Appl Biochem Biotechnol (2011) 165:1543–1555
DOI 10.1007/s12010-011-9374-0

Influence of Operational Parameters and Low Nickel Concentrations on Partial Nitrification in a Submerged Biofilter

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Received: 11 January 2011 / Accepted: 5 September 2011 /
Published online: 17 September 2011

Abstract The effect of Ni^{2+} concentrations on ammonium oxidation was studied in a batch and partial bionitrification reactor (PBNR). The nitrification rates up to the concentration of 0.1 mg Ni^{2+}/l were close to those without Ni^{2+}. After testing the operational conditions in the PNBR, the highest NO_2–N/NO_x–N ratio was achieved at the DO concentrations of 2.0 mg/l and pH 9.00. The PNBR was operated at steady state (NH_4–N loading rate and NO_2–N/NO_x–N ratio were 405 g m^{-3} day^{-1} and 0.74, respectively) before exposure to Ni^{2+}. The removal efficiency of NH_4–N and NO_2–N/NO_x–N ratio in the effluent waters was increased by adding low concentrations of heavy metals to the PBNR. The average number of aerobic mesophilic bacteria at the biofilm surface and in the water in the void volume of PNBR were 1.0×10^4 CFU/g and 1.4×10^5 CFU/ml, respectively.

Keywords Partial nitrification · NO_2–N/NO_x–N ratio · Ni^{2+}

Introduction

Biological nitrogen removal (BNR) is a widely applied technology in water and wastewater treatment facilities. Biological nitrification involves the conversion of ammonium-nitrogen (NH_4–N) to oxidised nitrogen compounds (NO_2–N and NO_3–N) by autotrophic bacteria, which is called *Nitrosomonas* and *Nitrobacter*, respectively. The BNR proceeds at low rate, because nitrite-oxidising bacteria (NOB) grow faster than the ammonium-oxidising bacteria (AOB) and aerobic, anoxic, operational conditions are needed for nitrification and denitrification processes, respectively. To cope with the operational difficulties of BNR, various kinds of biological reactor configurations was

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applied to enhance nitrogen removal efficiency. Biofilm systems have long solid retention times (SRT), favouring the functions of slow-grow organisms such as nitrifiers and are less sensitive to toxic compounds [1].

In contrast to the traditional BNR process, oxygen and carbon requirements are approximately 25% and 40% less in the nitritation/denitritation process, respectively [2], and denitrification rates with nitrite are 1.5 to 2 times greater than with nitrate [3].

The NO_2–N accumulation should be determined without affecting the AOB and the NOB must be adapted to high concentrations of NO_2–N. Anthonisen et al. [4] reported that unionised ammonia inhibited the nitrification reaction. Recent studies also suggested that free hydroxylamine, an intermediate of ammonia oxidation, might be a key factor that caused inhibition to nitrite oxidation [5]. In order to control the NO_2–N oxidation, environmental factors such as dissolved oxygen (DO) [6–8], temperature, [8], pH [8–10], and operational factors like SRT [11, 12] could be used.

The inhibitors for nitrification include heavy metal, toxicant, organic compounds, fulvic acids, oxidants, volatile fatty acids, and halide [13]. It is well known that nitrifying bacteria is sensitive to environmental factors such as pH, temperature, light, heavy metals, and organic solvents because of highly developed cell membrane folding [14].

Metals have been found in significant concentrations in various wastewater streams. Although a constant low-level exposure to metals does not typically affect microbial activity due to biomass acclimation, shock loads of metals can lead to complete failure of biological processes [15]. The nitrifying microorganisms are more susceptible to heavy metal inhibition than the microorganisms responsible for the oxidation of carbonaceous material [16, 17].

The great difference between the inhibition levels reported in various studies for the same metal due to the existence of different nitrifying species in each system [18–20]. The ranges of metal concentrations of inhibition to nitrification under pure culture were presented by Refs. [15, 18–21]. They observed that *Nitrosomonas* sp. was more sensitive than *Nitrobacter* sp. to nickel. Hu et al. [18] reported that nitrification inhibition was not a function of the total analytical metal concentration but strongly correlated with free cation concentration of $[Ni^{2+}]$ or $[Cd^{2+}]$. Exposure time to nickel also affects the ammonia oxidation [22]. The inhibitory effect and quantity of microorganisms depended on metal concentration and the type of microbial species present in sludge. Long-term application of metal to the continuous reactor caused the microbial shift from *Nitrosomonas* and *Nitrococcus* sp. to *Nitrosospira* sp. under gradually increased cadmium loading [23].

The impacts of nickel to microbial growth vary under different environmental conditions [24]. Concentrations of the metals that can be tolerated by nitrifiers in an activated sludge are much higher than those in pure cultures. Yetis and Gokcay [25] indicated that a nickel concentration up to 10 mg/l did not adversely affect the growth rate of microorganisms. Similarly, Lee et al. [19] studied the effects of nickel on a pure nitrification culture and concluded that there was no visible inhibition on nitrification until the nickel concentrations in the reactors were approximately 100 mg/l.

