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Using Excess Biomass Sludge Acclimated in Side Stream Partial Nitrification to Evaluation Partial Nitrification and Abrupt Cold Shock Effect in main stream

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Abstract

In this study biomass acclimation in a side stream partial nitrification sequencing batch reactor (SBR) was performed under optimum conditions (T 30ºC, SRT 9± 1 days and hydraulic retention time (HRT) 1.2 day). Excess sludge for solids retention time (SRT) regulation was added to another batch reactor (main stream) which performed under different conditions for partial nitrification evaluation and calculating temperature dependency factor. The results of main stream reactor showed that temperature was an important factor that affected specific ammonia oxidation rate (sAOR) after cold shock by about 98.5%. It was demonstrated that abrupt cold shock caused a significant reduction in the sAOR; up to 25, 55 and 78% for temperature reduction from 30 to 25, 20 and 15ºC, respectively. Therefore, the temperature dependency factor was calculated about 1.0965 and 1.106 based on indirect and direct comparison, respectively. NO_2/ NO_x ratio analysis showed that temperature, initial ammonia, MLVSS and time affected nitrite accumulation ratio by 49.7, 14.6, 3.7 and 30.7%, respectively. Results indicated that maximum NO_2/ NO_x ratio was about 94.9% and was reduced to 89.4% after 270 minutes.

Key words: AOB cultivation, Cold shock effect, Temperature dependency factor, Partial nitrification, Side stream reactor, Abrupt cold shock, Bio- augmentation

Highlights

- Using of excess biomass acclimated in partial nitrification process for SRT regulation could be an effective way to partial nitrification in different conditions.
- The effect of abrupt cold shock in partial nitrification process is much more than gradual shock.
- Unlike previous studies on temperature dependency factor determination, in present study this coefficient was calculated on partial nitrification instead of nitrification.
- The temperature dependency coefficient for partial nitrification process was calculated about 1.0965 and 1.106 for indirect and direct comparisons, respectively.

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Introduction

Nitrogen removal from wastewater is gaining a lot of attention due to its potential threat to environment and especially in water. Therefore treatment of nitrogen in ammonia form has become a significant field of research during the last decade (1 & 2). However, from environmental and economical point of view, Biological Nitrogen Removal (BNR) could be more interesting for treating strong nitrogen wastewater (1, 3 & 4). The conventional BNR involves two processes: (1) ammonium oxidation to nitrite and immediately to nitrate during the aerobic nitrification process and (2) the subsequent nitrate reduction to molecular nitrogen gas during anoxic denitrification (5). Needs of oxygen and organic matter in first and second processes, respectively, caused increase in the operational costs (5). Hence, to overcome these restrictions, some alternatives to the conventional BNR have been developed such as partial nitrification, simultaneous nitrification and denitrification, anaerobic ammonium oxidation (Anammox), and completely auto- trophic nitrogen removal over nitrite (Cannon) (5 & 6)

Some researchers have pointed the beneficial effects of performing BNR process via nitrite as compared to the traditional nitrification- denitrification through nitrate with the following: (2, 7- 9):

1) Saving 25% of the oxygen consumption implies 60% energy savings;

2) Reduce 40% chemical oxygen demand (COD) requirement in the denitrification;

3) Enhance nitrite reduction rates 1.5- 2 times in the denitrification stage.

4) Reduce 20% carbon dioxide emission during the denitrification

To perform partial nitrification, dissolved oxygen (DO), pH, ammonia concentration and temperature are most important. Some authors have reported that, at reduced dissolved oxygen concentrations, ammonia oxidizing biomass (AOB) is favoured over nitrite oxidizing biomass (NOB) (1, 7 & 9- 11). This is due to a greater oxygen affinity for the first step of nitrification. The critical values of DO recorded in previous studies were different and should be maintained about 0.8–1.5 mg/ L (3 & 9).

