Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 2, p. 189-197, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

Optimization of wastewater partial nitrification in Sequencing Batch Biofilm Reactor (SBBR) at fixed do level

Mehdi Hajsardar[1*], Seyed Mehdi Borghei[2], Amir Hessam Hassani[1], Afshin Takdastan[3]

[1]*Department of Environmental Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran*

[3]*Environmental Technologies Research Center, Ahvaz Jundishapour University of Medical Sciences, Ahvaz, Iran*

Article published on August 26, 2015

Key words: Fixed-Film bed reactor, SBBR, SND, NAR, Partial nitrification.

Abstract

A fixed-film bed reactor was studied to optimize partial nitrification in wastewater treatment. Nitrogen removal improvement was based on nitritation/denitritaion process in sequencing batch biofilm reactor (SBBR). Carbon source was added to the reactor at fixed dissolved oxygen (DO) concentration to modify simultaneous nitrification/denitrification (SND). In period 1, DO was declined from 1 to 0.5 mg/l during the reactor cycle. In period 2 and 3, DO was fixed at 0.5 mg/l and changing nitrite to nitrogen gas was achieved, directly, while nitrogen loading rate (NLR) and organic loading rate (OLR) were 0.18 kgN/m3d and 1.84 kgCOD/m3d, respectively. In period 2, the increment of nitrite accumulation rate (NAR) was observed but SND efficiency was reduced. C/N ratio was increased from 10 to 12.5 in period 3 to reach SND efficiency, at least, equal to the result of period 1. In period 3 NAR was 71.4 % and SND efficiency was 96%. Partial nitrification in SBBR at fixed DO level of 0.5 mg/l resulted in TN removal efficiency of 97.2 %. Effluent nitrite, nitrate and ammonium were 1.5, 0.6 and 0.7 mg/l, respectively, in period 3. In long term study of selected operation, SND efficiency was higher than 90 %.

***Corresponding Author:** Mehdi Hajsardar ✉ hajsardar@hotmail.com

Introduction

Biological nutrient removal has many advantages in contrast of other nutrient removal methods. (Metcalf and Eddy, 2003) Nitrogen removal from wastewater usually is done by nitrification and denitrification. (Chang *et al.*, 2011) Heterotrophic bacteria, namely *Pseudomonas*, are responsible for denitrification. This kind of bacteria needs a carbon source to convert nitrate to nitric oxide, nitrous oxide and nitrogen gas, respectively. (Metcalf and Eddy, 2003).

Many researches showed that simultaneous nitrification and denitrification (SND) would be achieved in one reactor by using some treatment processes. (Wang *et al.*, 2008; Ding *et al.*, 2011; Vijayalayan *et al.*, 2014; Ganesh *et al.*, 2015) In SND process, nitrification occurs on the outside of the biofilm while denitrification happens in the internal layer of biofilm. (Rittmann and Langeland, 2008) Direct equation of SND is presented in equation 1: (Wrage *et al.*, 2001).

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad \text{eq. 1}$$

In order to obtain SND condition, sequencing batch biofilm reactor (SBBR) could be used. SBBR is an SBR that is upgraded by adding fixed biofilm carriers. This way the capacity of the bioreactor to remove nutrients is increased. (Loukidou and Zouboulis, 2001; Rodgers *et al.*, 2008) Partial nitrification can improve SND efficiency in nitrogen removal. This process is achieved by fixing of pH, temperature, SRT, dissolved oxygen (DO) and online aeration balance. (Peng and Zhu, 2006; Sinha and Annachhatre, 2007) Partial nitrification provides 25 % less DO demand in nitrification and 40 % less carbon source demand in denitrificatin and could be combined with other processes such as Anammox. (Ciudad *et al.*, 2005; Cervantes, 2009; Wang *et al.*, 2015).

To remove nitrogen from wastewater by using mixed-culture, DO must be fixed between 0.5 to 2 mg/l. It is easier than providing pure-culture system. (Chu *et al.*, 2006; Chiu *et al.*, 2007) Two kinds of wastewater bacteria are important in partial nitrification. These two groups are ammonium oxidizing bacteria (AOB) and Nitrite oxidizing bacteria (NOB). AOB convert ammonium to nitrite at DO level of 3 mg/l and NOB convert nitrite to nitrate at a DO concentration of 1.1 mg/l. (Wiesman, 1994).