Most of the experimental study was carried out at high concentrations of Ni^{2+} to determine the inhibition effect to BNR. However, there is limited information on the low amount of Ni^{2+} affects on nitrifying bacteria. In this study, the DO and pH affects on NH_4–N oxidation and NO_2–N/NO_x–N ratio [(NO_2–N/(NO_2–N+ NO_3–N)] were investigated at various nitrogen loading rate (NLR). After determining the optimal operational and environmental conditions, considering the highest NO_2–N/NO_x–N ratio, low concentrations of Ni^{2+} affects on partial nitrification was investigated.

Materials and Methods

Feed Wastewater

The synthetic wastewater (medium solution) was prepared using tap water in addition to other chemicals for pH buffering and to provide the trace metals necessary to maintain bacterial growth. The medium solution was used for the batch and continuous experiments. The inorganic medium solution contained various concentrations of NH_4Cl with requested macro- and micronutrients (in mg/l): Na_2EDTA (4.83), $CuSO_4$ (0.0046), $ZnSO_4\ 7H_2O$ (0.023), $CoCl_2\ 6H_2O$ (0.0119), $Na_2MoO_4\ 2H_2O$ (0.066), $MgSO_4\ 7H_2O$ (36.97), $NaHCO_3$ (226), $CaCl_2\ 2H_2O$ (36.74), H_3BO_3 (1.0), $FeCl_3\ 6H_2O$ (0.316), and KH_2PO_4 (1920).

Enrichment of Microorganisms

The partial nitrification bioreactor (PNBR) was inoculated with microorganisms taken from the nitrification–denitrification parts of an aeration basin at the Kayseri Municipal Wastewater Treatment Plant and acclimatised to medium solution including NH_4–N. The inoculation conducted in a 5-l vessel lasted for approximately 1 month for microbial growth with daily replenishment of medium solution.

Batch Experiments

In order to determine the effects of Ni^{2+} concentrations on nitrification bacteria, batch experiments were carried out in 500-ml glass bottles, containing medium solutions and 50 mg NH_4–N/l. At the beginning of the batch experiments, the pH of mixed liquor was adjusted to 9.0 by using alkaline solution of 10 N NaOH and bicarbonate buffer was added considering the nitrification rate. After preparing the medium solution, the mixed liquor volatile suspended solids (MLVSS) analysis was carried out.

The concentrations of Ni^{2+} were varied between 0.0 and 2.0 mg/l in three batch units for each concentration. Three blank samples (without Ni^{2+}) were used to compare the results through all batch procedures. The total volume of liquor was 200 ml. The DO concentration was over 5.0 mg/l in the batch units at the beginning of the experimental study. The DO level in batch reactors was checked daily and aerated when the DO level was lower than 2.0 mg/l throughout the experimental periods. Acclimated microorganisms of about 50 mg/l were included for each batch unit, placed on a shaking incubator at 150 rpm and at a constant temperature of 35 °C.

The samples were withdrawn daily from batch units and filtered using 0.45 μm, white, 47-mm radius filters. The pH and DO level were checked, and concentrations of NH_4–N, NO_3–N, and NO_2–N in the clear samples were analysed at least three times. The batch experiment was completed when the concentrations of NH_4–N was lower than 10 mg/l for each Ni^{2+} concentrations.

The PNBR Set-up and Operation

Completely submerged PNBR consisted of a cylindrical stainless steel, 10-cm inner diameter and 30-cm height (Fig. 1). The PNBR was filled with 20-mm diameter pieces of plastic coils, which provided 0.4 m^2 resulting in 118 m^2 surface area/m^3 for bacterial growth.

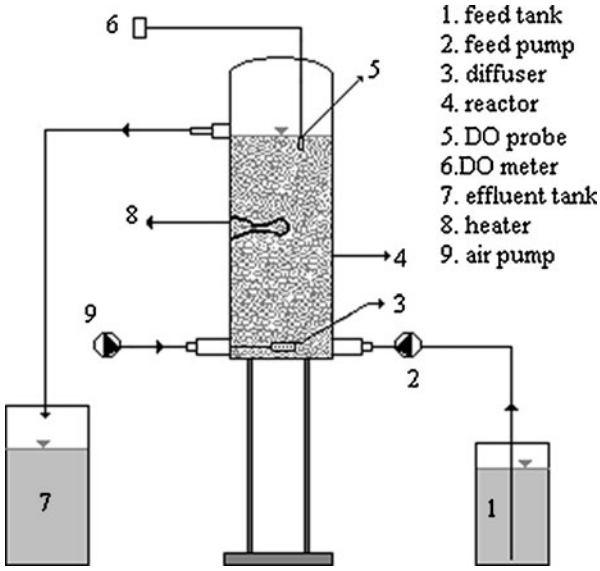

Fig. 1 Schematic diagram of the PNBR

The influent wastewater was pumped continuously to the bottom of PNBR using a peristaltic pump and discharged from the top of the reactor to the effluent tank. The PNBR was operated at 35 °C±1, an air diffuser was installed directly at the bottom and DO concentration was measured periodically at the top of the reactor by using a DO metre (YSI 5100). The PNBR had a liquid volume of 3.1 l.

