materials were added into 100 mL of distilled water in a sterile glass bottles and stirred at 280 rpm for 60 min to separate biomass attached to the surfaces (Gerhardt). Organic fraction of SS were determined as volatile suspended solid (VSS) in the samples.⁸ The total numbers of colony forming units of aerobic mesophilic bacteria were determined according to FDA, BAM.³⁹
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Inhibition of the ammonium oxidation. NH$_4^+$ increase in the concentrations of Cu$^{2+}$ determined at the concentration of 50 mg Cu$^{2+}$/L. Since copper is contained in the enzyme nitrite oxidoreductase, it was suggested the possibility that copper may stimulate the NOB. However, when the copper concentration was lower than 0.05 µg/L, incomplete nitrification was reported. Increase in nitrification efficiency in a sand filter was also determined for the influent concentration of 5.9 mg/L, which was the lower end value reported by Zhang et al. Although, the growth stimulation effect of copper was observed for the studied four strains of AOA, the effect of copper concentrations on the growth stimulation was different for each strain. In contrast, it was reported to have no any effect of copper on the growth of the AOB species N. europaea. Low concentration of Cu$^{2+}$ affect positively nitrification organisms and at the concentration of 5 mg/L, about 57% NH$_4^+$ efficiency increase was observed in this study.

Increase of NO$_2^-$ accumulation in the effluent might indicated that the AOB activity was promoted at low concentration of Cu$^{2+}$ while the NOB activity was not affected. It was reported that the activity of Nitrosomonas europaea in pure culture was stimulated by the concentration between 5-100 mg Cu$^{2+}$/L and 5-30 mg Cu$^{2+}$/L. At the concentration of 0.5 mg Cu$^{2+}$/L, the ammonium removal rate increased in the batch suspended growth reactor was reported. The ratio of NO$_2^-$/NO$_3^-$ was 0.74 for the synthetic wastewater without Cu$^{2+}$, while at the concentration of 5 mg Cu$^{2+}$/L, increased to 0.78, which was the highest level in this study (Fig. 3). At the concentration of 50 mg Cu$^{2+}$/L, the NO$_2^-$/NO$_3^-$ ratio dropped to 0.67, which was the lowest level in this study. When the initial Cu$^{2+}$ concentration in water was increased from 35 mg/L to 50 mg/L, NO$_2^-$ and NO$_3^-$ accumulation percent in the effluent decreased approximately 20% and 10%, respectively.

In a sand filter column study, addition of copper into the groundwater positively affect the nitrification process and increase of the ammonium removal was observed. The highest NO$_2^-$ accumulation of 320 mg/L in the fed-batch reactor was determined at the copper concentration of 0.25 mg/L. Since copper is contained in the enzyme nitrite oxidoreductase, it was suggested the possibility that copper may stimulate the NOB. However, when the copper concentration was lower than 0.05 µg/L, incomplete nitrification was reported. Increase in nitrification efficiency in a sand filter was also determined for the influent concentration of 5.9 mg/L, which was the lower end value reported by Zhang et al. Although, the growth stimulation effect of copper was observed for the studied four strains of AOA, the effect of copper concentrations on the growth stimulation was different for each strain. In contrast, it was reported to have no any effect of copper on the growth of the AOB species N. europaea. Low concentration of Cu$^{2+}$ affect positively nitrification organisms and at the concentration of 5 mg/L, about 57% NH$_4^+$ efficiency increase was observed in this study.
sequently. The process does not require any organic carbon source; therefore, it has been reported to have as a potential technical superiority and economic benefits when compared with conventional BNR process. The best NO\textsubscript{2}-N/NH\textsubscript{4}-N ratio for the ANAMMOX process was determined between 1.02 and 1.14.\textsuperscript{45,46} Cu\textsuperscript{2+} in the wastewater caused a deterioration of appropriate NO\textsubscript{2}-N/NH\textsubscript{4}-N ratio required for the Anammox process, which follow to the partial nitrification reactor. When the Cu\textsuperscript{2+} free wastewater fed to the SPBNR, the total maximum ANRR of 1.3 g/(m\textsuperscript{2}.day) was determined while the NiAR was 0.9 g/(m\textsuperscript{2}.day) (Fig. 4). At the concentration of 5 µg Cu\textsuperscript{2+}/L, the ANRR and NiAR increased to about 1.6 g/(m\textsuperscript{2}.day) and 1.5 g/(m\textsuperscript{2}.day), respectively. When the ANRR and NiAR increase percentages (about 30% and 61%) were compared, conclusion could be drawn that the AOB is more promoted than the NOB at the lowest Cu\textsuperscript{2+} concentration applied in this study. Further increase of Cu\textsuperscript{2+} concentrations in the feeding waters caused gradually decrease of ANRR and NiAR. At the highest applied Cu\textsuperscript{2+} concentration, the lowest value about 0.8 g/(m\textsuperscript{2}.day) and 0.6 g/(m\textsuperscript{2}.day) were observed for the ANRR and NiAR, respectively.

