Abstract—In this study after biomass acclimation in a side stream partial nitrification SBR under optimum conditions ($T=30\, ^\circ\mathrm{C}$, SRT=9±1 days and HRT=1.2 day) in order to superiority of ammonia oxidizing biomass over nitrite oxidizing biomass, excess sludge for solids retention time regulation was added to another batch reactor which had been performed under different conditions for partial nitrification evaluation. The results showed that temperature was an important factor affected specific ammonium oxidation rate after cold shock by about 98.5%, but specific nitrite oxidation rate has affected by temperature, time, and initial ammonium concentration by about 71, 21 and 5 percent, respectively. Results have also illustrated that temperature, time, initial ammonium and MLVSS concentration have affected NO$_2$/NO$_x$ ratio by about 50, 30, 15 and 3.5 percent respectively. This study showed that use of excess acclimated biomass could be an effective way to partial nitrification in different conditions for short times.

Keywords—AOB cultivation, biomass acclimation, cold shock, nitrite accumulation.

I. INTRODUCTION

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 by reaction of nitrification and subsequently denitrification [1-4].

Many ways have been described in literatures to achieve the BNR process via nitrite. Anthonisen et al. determined the effect of ammonia (NH$_3$) and nitrous acid (HNO$_2$) concentration upon ammonium oxidation and nitrite oxidation kinetics. They demonstrated that nitrite oxidizing biomass (NOB) was inhibited at concentrations higher than 0.2–2.8 mg HNO$_2$/L and/or 0.1–1.0 mg NH$_3$/L, while ammonia oxidizing biomass (AOB) was inhibited by unionized ammonia concentrations higher than 10–150 mg NH$_3$/L [1, 3, 5, 6].

Furthermore, many authors have reported that, at reduced dissolved oxygen concentrations, AOB is favoured over NOB activity due to a greater oxygen affinity for the first step of nitrification [2, 5-7]. The critical values of DO recorded in previous studies were different and by considering ammonium oxidation rate and nitrite accumulation ratio, DO concentrations should be maintained about 0.8–1.5 mg/L [3, 6].

Moreover, temperature is one of the important and serious factors in biological wastewater treatment especially in nitrification that affects reaction rate and growth rate of biomass [1, 6, 8]. Only at temperatures above 25 °C is it possible that the AOB can effectively out-compete the NOB [5, 6, 9-11]. Temperature effect on biological processes can be expressed by Arrhenius equation (Equation 1) and each biomass including AOB and NOB has their own temperature dependency factors [8, 12, 13]. Head and Oleszkiewicz evaluated cold shock effects upon first steps of nitrification rate by adding biomass into new environment (e.g. $T=10\, ^\circ\mathrm{C}$) which was acclimated in different temperatures (e.g. $T=30\, ^\circ\mathrm{C}$) [13]. Also, Hwang and Oleszkiewicz researched to the effect of cold-temperature shock on nitrification by using Equation 1 [14].

$$r_t = r_\infty e^{(T-T_\infty)}$$

Taguchi method is an experimental design and analyzing method which is used in many fields such as water and wastewater treatment [15-17]. In this method, some of experiments were done according to different levels of the studied factors. Then S/N and ANOVA analyzing, were used for calculating statistical index such as variance, F-ratio and percent influence of the variables. In the last steps, ignored experiments could be estimated [18].

In this study, partial nitrification in a main stream reactor has been investigated in different conditions by adding excess biomass for adjusting solids retention time (SRT) from a side stream Sequencing Batch Reactor (SBR) which was operated under optimum conditions for BNR via nitrite and biomass acclimation in partial nitrification phase. By using Taguchi method under different conditions including initial ammonium concentration, MLVSS and time, the effects of abrupt
temperature changing on partial nitrification and biomass activity which was acclimated in side-stream partial nitrification reactor have been studied. For this evaluations, specific ammonium oxidation rate (sAOR) and specific nitrite oxidation rate (sNOR) have been used as indexes to express ammonium and nitrite consumption, respectively and NO$_2$/NO$_x$ ratio has been used to explain nitrite accumulation percentage (Equation 2 to 4) [2, 3, 11, 14, 19, 20].

\[
\text{sAOR} = \frac{NH_4^+ \text{ Oxidize as } N}{MLVSS \times \theta_{am}} \times 1000
\]

\[
\text{sNOR} = \frac{NO_2^- \text{ Oxidize as } N}{MLVSS \times \theta_{an}} \times 1000
\]

\[
\frac{NO_2^-}{NO_x} = \frac{NO_2^- \text{ produce as } N}{(NO_2^- + NO_3^-) \text{ as } N} \times 100
\]

