STABILITY OF PARTIAL NITRITATION OF POULTRY SLAUGHTERHOUSE WASTEWATER IN A SEQUENTIAL BATCH REACTOR


TATIANE M. DE ASSIS¹, MARCOS V. SCHILICHTING², CARLA L. LOPES², AIRTON KUNZ³, SIMONE D. GOMES²

¹Corresponding author. Universidade Estadual do Oeste do Paraná - Unioeste/ Cascavel - PR, Brasil.
E-mail: tatianemassis@yahoo.com.br

ABSTRACT: The aim of this study was to monitor the stabilization of a sequential batch reactor (SBR) regarding the partial nitritation of poultry slaughterhouse wastewater, aiming at preparing it for a reactor with bacteria that promote anaerobic ammonia oxidation (anammox). A cylindrical reactor with a useful volume of 3.5 L, mechanical agitation (50 rpm), and suspended biomass was used in the experiment. The strategies adopted for nitrite-oxidizing bacteria (NOB) inhibition were cycle time (CT: 24, 16, 6, 5, 4 h and real-time monitoring), free ammonia accumulation, alkalinity restriction and low concentrations of dissolved oxygen (DO). The ammonification process, observed at all experimental phases, negatively influenced the control of partial nitritation process. Alkalinity restriction under low cycle times (4 and 5 h) conditions, together with low dissolved oxygen concentration, was a key factor for nitrite-oxidizing bacteria inhibition. Stability of partial nitritation was only reached by real-time monitoring, being the cycles stopped when 50% N–NH₄⁺ and 50% N–NO₂⁻ were present in the reactor.

KEYWORDS: anammox, deammonification, nitrite, nitrate, nitrifying.

INTRODUCTION

Biological nitrogen removal can occur by conventional method from autotrophic nitrification processes in which nitrifying bacteria oxidize ammonia to nitrite and then nitrite to nitrate, followed by the heterotrophic denitrification process, with conversion of nitrate to nitrite and, subsequently, to nitric oxide (NO), nitrous oxide (N₂O) and nitrogen gas (N₂). Recently, faster methods have been studied such as partial nitritation followed by final nitrogen removal by microorganisms with anammox (anaerobic ammonia oxidation) activity or short-cut denitrification (MADIGAN, 2010).

Nitrogen removal by anammox is an interesting treatment possibility since the bacteria involved have a greater capacity of transforming nitrogenous forms present in wastewater into nitrogen gas (N₂) (SCHEEREN et al., 2011). Researches on new nitrogen removal pathways are important because of the high cost of aeration systems, which are needed in wastewater with high loads (CHANG et al 2012). WANG et al (2011) indicated partial nitritation followed by denitrification, or anammox activity, as a viable solution regarding this aspect.

In order for the removal process to be possible via anammox, partial nitritation of wastewater is needed, so that the substrate presents in its composition a ratio of 1-mole ammoniacal nitrogen to 1.32-mole nitrite, i.e. 50% ammoniacal nitrogen and 50% nitrogen as nitrite (MADIGAN, 2010).

Partial nitritation of ammoniacal nitrogen to nitrite is promoted by nitrite-oxidizing bacteria (nitrifying) inhibition, which inhibits nitrate formation. The factors that exert a greater influence on this inhibition are pH, dissolved oxygen (DO), temperature and nitrifying bacteria (Nitrosomonas and Nitrobacter) doubling rate (PRA et al, 2012; VIANCELLI et al, 2011; SUN et al 2010, ZHU et al 2008).

The effect of pH on partial nitritation can be expressed in two ways, either the inhibitory action of the hydrogen [H⁺] and hydroxyl [OH⁻] ions on microorganism growth rate or the inhibitory action of free ammonia. According to ZHU et al. (2008), nitrite-oxidizing bacteria

² Universidade Estadual do Oeste do Paraná - Unioeste/ Cascavel - PR, Brasil.
³ Embrapa Suínos e Aves/ Concórdia - SC, Brasil.
Received in: 5-03-2016
Accepted in: 9-25-2016
present a lower growth rate when submitted to a high pH condition than ammonia-oxidizing bacteria, which favors partial nitrification.

Dissolved oxygen acts as a regulator of ammonia to nitrite conversion since it is the final electron acceptor in nitrification. In studies by VIANCELLI et al. (2011), in which partial nitrification and anammox activity occurred in the same reactor submitted to DO values lower than 0.5 mg L⁻¹, process viability was confirmed under low dissolved oxygen concentration. In experiments with complete nitrification, ANDRADE et al. (2010) assessed the influence of DO on nitrification speed and observed that nitrification rate is directly proportional to DO concentration.

