

Kinetic study on nitrification of ammonium nitrogen-enriched synthetic wastewater using activated sludge

Roumi Bhattacharya and Debabrata Mazumder 

ABSTRACT

Nitrification of ammonium nitrogen ($\text{NH}_4^+\text{-N}$)-bearing synthetic wastewater was performed in a batch-activated sludge reactor by varying the initial ammonium nitrogen concentration up to 400 mg/L at a pH of 8.1 ± 0.2 and temperature of $36 \pm 2^\circ\text{C}$ for developing the process kinetics using acclimatised biomass. Maximum ammonium nitrogen removal efficiency of 98.3% was achieved with initial ammonium nitrogen and mixed liquor suspended solids concentration of 235 mg/L and 2,180 mg/L, respectively, at 48 h batch period. Based on the experimental results, kinetic constants for ammonia nitrogen removal following Monod's approach were obtained as maximum substrate removal rate coefficient = 0.057 per day, yield coefficient = 0.336 mg volatile suspended solids/mg ammonium nitrogen, half velocity constant = 12.95 mg $\text{NH}_4^+\text{-N/L}$ and endogenous decay constant = 0.02 per day. Nitrification is a consecutive reaction with ammonium oxidation as the first step followed by nitrite oxidation. The overall rate of nitrite and nitrate formation was observed to be 1.44 per day and 0.34 per day, respectively.

Key words | activated sludge, nitrification kinetics, nitrification study, nitrogenous wastewater, overall reaction rate

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INTRODUCTION

Nitrogen in wastewater, when present beyond a certain limit in the form of ammonium or ammonia, leads to several environmental problems and is thus objectionable. Being a nutrient, removal of nitrogen is generally carried out in various biological processes by conversion of ammonium to nitrate via nitrite. This pathway is termed as nitrification, where the two consecutive steps of ammonia and nitrite oxidation are brought about by two different groups of nitrifiers: *Nitrosomonas* is the microbial genera responsible for ammonium oxidation and *Nitrobacter* and *Nitrospira* for nitrite oxidation. It is observed that *Nitrospira* dominates at low ammonium and low nitrite conditions (Blackburne *et al.* 2007). Nitrifiers are slow growing microorganisms and their growth rate is significantly affected by environmental factors, including pH, temperature, substrates concentration and presence of inhibitory compounds (Svenson *et al.* 2000). Efficient nitrification and successful optimisation of process design therefore demands effective control over operational conditions and appropriate understanding of growth kinetics of the nitrifiers.

Different kinetic parameters are basic requisites that must be measured to determine nitrification capacity of a

reactor; incorrect assessment of which may lead to uneconomical or even faulty process design. Development of kinetics is essential for fundamental understanding and quantitative analysis of biological processes facilitating the optimization of reactor volume and treatment period for a desired treatment capacity. Moreover, kinetic models describe the dynamics of a biological process, the effects of substrate concentration and other environmental parameters, including pH, temperature, inhibitory compounds etc. This needs to be developed to establish the relationship between substrate degradation rate and bacterial growth rate. The nitrifiers have a very slow growth rate and a high retention time is therefore required to attain requisite biomass concentration in the activated sludge reactor. A minimum sludge age from 2 to 20 days has been reported by previous researchers under various environmental conditions (Lawrence & Brown 1976; Stover *et al.* 1976). Maximum specific growth rate, describing the growth of biomass, is another governing kinetic parameter that determines the operational period of the system, below which nitrifiers would be washed out (Antoniou *et al.* 1990).

The exact order and rate of nitrification in an activated sludge reactor has been researched from a long time, although there are disagreements regarding the exact results obtained. This is because nitrification reaction is extremely sensitive to temperature, substrate concentration, pH and biomass in the reactor. Numerous experimental observations as carried out by earlier researchers showed that overall nitrification reaction is of first-order because the reaction rate is dependent on concentration of nitrifying organisms as well as substrate concentration. When both oxidation kinetics are considered separately, it is observed that they are zero-order reactions (Huang & Hopson 1974; Poduska & Andrews 1975). The experiments carried out by Poduska & Andrews (1975) demonstrated that ammonium oxidation reaction is essentially of zero-order for concentrations ≥ 1.0 mg/L at a temperature of 23 ± 1 °C. Ammonium oxidation is investigated by various researchers to strictly follow zero-order reactions (Wild *et al.* 1971; Kiff 1972; Poduska & Andrews 1975; Wong-Chong & Loehr 1975).

