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The Effect of Limited Ammonium Variations on Biological Nitrogen Removal Process

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ABSTRACT

The aim of this study was to investigate the effect of varying limited ammonium concentrations on the heterotrophic denitrification process responsible for biological nitrogen removal. The system performance was evaluated with gradually decreasing ammonium-nitrogen concentrations from 20 mgNH_4^+-N $L^{-1}$to inexistent ammonium inlet at 100 mgNO_3^- L^{-1}. Results of this study indicated that the total nitrogen removal efficiency reached maximum level at influent ammonium-nitrogen of 5 mg L^{-1}due to the absence of residual ammonium at the end of the reaction, although varying ammonium concentrations did not noticeably affect the nitrate removal efficiency. Additionally, nitrate consumption rate had a tendency to increase with the limitation of influent ammonia and the nitrate consumption rate reached maximum level at operational condition where ammonium was not present, corresponding to 85.4 mgNO_3^--N gMLSS$^{-1}h^{-1}$. The maximum ammonium consumption rate have attained with influent ammonium-nitrogen of 5 mg L^{-1}, being 18.4 mgNH_4^+-N gMLSS$^{-1}h^{-1}$

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Biyolojik Azot Giderim Prosesinde Sınırlı Amonyum Değişiminin Etkisi

ÖZET

Bu çalışmanın temel amacı, biyolojik azot gideriminden sorumlu heterotrofik denitrifikasyon prosesinde değişen sınırlı amonyum konsantrasyonlarının etkisini araştırmaktır. Sistem performansı 100 mg NO_3^- L^{-1} sabit giriş nitrat konsantrasyonunda 20 mgNH_4^+-N L^{-1}'den kademeli olarak azalan amonyum azotu konsantrasyonları ile değerlendirilmiştir. Bu çalışmanın sonuçları, amonyum konsantrasyonlarındaki değişimin nitrat giderme etkinliğini belirgin bir şekilde etkilememesine rağmen, reaksiyonun sonunda kalıntı amonyum bulunmaması nedeniyle 5 mg L^{-1}'lik giriş amonyum-azotu konsantrasyonunda toplam azot giderim veriminin maksimum seviyeye ulaştığını göstermiştir. Buna ek olarak, nitrat tüketim hızı giriş amonyum konsantrasyonun sınırlanması ile artış eğiliminde iken, amonyum bulunmayan işletim koşulunda nitrat tüketim hızı, 85,4 mg NO_3^--N gMLSS^{-1} sa^{-1}olarak maksimum seviyeye ulaşmıştır. Maksimum amonyum tüketim oranı, 5 mgL^{-1}'lik amonyum azotuyla, 18,4 mg NH_4^+-N gMLSS sa^{-1} olarak elde edilmiştir.

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Anahtar Kelimeler
Amonyum tüketimi, biyolojik azot giderimi, heterotrofik denitrifikasyon, nitrat tüketim hızı

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INTRODUCTION

The increase of nitrate level in the receiving environment causes environmental and health related serious hazards. When the nitrate discharges into the receiving environment in high concentrations, it causes toxic algal blooms and eutrophication (Ghafari et al., 2008). This environmental condition poses a great risk to environment, nature and living health. Additionally, nitrate is determined as one of the harmful pollutants because it would cause blue baby disease, especially in infants. Therefore, it is known that many different

chemical treatment techniques were studied to remove nitrate from wastewaters including treatment by aluminum-iron alloys (Xu et al., 2012; Hou et al., 2015), electrocoagulation-flocculation (Elazzouzi et al., 2017), and electrodialysis (Arahman, et al., 2017). However biological nitrate removal processes are generally accepted and widely applied in existing wastewater treatment plants due to cost-effective and efficient for high strength wastewater compared to chemical treatment methods (Wang and Yang, 2004; Burgin and Hamilton, 2007; Wang and Chu, 2016). In the denitrification process, nitrate is used as electron acceptor source, while organic or inorganic substances are used as electron donor and carbon source by the bacteria (Burgin and Hamilton, 2007; Ghafari et al., 2008). Biological denitrification process can be carried out autotrophically and heterotrophically (Zhao et al., 2011). Inorganic carbon sources such as carbon dioxide (CO_2), bicarbonate (HCO_3) are used by autotrophic microorganisms, while hydrogen compounds containing iron and sulfur are preferred as energy source in autotrophic denitrification (Karanasios et al.,2010). However, heterotrophic denitrification bacteria, which use organic carbon metabolites as carbon and energy source, are the most common bacteria in nature (Van Rijn et al., 2006). It has been reported that heterotrophic denitrification is much more economical, especially on a large scale, and resistant to shock nitrate loads (Ovez et al., 2006). Additionally, this process appears more attractive for engineering applications due to the ease of enrichment of heterotrophic culture (Schipper et al., 2010; Liu et al., 2009).

