Mathematical Exposition of Simultaneous Nitrification and Aerobic Denitrification

S.G. Kanyenda, S.K.O. Ntwampe, N. Mpongwana, B. Godongwana

Abstract—Traditional nitrogen removal techniques include sequential nitrification and aerobic denitrification processes. Recent technologies on nitrogen removal have shown that nitrification and denitrification processes can occur simultaneously in one bioreactor. However, models that ascertain the stoichiometric conversion ratios have not been developed for such systems. In this study simultaneous nitrification and aerobic denitrification data was used in models to describe the substrate consumption and product formation, with simplification using Simpson's rule being used to develop a suitable model. The stoichiometric conversion ratio from NH$_4$N to NO$_3$-N to NO$_2$-N where approximately found to be ranging from 0.35 - 0.05 mol: 0.677 – 0.35mol: 1 mol, respectively, indicating that the biological stoichiometric ratios of the simultaneous nitrification and aerobic denitrification processes are not equivalent as previously suggested in theoretical chemical stoichiometric ratios associated with traditional nitrification and denitrification.

Keywords— Aerobic denitrification, Biological Stoichiometry, Nitrification, Simultaneity.

I. INTRODUCTION

Biological nitrification-denitrification is the most commonly used process for total nitrogen removal from wastewater. This type of biological treatment of wastewater is important to minimise and avoid ecological degradation and deterioration of raw/fresh water sources used for drinking, industrial, agricultural and recreational purposes, which can culminate in potential hazards to humans or animals [9]. Such treatments have become common and a core activity for wastewater treatment plants. Biological nitrogen removal is generally preferred over physicochemical methods due to lower chemical/energy requirements, and being environmental benign [4], [2]. Nitrification is the biological oxidation of NH$_4$-N or NH$_3$ by means of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB) to nitrite first and then nitrates [1], [5]. Traditionally denitrification is a microbially facilitated process of nitrate reduction performed by heterotrophic facultative anaerobic bacteria that will produce nitrogen gas through a series of intermediate gaseous nitrogen oxides [7].

Traditional nitrogen removal in wastewater treatment plants occurs in separate aerobic and anaerobic stages sequentially, meaning that the nitrification process occurs in the first aerobic bioreactor and then the second bioreactor is used for anaerobic denitrification. This is because it is believed that denitrification can only occur under completely anaerobic and/or anoxic conditions, leading to an observation repudiating this theory by [12], indicating that both nitrification and aerobic denitrification can occur or took place in a single bioreactor under identical environmental conditions, a process currently known as simultaneous nitrification and denitrification (SND). Newly developed SND systems tend to be cheaper as they use less bioreactor volume, has reduced plant footprint, produces less sludge, and have lower energy intensity. Furthermore, SND systems are characterized by increased nitrogen removal rates and decreased reaction time. The maintenance of a population balance is also not necessary in SND as the two reactions (nitrification and denitrification) proceed under identical reaction conditions [8].

The SND reactions are a combination of the nitrification and denitrification reactions due to their simultaneous occurrence (Eq. 1 and 2.) as reported by [6].

\[
\begin{align*}
\text{NH}_3 + O_2 + H_2O & \rightarrow NO_3^- + 5 H^+ + 4 e^- \quad (1) \\
2 \text{NO}_3^- + 10 e^- + 12 H^+ & \rightarrow N_2 + 6 H_2O \quad (2)
\end{align*}
\]

With the overall reaction being – see Eq. 3:

\[
\text{NH}_3 + O_2 + \text{NO}_3^- + 6 e^- + 7 H^+ \rightarrow N_2 + 5 H_2O \quad (3)
\]

This study developed a model with which the understanding of the stoichiometry of simultaneous nitrification and aerobic denitrification is studied, elucidating changes in the NH$_4$+, NO$_3$- and NO$_2$- concentrations at particular time intervals due to changes in the metabolism of the microbes responsible for their oxidation. The model assessment determined whether the reactions are in series or parallel (NH$_4$+ is being degraded to both NO$_3$- and NO$_2$ simultaneously).