Aims of this study are optimization of wastewater partial nitrification by fixing DO concentration in a fixed-film bed reactor and survey the quality of using internal carbon source to complete nitrogen removal process. Presented method is classified as a biological nutrient removal.

This process was studied in an SBR that was upgraded to an SBBR. The results of this optimization are depended on C/N ratio because C/N affects on both TN removal efficiency and SND efficiency while DO concentration is fixed. So, C/N was studied as a significant factor to increase TN removal efficiency and SND efficiency, too.

In this study, as presented in Fig. 1, nitrification process was stopped on the step of nitritation (changing ammonium to nitrite) and nitrite was not allowed to be changed to nitrate. Then, after entering of internal carbon source, by denitritation, nitrite changed to nitrogen gas directly.

Internal carbon source was synthetic wastewater that was added to the reactor twice a cycle. In the other words, nitratation (changing nitrite to nitrate) and denitratation (changing nitrate to nitrite) was eliminated by fixing DO level in SBBR. Without nitrattation and denitratation, less oxygen and carbon source are needed, respectively. DO fixing helped that the activity of AOB continued, normally, but the activity of NOB limited, so nitrite accumulation rate (NAR) was increased in the system. Hence, NAR was considered as a suitable index to survey partial nitrification in SBBR. Higher NAR indicated lower activity of NOB but besides NAR increasing, maximizing of TN removal was one of the aims of the study.

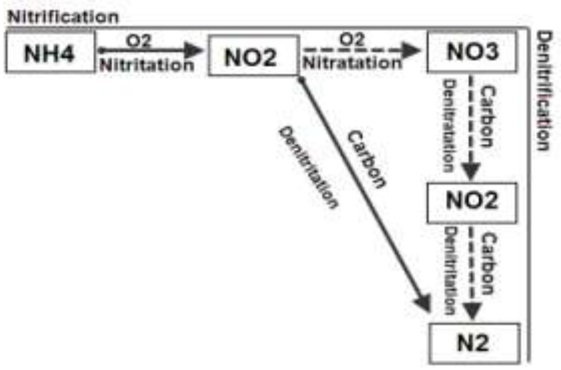


Fig. 1. Nitritation/Denitritation process concept for nitrogen removal.

Materials and methods

SBBR

An SBBR was made by adding fixed biofilm carriers (Kaldnes, K_2) to a cylindrical reactor, which was made of Plexiglas (table. 1). Biofilm media was made of high density polyethylene (HDPE) and its effective special area was 369.6 m^2/m^3. The bed was fixed between two plates which had holes allowed wastewater to pass. Fig. 2 shows a schematic representation of SBBR. Also, a sequencing batch reactor (SBR) without any biofilm carrier was used as the blank.

Table 1. SBBR properties.

Diameter (m)	Height (m)	Total volume (L)	Working volume (L)	Exchange ratio (%)	Packing media volume (L)	Height of bed from bottom (m)
0.24	0.68	31	28	100	12	0.3

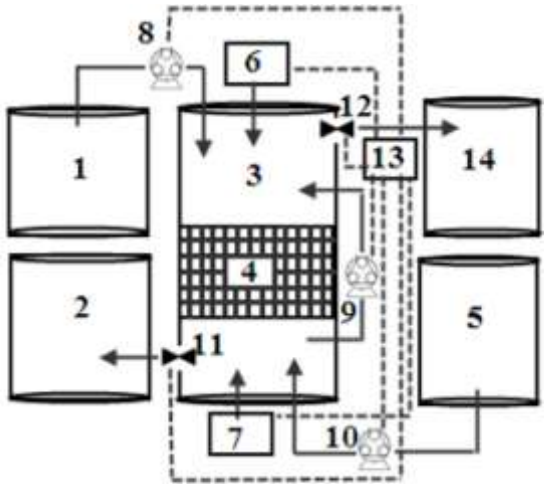


Fig. 2. Schematic diagram of SBBR. (1- influent tank; 2- effluent tank; 3- reactor; 4- fixed bed; 5- back wash tank; 6, 7- aerators; 8, 9, 10- pumps; 11, 12- solenoid valves; 13- timer; 14- back wash effluent tank.).