The most important parameters affecting heterotrophic denitrification performance are temperature, hydraulic retention time (HRT), external carbon source, and dissolved oxygen (DO) concentration. The denitrification efficiency reached to approximately 92% at high temperatures (≥20 °C) and low HRT levels (3-6 h) while it was 82% at low temperatures (10-15°C) and high HRTs (13-17 h) (Chu and Wang, 2013).It is also very important to select an appropriate carbon source in the denitrification process because this will increase the effectiveness of the process. The different external carbon and electron donor sources included methanol, glucose, lactate, butyrate, and acetate are commonly used for denitrification to improve denitrification rates and process stability (Modin et al., 2007; Ge et al., 2012). Additionally, it is known that the DO levels should be below 1 mg L^{-1} in order to successfully take place of denitrification process (Dabkowski, 2008; Modin et al., 2010). Nitrogen form is very important on anoxic denitrification microorganisms. Ammonium ions have been found to be the preferred form of nitrogen for assimilation by microbes (Burger and Jackson, 2003; Cai et al., 2013; Feng et al., 2017). If we accept $C_5H_7O_2N$ as being representative of biomass, we can see that carbon and nitrogen are the reduced elements that will house those electrons (Machado, 2011). Nitrogen form in biomass is in the -III state. If the nitrogen available for biomass synthesis is also in the –III state (as in ammonia), no electrons will be required to reduce it, and the electrons captured through synthesis will all be associated with the carbon. Most microalgae and microorganisms generally use nitrate when the ammonium is low or depleted in the wastewater (Cai et al., 2013). However, the effect of operational conditions which ammonium is also used as an external nitrogen source on heterotrophic denitrification process performance has still not been determined and detailed studies have been lack of process operation. Therefore, the main purpose of this study was to evaluate nitrogen removal ability of bacteria responsible for heterotrophic denitrification under different influent ammonium-nitrogen concentrations. The experimental results could provide useful information about how to select an appropriate level of influent ammonium concentration, as well as nitrogen source form used for growth by denitrifiers to enrich cultures with high nitrate removal capacities.

MATERIAL and METHODS

Synthetic wastewater and inoculum

The inoculum culture was enriched using sample taken from anaerobic tank Gaziantep Wastewater Treatment Plant in Gaziantep, Turkey and microbial cultures were adapted for 40 days. The SBR was fed with synthetic wastewater containing sodium nitrate ($NaNO_3$), sodium acetate ($CH_3COONa \cdot 3H_2O$), ammonium chloride (NH_4Cl), macro and micronutrients. Acetate and nitrate concentrations were kept constant at 240 mg $DOCL^{-1}$ and 100 mg $NO_3{}^{-}L^{-1}$during all study periods, respectively. The characteristic of synthetic wastewater is showed in the Table 1.

Table 1. Synthetic wastewater composition

Chemicals	Concentrations (mg L^{-1})
NH_4Cl	76.40-19.10
$CH_3COONa \cdot 3H_2O$	136.80
$NaNO_3$	137.08
$MgCl_2.6H_2O$	206.20
$CaCl_2.2\ H_2O$	67.50
KH_2PO4	12.20
$Na_2HPO_4.\ 2H_2O$	6.30
$FeSO_4.7H_2O$	0.40
H_3BO_3	0.15
$MnCl_2.4\ H_2O$	0.083
$CuSO_4.5\ H_2O$	0.025
$ZnCl_2$	0.008
$NaCl_2.6H_2O$	0.015

Reactor design and operation

Lab-scale SBR study was conducted with active working volume of 2 L glass reactor. The batch system

was operated with a 3-hour cycle time and the SBR system is shown in Figure 1.

The synthetic wastewater was regularly prepared daily and continuously purged with nitrogen gas for ten minutes to eliminate the oxygen leakage before it was added the reactor. Sludge age was kept constant for 40 days. The SBR was operated at room temperature without pH control during the experiments. Mixed liquid suspended solid (MLSS) concentration was kept at 2500±300 mgL^{-1} level during SBR operation.

The performance of the fed-batch reactor was investigated on four different operational conditions containing reduced influent NH_4^+-N concentrations (Table 2).