GERARDI (2006) observed that a difference in doubling time between the two nitrifying bacteria groups influences inhibition process. In treatment systems, Nitrosomonas (nitrifying) population size is greater than Nitrobacter (nitrifying) because Nitrosomonas obtain more energy from ammonium ion oxidation; on the other hand, Nitrobacter obtains its energy from nitrite ion oxidation. Therefore, nitrifying bacteria have a shorter generation time and are able to increase rapidly in number when compared to nitrifying bacteria. In order to reduce the growth rate of nitrifying bacteria, several authors have maintained the reactor temperature around 35 °C since high temperature reduces microorganism growth rate (ZHU et al. 2008; WANG et al. 2011; PRÁ et al. Al. 2012).

Sequential batch reactor (SBR) has been shown a viable option in nutrient removal due to ease of operation and low cost (DALLAGO et al, 2012; ANDRADE et al 2010; MEES et al, 2013).

In this context, the aim of this study was to assess the stability of partial nitrification of poultry slaughterhouse wastewater in a sequential batch reactor (SBR) using as variables the pH, presence of free ammonia, alkalinity supply, DO concentration and cycle time.

**MATERIAL AND METHODS**

**Wastewater**

Wastewater was collected at the exit of an anaerobic lagoon from the wastewater treatment system of a poultry slaughterhouse located in the western region of Paraná State. After collection, wastewater was homogenized, conditioned in 2 L polyethylene containers and stored in a freezer at −20 °C. Before storing process, wastewater was characterized according to physical and chemical parameters presented in Table 1.

**TABLE 1. Characteristics of poultry slaughterhouse wastewater and respective methods used.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Poultry wastewater*</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>TKN (mg L⁻¹)</td>
<td>98 ± 11</td>
<td>4500–Norg C – Semi-micro Kjeldahl (APHA, 2005)</td>
</tr>
<tr>
<td>N–NH₄⁺ (mg L⁻¹)</td>
<td>64 ± 10</td>
<td>4500–NH₃ F – Colorimetric (APHA, 2005)</td>
</tr>
<tr>
<td>N–NO₂⁻ (mg L⁻¹)</td>
<td>**</td>
<td>Kit HACH – Colorimetric</td>
</tr>
<tr>
<td>N–NO₃⁻ (mg L⁻¹)</td>
<td>**</td>
<td>Kit HACH – Colorimetric</td>
</tr>
<tr>
<td>COD (mg L⁻¹)</td>
<td>75 ± 25</td>
<td>5220 D – Colorimetric</td>
</tr>
<tr>
<td>pH</td>
<td>6.7 ± 2</td>
<td>Potentiometric (APHA, 2005)</td>
</tr>
<tr>
<td>TS (mg L⁻¹)</td>
<td>204 ± 135</td>
<td>2540 B – Gravimetric (APHA, 2005)</td>
</tr>
<tr>
<td>VSS (mg L⁻¹)</td>
<td>67 ± 31</td>
<td>2540 E – Gravimetric (APHA, 2005)</td>
</tr>
<tr>
<td>Alkalinity (mgCaCO₃ L⁻¹)</td>
<td>407 ± 183</td>
<td>2320 B – Titrimetric (APHA, 2005)</td>
</tr>
</tbody>
</table>

*Means ± standard deviation. **Nitrite and nitrate presented values close to zero. TKN: total Kjeldahl nitrogen; COD: chemical oxygen demand; TS: total solids; VSS: volatile suspended solids.

Free ammonia concentration in the reactor was determined by [eq. (1)], according to ANTHONISEN et al. (1976):
Free ammonia (mg L\(^{-1}\)) = \( (17/14)^*((N-\text{NH}_4^+)*10^{\text{pH}})/e^{(6.344/273 + ^\circ\text{C}) + 10^{\text{pH}}} \) \tag{1}

where,

\( N-\text{NH}_4^+ \) is the ammoniacal nitrogen concentration in the reactor;

\( \text{pH} \) is the pH value in the reactor, and

\( ^\circ\text{C} \) is the temperature in the reactor.