II. MATERIAL & METHODS

A. Substrate and Acclimation Process

For preparation of synthetic wastewater, effluent from the wastewater treatment plant (WWTP) of Shahin-Shahr city in Isfahan province of Iran was used and regulated by addition of about 900 mg N/L as NH$_4$Cl, 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$_2$PO$_4$ [1, 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 and 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 I. The lab-scale SBR and other automatic equipment for regulation of temperature and DO, filling and decanting, external carbon source injection are shown in Figure 1-a.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>unit</th>
<th>value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH$_4^+$ as N</td>
<td>mg N/L, cycle</td>
<td>250</td>
</tr>
<tr>
<td>HRT</td>
<td>day</td>
<td>1.2</td>
</tr>
<tr>
<td>pH</td>
<td>-</td>
<td>7.5-8.5</td>
</tr>
<tr>
<td>DO</td>
<td>mg/L</td>
<td>0.8-1.2</td>
</tr>
<tr>
<td>T</td>
<td>°C</td>
<td>30±1</td>
</tr>
<tr>
<td>SRT</td>
<td>day</td>
<td>9±1</td>
</tr>
<tr>
<td>Cycle</td>
<td>Number/day</td>
<td>3</td>
</tr>
<tr>
<td>sub cycle (aerobic-anoxic)</td>
<td>Number/cycle</td>
<td>3</td>
</tr>
</tbody>
</table>

B. Batch tests and different conditions

After start-up of side stream partial nitrification SBR (steady state conditions), some of excess sludge for SRT regulation was added to a batch reactor that operated under different conditions for partial nitrification evaluation (Figure 1-b). Experiments have been done at different conditions by changing initial ammonium, MLVSS, time and temperature based on Taguchi method. These factors and their levels are summarized in Table II.

C. 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. For data analysis, based on experimental design and L-16 array, S/N (signal/noise) analysis and ANOVA by Taguchi method and QT4 program were done and contribution of any factors on results was calculated and finally eliminated experiments have been estimated [21].

D. Analytical methods

NH$_4^+$-N was measured by Nesler and spectro-photometric method in 410 nm. (DR 4000, Hach Co., USA) [21]. Nitrite and nitrate were measured by spectro-photometric method (DR 4000, Hach Co., USA) [22]. MLVSS were measured according to the Standard Methods [21]. 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, sNOR and NO$_2$/NO$_x$ ratio were calculated based on Equations 4-6.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Temperature(°C)</td>
<td>30±1, 25±1, 20±1, 15±1</td>
</tr>
<tr>
<td>B: Initial ammonium(mg NH$_4^+$-N$^{-1}$)</td>
<td>50±5, 75±5, 100±5, 150±5</td>
</tr>
<tr>
<td>C: MLVSS(mg VSSL$^{-1}$)</td>
<td>1250±10, 1000±10, 750±10, 500±10</td>
</tr>
<tr>
<td>C: Time(minute)</td>
<td>60, 120, 180, 270</td>
</tr>
</tbody>
</table>
III. RESULTS AND DISCUSSION

A. Partial nitrification & AOB cultivation in side stream SBR

Ammonium and nitrite oxidation and nitrate formation in an 8 hours cycle and under steady state conditions are shown in Figure 2.

The average indexes of sAOR, sNOR and NO$_2$/NO$_x$ ratio in any cycle after steady state conditions were calculated to be 14.83±1.92 mg N–NH$_4^+$ /gr VSS. hr, 1.25±0.40 mg N–NO$_2^-$ /gr VSS. hr and 91.2±1.3%, respectively. Dosta et al. have reached sAOR about 19 mg N–NH$_4^+$ (g VSS h)$^{-1}$ and NO$_2$/NO$_x$ ratio up to 95% for T=30ºC and 250-300 mg N–NH$_4^+$ /L [3]. These results showed that this approach caused partial nitrification and nitrate production inhibition which is in agreement with other studies [3]. By this strategy, NOB growth rate was inhibited based on high temperature, high free ammonia, and pH adjustment in anoxic phase and low DO concentration which is in good agreement with those reported in other researches [1, 3, 6, 10, 11]. Therefore, excess sludge from this reactor had more AOB than NOB and thus was suitable for partial nitrification in the other reactor.

B. Partial nitrification evaluation in different conditions

1) sAOR analysis

Analysis of sAOR demonstrated that temperature, initial ammonium, MLVSS and time affected this index by 98.5, 0.09, 0.05 and 1.3 percent, respectively. These results showed that temperature had serious effects on sAOR and by reduction of temperature; excessive reduction in sAOR has been acquired. Other factors except time had not important effects on this index (Figure 3).