Firstly, SBR was performed with 20 $mgNH_4^+$-NL^{-1} influent ammonium concentration at the constant nitrate concentration of 100 $mgNO_3^-L^{-1}$ (period I). Further, ammonium concentration was decreased to 10 and 5 $mgNH_4^+$-NL^{-1} in the period II and III, respectively, to evaluate the effect of limited ammonium concentration on heterotrophic denitrification process performance. The rest of the study was carried out at the ammonium-free conditions (period IV). All assays were conducted twice and mean values were presented.

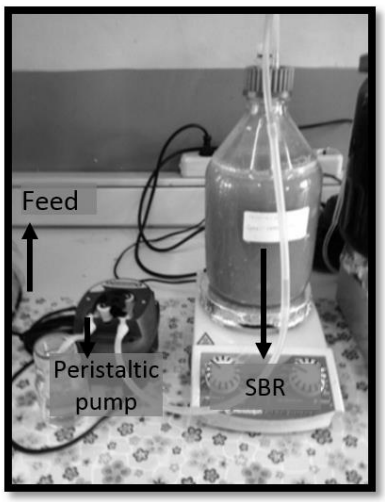

Figure 1. Denitrification reactor system

Table 2. Summary of operational conditions of SBR

Periods	Influent DOC concentration (mg L^{-1})	Influent NO_3^- concentration (mg L^{-1})	Influent NH_4^+-N concentration (mg L^{-1})
I	240±20	100±5	20
II	240±20	100±5	10
III	240±20	100±5	5
IV	240±20	100±5	0

Analytical methods

All liquid samples were firstly centrifuged using Eppendorf Centrifuge device (Centrifuge Eppendorf 5415R, Hamburg, Germany) at 4000 rpm for 5 min, and then they were filtered through 0.45 µm syringe filter before the ammonium, nitrate, nitrite, and DOC ammonium measurements. DOC concentration was measured using a total organic carbon device (Shimadzu TOC-V_{CPN}, Kyoto, Japan). The device was calibrated by preparing a solution potassium hydrogen phthalate ($KHC_8H_4O_4$) and sodium hydrogen carbonate ($NaHCO_3$) based on the approximately DOC value of the samples. Anion and cation measurements were performed on ICS-5000 model ion chromatography instrument (Dionex, Sunnyvale, CA, USA) equipped with IonPac® AG9-HC guard and AS9HC analytical column. A mixture eluent containing 9mM Na_2CO_3 and 20mM metano-sulphonic acidion chromatograph was passed through the device at a flow rate of 1 ml min^{-1}. All anions and cations were measured with a single injection. pH changes were observed with a multimeter (340i, WTW, Oslo, Norway). An UV-Vis spectrophotometer (HACH, DR 5000, Loveland, USA) was used at absorbance wavelength of 600 nm to determine the MLSS concentration based on dose-absorbance response curves in the mixed liquid samples taken from lab scale denitrification reactor.

RESULT and DISCUSSION

Nitrogen profiles and consumption rate

The profiles of varying nitrogen forms as a function of the operation period are shown in Figure2.

In the first two periods, influent ammonium-nitrogen concentration was 20±3 and 10±1 mgL^{-1}. The percent ammonium-nitrogen removals was 54% and 35% at the end of the cycle time of 3 hour, corresponding to 9.2 and 6.5mgL^{-1} effluent NH_4^+-N concentrations in period I and II, respectively (Figure 2A). These results indicated that ammonium could not be completely consumed by microorganisms responsible for denitrification in these operational conditions. However, nitrate was almost completely consumed in both periods, corresponding to about 3.5mgL^{-1} effluent nitrate concentration (Figure 2B). The nitrite formation occurred at the beginning of the reaction time indicating nitrate reduction took place. Afterwards, the amount of formed nitrite decreased sharply because microorganisms used nitrite as electron acceptors after nitrate consumed quickly in the system. Effluent nitrite concentration was not changed by decreasing ammonium-nitrogen concentration from 20 to 10 mgL^{-1} and reached about 3 $mgNO_2^-L^{-1}$at the end of the cycle time (Figure 2C). In the period III, influent ammonium-nitrogen concentration was decreased stepwise to 5 mg L^{-1} and it can be seen in Figure 2A that ammonium was never observed in the effluent liquid samples of SBR as of the first ten minutes of the reaction time.