Analytical methods

In this study Mixed liquor suspended solids (MLSS), Total suspended solids (TSS) and Chemical oxygen demand (COD) were determined according to the standard methods for the examination of water and wastewater (APHA, 2005). Common forms of nitrogen including ammonium, nitrate and nitrite were analyzed with a spectrophotometer (HACH, Model DR 5000, USA). Ammonium and nitrate were determined according to Salicylate method and Cadmium reduction method, respectively. Nitrite was analyzed according to Ferrous sulfate method and Diazotization methods. Total Kjeldahl nitrogen (TKN) was started by using infra-red rapid digestor (behr Labor-Technik GmbH, Model, behrotest® InKjel 450 P, Germany), steam distillation unit (behr Labor-Technik GmbH, Model, behrotest® S 4 Germany), then, Manual titration showed the result. Organic nitrogen was determined from the difference between TKN and ammonium levels. DO, pH and oxidation-reduction potential (ORP) were analyzed with a portable DO Meter (WTW GmbH, Model ProfiLine Oxi 3210, Germany), a portable pH meter (HANNA instruments, Model, HI 991001, USA) and a portable ORP meter (EUTECH, Model, Oakton ORPT estr 10, Singapore), respectively.

Reactor start-up and operation

At first, 5 l seed was brought from return activate sludge of Choneybe wastewater treatment plant (Ahvaz, Iran), then, 40 days was needed for microorganisms to adapt themselves to the new environment. Synthetic wastewater was added to fill the reactor in a few days. The concentration of COD, total nitrogen (TN), ammonium, organic nitrogen, TKN, nitrate and nitrite in Synthetic wastewater were 1000, 100, 65, 34, 99, 0.5 and 0.3 mg/l respectively. At the beginning, the reactor was operated at a nitrogen loading rate (NLR) of 0.027 kgN/m3d and an organic loading rate (OLR) of 0.27 kgCOD/m3d. During the start-up, NLR and OLR were increased to 0.18 kgN/m3d and 1.84 kgCOD/m3d, respectively, by increasing the concentration of influent nitrogen and COD and reducing the retention hydraulic time (HRT). HRT and solid retention time (SRT) were 24 h and 55 d, respectively. The concentration of influent and effluent ammonium, also, applied NLR during the start-up of the reactor is described in Fig. 3. The operation of SBBR was divided into three periods: period 1, period 2 and period 3. The operation of a cycle of SBBR after reaching steady flow is presented in Fig. 4. It is noticed that the difference between period 2 and period 3 was the amount of C/N ratio.

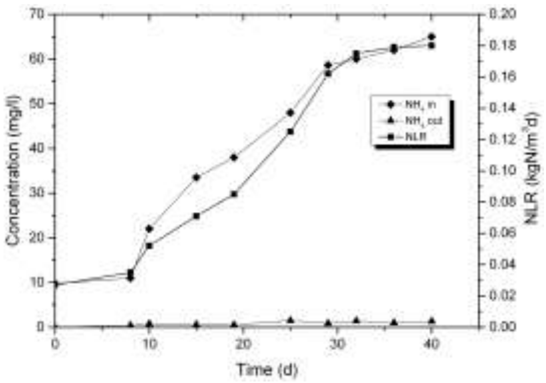


Fig. 3. NLR and the concentration of influent and effluent ammonium during start-up of the reactor.

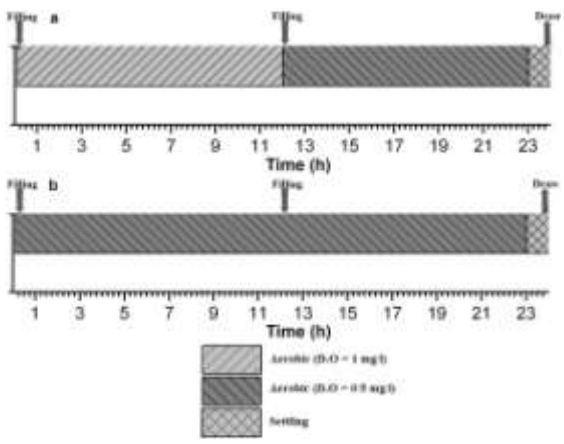


Fig. 4. SBBR cycle: (a) during period 1 (b) during period 2 and period 3.