**Inoculum**

The reactor was inoculated with nitrifying biomass from a sequential batch reactor (SBR) operated with bovine slaughterhouse wastewater. In the reactor inoculation, 25% of inoculum and 75% poultry slaughterhouse wastewater (v/v) were used. Reactor biomass concentration expressed in volatile suspended solids (VSS), was maintained at 2700 mg L\(^{-1}\), according to the condition used by MEES et al. (2013).

**Experimental apparatus and operating conditions**

Experimental apparatus consisted of a reactor, an aerator coupled to a flow meter to control airflow rate, a heater, a temperature controller and a mechanical stirrer with rotating blades with rotation set at 50 rpm.

SBR reactor was assembled in bench scale and manufactured of cylindrical polyethylene (18.5 cm in diameter and 20.5 cm in height), with a total volume of 5.5 L and a working volume of 3.5 L. In each batch, 2.625 L of wastewater were added to the reactor, in which was maintained 875 mL of the previous batch as inoculum. The reactor was operated with suspended biomass.

Batch reactor operating cycle was composed of the following phases: feeding, reaction (aerobic), sedimentation and discharging. Between one and another cycle, the system was kept at rest for immediate analysis of \( N-\text{NH}_4^+ \) and alkalinity because, when necessary, initial alkalinity was adjusted to the next batch. Alkalinity correction was carried out with sodium bicarbonate solution at a ratio of 0.61 mg NaHCO\(_3\) per mg \( N-\text{NH}_4^+ \) to be oxidized, as described by LAMENGO NETO & COSTA (2011). In addition to sampling for alkalinity correction, collected samples were analyzed for physical and chemical parameters, as presented in Table 1.

During the start-up, the reactor was operated with a 24-hour cycle time (CT), with a supply of 3.0 mg L\(^{-1}\) of oxygen and calcium bicarbonate to maintain a pH range between 7.0 and 8.0. The reactor was maintained under these conditions for 30 days.

At the end of reactor start-up period, strategies were adopted to inhibit nitrite-oxidizing bacteria, aiming at allowing the oxidation of only 50% \( N-\text{NH}_4^+ \) to \( N-\text{NO}_2^- \) (Table 2).
TABLE 2. Strategies of nitrite-oxidizing bacteria inhibition during sequential batch reactor operation.

<table>
<thead>
<tr>
<th>Experimental phase</th>
<th>Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>CT reduction from 24 to 16 hours, pH control in the range between 7.4 and 7.8, and dissolved oxygen (DO) with an average concentration of 1.0 mg L(^{-1}).</td>
</tr>
<tr>
<td>Phase II</td>
<td>CT reduction to 6 hours, pH control in the range between 7.4 and 7.8, and DO according to Phase I.</td>
</tr>
<tr>
<td>Phase III</td>
<td>CT reduction to 5 hours, inhibition by free ammonia by using a pH range between 8.0 and 9.0, and DO according to Phase I.</td>
</tr>
<tr>
<td>Phase IV</td>
<td>CT reduction to 4 hours, sufficient alkalinity supply for oxidation of only 50% N–NH(_4^+), according to [eq. (2)], and oxygen restriction with an average concentration of 0.5 mg L(^{-1}).</td>
</tr>
<tr>
<td>Phase V</td>
<td>Variable CT, with hourly analysis of N–NH(_4^+), N–NO(_3^−) and N–NO(_2^−) and CT interruption when reaching the proportion required. DO concentration varied between 0.1 and 0.3 mg L(^{-1}). Alkalinity supply was sufficient to promote the oxidation of only 50% N–NH(_4^+) to N–NO(_2^−), according to [eq. (2)].</td>
</tr>
</tbody>
</table>

In order to promote the oxidation of only 50% N–NH\(_4^+\) to N–NO\(_2^−\), in the phases IV and V the alkalinity supply was calculated by [eq. (2)]

\[
\text{Additional alkalinity (mg) = } [(7.14*A*X) − (B*Y)]*V \tag{2}
\]

where,

- 7.14 is the mass of CaCO\(_3\) (mg) required for oxidation of 1.0 mg of ammoniacal nitrogen,
- A is the N–NH\(_4^+\) fraction to be oxidized (in this study, 0.5),
- X is the initial concentration of ammoniacal nitrogen in the wastewater (mg L\(^{-1}\)),
- B is the alkalinity fraction required to oxidize A,
- Y is the initial concentration of alkalinity (mg L\(^{-1}\)), and
- V is the reactor volume (L).