Additionally, nitrite accumulation was not observed and the available nitrate in the system was wholly consumed at the end of 15-minute reaction time under this operational condition, indicating complete denitrification. In the last working period, nitrate was used only as both electron acceptor and nitrogen source required for microbiological growth of denitrifying bacteria. Nitrate removal efficiency of 96.5% was obtained at the end of the reaction time of 3h (Figure 2B). The approximate nitrite accumulation of 4 mgNO_2-L^{-1} was observed as a results of activity of denitrification microorganisms in anoxic environments (Figure 2C).

Ammonium and nitrate conversion rates were also determined for the four operational conditions tested in Figure 3A and 3B, respectively. Thereby, ammonium and nitrate measurements were used to define amount of ammonium-nitrogen or nitrate-nitrogen oxidized by one gram of mixed liquor suspended solid per minute and it was determined how the ammonia oxidation rate of the cultures changed. Figure 3 illustrates the changing ammonium and nitrate conversion rates under each of the operational conditions.

The ammonium and nitrate consumption rates of the cultures increased stepwise as the influent ammonium limited. The maximum ammonium consumption rate were attained with influent ammonium-nitrogen of 5 mg L^{-1}, being 18.4 mg NH_4^+-N $gMLSS^{-1}$ h^{-1}. Nitrate consumption rate had a tendency to increase with the limitation of influent ammonia, and the nitrate consumption rate reached maximum level at operational condition which ammonium is not present since nitrate was used as electron acceptor and sole nitrogen source required for microbiological growth, corresponding to 85.4 mg NO_3-N $gMLSS^{-1}h^{-1}$. Panthi and Wareham (2008) studied the effect of different arsenite concentrations on performance of denitrification process using volatile fatty acids as an external carbon source. The maximum specific denitrification rate of 14.16 mg NO_3-$NgVSS^{-1}h^{-1}$ (0.34 g NO_3-$NgVSS^{-1}d^{-1}$) was achieved at the constant carbon-to-nitrogen ratio of 3.0 and total cycle time of 4h in SBR without arsenite, corresponding to 6 times lower consumption rate compared to our study results. According to our study, the operational condition containing influent ammonium-nitrogen of 5 mg L^{-1} (period III) was found optimum for denitrification process when both the effluent nitrogen contaminants (Figure 2) and the ammonium/nitrate conversion rates (Figure 3) were taken into consideration.