Since NO_2–N accumulations can be controlled by free ammonia (FA) concentrations at high pH, a series of NH_4–N oxidation experiments were performed under various pH values. The initial pH value of feeding solution was adjusted to be 7.5, 8.0, 8.5, and 9.0 at DO concentration of 1.0±0.2 mg/l. The DO concentration effects on NO_2–N accumulation and NH_4–N oxidation was also tested for 1.0±0.2 and 2.0±0.2 mg/l at a constant pH value.

The NLR was increased gradually by changing influent NH_4–N concentration of PNBR. After determining the optimal conditions, considering NO_2–N/NO_x–N ratio and NH_4–N removal efficiency, a heavy metal experimental study was carried out at constant experimental conditions by varying the concentration of Ni^{2+} between 0.01 and 0.1 mg Ni^{2+}/l.

Analytical Methods

Samples were withdrawn daily from the PNBR effluents and filtered using 0.45 μm, white, 47-mm radius filters. All samples were tested for NH_4–N, NO_3–N, and NO_2–N concentrations with the Merck photometer (Nova 60 Model) using analytical kits: NH_4–N (14752), NO_2–N (14776), and NO_3–N (14773). The metals in the feeding solution were determined by inductively coupled plasma atomic emission spectroscopy, and the analysis of samples was carried out at ambient temperature.

The samples of biofilm covering filling materials from different height and water in void volume of the PNBR were removed, and a biomass amount and identification test was carried out at the end of the experimental study. The filling materials were introduced in sterile glass bottles with 100 ml of distilled water and stirred at 280 rpm for 1 h to separate the biomass from the attached surface. The amount of attached solids and volatile solids

(VSs) were determined by vacuum filtration of 100 ml solutions through pre-weight 0.45 μm filter and drying the filter paper in an oven at 103 °C temperature and then at 550 °C until constant weight [26] is obtained. The total numbers of colony-forming units of aerobic mesophilic bacteria were determined according to FDA, BAM [27]. Bacterial identification was carried out using test kits of Phoenix P MIC ID 51 and Phoenix N MIC ID 55 in the BD Phoenix 100 system in the microbiological laboratory at Cumhuriyet University.

Results and Discussion

The PNBR was operated for about 1.5 years at various ammonia and Ni^{2+} loadings to determine the optimal operating conditions and Ni^{2+} affects on nitrifying bacteria.

Start-up Period of the PNBR

The objective of the start-up period was to promote microbial growth for complete ammonium oxidation. Microbial attachment and biofilm formation was facilitated by operating reactor in a batch mode with water recycling. In the first 2 weeks of the start-up period, 80% of NH_4–N was completely removed between feeding periods. While NO_2–N was not detected at the beginning of the operation, nitrification was quickly completed to NO_3–N. After the start-up period, the PBNR was operated in a continuous mode and the NLR was increased gradually. At low NLR, NH_4–N was completely converted to NO_3–N and then NO_2–N concentration increased with increasing the NLR.

The PNBR was operated at various pH values, DO concentrations, and NLRs to determine optimal operational condition to achieve the highest NO_2–N/NO_x–N ratio and NH_4–N removal efficiency at the temperature of 35 °C. Variation of the NLR was provided with changing influent NH_4–N concentrations at constant flow rate of 4 l/day.

Effects of the NLR on NO_2–N/NO_x–N ratio and NH_4–N Removal Efficiency

During the start-up period, the PBNR was operated under conditions of pH=7.5 and DO= 2.0 mg/l at various NLR. At first, the decrease of NH_4–N was almost completed by a significant accumulation of NO_3–N and a small fraction of NO_2–N. After the nitrification was established, the NLR was gradually increased from about 65 to 215 $g m^{-3}$ day^{-1} by varying the influent NH_4–N concentration in the feeding wastewater. This change resulted in incomplete oxidation of NH_4–N and the effluent NH_4–N and NO_2–N concentration increased. The purpose of this exercise was to determine the maximum NLR, NH_4–N removal efficiency, and NO_2–N accumulation potential under operational conditions.

The lowest NO_2–N/NO_x–N ratio was observed when the NLR was lower than 200 g NH_4–N/m^3.day. Increasing the NLR from 200 to about 215 kg m^{-3} day^{-1}, the NO_2–N/NO_x–N ratio sharply increased to about 0.52 and also NH_4–N effluent concentrations while the NO_3–N concentration was decreased, simultaneously (Fig. 2).

The average NH_4–N removal efficiency was about 95% and 65% at the NLR of 205 and 215 $g m^{-3}$ day^{-1}, respectively. As shown in Fig. 2, it was evident that the PNBR was unable to provide a high NH_4–N removal efficiency when the NLR was higher than 200 g NH_4–N m^{-3} day^{-1} under operational conditions. Although the partial nitrification considering NO_2–N/NO_x–N ratio remained very efficient at high NLR, effluent concentration of NH_4–N was too high.