Results and discussion

The results of the blank reactor (SBR) showed that TN removal efficiency was 65 %. The blank had a cycle similar to SBBR but biofilm carriers were not added to the reactor. SND efficiency according to equation 2, (Zeng *et al.*, 2003) was 59 % and NAR according to equation 3, was 2.9 % in the blank. SND

$$\text{Efficiency (\%)} = \frac{Denitrification}{Nitrification} = \frac{NH_4(tot) - NO_x(acc)}{NH_4(tot)} \times 100 \quad \text{eq. 2}$$

Where:

SND efficiency: the efficiency of simultaneous nitrification/ denitrification (%)

NH_4 (tot): the concentration of inlet ammonium (mg/l) NO_x (acc): the concentration of sum of outlet nitrate and nitrite (mg/l)

$$\text{NAR (\%)} = \frac{NO_2}{NO_2 + NO_3} \times 100 \quad \text{eq. 3}$$

Where:

NAR: nitrite accumulation rate (%)

NO_2: the concentration of outlet nitrite (mg/l)

NO_3: the concentration of outlet nitrate (mg/l)

Period 1

In period 1 SBBR was operated with two aeration mode. At the first, 12 hours at DO level of 1 mg/l and then 11 hours at DO level of 0.5 mg/l. As presented in table 2, NAR was 13.63 %. NAR was increased by 10.65 % in comparison to the blank and it was shown that the activity of NOB was limited due to DO limitation in depth of the biofilm and less nitrite was changed to nitrate. Ammonium, nitrate and nitrite in the effluent were 0.8, 1.9 and 0.3 mg/l, respectively, in period 1 that met the standards recommended by Iran department of environment. (DOE, 1994) In period 2, TN removal efficiency was 97 % and it was 32 % higher than the blank. Also, SND efficiency was 96.6 % that was 37.6 % higher than the blank. Fig. 5.a shows the evolution of ammonium, nitrate and nitrite during a typical cycle of SBBR in period 1. As seen, after 12 hours of aerobic reaction (DO of 1 mg/l), the concentration of nitrate and ammonium were shifted instantly because of entering wastewater as internal carbon source. In second aerobic step (DO of 0.5

mg/l) that lasted 11 hours, denitritation was happened in last 4 hours and in 1 hour anoxic settling.

Table 2. General results of SBBR and SBR.

Reactor	SBR	SBBR	SBBR	SBBR
Period	Blank	Period 1	Period 2	Period 3
SND efficiency (%)	59	96.6	92	96.7
NAR (%)	2.9	13.6	68	71.4
C/N	10	10	10	12.5
NLR (kgN/m3d)	0.18	0.18	0.18	0.18
OLR (kgCOD/m3d)	1.84	1.84	1.84	2.29
TN removal efficiency (%)	65	97	94	97.2
DOi+ DOii+ (mg/l)	10.5	10.5	0.5 0.5	0.5 0.5
Fixed-Film media	No	YES	YES	YES

+DOi: DO concentration in first aerobic step; DOii: DO concentration in second aerobic step.

Fig. 5.b shows ORP curve that was drawn versus time in a typical cycle of SBBR in period 1. ORP curve versus time is rising under aerobic condition until inflection point which is named α and indicates end of nitrification. (Paul, 1998) In this study nitrification was not completed because of partial nitrification and when ammonium was changed to nitrite, nitrification was stopped by fixing DO. Fig. 5.b shows that no inflection point was observed in ORP curve in period 1. Neither when DO was 1 mg/l nor when DO was 0.5 mg/l, α point was identified. This, approved that partial nitrification was achieved in SBBR to remove common nitrogen forms such as ammonium, nitrate and nitrite.

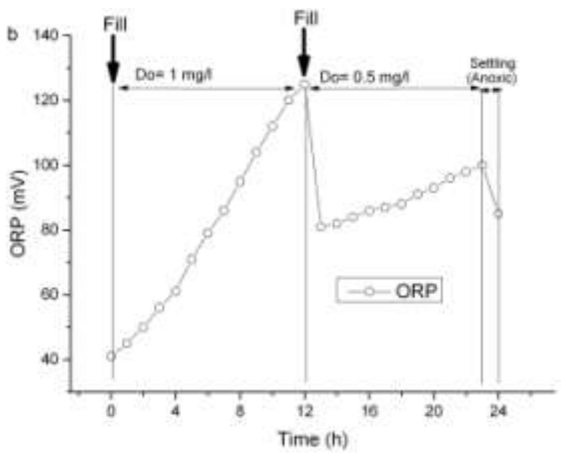


Fig. 5. (a) Ammonium, nitrate and nitrite in a typical cycle of SBBR in period 1 (b) ORP curve versus time in a typical cycle of SBBR in period 1.