Each experimental phase lasted 14 days, seven of them for microorganism adaptation to the new conditions and the other seven days for taking samples in all operating cycles, thus justifying the different numbers of cycles occurred at each experimental phase.

At phase V, CT was not previously fixed as at the other phases. A differentiated strategy was chosen, which consisted of performing 12 cycles with real-time monitoring, closing each cycle until such time as the proportion of 50% N–NH\(_4^+\) and 50% N–NO\(_2^−\) is reached.

In order to demonstrate the results, temporal profiles are presented, which allow visualizing the ammoniacal nitrogen conversion into nitrite and nitrate during the tests.

RESULTS AND DISCUSSION

During the start-up period, the reactor was operated with a 24-hour cycle time, presenting 99% average conversion from N–NH\(_4^+\) to N–NO\(_3^−\), confirming that the biomass used in the experiment was active.

Phase I

Results concerning reactor operation under conditions established at experimental phase I (CT: 16 h; pH: 7.4 to 7.8; DO: 1.0 mg L\(^{-1}\)) are shown in Table 3.
TABLE 3. Characterization of reactor affluent and effluent at phase I.

<table>
<thead>
<tr>
<th></th>
<th>DO (mg L(^{-1}))</th>
<th>pH</th>
<th>TA (mg L(^{-1}))</th>
<th>Temp. (°C)</th>
<th>N–NH(_4^+) (mg L(^{-1}))</th>
<th>N–NO(_2^-) (mg L(^{-1}))</th>
<th>N–NO(_3^-) (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affluent</td>
<td>Mean</td>
<td>0.60</td>
<td>7.67</td>
<td>613.33</td>
<td>25.90</td>
<td>52.26</td>
<td>6.47</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.31</td>
<td>0.14</td>
<td>144.26</td>
<td>2.32</td>
<td>6.57</td>
<td>6.32</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>52.10</td>
<td>1.77</td>
<td>23.52</td>
<td>8.96</td>
<td>12.58</td>
<td>97.68</td>
</tr>
<tr>
<td>Effluent</td>
<td>Mean</td>
<td>4.48</td>
<td>7.70</td>
<td>261.01</td>
<td>34.83</td>
<td>16.94</td>
<td>14.00</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.96</td>
<td>0.32</td>
<td>71.22</td>
<td>0.84</td>
<td>20.10</td>
<td>11.50</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>43.81</td>
<td>4.13</td>
<td>27.29</td>
<td>2.42</td>
<td>118.68</td>
<td>82.14</td>
</tr>
</tbody>
</table>

TA: total alkalinity; Temp.: temperature; SD: standard deviation; CV: coefficient of variation.

A nitrate increase in the reactor effluent can be observed, with the three combined nitrogen forms in the effluent higher than the N–NH\(_4^+\) values in the affluent, indicating the existence of heterotrophic activity in the reactor (ammonification), in which organic nitrogen was converted into ammoniacal nitrogen, providing more substrate for nitrification during batching. MOREIRA et al. (2013) observed average values for the constants k\(_1\) (nitritation) and k\(_2\) (nitratation) of 0.0043 ± 0.00315 min\(^{-1}\) and 0.02305 ± 0.00105 min\(^{-1}\), respectively, indicating that the conversion of ammoniacal nitrogen into nitrite is a limiting stage of the process since its speed is five times lower than the conversion rate from nitrite to nitrate, confirming the favoring of nitrate generation with cycle time increasing.

Phase II

In the Table 4 is shows the characterization of reactor affluent and effluent during the its operation under conditions established at experimental phase II (CT: 6h; pH: 7.4 to 7.8; DO: 1.0 mg L\(^{-1}\)).

TABLE 4. Characterization of reactor affluent and effluent at phase II.

<table>
<thead>
<tr>
<th></th>
<th>DO (mg L(^{-1}))</th>
<th>pH</th>
<th>TA (mg L(^{-1}))</th>
<th>Temp. (°C)</th>
<th>N–NH(_4^+) (mg L(^{-1}))</th>
<th>N–NO(_2^-) (mg L(^{-1}))</th>
<th>N–NO(_3^-) (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affluent</td>
<td>Mean</td>
<td>1.72</td>
<td>7.60</td>
<td>606.63</td>
<td>22.63</td>
<td>44.51</td>
<td>12.03</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.58</td>
<td>0.07</td>
<td>115.56</td>
<td>4.35</td>
<td>4.49</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>91.87</td>
<td>0.96</td>
<td>19.05</td>
<td>19.23</td>
<td>10.08</td>
<td>18.67</td>
</tr>
<tr>
<td>Effluent</td>
<td>Mean</td>
<td>1.70</td>
<td>7.89</td>
<td>539.08</td>
<td>33.85</td>
<td>21.77</td>
<td>49.39</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.83</td>
<td>0.28</td>
<td>72.75</td>
<td>1.37</td>
<td>7.75</td>
<td>16.17</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>107.98</td>
<td>3.57</td>
<td>13.49</td>
<td>4.03</td>
<td>35.60</td>
<td>32.73</td>
</tr>
</tbody>
</table>