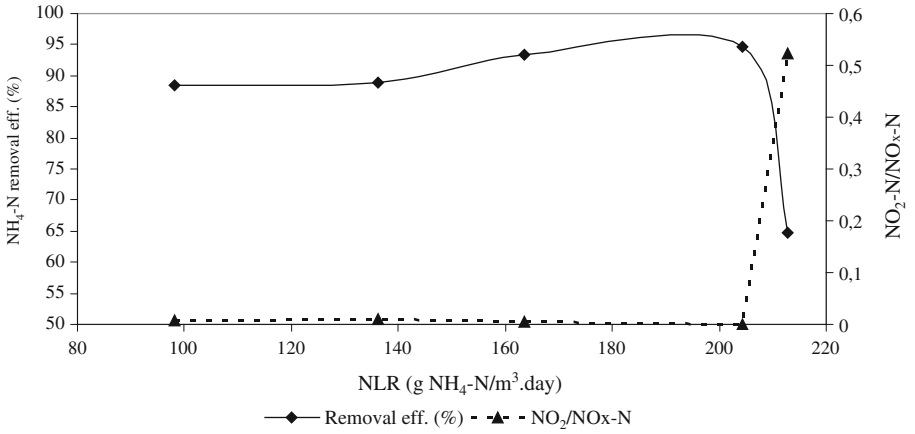

Fig. 2 The NLR effects on NO_2–N/NO_x–N ratio and NH_4–N removal efficiency

In the literature, several strategies such as high pH and low DO concentration for obtaining NO_2–N as the main product of nitrification are proposed: to use pure *Nitrosomonas* cultures and inhibition of NOB or to manipulate the oxygen concentration in the reactor [28]. The effect of pH and DO concentrations on partial nitrification in the PBNR was investigated to achieve high NO_2–N/NO_x–N ratio.

Effects of pH Variation on the NO_2–N/NO_x–N Ratio

Anthonisen et al. [4] reported that the AOB and NOB were inhibited 10–150 mg/l and 0.1–1.0 mg/l of FA and all nitrifying bacteria were inhibited above 0.2 mg/l of free nitric acid. The FA and free nitric acid (FNA; HNO_2) concentrations were estimated using Eqs. 1 and 2 [4]. Since FA achieves the NO_2–N accumulation, the feeding solution of pH varied between 7.5 and 9.0 at the temperature and DO concentrations of 35±1.0 °C and 2.0 mg/l, respectively.

$$\mathrm{FA(mg/l)} = \frac{17}{14}\,\frac{\Sigma\mathrm{NH_4-N(mg/l)} \times 10^{\mathrm{pH}}}{e^{6344/(273+T)} + 10^{\mathrm{pH}}} \tag{1}$$

$$\mathrm{HNO_2(mg/l)} = \frac{47}{14}\,\frac{\Sigma\mathrm{NO_2-N(mg/l)}}{\exp^{-(2300/273+T)} \times 10^{\mathrm{pH}}} \tag{2}$$

Figure 3 shows a clear effect of pH and NLR on the NO_2–N/NO_x–N ratio, which reveals a significant influence on NO_2–N accumulation even at DO concentration as high as 2.0 mg/l. Although partial nitrification in the biofilms has been mainly reported for low DO concentrations in the bulk phase, it has also been observed at the DO concentration of 6.8 mgO_2/l in a rotating biological contactor [29] and about 2.0 mg/l in a fluidised-bed biofilm reactor [30].

Although the efficiency of NO_2–N production was limited at low pH, increasing the pH value from 7.5 to 9.0, this increased the NO_2–N concentration in the effluent water from 48 to about 100 mg/l.

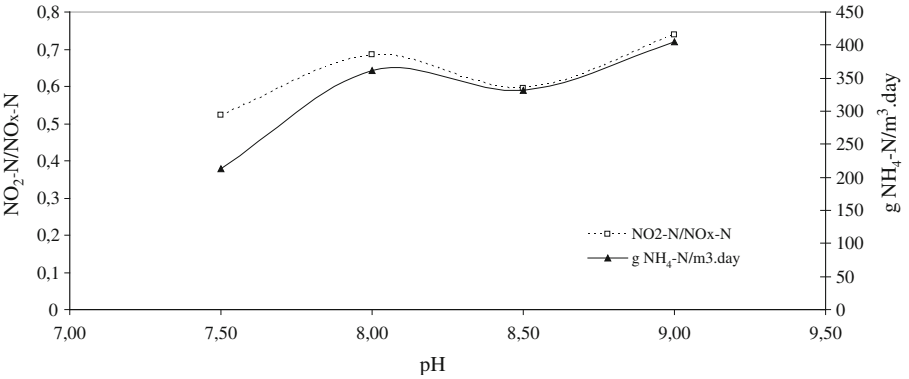

Fig. 3 Influence of the pH value on NO_2–N/NO_x–N ratio

Regarding NOB, the values shown in Fig. 4, the activity of microorganisms are clearly dependent on the FA. Because of the increase in pH value from 7.5 to 9.0 and also the NLR from 213 to 405 g NH_4–N m^{-3} day^{-1}, the NO_2–N/NO_x–N ratio and concentrations of FA was increased from 52% to 74% and 7.9 to 231 mg/l, respectively. The calculated FNA level in reactor was below the inhibition level of 0.2 mg/l for all applied pH levels.