Period 2

DO was constant (0.5 mg/l) in whole aeration time of period 2. At first, nitritation was happened and influent ammonium concentration of 65 mg/l was converted to nitrite. Then, before changing to nitrate, synthetic wastewater was added to SBBR as internal carbon source. Therefore, nitrite was changed to nitrogen gas by denitritation. Nitrate concentration was 3.3 mg/l at the end of the cycle of period 2.

Effluent nitrite was higher than effluent nitrate in this period and it was shown that partial nitrification was achieved. NAR was determined 68 % in period 1. It was 54.41 % higher than determined NAR in period 1. On the other hand, SND efficiency and TN removal efficiency were decreased in period 2 in contrast of period 1. SND efficiency and TN removal efficiency were 92 % and 94 %, respectively.

The effluent Ammonium and nitrate concentration were 1.15 and 0.55 mg/l, respectively, in period 2. These two parameters were higher than period 1. Fig. 6.a shows the evolution of ammonium, nitrate and nitrite during a typical cycle of SBBR in period 2. As Fig. 6.a describes, nitrite was decreased at the end of first aerobic phase (DO of 1 mg/l) similar to period 1 but when carbon source was added, nitrite raised in second aeration phase (DO of 0.5 mg/l) of period 2. The concentration of nitrite reached to 19 mg/l after 17 hours from the start of the cycle.

This was because of the limitation of the activity of NOB and less nitrite was changed to nitrate in period 2. This is why NAR was increased to 68 % in this period. Also, fig 6.b shows that no inflection point of α point was detected in ORP curve in period 2 similar to period 1. It showed that nitrification was not completed in period 2, too, and partial nitrification was happened. The results of period 2 showed that nitritation/ denitritation can be a short-cut way for nitrogen removal through increment of NAR but 3 % decreasing of TN removal efficiency rather than period 1, must be remedied.

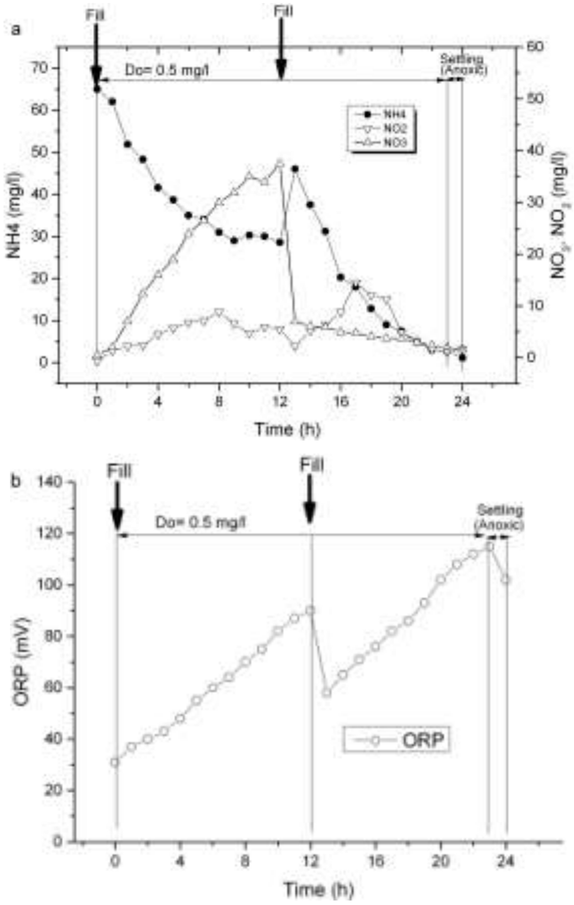
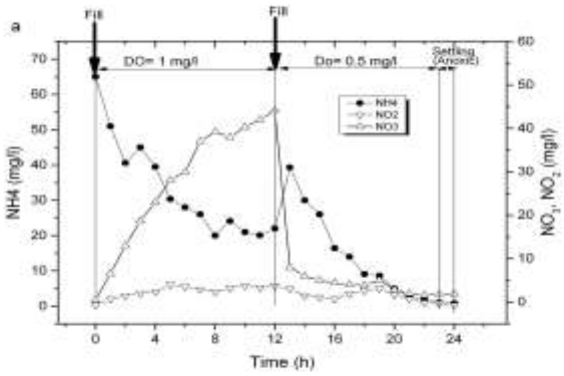


Fig. 6. (a) Ammonium, nitrate and nitrite in a typical cycle of SBBR in period 2 (b) ORP curve versus time in a typical cycle of SBBR in period 2.