II. MATERIALS AND METHODS

A. Bioreactor Conditions for Simultaneous Nitrification and Aerobic Denitrification

The performance of simultaneous nitrification and aerobic denitrification by Chryseobacterium sp. R31 was conducted in inorganic basal medium which consisted of 14g/L K$_2$HPO$_4$, 6g/L KH$_2$PO$_4$, 0.2g/L MgSO$_4$-7H$_2$O, 1.251 g/L glucose, 0.191 g/L ammonium chloride and trace mineral solution 2 mL at pH 7. The trace mineral solution consisted of, EDTA 0.01g/L, ZnSO$_4$-7H$_2$O 0.0001g/L; CaCl$_2$-2H$_2$O 0.1g/L; MnCl$_2$-2H$_2$O 0.008g/L; FeCl$_3$-6H$_2$O 0.71g/L; (NH$_4$)$_2$Mo$_7$O$_{24}$ 0.00011g/L; CuSO$_4$-5H$_2$O 0.0001g/L; CoCl$_2$-6H$_2$O 0.2g/L. It was incubated

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at 37°C with shaking (100 rpm) for 48 h. Aliquots were removed from each Erlenmeyer flask at 4-hour intervals to determine NH₄⁺, NO₃⁻ and NO₂⁻.[8] A 100 mL of HNM with 0.031 g NH₄Cl, 0.047 g nitrate, and 0.27 g sodium succinate at a pH value of 7.2, was also inoculated with Alcaligenes sp. TB and cultivated at 30 °C, and 120 rpm. The HNM consisted of sodium succinate, 4g/L, KH₂PO₄ 0.5g/L, K₂HPO₄ 0.5g/L, MgSO₄·7H₂O 0.1g/L, CaCl₂ 0.02g/L and trace element solution, 1 mL. Trace element solution contains the following (g/L): CuSO₄ 1.0, FeSO₄ 1.0, MnSO₄ 5.0, Na₂MoO₄ 1.0 and ZnCl₂ 2.0. Samples were collected every 4 h to determine NH₄⁺, NO₃⁻ and NO₂⁻ [3].

B. Model Development

Given the chemical stoichiometric ratios of nitrification and denitrification, 1 mol of NH₄⁺ reacts with dissolved oxygen to produce 1 mol of NO₃⁻ which is further reduced to 1 mol of NO₂⁻ such that [10] – see Eq. 4:

\[
\frac{d(NH_4^+)}{dt} = a \int_0^{\infty} C(t) \, dt - b \int_0^{\infty} C(t) \, dt + c \int_0^{\infty} C(t) \, dt
\]

(4)

**TABLE I: DEFINING THE PROPOSED MODEL (4)**

<table>
<thead>
<tr>
<th>Conversion</th>
<th>NH₄⁺</th>
<th>NO₂⁻</th>
<th>NO₃⁻</th>
<th>N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disappearance/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degraded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formation/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disappearance</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formation/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disappearance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

whereby, a, b, c are stoichiometric coefficients.

And, \( a \int_0^{\infty} C(t) \, dt \) can be solved using Simpson’s rule to determine the concentration of either NO₂⁻, NO₃⁻, N₂ per time interval. Hence, integrals can be solved easily by dividing the curve into smaller trapezoids to increase accuracy and using the Simpson’s rule as shown in Eq. 5:

\[
\int_0^{14} f(x) dx = \frac{h}{3} [f(x_0) + 4f(x_1) + 2f(x_2) + 4f(x_3) + 2f(x_4) + \cdots + 4f(x_{n-1}) + f(x_n)]
\]

(5)

For which the given curve \( C(t) \) where the experiment was run from \( t = x_0 \) to \( x_n \) can be used to describe how the concentration \( C(t) \) varies with time, whereby (see Eq. 6):

\[
\int_{x_0}^{x_0+x_n} f(x) \, dx = \frac{x_n-x_0}{3n} \left[ f(x_0) + 4g(x_1) + 2g(x_2) + 4g(x_3) + 2g(x_4) + \cdots + 4g(x_{n-1}) + 2g(x_n) \right]
\]

(6)

Where \( f(X_0) \) represents the concentration \( C(t) \) when \( t = 0 \) and \( n \) is the number of trapezoids, and \( f(X_n) \) represents the concentration \( C(t) \) at \( t = X_n \), used for the mathematical exposition of the curve/experimental data.

III. RESULTS AND DISCUSSION

Experimental data, e.g. Figure 1 and 2 by [8] and [3] was used to develop the model. Figure 1 shows the profile of NH₄⁺ degradation by Chryseobacterium sp. R31 isolate where 95.87% of NH₄⁺ was consumed after 48h, the ammonium degradation/utilization rate was high within the first 32h after which it declined. The simultaneous accumulation of NO₂⁻ and NO₃⁻ with time is also shown in Figure 1. Decreases in the NO₂⁻ and NO₃⁻ concentrations ascertained the denitrification process and was associated with their utilization as nitrogen source after the exhaustion of ammonium [8]. NH₄⁺ utilization by Alcaligenes sp. TB is shown in Figure 2, where 200mg/L of NH₄⁺ was completely removed. The Alcaligenes sp. TB has better ammonium removal properties compared to the results in Figure 1 by Kundu et al. [8], [3].