Moreover, temperature is one of the important and serious factors in biological wastewater treatment especially in nitrification process. This factor affects reaction rate and growth rate of biomass (9, 12 & 13). Only at temperatures above 25°C it is possible that the AOB effectively compete NOB (7, 9 & 14- 16). To overcome this problem, bio- augmentation in side stream reactor could be used. Bio-augmentation is one of the possible methods to promote the nitrification via nitrate or nitrite in main stream (17- 19). In wastewater treatment plants, high ammonia reject water could be used to enhance nitrification activity by or without bio-augmentation. Several strategies have been proposed such as directly mixing reject water with the return sludge (Which is commonly used) or adding excess sludge from the reject water treatment reactor, which is known as the bio- augmentation process (17 & 19). By using recent strategy (bio- augmentation) and under optimum conditions (e.g. T= 30^{o}C), AOB could be cultivated. Then excess sludge can be used in other conditions (e.g. low temperature) for partial nitrification. In this case, effect of temperature after cold shock on process rate can be expressed by Arrhenius equation (Equation 1) ; where, r_T and r_{T0} are nitrification rate in T and T_0 as the new and pervious temperature, respectively. Each biomass including AOB and NOB has its own temperature dependency factor (θ) (13, 19 & 20). Different temperature dependency factor which was calculated in granular or abrupt cold shock is summarised in Table 1 for nitrification process.

$$r_T = r_{T_0}\theta^{(T-T_0)} \quad (1)$$

Table 1- Temperature dependency factor in different study (19, 21)

Researchers	dependency factor (/ ° C)
Downing and Hopwood	1.127
US environmental protection agency	1.103
Painter and Default	1.0756
Biowin Default	1.096
Jones	1.072
Head and Oleszkiewicz	1.088
Hwang and Oleszkiewicz	1.116

Head and Oleszkiewicz evaluated cold shock effects upon first steps of nitrification rates (ammonia oxidation) (19). They added biomass which was acclimated in different temperatures (e.g. T= 30ºC) into new environment (e.g. T= 10ºC). Then, they determined the impact of sudden decrease in temperature on first step of nitrification rates. In their study, biomass which was produced during warm nitrification process was added into cold SBR at various hydraulic retention times. They showed that the average decreases in nitrification rates were 58, 71 and 82% for biomass cooled to 10°C when the biomass was acclimated in 20, 25 and 30°C, respectively (19). Also, Hwang and Oleszkiewicz evaluated effect of cold temperature shock on nitrification rate by using Equation 1 (21). They investigated the effect of temperature on nitrification rate by a sharp temperature decreasing. In addition, they compared this effect with gradual temperature decreasing. They found that the immediate temperature decreasing by 10 degrees (ºC), led to a 20% larger decreasing in nitrification rate than predicted by 1.072 which is more accepted as temperature dependency factor (21). In both studies, direct comparison was used to determined temperature dependency coefficient. In direct comparison, the effects of other factors except temperature which affect the process aren’t eliminated. For the resolution of this problem, many experiments were defined and were done to calculate real dependency coefficient. To perform these experiments, Taguchi method was used.

Taguchi method is an experimental design (fractional) and analysing method which is used in many fields such as water and wastewater treatment (22 & 23). In this method, some of experiments were done according to different levels of the studies factors. Then S/ N and ANOVA analysing were used to calculatr statistical index such as variance, F- ratio and percent influence of the variables. In the last step, ignored experiments could be estimated (24). Finally and for temperature dependency factor of calculating by indirect method, affecting all factors except temperature was ignored.

In this work, after partial nitrification in a side stream SBR for partial nitrification and AOB cultivation, a series of batch partial nitrification tests with various combinations were conducted in a main stream reactor. Four factors included temperature, initial ammonia, MLVSS and time were considered. Thus the objectives of this research were threefold: (1) study the effect of mentioned factors on partial nitrification especially after cold shock; (2) nitrite accumulation investigation; and (3) temperature dependency coefficient calculation by direct and indirect comparison for partial nitrification.