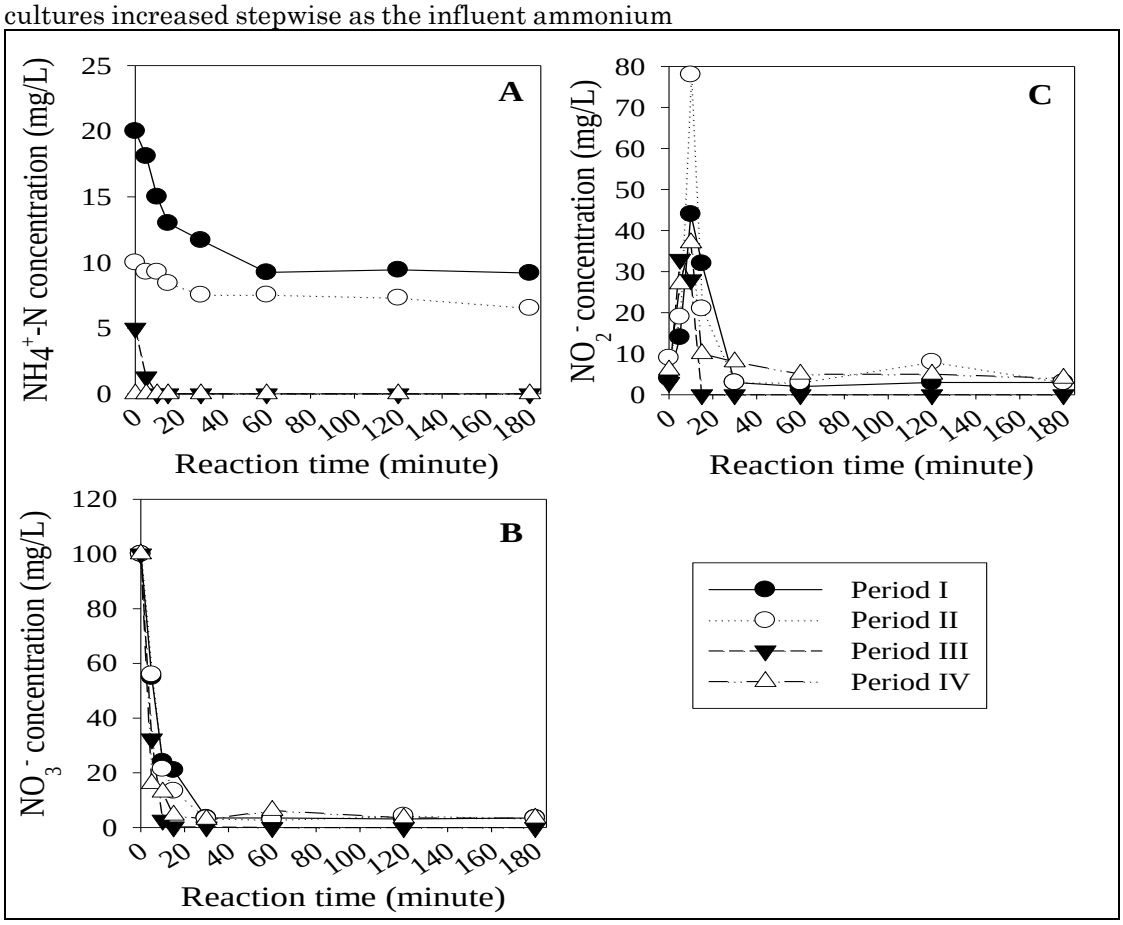

Figure 2. The profile of varying nitrogen forms

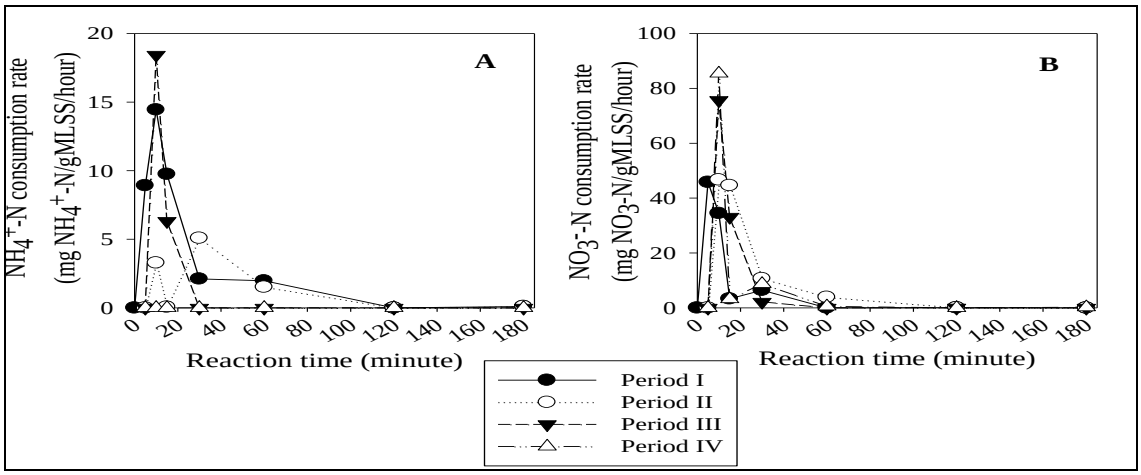

Figure 3. The profile of ammonium (A) and nitrate (B) consumption rates

DOC and pH profiles

In this study, acetate was used as the electron donor and carbon source for conventional denitrification process and acetate consumption was assessed by the DOC profile. Figure 4A shows the DOC removal performance of the SBR under varying influent ammonium concentrations. The influent DOC concentration was kept constant at 240 mgDOC L^{-1} during all study periods. In the first two periods, DOC removal yields reached about 15% at the end of the cycle time. The consumed carbon amount as a result of heterotrophic denitrification process was higher than stoichiometric value (24.2 mgDOC L^{-1}), corresponding to about 36 mgDOCL^{-1} of carbon amount consumed because fraction of substrate was used toward cell growth and maintenance energy. Maximum DOC removal efficiency was observed parallel to the increase in denitrification efficiency at the first 30 minute of total reaction times in the period III, corresponding to 40% removal efficiency (Figure 2 and 4A). The increase in DOC concentration during the remaining reaction time can be explained by endogenous respiration of microbial due to the absence of nitrogen in the system (Figure 2 and 4A). Afterwards, SBR was operated with ammonium absence condition in the last period and DOC formation was observed after the first ten minutes of reaction time. This result can be explain that most of the available nitrate in the system was used for microbial growth and operating conditions containing limited nutrients formed by nitrate depletion at the end of the first twenty minute reaction period caused the formation of an endogenous phase for microorganisms.