As shown in Figure 3, by time increasing, sAOR has improved slightly which is indicated that adding biomass in to new environment caused a primary shock effect. Optimum levels and other parameter for bigger sAOR as an aim of partial nitrification after ANOVA are shown in Table III and effects of all consideration factors on sAOR after S/N analyzing are shown in Figure 4.

The average reduction in nitrification rates with the sudden decrease in temperature after cold shock from 30ºC in to temperature of 25, 20 and 15ºC were about 25, 55 and 78 percent, respectively which is in agreement with other studies [13, 14]. Head and Oleszkiewicz have demonstrated that adding nitrify biomass from 30 to 10ºC, caused about 82% reduction in nitrification rate which is near 78% decrease in the present study [13]. Geo et al. observed that sAOR decreased by 1.5 times with the temperature decreasing from 25 to 15 ºC [23].

2) NO$_2$/NO$_x$ ratio analysis

Analysis of NO$_2$/NO$_x$ ratio as an important index for demonstrating nitrite accumulation ratio showed that temperature, initial ammonium, MLVSS and time affected NO$_2$/NO$_x$ ratio by 49.7, 14.6, 3.7 and 30.7 percent,
respectively. Optimum levels and other parameter for bigger NO₂/NOₓ as an aim of partial nitrification after ANOVA are shown in Table III and effects of all consideration factors on NO₂/NOₓ ratio after S/N analyzing are shown in Figure 4.

These consequences explained that temperature reduction, time increasing, low initial ammonium and high MLVSS caused NO₂/NOₓ ratio reduction. This reduction exhibited that NOB activity had recovered based on high SRT and low free ammonia beside temperature reduction. This results appeared that maximum NO₂/NOₓ ratio has been happened at 30°C by initial ammonium about 150 mg N-NH₄⁺/L and time less than 60 minutes by about 94.9% which is in good agreement with side stream partial nitrification results and with those reported in the other studies [3, 11]. The results showed that time elapsed to 120 minutes didn’t have important effects on NO₂/NOₓ ratio, but bigger time up to 270 minutes, had serious effects on NO₂/NOₓ ratio and was reduced it to 89.4% which is showed that NOB has recovered its activity by time.

3) sNOR analysis

Analysis of sNOR appeared that temperature, initial ammonium, MLVSS and time affected sNOR by 71.2, 5.2, 1.6 and 21 percent, respectively. These outcomes indicated that temperature reduction, time decrease and high initial ammonium have significant effect on sNOR reduction. Optimum levels and other parameter for lower sNOR after ANOVA are shown in Table III and effects of all consideration factors on sNOR ratio after S/N analyzing are shown in Figure 4.

IV. CONCLUSION

In this study a side stream SBR was used for partial nitrification and biomass acclimation. Nitrogen was completely removed with three cycles per day, temperature about 30°C, SRT 9±1 days and HRT 1.2 days. Under steady state conditions, sAOR, sNOR and NO₂/NOₓ were about 14.83±1.92 mg N-NH₄⁺/gr VSS. hr, 1.25±0.4 mg N-NO₂⁻/gr VSS. hr and 91.2±1.3 percent, respectively, which is showed that partial nitrification and AOB acclimation were happened. Moreover, after biomass acclimation for partial nitrification in side stream SBR, excess sludge from this reactor has been added to another batch reactor which was used in different conditions based on L-16 array. Factors which were evaluated in this research were temperature, initial ammonium, MLVSS and time. The results of experiments showed that by abrupt temperature decreases, sAOR, sNOR and NO₂/NOₓ ratio were decreased but effects of temperature in any factors were different. Outcomes displayed that temperature affected these indexes by 98.5, 71.3 and 49.7, respectively. Furthermore, initial ammonium has influenced only sNOR and NO₂/NOₓ ratio by 5.2 and 14.6 percent, respectively, so that initial ammonium increasing caused NO₂/NOₓ increases and sNOR decreasing. Besides, time also was seriously affected only sNOR and NO₂/NOₓ by 21 and 30.7 percent, respectively. Time elapsing caused sNOR increasing accordingly with NO₂/NOₓ ratio decreasing.

Fig. 4 Effects of temperature, NH₄⁺, MLVSS and time on sAOR, sNOR and NO₂/NOₓ ratio after S/N analyzing (a-temperature effects, b-NH₄⁺ effects, c-time effects and d-MLVSS effects)
This study showed that use of excess acclimated biomass 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.

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REFERENCES