As expected, the experimental studies confirmed that the NO_2–N accumulation could be achieved by regulating pH to control FA concentration. But the threshold inhibition concentrations of FA found in the literature were different [13]. The FA concentration is not only factor for NO_2–N accumulation [12]. Kim et al. [31] reported that the NO_2–N accumulation rate (relative NO_2–N accumulation) was largely determined by temperature with no dependence on FA concentrations. Kim et al. [31] explained that the NO_2–N accumulation is not possible on a long-term basis, using pH as a key parameter. The AOB dominate over NOB at DO concentrations was below 1.0 mg/l [32]. Concerning the role of FA concentration on NO_2–N accumulation, it was observed that the NO_2–N accumulation rate increased significantly with increase in temperature. However, there was not a significant difference in the NO_2–N accumulation rate among different FA concentration at

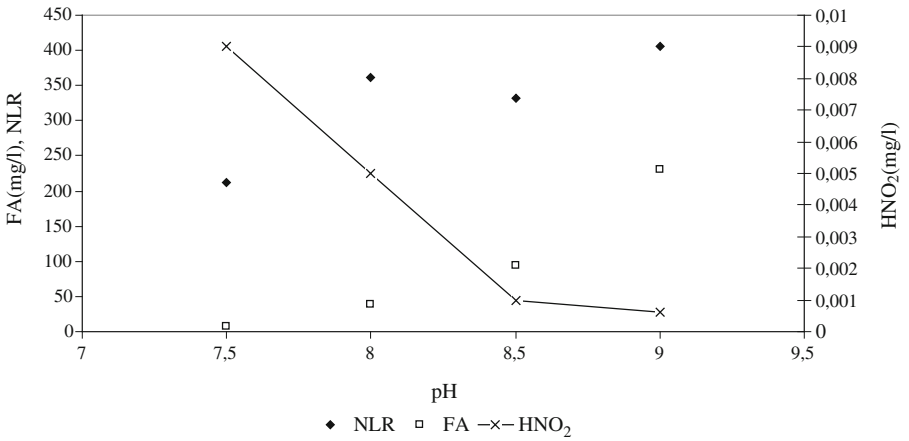

Fig. 4 Effect of pH on FA and HNO_2 under various NLR

the certain temperature [31]. The accumulation of NO_2–N can be attributed mainly to the increase of temperature but FA inhibition of NOB [31] and limitation of DO in the biofilm process [32–34]. As a result, owing to the combined DO limitation and NH_3–N inhibition, and temperature on NOB, NO_2–N accumulation was achieved throughout the experimental studies.

According to these experimental studies under operating conditions, the highest NO_2–N/NO_x–N ratio was obtained at pH 9.00, DO=2.0 mg/l, and 405 g NH_4–N m^{-3} day^{-1}.

The DO Concentrations Effects on NO_2–N/NO_x–N Ratio

The DO half-saturation coefficients of AOB and NOB are 0.2–0.4 and 1.2–1.5 mg/l, respectively. Therefore, low DO concentration is more restrictive for the growth of NOB than AOB, which will result in NO_2–N accumulation [28]. The effects of DO on NO_2–N accumulation were tested for DO concentrations of 1.0 and 2.0±0.2 mg/l at constant temperature and pH of 35 °C and 9.0, respectively.

Under operational conditions of 1.0 mg/l DO concentration and the NLR of 341 g m^{-3} day^{-1}, the removal efficiency of NH_4–N and effluent concentrations of NO_2–N was 43% and 57 mg/l, respectively. As can be seen in Fig. 5, the concentrations of 100 mg NO_2–N/l was