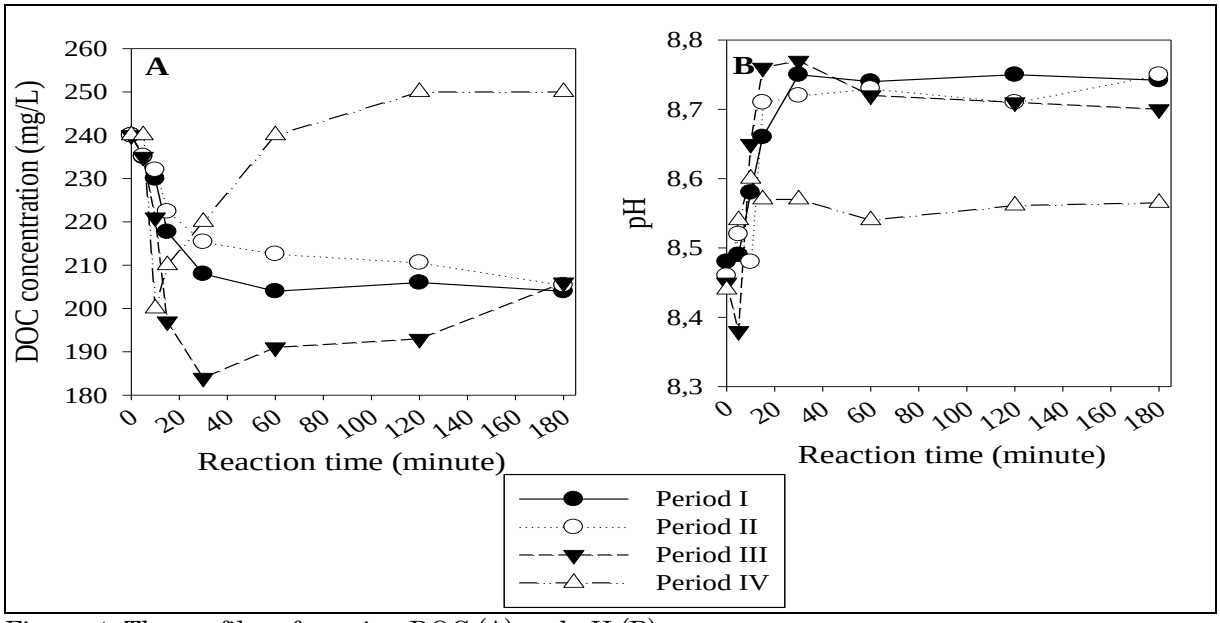

Figure 4. The profiles of varying DOC (A) and pH (B)

The pH was uncontrolled during reactor operation to observe the pH variations through biochemical reactions. The denitrification process, which is reduction of nitrate to nitrogen gas, is known to raise alkalinity thereby rising reactor pH. Hence, reduction of nitrate into nitrite is the main reaction responsible for the increase in pH level as was observed by other authors (Zhao et al., 2011). pH profile also showed almost similar behavior to total nitrogen removal profile and pH level of the reactor had a tendency to increase through reaction time (Figure 4B). However, a decrease in the pH profile was observed towards the end of the reaction in the period III indicating that denitrification was completed before reaction time ended and endogenous respiration started. At the ammonium-free conditions where nitrate was used for denitrification and microbial growth, pH remained at the lowest level as observed in DOC profile.

CONCLUSIONS

In this study, the effect of influent ammonium-nitrogen concentration on heterotrophic denitrification was investigated in the sequencing batch reactor. The high ammonium levels adversely affected the total nitrogen removal efficiency and incomplete ammonium consumption was occurred. The influent ammonium limitation in the SBR resulted in increasing NH_4^+-N and NO_3^--N consumption rates, corresponding to 18.4 mg NH_4^+-N $gMLSS^{-1}h^{-1}$ and 85.4 mg NO_3-N $gMLSS^{-1}h^{-1}$ maximum consumption rates. However, an increase in the effluent DOC concentration was observed at the ammonium-free conditions due to endogenous respiration arising from the absence of nitrogen. Additionally, the pH change was observed and the pH level increased during the reaction due to alkalinity formation and hydroxyl ion production resulted from the denitrification process. The results showed that influent ammonium played an important role on denitrification performance in the SBR and the influent ammonium concentration of 5 mgL^{-1} was found a favorable level for denitrification process.

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