For investigation of these objectives, specific ammonium oxidation rate (sAOR) and NO_2/ NO_x ratio were used (Equation 2 and 3) (1, 3, 14, 21, 25 and 26). sAOR was used to express nitrification rate and for evaluation of abrupt cold shock effects. Moreover, NO_2/ NO_x ratio was applied to explain nitrite accumulation percentage and partial nitrification evaluation.

$$\text{sAOR}\ (\text{mg N–}NH_4^+/\ \text{gr VSS.hr}) = \frac{NH_4^+\ \text{Oxidize as N}}{\text{MLVSS} \times \theta_{ox}} \times 1000 \quad (2)$$

$$NO_2^-/NO_x^-\ (\%) = \frac{NO_2^-\ \text{produce as N}}{(NO2^- + NO3^-)\ \text{as N}} \times 100 \quad (3)$$

Material and Method

Substrate and Acclimation Process: Synthetic wastewater which was used in this study is similar to reject water of wastewater treatment plant. For synthetic wastewater preparation, effluent from the wastewater treatment plant (WWTP) of Shahin- Shahr city in Isfahan province of Iran was used. This wastewater regulated by addition of about 900 mg N/ L as NH_4Cl, 1500 $mgCODL^{-1}$ as beef extract, 1-1.1/ 1 mol HCO_3^-/ mol N ratio as $NaHCO_3$, 20 mg P/ L as KH_2PO_4 (2 & 3).

The SBR which was operated in this study was inoculated with sludge from a secondary reactor of the municipal WWTP. After about one month of acclimation with no sludge withdrawal, the biomass was mixed with the secondary sludge from the WWTP for simultaneous denitrification. Then, final sludge inserted into SBR as a side stream process to treat synthetic wastewater in 8 hours cycle's under optimum conditions for partial nitrification based on Table 2. The lab- scale SBR and other automatic equipment for regulation of temperature and DO, filling and decanting, external carbon source injection are shown in Fig. 1- a.

Table 2- Optimum conditions used in side stream SBR reactor for partial nitrification (3)

Parameter	unit	value
NH_4^+as N	mg N/ L. cycle	250
HRT	day	1.2
pH	-	7.5- 8.5
DO	mg/ L	0.8- 1.2
T	ºC	30± 1
SRT	day	9± 1
Cycle	Number/ day	3
sub cycle (aerobic-anoxic)	Number/ cycle	3

Batch tests and different conditions: After steady state conditions in the side stream SBR, some of excess sludge for SRT regulation was added to a batch reactor that operated under different conditions for partial nitrification evaluation including abrupt cold shock effect assessment (Fig. 1- b). Experiments were done at different conditions by changing in initial ammonia, MLVSS, time and temperature based on Taguchi design. These factors and their levels that cover a wide range of conditions are summarized in Table 3.

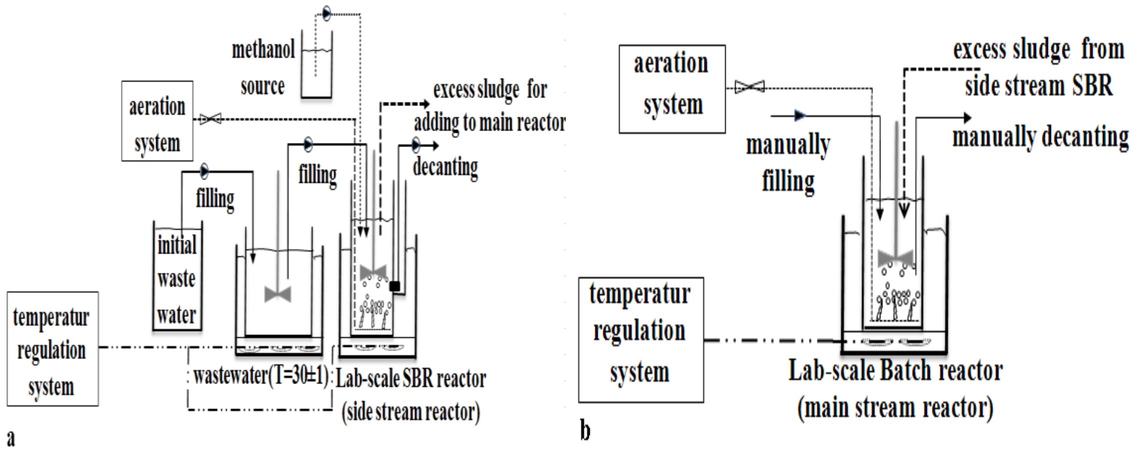

Fig. 1- a) Lab- scale side stream SBR reactor, b) Lab- scale main stream batch reactor