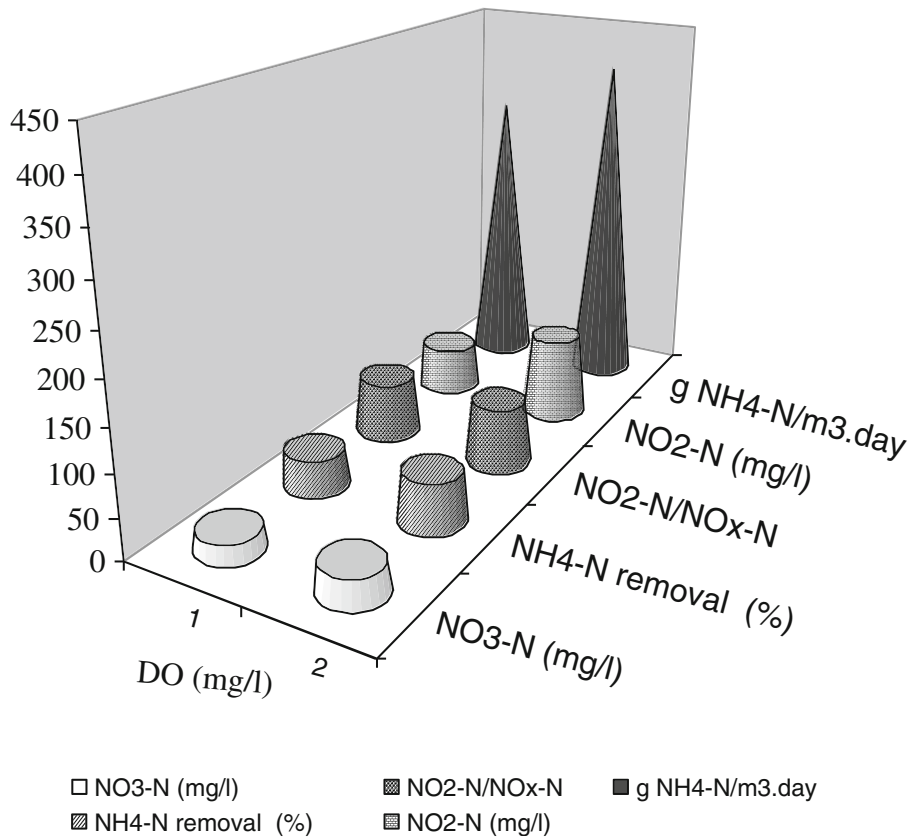

Fig. 5 The DO effects on partial nitrification

achieved at 2.0 mg DO/l and the NLR of 405 $g m^{-3}$ day^{-1}. Increasing DO concentration from 1.0 to 2.0 mg/l increased the NO_2–N accumulation, NH_4–N removal efficiency, and also the NLR.

As a result of the change of DO concentration from 1.0 to 2.0 mg/l in the PNBR, improvement of the NO_2–N production efficiency and the NLR were about 42% and 16%, respectively. As expected, the higher DO concentration caused a higher nitrification rate and similar results were obtained in a biofilm system [30, 35, 36]. Various level of NO_2–N accumulation has been reported for different reactor configurations by controlling of DO concentration. In a fluidised-bed reactor, 34% of NO_2–N accumulation and 40% of NH_4–N oxidation was achieved [37]. Garrido et al. [35] achieved high NO_2–N accumulation (50%) at the DO concentration between 1 and 2 mg/l. Bernet et al. [38] observed about complete conversion of NH_4–N at the DO concentration of 0.5 mg/l with above 90% of NO_2–N/NO_x–N ratio. Aslan and Dahab [30] operated at high DO concentration and about 72% NO_2–N/NO_x–N ratio was achieved in the fluidised-bed biofilm nitritation reactor. Ann et al. [39] and Aslan and Dahab [30] observed 80% NO_2–N ratio and above 66% of the NO_2–N production efficiency at high DO concentration, respectively. This indicates that the DO was not only the limiting factor for high NO_2–N accumulation and it was probably high concentration of FA.

The initial FA concentrations were far above the threshold concentration of inhibition at which the inhibition of NO_2–N began for the 2.0 mg DO/l concentration in the PNBR. It was assumed that the highest NO_2–N accumulation was achieved under operational conditions by DO level which is likely that is the limiting substrate when the NH_4–N load is high.

Yang and Alleman [5] reported that the DO level alone did not appear to be dominant factor behind NO_2–N build-up, and its correlation with FA concentration alone was also erratic. High NO_2–N accumulation was observed when the ratio of DO/FA was below 5 [12, 36].

The PNBR was operated at steady state (NH_4–N loading rate and NO_2–N/NO_x–N ratio were 405 $g m^{-3}$ day^{-1} and 0.74, respectively) before exposure to heavy metals.

Effects of Nickel on Nitrification in a Batch Unit

In order to determine Ni^{2+} affects on NH_4–N elimination, experiments were carried out until reaching 97% NH_4–N removal efficiency (effluent concentrations was lower than 2.0 mg NH_4–N/l). When the pH was lower than the 8.00 in a day during the batch experimental period, the pH was increased to 9.0 by adding NaOH solution. Due to the conversion of NH_4–N to NO_2–N and NO_3–N, the concentrations of NH_4–N decreased continuously in the batch units.

Batch experiments at several Ni^{2+} concentrations were carried out to highlight the differences between nitrification rates with and without nickel. It can be seen in Fig. 6 that nitrification rates was high without and with at low concentrations of Ni^{2+}. In the batch assays, the NH_4–N oxidation decreased significantly as the applied Ni^{2+} concentration to the nitrifying biomass increased. The average nitrification rates of 1.516 and 1.523 mg NH_4–N/mg MLVSS day was determined for the blank sample and 0.1 mg Ni^{2+} concentrations, respectively. Concentrations of NH_4–N had a quick decrease at low concentrations of Ni^{2+} ($\leq$0.1 mg/l) and after 4 days, removal efficiency and nitrification rates of 97% and 1.52 NH_4–N mg^{-1} MLSS day^{-1} was achieved, respectively. The small differences of the nitrification rate for low concentrations of nickel might be caused stimulation effects.