The ANRR and NiAR losses were determined about 8% and 18% at the Cu\textsuperscript{2+} concentration of 10 µg/L, respectively (Fig. 5). However, for both the ANRR and NiAR, approximately equal losses about 24% and 37% were observed at the inlet concentrations of 35 and 50 µg Cu\textsuperscript{2+}/L. Aslan and Gurbuz\textsuperscript{7} reported that Nitrosomonas sp. was equally or more sensitive than Nitrobacter sp. to copper and nickel. For the low concentrations of Cu\textsuperscript{2+}, it could be drawn from the experimental results that Nitrosomonas sp. are less sensitive than Nitrobacter sp. However, both AOB and NOB were inhibited approximately equal levels when the Cu\textsuperscript{2+} concentration was higher than 10 µg/L.

FA concentrations between 10-150 mg/L and 0.1-1.0 mg/L negatively affect the AOB and NOB activity, respectively, while all nitrifying bacteria are inhibited when the FNA concentration is higher than 0.2 mg/L.\textsuperscript{13} Concentration of FNA in the SPBNR was lower than the proposed inhibition concentration of 0.2 mg/L. The calculated FA concentration was about 160 mg/L. For a long term basis, it was reported that the pH parameter could not be applicable for the accumulation of NO\textsubscript{2}-N, because of the adaptation of NOB to high NH\textsubscript{3} concentrations.\textsuperscript{47} The SPBNR was operated at the pH of about 9.0 more than 1.5 years. Because the calculated FA concentration was constant throughout the experimental studies, increase of the NO\textsubscript{2}-N/NO\textsubscript{x}-N ratio could not be attributed to the FA.

Previous experimental studies have reported that the toxic effects of heavy metals correlated well with the pH level of water. The pH of influent wastewater has been considered as the most important operating parameter affecting metal solubility and toxicity. An increase in pH can reduce the availability of heavy metal toxicity to microorganisms in the biological process.\textsuperscript{48} Adsorbed and free copper are considered as the main copper forms causing the inhibition of nitrification organisms.\textsuperscript{49} The nitrification inhibition was reported as correlated positively with the adsorbed copper and Cu(NH\textsubscript{3})\textsubscript{4}\textsuperscript{2+}.\textsuperscript{50} Increase of copper toxicity was observed by increasing ammonia concentration in the water due to the Cu(II)-amine species.\textsuperscript{50} Concentrations of ammonia and copper species in the dilute aqueous system can be estimated by using some model.\textsuperscript{51} Considering the pH and ammonia effect on the amine species, it was assumed that the main fractions of Cu\textsuperscript{2+} were adsorbed copper and Cu(NH\textsubscript{3})\textsubscript{2}\textsuperscript{4+} species which were the reason of nitrification inhibition in the SPBNR. The same observation was reported by Lee et al.\textsuperscript{52} Experimental studies performed previously show that there are some contradictory results on the effects of Cu\textsuperscript{2+} on nitrification process.\textsuperscript{18,22} Although, increase of Cu\textsuperscript{2+} dose