Table 3- Factors & its levels in partial nitrification batch tests

Factor \ Levels	1	2	3	4
Factor A:Temperature (ºC)	30± 1	25± 1	20± 1	15± 1
Factor B:Initial ammonia (mg NH_4^+- NL^{-1})	50± 5	75± 5	100± 5	150± 5
Factor C:MLVSS (mg $VSSL^{-1}$)	1250± 10	1000± 10	750± 10	500± 10
Factor C:Time (minute)	60	120	180	270

Design of experiments and data analysis: In order to reduce batch tests, experimental design by Taguchi method was used. Based on this method, the factors and their levels, an L- 16 array were designed and selected.

Based on experimental design and L- 16 array, S/ N (signal/ noise) analysis and ANOVA by Qualitek4 (QT4) software were done and contribution of any factors on results was calculated. Finally eliminated experiments were estimated (24)

Analytical methods: NH_4^+- N was measured by Nesler and spectro-photometric method in 410 nm. (DR 4000, Hach Co., USA) (27). Nitrite and nitrate were measured by spectro- photometric method (DR 4000, Hach Co., USA) (28). MLVSS were measured according to the Standard Methods (27). DO concentration and pH were measured by DO meter (Oxi 340i- WTW) and pH meter (pHs- 25cw; microprocessor pH/ mv meter, LIDA, China), respectively. sAOR and NO_2/ NO_x ratio were calculated based on Equations 2 and 3.

Results

Partial nitrification & AOB cultivation in side stream SBR: Partial nitrification was done under steady state conditions based on optimum environment which was summarized in Table 1. Based on this strategy, the results of ammonia and nitrite oxidation and nitrate formation in the 8 hours cycle and under steady state conditions are shown in Fig. 2.

Main stream process evaluation in different conditions: Excess daily sludge from side stream SBR was added to another batch reactor (main stream reactor) in different conditions based on Table 3 and L- 16 array. Then sAOR and NO_2/ NO_x

ratio were calculated and analysis by S/ N and ANOVA as follows:

sAOR analysis results: Table 4 shows results of sAOR analysis in main stream reactor. Also Fig. 3 shows sAOR after cold shock with and without removing the effects of other factors except temperature (indirect and direct compassion). Moreover, this figure illustrate reduction on sAOR by θ=1.072 as an accepted temperature dependency coefficient. Based on sAOR reduction, exponential trend line was used to calculate temperature dependency coefficient (Fig. 4).

NO_2/ NO_x ratio analysis results: Results of NO_2/ NO_x ratio analysis as an important index for demonstrating nitrite accumulation ratio and partial nitrification after ANOVA are shown in Table 4. Also Fig. 5 displays the effect of time and temperature on NO_2/ NO_x ratio after adding excess sludge to main stream reactor.