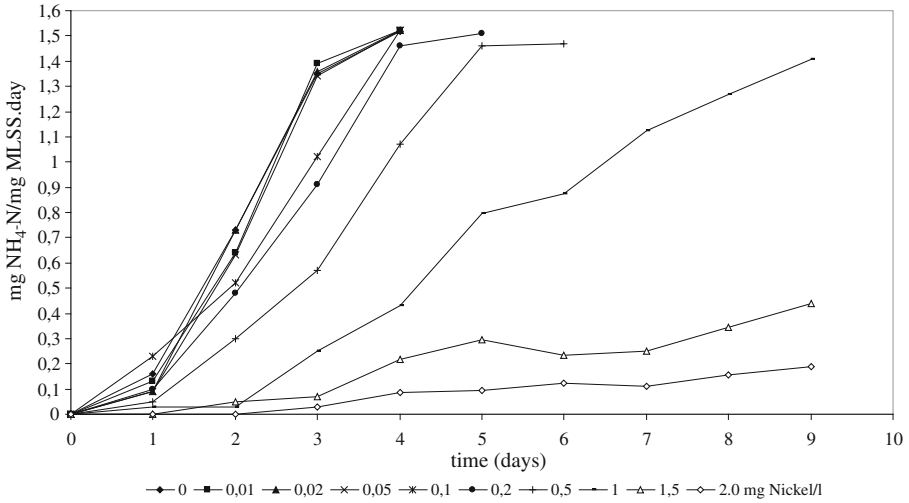

Fig. 6 The average nitrification rates with reaction time at various Ni^{2+} concentrations in a batch unit

At the concentration of 1.0 Ni^{2+} mg/l, nitrification appeared to be partially suppressed, indicated by a drop in effluent NO_3–N and increase in NH_4–N concentration. Although, the target NH_4–N removal was achieved in long reaction times for high concentrations of Ni^{2+}, increasing concentrations from 0.1 to 0.2, 0.5, and 1.0 mg Ni^{2+}/l, increase the reaction times from 4 to 6 and 9 days, respectively. Further increase of Ni^{2+} concentrations to 2 mg/l, nitrification rates was sharply decreased to 0.19 mg NH_4–N mg^{-1} MLSS day^{-1} and about 12% NH_4–N removal efficiency was achieved in 9 days of reaction times.

The results of batch experimental studies indicated that nitrification rates up to 0.1 mg Ni^{2+}/l concentration was close to one without Ni^{2+} and increasing the concentration of Ni^{2+} in the batch unit, inhibiting effect of Ni^{2+} on nitrification occurred. At the concentration of 2.0 mg Ni^{2+}/l, nitrification appeared to be partially suppressed, indicated by a drop in effluent NO_x–N and increase in NH_4–N concentrations at the end of batch tests.

The PBNR Run with Various Concentrations of Nickel

The PNBR was operated about a year to determine optimal operating conditions, considering NH_4–N removal efficiency and NO_2–N/NO_x–N ratio. Because the highest NH_4–N removal efficiency and NO_2–N/NO_x–N ratio were achieved under operational conditions of 35 °C, pH at 9.0 of feeding solution, and 2.0 mg/l of DO concentrations at the top of PNBR, the experimental set-up was adjusted to these operational conditions for determining low concentrations of Ni^{2+} effects on partial nitrification. Prior to adding Ni^{2+} in the feeding wastewater, the PNBR was operated about 2 months to achieve steady state at optimal operating conditions. During the experimental studies, the PNBR effluents were collected to measure concentrations of NH_4–N, NO_2–N, and NO_3–N under steady-state conditions.

The PNBR was operated at NLR of about 405 g NH_4–N m^{-3} day^{-1} by applying about 4 l/day flow rate, 250 mg NH_4–N/l and various Ni^{2+} concentrations to determine the changes in NH_4–N removal and NO_2–N/NO_x–N ratio. The removal efficiency of NH_4–N and NO_2–N/NO_x–N ratio in the absence of Ni^{2+} was about 58% and 0.74, respectively.