Period 3

In period 3, C/N ratio was increased to increase TN removal efficiency and SND efficiency at constant Do level of 0.5 mg/l. In other words, the purpose of study in period 3 was to reach TN removal efficiency and SND efficiency, at least, equal to the results of period 1 that DO was decreased in two steps from 1 to 0.5 mg/l. As seen in Fig.7, by increasing C/N ratio from 10 to 12.5 in SBBR operation, TN removal efficiency increased from 94 to 97.2 %. Too High C/N ratios, for example 22, would decrease TN removal in SBBR operation down to 38 %. (Ding *et al.*, 2011). NAR was 71.4 % in period 3 that was the maximum determined NAR in this study. Also, SND efficiency was 96.7 % in this period and unlike the results of period 2, SND efficiency was higher than period 1. The increment of SND efficiency was because of fixing the C/N ratio. At higher C/N ratios, there is more organic matter as electron donor and less nitrate as electron acceptor so there is less challenge for acceptation of electron and denitritation is more successful. (Metcalf and Eddy, 2003). Long term results of selected method are presented in Fig. 8. To study the long term effect of partial nitrification, SBBR was operated for 30 day in period 3 at the C/N ratio of 12.5. NAR, SND efficiency and TN removal efficiency were not below 65, 90 and 93 %, respectively, in 30 days operation of the reactor at DO level of 0.5 mg/l. The best results of long term study in period 3 were detected at days number 1, 2, 3, 6, 7, 11, 12, 18 and 23 when TN removal efficiency was higher than the result of period 2 (97 %).



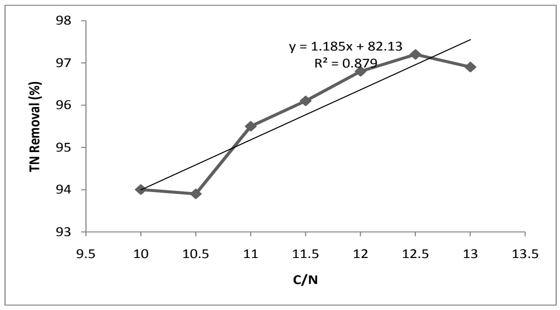


Fig. 7. TN removal efficiency vs. C/N ratio in period 3.

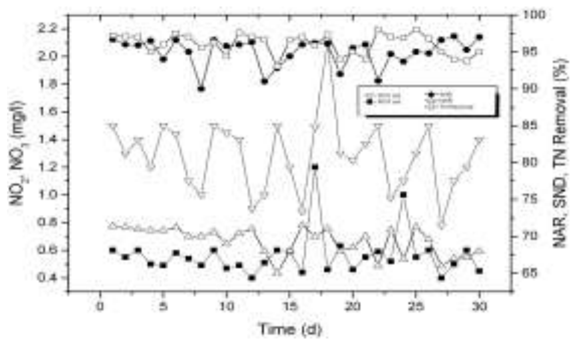


Fig. 8. NAR, SND efficiency, TN removal efficiency, outlet nitrate and outlet nitrite in long term study of period 3.

ORP curve in period 3 (Fig. 9) had no inflection point of α and it was because of partial nitrification similar to previous periods. Indeed, pH curve in Fig. 9 indicates that nitrification was not completed in this period.

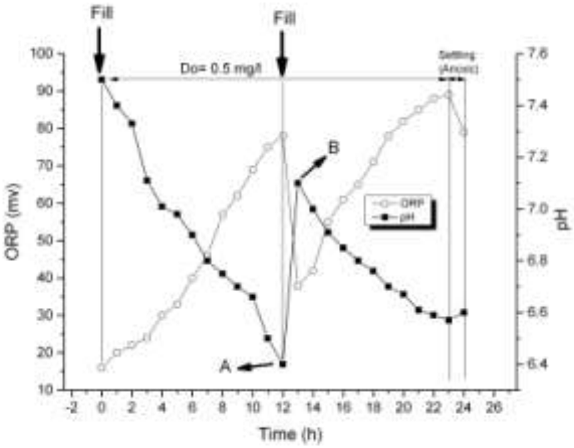


Fig. 9. ORP curve and pH curve versus time in a typical cycle of SBBR in period 3.

