

Mathematical Exposition of Simultaneous Nitrification and Aerobic Denitrification

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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 $\text{NH}_4\text{-N}$ to $\text{NO}_3\text{-N}$ to $\text{NO}_2\text{-N}$ where approximately found to be ranging from 0.035 - 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.

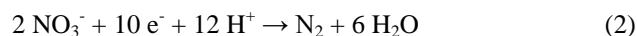
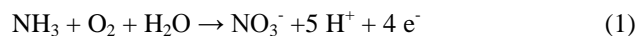
I. INTRODUCTION

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 $\text{NH}_4^+\text{-N}$ 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].

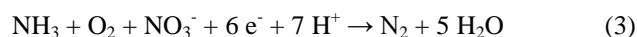
Traditional nitrogen removal in wastewater treatment plants occurs in separate aerobic and anaerobic stages sequentially, meaning that the nitrification process occurs in the first aerobic bioreactor and then the second bioreactor is used for anaerobic

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].



With the overall reaction being – see Eq. 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_2HPO_4 , 6g/L KH_2PO_4 , 0.2g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 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, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.0001g/L; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1g/L; $\text{MnCl}_2 \cdot 2\text{H}_2\text{O}$ 0.008g/L; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.71g/L; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ 0.00011g/L; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.0001g/L; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 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:

$$\frac{d(NH_4^+)}{dt} : a \int_0^\infty C(t) dt : b \int_0^\infty C(t) dt : c \int_0^\infty C(t) dt \quad (4)$$

TABLE I: DEFINING THE PROPOSED MODEL (4)

$\frac{d(NH_4^+)}{dt}$	$a \int_0^\infty C(t) dt$	$b \int_0^\infty C(t) dt$	$c \int_0^\infty C(t) dt$
NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	N ₂
Disappearance/ Degraded	Formation/ Disappearance	Formation/ Disappearance	formation

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:

$$\int_0^{14} f(x) dx = \frac{h}{3} [f_0 + 4f_1 + 2f_2 + 4f_3 + 2f_4 + \dots + 4f_{n-1} + f_n] \quad (5)$$

For which the given curve C(t) where the experiment was run from t = x₀ to x₆, can be used to describe how the concentration C(t) varies with time, whereby (see Eq. 6):

$$\int_{x_0}^{x_6} c(t) dt = \frac{x_6-x_0}{3n} [f(x_0) + 4f(x_1) + 2f(x_2) + 4f(x_3) + 2f(x_4) + 4f(x_5) + 2f(x_6)] \quad (6)$$

Where f(X₀) represents the concentration C(t) when t = 0 and n is the number of trapezoids, and f(X₆) represents the concentration C(t) at t = X₆, 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 Figure1. 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].

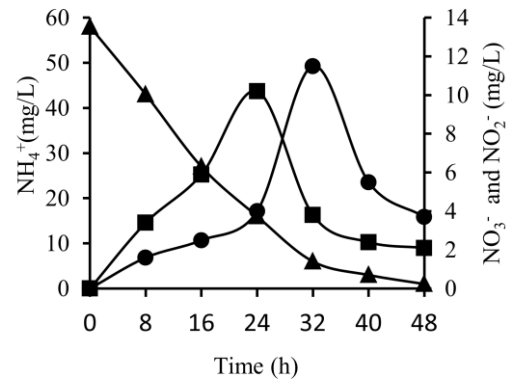


Fig. 1: Time profile of NH₄⁺ oxidation by *Chryseobacterium* sp. R31. NH₄⁺ (filled triangles), NO₂⁻ (filled squares), and NO₃⁻ (filled circles).

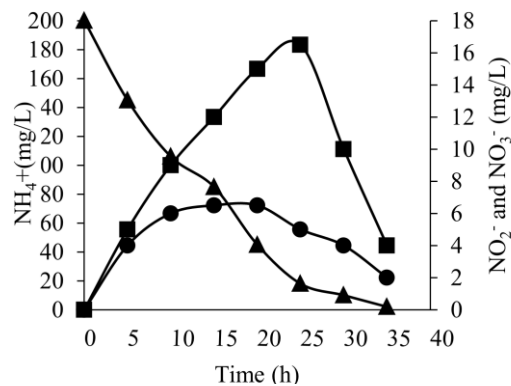


Fig 2: NH₄⁺ degradation by *Alcaligenes* sp. TB characteristics of group (I) NH₄⁺ (filled triangles), NO₂⁻ (filled squares), and NO₃⁻ (filled circles).

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.

TABLE II: DATA DERIVED FROM FIGURE 1

Time (h)	NH ₄ ⁺ (mg/L)	NO ₃ ⁻ (mg/L)	NO ₂ ⁻ (mg/L)
0	200	0	0
5	145	4	5
10	106	6	9
15	85	6.5	12
20	45	6.5	15
25	18	5	16.5
30	10	4	10
35	2	2	4

The concentration of NH₄⁺ degraded over a period of 35 h was computed, and the concentrations of NO₃⁻ and NO₂⁻ 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 (NH₄⁺): 2.4 mmol/L.h (NO₃⁻): 6.9 mmol/L.h (NO₂⁻), which were translated into 0.05 (NH₄⁺): 0.35 (NO₃⁻): 1 (NO₂⁻). 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 (NH₄⁺): 1.287 mmol/L.h (NO₃⁻): 1.9 mmol/L.h (NO₂⁻) translated to ratios of 0.035 (NH₄⁺): 0.0677 (NO₃⁻): 1 (NO₂⁻), which indicated that SND can be specie dependent with bioreactor conditions being highly influential in the determination of SND processes. The rate and ratio of NH₄⁺ 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 NH₄⁺ removal by *Pseudomonas alcaligenes* AS-1 which was 0.064 mmol/L.h [8]. It was reported that the NH₄⁺ removal by *Bacillus* sp. LY was 0.024 mmol/L.h, a lower rate of removal than that observed for *Alcaligenes* sp. TB.

TABLE III: DATA EXTRACTED FROM FIGURE 2

Time (h)	NH ₄ ⁺ (mg/L)	NO ₃ ⁻ (mg/L)	NO ₂ ⁻ (mg/L)
0	58	0	0
8	43	3.4	1.6
16	27	5.9	2.5
24	16	10.2	4
32	6	3.8	11.5
40	3	2.4	5.5
48	1	2.1	3.7

The average ratios for the ammonium degradation to nitrite to nitrate were from 0.035 - 0.05 (NH₄⁺): 0.677 - 0.35 (NO₃⁻): 1 (NO₂⁻).

These stoichiometric ratios indicated that the conversion reactions from NH₄⁺ to NO₂⁻ and NO₃⁻ were hypothetically parallel, meaning that NH₄⁺ was degraded to NO₂⁻ and NO₃⁻ 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 NH₄⁺ degradation to NO₃⁻ to NO₂⁻ was found to be ranging from; 0.035 - 0.05 (NH₄⁺): 0.677 - 0.35 (NO₃⁻): 1 (NO₂⁻) meaning that for 0.035 moles of NH₄⁺ consumed 0.0677 moles of NO₃⁻ are produced.

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