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Fig. 1: Time profile of NH₄⁺ oxidation by Chryseobacterium sp. R31. NH₄⁺ (filled triangles), NO₂⁻ (filled squares), and NO₃⁻ (filled circles).

Fig. 2: NH₄⁺ degradation by Alcaligenes sp. TB characteristics of group (I) NH₄⁺ (filled triangles), NO₂⁻ (filled squares), and NO₃⁻ (filled circles).

As per the theoretical stoichiometric analysis of both nitrification and denitrification, a mole of NH₄⁺ degraded produces a mole of NO₃⁻ that will further be degraded to a mole of NO₂⁻ based on chemical stoichiometric ratio conversions given that the reactions are hypothetically assumed to be in series. However, with the SND processes, it was observed that the quantity of NO₃⁻ produced inadequately describes the molar quantity of NH₄⁺ degraded as it is also a nitrogen source for biological growth, i.e. a nutrient which is also required by microorganisms for metabolic activities. Thus, Table 1 and 2 indicates the data derived for use in the further analyses of the phenomena observed.
The concentration of NH$_4^+$ degraded over a period of 35 h was computed, and the concentrations of NO$_3^-$ and NO$_2^-$ over the same period of time were determined as elucidated in Eq. 6. Thus, the ratio of the ammonium degraded to nitrite or nitrate was, 0.3142 mmol/L.h (NH$_4^+$): 2.4 mmol/L.h (NO$_2^-$): 6.9 mmol/L.h (NO$_3^-$), which were translated into 0.05 (NH$_4^+$): 0.35 (NO$_2^-$): 1 (NO$_3^-$). Similarly, as shown in Fig. 2, for an experiment conducted for a longer experimentation period (48 h), the conversion ratios were 0.066 mmol/L.h (NH$_4^+$): 1.287 mmol/L.h (NO$_2^-$): 1.9 mmol/L.h (NO$_3^-$) translated to ratios of 0.035 (NH$_4^+$): 0.0677 (NO$_2^-$): 1 (NO$_3^-$), which indicated that SND can be species dependent with bioreactor conditions being highly influential in the determination of SND processes. The rate and ratio of NH$_4^+$ removal by *Chryseobacterium* sp. R31 was supposedly lower than the one by *Alcaligenes* sp. TB; however, the observation was approximately similar to the rate of NH$_4^+$ removal by *Pseudomonas alcaligenes* AS-1 which was 0.064 mmol/L.h [8]. It was reported that the NH$_4^+$ removal by *Bacillus* sp. LY was 0.024 mmol/L.h, a lower rate of removal than that observed for *Alcaligenes* sp. TB.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>NH$_4^+$ (mg/L)</th>
<th>NO$_2^-$ (mg/L)</th>
<th>NO$_3^-$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>200</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>145</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>106</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
<td>6.5</td>
<td>12</td>
</tr>
<tr>
<td>20</td>
<td>45</td>
<td>6.5</td>
<td>15</td>
</tr>
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<td>10</td>
</tr>
<tr>
<td>35</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE II: DATA DERIVED FROM FIGURE 1</th>
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</thead>
</table>

The average ratios for the ammonium degradation to nitrite to nitrate were from 0.035 - 0.05 (NH$_4^+$): 0.677 – 0.35 (NO$_3^-$): 1 (NO$_2^-$).

These stoichiometric ratios indicated that the conversion reactions from NH$_4^+$ to NO$_2^-$ and NO$_3^-$ were hypothetically parallel, meaning that NH$_4^+$ was degraded to NO$_2^-$ and NO$_3^-$ simultaneously. The biological stoichiometric ratios also differed from the theoretical/chemical stoichiometric ratios on conversion from one nitrogenous compound to another [11], depending on bioreactor physiological parameters. This study is one of the preliminary studies associated with the elucidation of the SND via mathematical modeling.

### IV. CONCLUSIONS

From the results observed using the model developed to describe the biological conversion and/or stoichiometric ratio in SND, the average ratios for the NH$_4^+$ degradation to NO$_2^-$ was found to be ranging from; 0.035 - 0.05 (NH$_4^+$): 0.677 – 0.35 (NO$_3^-$): 1 (NO$_2^-$) meaning that for 0.035 moles of NH$_4^+$ consumed 0.0677 moles of NO$_3^-$ are produced.

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### REFERENCES


Sphiwe G Kanyenda was born in Zimbabwe on the 4th of September 1993. She obtained her Baccalaureus Technologiae’ in Biomedical Biotechnology in 2017 and the National Diploma in Biotechnology in 2016 at the Cape Peninsula University of Technology, Cape Town, South Africa and is currently a Master of Engineering: Chemical Engineering student, at the Cape Peninsula University of Technology, Cape Town, South Africa.