A few researches have studied pH profile under aerobic condition to find a minimum point which is named Ammonia Valley and shows the end of nitrification. Before Ammonia Valley pH is decreasing and after that pH is rising. Another point that has been studied in pH curve under anoxic condition is named Nitrate Apex and corresponds to the end of nitrate consumption. Before Nitrate Apex pH profile is rising and after this point pH is decreasing. (Martin de la Vega *et al.*, 2012; Zanetti *et al.*, 2012) Point A and point B in Fig. 9 could not be Ammonia Valley and Nitrate Apex, respectively. Rising of pH profile after point A happened because of second filing event of SBBR. Besides, inflection point of B was not happened under anoxic condition. These evidences showed that partial nitrification at DO level of 0.5 mg/l and C/N ratio of 12.5 had the main role in increment of nitrogen removal efficiency.

Presented method in this study provided TN removal efficiency of higher than 97%. It was an easier way for nitrogen removal in contrast of a number of new researches that considered partial nitrification as a part of their study. Reported TN removal efficiency was 98% by applying one hour aeration over a four-hour cycle by using simultaneous nitrification-anammox-denitrification (SNAD) and considering periodic aeration cycles. (Abbassi *et al.*, 2014) TN removal efficiency was reported 96% when sufficient zinc (50-100 mg/l) was present in wastewater treated by simultaneous partial nitrification, anammox and denitrification process in SBBR. (Daverey *et al.*, 2014).

Also, in this study required condition for SND was provided by adding internal carbon source through two filling events and using biofilm carries in SBBR. In a research to survey SND, it was reported that TN removal efficiency was 54% with no external carbon source and it was 70% by adding external carbon source of 20 mg/l total organic carbon (TOC). (Vijayalayan *et al.*, 2014) It was reported that using a biofilm reactor to reach SND without adding external carbon source resulted in 88.9 ± 6.1% TN removal efficiency. (won *et al.*, 2015).

Accordingly, the results of this study to reach partial nitrification and providing SND condition in SBBR were almost equal to the results of other new researches. Indeed, the results were better than a number of other studies while presented method had less difficulty in operation.

Conclusion

Nitrogen removal efficiency was improved by upgrading SBR to SBBR through adding fixed biofilm carriers. SND efficiency was considered as indicator of SND process quality in SBBR. SND efficiency was optimized by using partial nitrification method and considering twice filling events per cycle of the reactor as adding internal carbon source. Nitritation/ Denitritation at constant DO concentration during a cycle of SBBR was a short-cut way that increased NAR by limiting NOB activity. Constant DO level increased NAR but decreased SND efficiency and TN removal efficiency rather than when DO was reduced during a cycle. Increasing C/N ratio at constant DO level resulted in optimization of SND efficiency and TN removal efficiency. NAR, SND efficiency and TN removal efficiency were maximized at reasonable maximum C/N ratio and fixed reasonable minimum DO level, simultaneously.

References

Abbassi R, Yadav Ak, Huang S, Jaffe PR. 2014. Laboratory study of nitrification, denitrification and anammox processes in membrane bioreactors considering periodic aeration. Journal of Environmental Management **142,** 53-59.

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association/American Water works Association/ Water Environment Federation, Washing-ton DC, USA: APHA-AWWA-WEF Publishing.

Cervantes FJ. 2009. Environmental technologies to treat nitrogen pollution, Principles and engineering. London, UK: IWA Publishing.

Chang CY, Tanong K, Xu J, Shon H. 2011. Microbial community analysis of an aerobic nitrifying-denitrifying MBR treating ABS resin wastewater. Bioresource Technology **102,** 5337-5344.

Chiu YC, Lee LL, Chang CN, Chao AC. 2007. Control of carbon and ammonium ratio for simultaneous nitrification and denitrification in a sequencing batch bioreactor. International Biodeterio-ration and Biodegredation **59,** 1-7.

Chu L, Zhang X, Yang F, Li X. 2006. Treatment of domestic wastewater by using a microaerobic membrane bioreactor. Desalination **189,** 181-192.