TA: total alkalinity; Temp.: temperature; SD: standard deviation; CV: coefficient of variation.

At phase II, a higher nitrate concentration was observed in the reactor affluent when
compared to phase I due to nitrate accumulation from the previous batch. The occurrence of complete nitrification was also observed, demonstrating that cycle time reduction from 16 to 6 hours, while maintaining the other operating conditions, was insufficient to promote partial nitrification. TKN average values in reactor affluent and effluent were 252.68 ± 66.57 mg L$^{-1}$ and 24.84 ± 10.76 mg L$^{-1}$, respectively, and COD values were 45.26 ± 7.46 and 11.67 ± 7.69 mg L$^{-1}$, respectively. BRESSAN et al. (2011) observed nitrate formation in the second month of reactor operation. The authors attributed nitrate accumulation to the cellular growth of nitrifying bacteria, making ammonia oxidation process faster. The authors worked around this situation with CT reduction, a measure also adopted in this study.

**Phase III**

At experimental phase III (TC: 5 h; pH: 8 to 9; DO: 1 mg L$^{-1}$), pH range from 8 to 9 was used in order to inhibit nitrite-oxidizing bacteria by free ammonia (SUN et al, 2010). In the Table 5 is show concentration values of nitrogenous forms in reactor affluent and effluent during the operation under phase III conditions.

**TABLE 5.** Results of nitrogenous forms in reactor affluent and effluent at phase III.

<table>
<thead>
<tr>
<th></th>
<th>N–NH$_4^+$ (mg L$^{-1}$)</th>
<th>N–NO$_2^-$ (mg L$^{-1}$)</th>
<th>N–NO$_3^-$ (mg L$^{-1}$)</th>
<th>Free ammonia (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affluent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>46.30</td>
<td>1.90</td>
<td>26.83</td>
<td>–</td>
</tr>
<tr>
<td>SD</td>
<td>7.90</td>
<td>9.90</td>
<td>9.90</td>
<td>–</td>
</tr>
<tr>
<td>CV (%)</td>
<td>17.00</td>
<td>105.00</td>
<td>36.90</td>
<td>–</td>
</tr>
<tr>
<td><strong>Effluent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>15.10</td>
<td>7.20</td>
<td>77.00</td>
<td>2.84</td>
</tr>
<tr>
<td>SD</td>
<td>14.52</td>
<td>11.60</td>
<td>32.70</td>
<td>3.32</td>
</tr>
<tr>
<td>CV (%)</td>
<td>96.20</td>
<td>160.60</td>
<td>42.50</td>
<td>116.10</td>
</tr>
</tbody>
</table>

SD: standard deviation; CV: coefficient of variation.

In the Table 5 can be observed free ammonia formation in the reactor. Inhibition of bacteria that oxidize nitrite to nitrate can occur at free ammonia concentrations from 0.1 mg L$^{-1}$, but according to ANTHONISEN et al. (1976), this inhibition effectively manifests for *Nitrosomonas* at initial free ammonia concentration in the range from 10 to 150 mg L$^{-1}$. Such concentration was not reached in this study.

At 5-hour cycle time (phase III), parameter values in reactor affluent and effluent were, respectively, 60.27 ± 7.36 and 30.94 ± 13.72 mg L$^{-1}$ for TKN, 50.86 ± 9.75 and 37.26 ± 11.35 mg L$^{-1}$ for COD, 8.58 and 8.72 for pH and 1367 and 1310 mg L$^{-1}$ for alkalinity.

SUN et al. (2010) obtained satisfactory results when using free ammonia as an inhibitor of nitrite-oxidizing bacteria in a study with sanitary sewage with an effluent concentration of 2444 mg L$^{-1}$ of ammoniacal nitrogen and 15 mg L$^{-1}$ of free ammonia. The authors aforementioned obtained partial nitrification efficiency of 76%. Comparing that result to those found in this study, the initial low ammoniacal nitrogen concentration in this study may promoted a negative influence on that inhibition strategy.