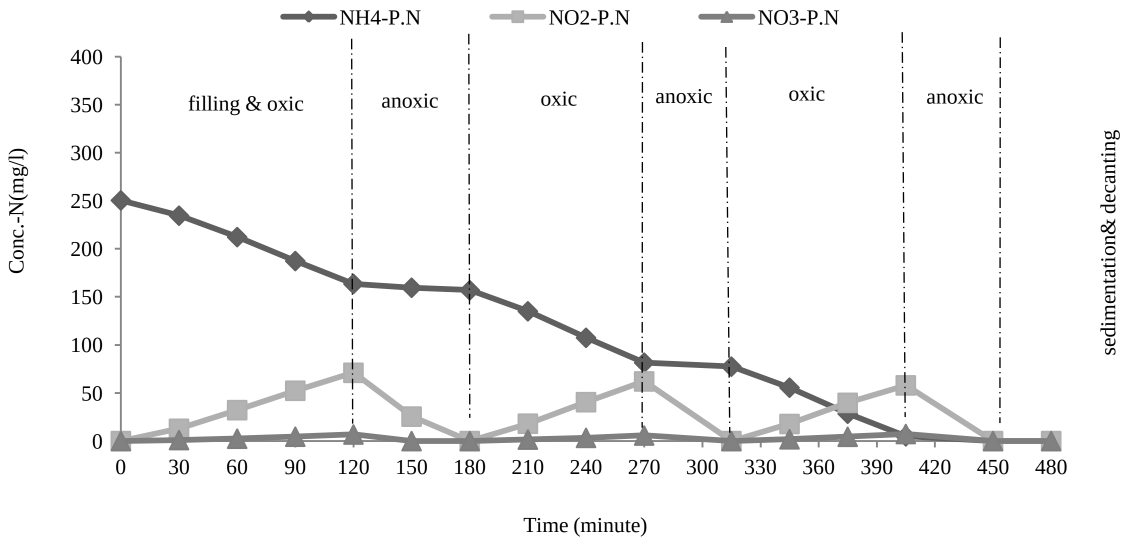

Fig. 2- Side stream partial nitrification results after steady state conditions

Table 4- ANOVA & S/ N Analysis results of main stream reactor after excess sludge adding

indexes / Factors	sAOR					NO_2/ NO_x				
	DOF (f)	variance	F- Ratio (F)	Percent influence	Optimum levels	DOF (f)	variance	F- Ratio (F)	Percent influence	Optimum levels
Temperature	3	133.2	16964.6	98.5	1	3	0.35	187.8	49.7	1
Initial ammonia	3	0.13	16.3	0.09	4	3	0.1	56.05	14.6	4
MLVSS	3	0.09	11.1	0.06	4	3	0.03	14.8	3.7	4
Time	3	1.8	229.6	1.3	4	3	.22	116.4	30.7	1
Other/ error	3	0.007	-	0.03	-	3	0.001	-	1.3	-