Ciudad G, Rubilar O, Munoz P, Ruiz G, Chamy R, Vergara C, Jeison D. 2005. Partial nitrification of high ammonia concentration wastewater as a part of a shortcut biological nitrogen removal process. Process Biochemistry **40,** 1715-1719.

Daverey A, Chen YC, Sung S, Lin JG. 2014. Effect of zinc on anammox activity and performance of simultaneous partial nitrification, anammox and denitrification (SNAD) process. Bioresource Technology **165,** 105-110.

Ding D, Feng C, Jin Y, Hao C, Zhao Y, Suemura T. 2011. Domestic sewage treatment in a sequencing batch biofilm reactor (SBBR) with an intelligent controlling system. Desalination **276,** 260-265.

DOE. 1994. Iranian effluent discharge standards. Research department of environmental protection organization of Iran (DOE), Tehran, Iran: DOE Publishing.

Ganesh R, Sousbie P, Torrijos M, Bernet N, Rmanujam RA. 2015. Nitrification and denitrification in a sequencing batch reactor treating tannery wastewater. Clean Technologies and Environmental Policy **17,** 735-745.

Loukidou MX, Zouboulis Al. 2001. Comparison of two biological treatment process using attached-growth biomass for sanitary landfill leachate treatment. Environmental Pollution **111,** 273-281.

Martin de la Vega PT, Martinez de Salazar E, Jaramillo MA, Cros J. 2012. New contributions to the ORP and DO time profile characterization to improve biological nutrient removal. Bioresource Technology **114,** 160-167.

Metcalf and Eddy. 2003. Wastewater engineering: Treatment and reuse. Fourth ed. Boston, USA: McGraw-Hill Publishing.

Paul R, Pisson-Saune S, Mauret M, Cantet J. 1998. Process state evaluation of alternating oxic-anoxic activated sludge using ORP, Ph and DO, Water Science and Technology **38,** 299-306.

Peng Y, Zhu G. 2006. Biological nitrogen removal with nitrification and denitrification via nitrite pathway. Applied Microbiology and Biotechnology **73,** 15-26.

Rittmann BE, Langeland WE. 1985. Simultaneous denitrification with nitrification in single-channel oxidation ditches. Journal of Water Pollution Control Federation **57,** 300-308.

Rodgers M, Wu G, Zhan X. 2008. Nitrogen and phosphorus removal from domestic strength synthetic wastewater using an alternating pumped flow sequencing batch biofilm reactor. Journal of Environ-mental Quality **37,** 977-982.

Sinha B, Annachhatre A. 2007. Partial nitrification-operational parameters and microorganisms involved. Reviews in Environmental Science and Bio/Technology **6,** 285-313.

Vijayalayan P, Thanh BX, Visvanathan C. 2014. Simultaneous nitrification denitrification in a Batch Granulation Membrane Airlift Bioreactor. Interna-tional Biodeterioration and Biodegradation. **95,** 139-143.

Wang J, Peng Y, Wang S, Gao Y. 2008. Nitrogen removal by simultaneous nitrification and denitrification via nitrite in a sequencing hybrid biological reactor. Chinese Journal of Chemical Engineering **16,** 778-784.

Wang Y, Wang Y, Wei Y, Chen M. 2015. In-situ restoring nitrogen removal for the combined partial nitritation-anammox process deteriorated by nitrate build-up. Biochemical Engineering Journal **98,** 127-136.

Wrage N, Velthof GL, van Beusichem ML, Oenema O. 2001. Role of nitrifier denitrification in the production of nitrous oxide. Soil Biology and Biochemistry **33,** 1723-1732.

Wiesman U. 1994. Biological nitrogen removal from wastewater. Advances in Biochemical Engineering/ Biotechnology **51,** 113-154.

Won SG, Jeon DY, Kwag JH, Kim JD, Ra CS. 2015. Nitrogen removal from milking center wastewater via simultaneous nitrification and denitrification using a biofilm filtration reactor. Asian-Australasian Journal of Animal Sciences **28,** 896-902.

Zanetti L, Frison N, Nota E, Tomizioli M, Bolzonella D, Fatone F. 2012. Progress in real-time control applied to biological nitrogen removal from wastewater. A short review. Desalination **286,** 1-7.

Zeng RJ, Lemaire R, Yuan Z, Keller J. 2003. Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. Biotechnology and Bioengineering **84,** 170-178.