In a similar research, aiming at nitrification via nitrite, QUEIROZ et al. (2011) worked with an initial free ammonia concentration of approximately 12 mg L$^{-1}$, an internal reactor temperature of 35 ºC and an initial concentration of ammoniacal nitrogen varying between 200 and 500 mg L$^{-1}$. The authors observed that such free ammonia concentration was insufficient to inhibit nitrification, even though within the parameters recommended by the literature. Thus, these results were attributed to microorganism adaptation. Free ammonia concentrations present in this study also evidenced such adaptation behavior of bacteria to free ammonia concentrations present in the substrate.

**Phase IV**

In the Table 6 is shown the characterization of reactor affluent and effluent during the
operation under experimental phase IV conditions (CT: 4 h; DO: 0.5 mg L\(^{-1}\)).

**TABLE 6. Characterization of reactor affluent and effluent at phase IV.**

<table>
<thead>
<tr>
<th></th>
<th>DO (mg L(^{-1}))</th>
<th>TA (mg L(^{-1}))</th>
<th>SA (mg L(^{-1}))</th>
<th>pH</th>
<th>(\text{N}--\text{NH}_4^+) (mg L(^{-1}))</th>
<th>(\text{N}--\text{NO}_2^-) (mg L(^{-1}))</th>
<th>(\text{N}--\text{NO}_3^-) (mg L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Affluent</strong></td>
<td>Mean</td>
<td>0.76</td>
<td>213.69</td>
<td>254.20</td>
<td>7.19</td>
<td>46.01</td>
<td>43.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.22</td>
<td>32.25</td>
<td>121.35</td>
<td>0.35</td>
<td>5.98</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>3.46</td>
<td>6.62</td>
<td>2.09</td>
<td>20.18</td>
<td>7.69</td>
<td>1.06</td>
</tr>
<tr>
<td><strong>Effluent</strong></td>
<td>Mean</td>
<td>–</td>
<td>166.81</td>
<td>–</td>
<td>7.45</td>
<td>31.64</td>
<td>17.52</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>–</td>
<td>56.40</td>
<td>–</td>
<td>0.31</td>
<td>10.15</td>
<td>15.85</td>
</tr>
<tr>
<td></td>
<td>CV (%)</td>
<td>–</td>
<td>2.96</td>
<td>–</td>
<td>24.10</td>
<td>3.11</td>
<td>1.10</td>
</tr>
</tbody>
</table>

TA: total alkalinity, SA: supplemented alkalinity CaCO\(_3\).

Nitrifying bacteria activity was inhibited from strategies used in this experimental phase (IV), in which additional alkalinity started being provided, calculated as Equation 2. However, such factors were still not sufficient to maintain stable partial nitritation, represented by 50% N--NH\(_4^+\) and 50% N--NO\(_2^-\).

At four-hour cycle time (phase IV), affluent and effluent concentrations of TKN were 69.92 ± 29.50 and 35.93 ± 11.30 mg L\(^{-1}\), respectively, and 52.44 ± 10.24 and 28.53 ± 6.62 mg L\(^{-1}\) for COD, also demonstrating a heterotrophic activity in the reactor.

SCHREEN et al. (2011) and GALÍ et al. (2010), when carrying out studies with partial denitritation implementation, observed that high concentrations of ammoniacal nitrogen facilitated the process maintenance. The authors commented that the critical point for partial nitrification maintenance from ammonia to nitrite was low ammoniacal nitrogen concentration in the affluent. ZHU et al. (2008) also reported difficulties in maintaining partial nitritation in affluents with low ammoniacal nitrogen concentrations (close to 50 mg L\(^{-1}\)) due to low ammonia saturation coefficient.

When comparing the results of this study with those of other authors, a possible conclusion is that low ammonia concentration in the affluent may have promoted a negative influence on the establishment and maintenance of partial nitration in the reactor. SUN et al. (2010) and PRÁ et al. (2012) were successful in partial nitritation when working with ammonia concentrations of 2444 mg L\(^{-1}\) and approximately 1343.56 mg L\(^{-1}\), respectively.