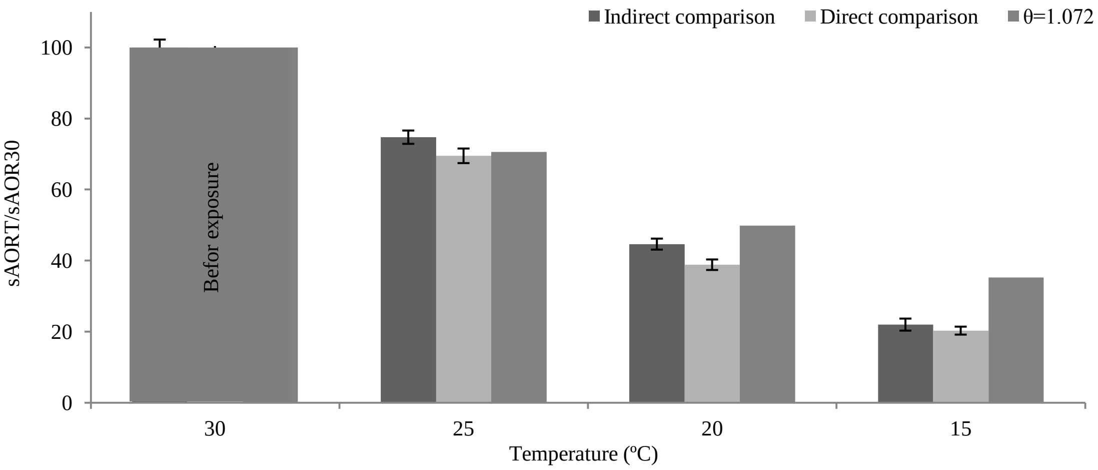

Fig. 3- sAOR reduction after cold shock in direct and indirect comparison

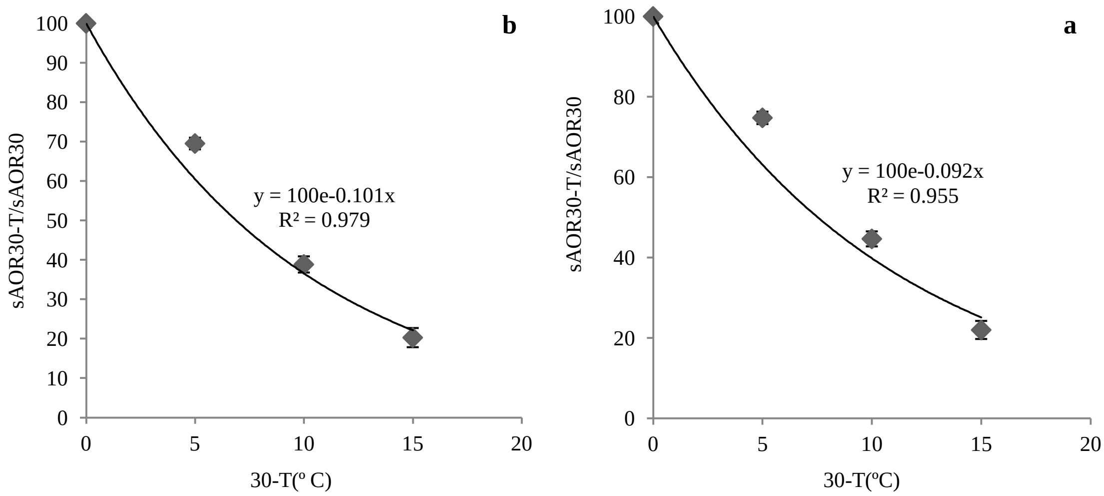

Fig. 4- Exponential trend line for temperature dependency factor calculating (a- indirect and b- direct)

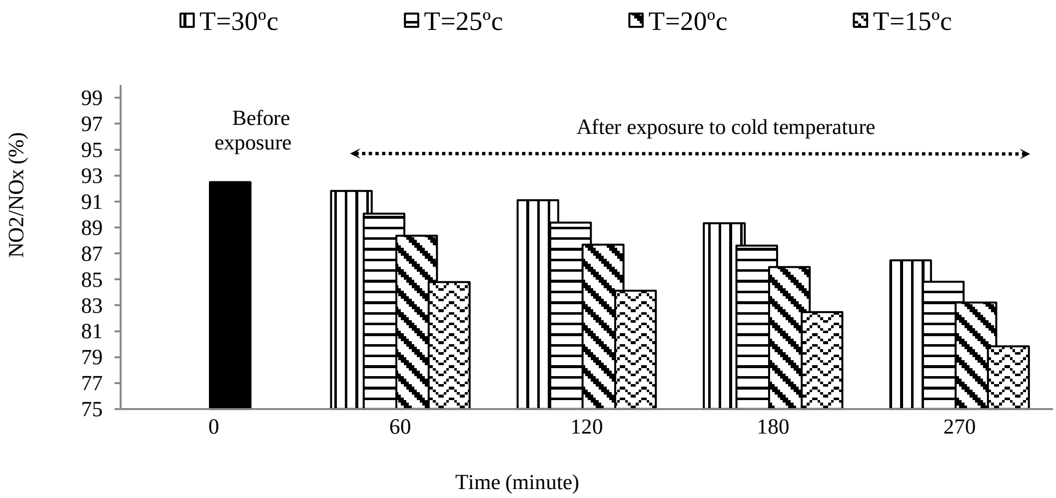

Fig. 5- Effect of temperature & time on NO_2/ NO_x ratio after biomass adding to new environment

Discussion and Conclusion

Side stream results: The average of sAOR and NO_2/ NO_x ratio in any cycle of side stream reactor after steady state conditions were calculated to be 14.83± 1.92 mg N- NH_4^+ (gr VSS.hr) $^{-1}$ and 91.2± 1.3%, respectively. These results showed that this approach caused partial nitrification and nitrate production inhibition which is in agreement with other studies (3). Dosta et al. have reached sAOR about 19 mg N- NH_4^+ (gr VSS.hr) $^{-1}$ and NO_2/ NOx ratio up to 95% for T=30ºC and 250- 300 mg N- NH_4^+/ L (3). Results suggested the superiority of AOB on NOB based on high NO_2/ NO_x ratio. Therefore, excess sludge from this reactor had more AOB than NOB and thus was suitable for partial nitrification in another reactor under different condition and for temperature dependency coefficient calculation in partial nitrification instead of nitrification.