The removal efficiency of NH_4–N and NO_2–N/NO_x–N ratio was increased by adding low concentrations of Ni^{2+} (Fig. 7). Because low concentrations of Ni^{2+} (≤0.05 mg/l)

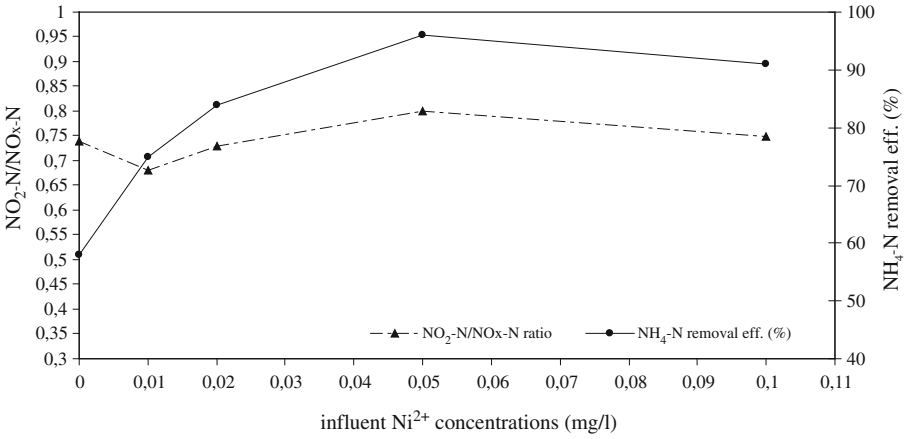

Fig. 7 The NO_2–N/NO_x–N ratio variation at different Ni^{2+} concentrations in the PNBR

stimulate the nitrification organisms, NH_4–N removal efficiency and NO_2–N/NO_x–N ratio was increased to about 96% and 0.8, respectively. The NO_2–N/NO_x–N ratio was decreased from 0.74 to 0.68 while the removal efficiency of NH_4–N increasing for 0.01 mg/l concentrations of Ni^{2+}. It was assumed that NH_4–N removal efficiency was increased in the effluent water due to the low concentrations of Ni^{2+} positively affects the *Nitrosomonas* sp. A similar result was observed at the batch experiments. Bagby and Sherrard [40] reported that relatively low concentrations of heavy metals may serve to stimulate biological systems as indicated by the rate of biological reaction. However, further increase in heavy metal concentration decreases the reaction rate and results in failure of the biological activity. Further increase the concentrations of Ni^{2+} to 0.1 mg/l caused little decrease of removal efficiency (from 96% to 91) and NO_2–N/NO_x–N ratio (from 0.8 to 0.75). The experimental results confirm the study of Temel [41] where the inhibition effect of heavy metals on the nitrification was evaluated. Temel [41] explained that the activity of nitrite oxidisers is inhibited at low concentrations of Ni^{2+} (0.15 mg/l) while that of ammonia oxidisers had not been affected.

The observed inhibition as a function of Ni^{2+} concentration was lower in the PNBR than was observed in the batch unit. The differences in inhibition of metal concentration between the batch and continuous reactors might be caused by exposure times and accumulation of Ni^{2+} in the continuous flow reactor. This finding was confirmed by earlier observation [15]. Hu et al. [22] reported that nitrification inhibition increased from approximately 35% to 60–65%, with the increase of exposure time to nickel from 1 to 8–25 h. Nickel inhibited ammonium oxidation but not nitrite oxidation up to total analytical concentrations of approximately 1.0 mM [20].

Ammonium consumption in the PNBR was calculated using following equation:

$$\begin{aligned}\text{Ammonium consumption(mg)} = {} & \text{Total water volume(l)} \\ & \times \text{Total nitrogen}[(NO_3 - N + NO_2 - N = NO_x - N)] \\ & \times (\text{influent} - \text{effluent})(\text{mg/l})\end{aligned}$$

A yield coefficient (Y) of microorganisms for *Nitrosomonas* and *Nitrobacter* species was proposed as between 0.05–0.29 and 0.02–0.08 mg VSs/mg NH_3–N by Eckenfelder [42] and it was determined as 0.09 mg VSs/mg NH_4–N in the PNBR. Based on the measurement

in this study, the reduction of 1 g NH_4–N theoretically produces about 0.1 g new cells. The relation was apparent that consumed NH_4–N (in mg) correlated well with conversion of nitrogen to microorganisms proposed by [42].

The average number of aerobic mesophilic bacteria at biofilm surface and water in the void volume of PNBR were 1.0×10^4 CFU/g and 1.4×10^5 CFU/ml, respectively. Identification tests of bacteria in the PNBR showed the following predominance of species: *Acinetobacter lwoffii/haemoltyticus, Acinetobacter baumanni*, *Achromobacter* sp., *Paenibacillus alveti, Stenotrophomonas maltophilia*.

Conclusions

Experiments were carried out in the submerged PNBR system to determine the highest NO_2–N/NO_x–N ratio under various experimental conditions. The highest NO_2–N/NO_x–N ratio of 0.74 was obtained under operational conditions of NLR, pH, and concentrations of DO at 405 $g m^{-3}$ day^{-1}, 9.0 and 2.0 mg/l, respectively. Although the results of experimental studies indicated that activity of nitrification organisms is inhibited at high concentrations of Ni^{2+}, low concentrations of Ni^{2+} stimulate nitrification organisms under operational conditions. The concentration of Ni^{2+} effects on nitrification in the continuous reactor was different in the batch unit. The differences might be caused by exposure times and accumulation of Ni^{2+} in the reactor.

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

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