**Phase V – Real-time monitoring of partial nitritation**

At phase V (CT: variable; DO: 0.1 to 0.3 mg L\(^{-1}\)), the last experimental phase and that which received alkalinity supplementation according to Equation 2, the ideal cycle time for wastewater partial nitritation was investigated. For this, 12 cycles were carried out, in which wastewater was sampled at each hour and performed analyses of ammoniacal nitrogen, nitrite, and nitrate. The cycle was stopped at the time the ratio of approximately 50% nitrite to 50% ammoniacal nitrogen (partial nitritation) was identified. Results of N--NH\(_4^+\), N--NO\(_2^-\) and N--NO\(_3^-\) for reactor affluent and effluent at phase V cycles are shown in Figure 1.
FIGURE 1. Concentration of nitrogenous forms in reactor affluent and effluent at phase V cycles.

The cycle time of the 12 cycles performed at phase V presented variation, with an average of 6.03 hours and a modal value of 6.5 hours.

As demonstrated in this study, BLACKBURNE et al. (2007) also used low DO concentration in a nitrifying reactor operated under the continuous system. Enrichment of ammonia-oxidizing bacteria was achieved with DO concentration of 0.4 mg L\(^{-1}\).

SUN et al. (2010) observed that in several studies partial nitritation was maintained from the application of real-time control in SBR reactors. GU et al. (2012) assessed the performance of partial nitritation process under low-temperature conditions in a sequential batch reactor with real-time control and obtained 90% nitrite accumulation rate.

Results of TKN and COD obtained at this phase also reinforce ammonification occurrence in the reactor from COD consumption. TKN and COD concentrations in reactor affluent and effluent were 71.53 ± 6.05 and 41.06 ± 7.36 mg L\(^{-1}\) and 81.19 ± 11.39 and 43.05 ± 11.49 mg L\(^{-1}\), respectively.

In the Figure 2 is shown the nitrogenous species concentrations, total ammoniacal nitrogen (N–TAN) in reactor affluent and effluent, nitrite and nitrate in the effluent (Figure 2a) and N–NH\(_4^+\):NO\(_2^-\) ratio values (Figure 2b) for all the tests carried out at experimental phases from I to V.

FIGURE 2. Reactor behavior for all cycles of phases from I to V: a) nitrogen species concentrations in reactor affluent and effluent; b) N–NH\(_4^+\):NO\(_2^-\) ratio values at the end of each cycle.

To be considered partial nitritation, N–NH\(_4^+\):NO\(_2^-\) ratio should have a value close to 1, as shown in Equation 3 (MADIGAN, 2010):
Stability of partial nitritation of poultry slaughterhouse wastewater in a sequential batch reactor

\[
\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.26\text{NO}_3^- + 0.066\text{CH}_2\text{O}_0.3\text{N}_{0.15} + 2.03\text{H}_2\text{O} \quad (3)
\]

According to the profiles presented in Figure 2, mainly regarding N–NH\textsuperscript{4+}:NO\textsubscript{2}– ratio (Figure 2b), partial nitritation process stabilization was not reached during the study. However, cycles in which N–NH\textsuperscript{4+}:NO\textsubscript{2}– ratio reached values close to 1 were observed at all studied phases, indicating that modifications in inhibition strategies reached their initial effect, followed by microorganism adaptation to the changes promoted. In addition, at phase V, in which real-time monitoring of partial nitritation was promoted, N–NH\textsuperscript{4+}:NO\textsubscript{2}– ratio values were more stable and closer to those recommended.

CONCLUSIONS

Ammonification process, observed at all experimental phases, negatively influenced partial nitritation process control. Alkalinity restriction together with low dissolved oxygen concentration under low cycle time (4 and 5 h) conditions, was decisive factors for nitrite-oxidizing bacteria inhibition. Partial nitritation stability was only achieved by real-time monitoring, in which cycles were stopped when 50% N–NH\textsuperscript{4+} and 50% N–NO\textsubscript{2}– were present in the reactor.

ACKNOWLEDGEMENTS

To CAPES for granting a scholarship.

REFERENCES


BRESSAN, C.R.; SOARES, H.M.; KUNZ, A. Limiting alkalinity to control partial nitritation in a sequencing batch reator (SBR) in order to adequate an effluent to ANAMMOX processes. In: II SIMPÓSIO INTERNACIONAL SOBRE GERENCIAMENTO DE RESÍDUOS AGROPECUÁRIOS E AGROINDUSTRIAIS, 2., 2011, Foz do Iguaçu. Anais...