sAOR: Analysis of sAOR in main stream reactor demonstrated that temperature, initial ammonia, MLVSS and time affected this index by 98.5, 0.09, 0.05 and 1.3%, respectively (Table 4). These results showed that temperature had serious effects on sAOR and by reduction of temperature; excessive reduction in sAOR was acquired. The average reduction in nitrification rate (sAOR) with the sudden decrease in temperature after cold shock from 30 to 25, 20 and 15ºC were about 25, 55 and 78%, respectively (Fig. 3). For more investigation, the Arrhenius equation (Equation 1) was used by considering 1.072 values as the accepted temperature correction coefficient for gradual temperature change. The sAOR would possibly be decreased after cold shock by 29, 50 and 65% at 25, 20 and 15ºC, respectively (Fig. 4). Comparison of these results shows that in sudden temperature decreases, further reductions will occure in a process rate. Hwang and Oleszkiewicz revealed that by adding nitrifying biomass from 20 to 10ºC, a deeper decrease in nitrification rate as predicted by 1.072 value (about 20%) (21) would be induced. They also demonstrated that adding nitrify biomass from 30 to 10ºC, caused 82% reduction in nitrification rate which is close to 84% decrease estimated in the present study for temperature reduction from 30 to 10ºC. Guo et al. (29) observed that sAOR decreased by 1.5 times when the temperature is decreasing from 25 to 15ºC. This is in agreement with 55% reduction from 30 to 20ºC in the present study.

By using Equation 1, $sAOR_{30}$ and sAORT as r_{T0} and r_T, respectively, and by using indirect comparison, the temperature dependency factor is calculated about 1.0965 which is closed to Biowin Default (Table 1). Because the effect of temperature on sAOR was bigger than the other factors, direct comparison can also be used. By direct comparison the temperature dependency factor is about 1.106 which has slight differences with the previous value and closed to Hwang and Oleszkiewicz (21). They used direct comparison to calculate this coefficient. This means that direct comparison may produce different results based on the effect of other factors except temperature.

NO_2/ NO_x: Analysis of NO_2/ NO_x ratio showed that temperature, initial ammonia, MLVSS and time affected NO_2/ NO_x ratio by 49.7, 14.6, 3.7 and 30.7%, respectively (Table 4). These consequences explained that temperature reduction, time increasing, low initial ammonia and high MLVSS caused NO_2/ NO_x ratio reduction. This reduction indicated that NOB activity had recovered based on low free ammonia and temperature reduction. These results appeared that maximum NO_2/ NO_x ratio has been happened at 30ºC by initial ammonia about 150 mg N- NH_4^+/ L and time less than 60 minutes by about 94.9%. This is in good agreement with side stream partial nitrification results in the present study and with those reported in other studies (3 & 14). Guo et al. (29) observed that nitrite accumulation ratio was always above 90% which is in agreement with present results.

The results showed that time increasing to 120 minutes didn't have important effects on NO_2/ NO_x ratio. Bigger time up to 270 minutes had serious effects on NO_2/ NO_x ratio and was reduced to 89.4% which showed that NOB has recovered its activity by time (Fig. 5). Therefore, using excess sludge that acclimated in partial nitrification process is a good idea to partial nitrification in main stream, especially in short times.

In this study a side stream SBR was used for partial nitrification and biomass acclimation. The SBR was perfumed with three cycles per day, temperature about 30ºC, SRT 9± 1 days and HRT 1.2 days. Under steady state conditions, sAOR and NO_2/ NO_x were about 14.83± 1.92 mg N–NH_4^+/ grVSS. hr and 91.2± 1.3%, respectively. These results indicated that partial nitrification and AOB acclimation were happened.