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Effects of Cu\textsuperscript{2+} concentrations on the ANRR and NiAR.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.png}
\caption{Losses of the ANRR ve NiAR at various Cu\textsuperscript{2+} concentrations.}
\end{figure}
caused significant decline of the NH$_4^+$ oxidation rate, it was reported that the specific NO$_2^-$ oxidation rate was affected less up to the concentration of 2 mM Cu$^{2+}$.\textsuperscript{35} On the other hand, Grunditz et al.\textsuperscript{53} reported that the AOB are almost equally or less inhibited than the NOB. Inhibitory effects of Cu$^{2+}$ concentration of 0.92 mg/L, which is about 12 times higher than that used by Juliastuti et al.\textsuperscript{18} on the nitrifying biofilms was not observed.\textsuperscript{54} The nitrifying organisms in the biofilms is able to resist toxicity at higher levels because the structural integrity.\textsuperscript{54,55} In this experimental study, effluent NO$_2$-N concentrations and the NiARs values steadily decreased with dosing Cu$^{2+}$ higher than 10 µg/L, while as could be predicted the NH$_4$-N concentrations increased. Significant rates differences between NO$_2$-N and NH$_4$-N variations were not observed for the tested concentrations of Cu$^{2+}$. Experimental results are not in consistent with some previous studies.\textsuperscript{15,23,31,56}

As mentioned above, the effects of Cu$^{2+}$ on biological nitrification process are varied under the bioreactor operational conditions, such as the initial NH-N concentration, pH, hydraulic retention time, sludge age, reactor types, dosing method and concentrations of metals, etc. Because of the different operational conditions, the toxicity and stimulatory concentrations of Cu$^{2+}$ on the nitrification organisms are varied.

In order to calculate yield coefficient (Y) of biomass, ammonium consumption in the SPBNR during the study was calculated by using Eq. (5).

\[
\text{Ammonium Consumption (mg) = } \sum \text{ applied water volume (L) } \times \sum \text{ nitrogen } [\text{NH}_4\text{-N + NO}_2\text{-N}] \text{ (influent - effluent)} \text{ (mg/L)} \tag{5}
\]

In this study, the biomass yield coefficient was determined as 0.12 mg VSS/mg NH$_4$-N, which correlated well with the value proposed between 0.05-0.29 and 0.02-0.08 mg VSS/mg NH$_4$-N for the nitrosomonas and nitrobacter species\textsuperscript{37}, respectively.

The average number of aerobic mesophilic bacteria at the biofilm surface and water in the void volume of SPBNR were $4.0 \times 10^4$ CFU/g and $2.0 \times 10^4$ CFU/mL, respectively.

4. Conclusion

Compared with the conventional BNR process, the partial nitrification has been considered as a cost effective due to the lower oxygen and carbon source needs. In this process, while the nitrite oxidation is restricted, the oxidation of ammonium should not be affected. Depending on the process to be applied (denitrification or ANAMMOX), the partial nitrification effluent could be contain nitrite, nitrate and ammonium. As can be predicted most of the municipal wastewaters contain low concentrations of heavy metals, such as Cu, Zn, Fe etc., which are necessary for the bacterial growth. Although, low concentrations of heavy metals stimulate the bacterial growth, inhibition of bacterial activity occurs at high concentrations. In this experimental study, in order to determine the effect of nitrite accumulation and ammonium oxidation in the partial nitrification process, low concentrations of Cu$^{2+}$ in the synthetic wastewaters was tested.

Experimental results indicated that the activity of nitrification organisms was stimulated at low Cu$^{2+}$ concentration. At the concentration of 5 mg Cu$^{2+}$/L, NH$_4$-N removal efficiency, NO$_2$-N/NO$_3$-N and NO$_2$-N/NH$_4$-N ratios elevated from 58%, 0.74, and 1.2 (control) to 91%, 0.78, and 2.6, respectively. However, it was observed that at higher copper concentrations, deterioration of the effluent water quality as a result of the inhibition of nitrification organism activity. Under the operational conditions of reactor, the positive effect of low copper concentration on the nitrification organisms caused the increase of ANRR and NiAR. At the lowest Cu$^{2+}$ concentration applied in this study, increase of the ANRR more than the NiAR might be indicated the AOB was more promoted than the NOB. Considering the equal ANRR and NiAR losses when the Cu$^{2+}$ concentration was higher than 10 µg/L, it could be concluded that the AOB and NOB were inhibited equally under the operational conditions.

In the partial nitrification process, Cu$^{2+}$ in the wastewater caused a deterioration NO$_2$-N/NH$_4$-N ratio, which important for the ANAMMOX process. Considering the effluent water quality of partial nitrification reactor, the denitrification process is observed as more suitable for the removal of NO$_2$-N and NO$_3$-N.

Acknowledgement

This study was supported by The Research Fund of Cumhuriyet University (CUBAP) under Grant No. M-327, Sivas, Turkey.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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