Moreover, after biomass acclimation in side stream SBR, excess sludge from this reactor has been added to another batch reactor (main stream) which was used in different conditions based on L- 16 array. Factors which were evaluated in this research were temperature, initial ammonia, MLVSS and time. The results of main stream experiments showed that by abrupt temperature decreases, sAOR and NO_2/ NO_x ratio were decreased but effects of temperature in any index were different. Outcomes displayed that temperature affected these indexes by 98.5and 49.7, respectively.

Furthermore, initial ammonia has influenced only NO_2/ NO_x ratio by 14.6%, so that initial ammonia increasing caused NO_2/ NO_x increases. Time also seriously affected only NO_2/ NO_x by 30.7%. Time elapsing caused NO_2/ NO_x ratio decreasing.

This study showed that use of excess acclimated biomass (bio- augmentation) in partial nitrification process for SRT regulation (predominance AOB against insufficient NOB) could be an effective way to partial nitrification in different conditions for short times usually less than 120 minutes. Also, it founded that temperature was an important factor that affected sAOR and NO_2/ NO_x ratio after clod shock. By using Equation 1 and indirect comparison (ignore effect of other factors except temperature), the temperature dependency factor is calculated about 1.0965 for estimating cold shock effect. By direct comparison, the temperature dependency factor is about 1.106.

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استفاده از زیست توده غنی شده در نیتریفیکاسیون مختصر جریان کنار گذر برای بررسی نیتریفیکاسیون مختصر و شوک کاهشی ناگهانی دما در جریان کنار گذر

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چکیده

در پژوهش حاضر، غنی‌سازی زیست‌توده در یک رآکتور ناپیوسته متوالی(SBR) جریان کنارگذر در فرآیند نیتریفیکاسیون مختصر و در شرایط بهینه انجام شد (دمای ۳۰ درجه سلسیوس، سن لجن ۱±۹ روز و زمان ماند ۱/۲ روز). لجن مازاد غنی شده مورد نظر با هدف تنظیم سن لجن(SRT) به رآکتور ناپیوسته دیگری (رآکتور جریان اصلی) که در شرایط متنوع بهره‌برداری می‌شد، منتقل شد تا فرآیند نیتریفیکاسیون مختصر در آن بررسی و ضریب وابستگی دمایی (ɵ) تعیین شود. نتایج رآکتور جریان اصلی نشان داد که دما با ۹۸/۵ درصد، مهم‌ترین عاملی بوده که نرخ ویژه اکسیداسیون آمونیوم را پس از شوک دمایی تحت تأثیر قرار داده است. نتایج نشان داد که شوک کاهش ناگهانی دمایی باعث کاهش شدید sAOR به مقدار ۲۸، ۵۵ و ۷۸ درصد به ترتیب برای کاهش دما از ۳۰ به ۲۵، ۲۰ و ۱۵ درجه سلسیوس شده‌است. بر این اساس، ضریب وابستگی دمایی بر اساس دو روش مقایسه‌ی غیر مستقیم و مستقیم به ترتیب معادل ۱/۰۹۶۵ و ۱/۱۰۶ بر حسب درجه سانتی‌گراد تعیین شد. همچنین، تحلیل نتایج NO2/NOx نشان داد که دما، غلظت آمونیوم اولیه، غلظت زیست‌توده (MLVSS) و زمان با ۴۹/۷، ۱۴/۶، ۳/۷ و ۳۰/۷ درصد، این شاخص را تحت تأثیر قرار داده است. نتایج گویای این است که حداکثر مقدار NO2/NOx پس از انتقال زیست توده به محیط جدید، حدود ۹۴/۹ درصد بوده که پس از ۲۷۰ دقیقه به ۸۹/۴ درصد کاهش یافته است.

واژه‌های کلیدی: کشت اکسایندگان آمونیوم، اثر شوک کاهش دمایی، ضریب وابستگی دمایی، نیتریفیکاسیون مختصر، رآکتور جریان جانبی، شوک کاهشی ناگهانی، غنی‌سازی زیستی

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