Wild *et al.* (1971) performed a batch nitrification study at very high biomass concentration and ammonium nitrogen concentration between 30 and 40 mg/L. Kiff (1972) developed nitrification kinetics in an activated sludge plant and confirmed it to be a zero-order reaction over an ammonia concentration range of 1.6–52.0 mg/L within a temperature range of 5–30 °C. Huang & Hopson (1974) conducted an experiment with ammonia nitrogen concentration of 2.5–673 mg/L to examine the best-fit model among zero-, first-order and autocatalytic growth reactions. From the experiments, nitrification reaction was confirmed to be of zero-order. Wong-Chong & Loehr (1975) studied the effect of temperature on nitrification kinetics in an activated sludge reactor with varying initial nitrogen concentration of 100–1,100 mg/L, maintaining a pH of 6.0–8.5. The studies confirmed that both ammonia and nitrite oxidation follow zero-order reaction kinetics for substrate depletion and, within the given range of influent ammonium concentration, the reaction rate was independent of substrate concentration. A study was conducted by Charley *et al.* (1980) using a deep shaft-type reactor to determine the best-fit kinetics of nitrification reaction separately, with ammonia concentrations varying between 0.5 and 100 mg/L and nitrite concentrations 0.5–50 mg/L. It was demonstrated that for nitrite nitrogen concentrations up to 20 mg/L, nitrite oxidation follows first-order reaction kinetics.

The growth of nitrifiers follows Monod's Equation and several studies have been conducted to show this, including Downing & Hopwood (1964), Knowles *et al.* (1965),

Shah & Coulman (1978). Knowles *et al.* (1965) developed a computer-based empirical model to describe nitrification kinetics following Monod's approach and later validated the model using experimental data. Results have confirmed that nitrification reaction is fairly validated using this model, where reaction rate is proportional to substrate concentration until maximum substrate removal rate coefficient (μ_{\max}) is reached, and thereafter the kinetics follows zero-order. Most of the earlier research works had been conducted in a temperature range of 5–35 °C and it was observed that optimum nitrification kinetics for ammonia oxidation was obtained around 15–20 °C under pre-examined environmental conditions (Wild *et al.* 1971).

Most of the above studies on nitrification kinetics were conducted between 5 °C and 30 °C. Nitrification reactions are strictly temperature dependent and it is not recommended to follow the same kinetics in warm regions where average temperature varies between 35 °C and 38 °C. Establishment of proper nitrification kinetics at this temperature conditions for optimisation and efficient performance of nitrifying reactors is therefore required. Moreover, to date, there have been no proper nitrification kinetics developed for industrial wastewaters with high nitrogen and low carbon content that can define the conversion of ammonium nitrogen to nitrite nitrogen. Wastewaters from tanneries, pig farms, slaughterhouses and fertiliser industries (Obaja *et al.* 2005; Goberna *et al.* 2010; Keluskar *et al.* 2013), as well as sludge dewatering effluents, effluents from anaerobic digestion and landfill leachate (Wett & Rauch 2003; Guštin & Marinšek-Logar 2011; Dębowski *et al.* 2017) fall under this category where nitrogen is generally present as ammonium nitrogen. The main problem in maintaining high nitrification efficiency when treating low carbon: nitrogen wastewaters is fluctuation in influent concentration and flow, which may also affect to the dissolved oxygen level in the reactor (Campos *et al.* 2007).

With this challenge in background, the present work aims to investigate and compare the reaction order along with the development of proper nitrification kinetics of wastewaters with low carbon: nitrogen ratios in an activated sludge process and compare the results with those obtained from previous literature sources under different experimental conditions. Accordingly, the study has established nitrification kinetics using both the Monod type and the consecutive oxidative reaction approach for a synthetic nitrogenous wastewater sample characterized by high ammonium nitrogen concentrations without any organic carbon content under warm environmental conditions.

MATERIALS AND METHODS

Acclimatisation of nitrifying biomass

The microbial culture used in the study was isolated from root-level soil collected from pea cultivation farms. The microorganisms were acclimatised to high nitrogen- and low carbon-containing synthetic wastewater over a period of 60 days in a batch-activated sludge reactor, in the Environmental Engineering Laboratory Indian Institute of Engineering Science and Technology Shibpur. For that purpose, two different synthetic samples were prepared, one having a chemical oxygen demand (COD) of 10 g/L (Feed 1) and the other having ammonium nitrogen concentration of 10 g/L without any organic carbon source (Feed 2). The compositions of the feeds are given in Tables 1 and 2, respectively.

At the beginning of acclimatisation, 90 mL of Feed 1 and 10 mL of Feed 2 were added to the reactor. After ammonium nitrogen had been degraded to a satisfactory level, the amount of nitrogenous feed (Feed 2) was increased by 5 mL in each step with a reciprocal decrease in the amount of Feed 1 by 5 mL, aggregating a total volume of 100 mL in each step. The process was continued until the feed contained entire volume, i.e. 100 mL of nitrogenous wastewater (Feed 2). During the acclimatisation period, pH and temperature of the reactor was maintained at 8.1 ± 0.2 and 36 ± 2 °C, respectively. At the same time, COD, nitrate and ammonium nitrogen concentrations of the reactor were monitored daily.

Batch studies for development of kinetics

After successfully acclimatising the biomass, several batch studies were undertaken to develop nitrification kinetics. From available literatures, it has been confirmed that the nitrification rate is maximum within the pH range of

Table 1 | Composition of synthetic wastewater used as carbonaceous feed (Feed 1)

Constituents	Amount (in g/L)
Dextrose	10
Ammonium sulfate	3.77
Potassium dihydrogen phosphate	0.175
Dipotassium hydrogen phosphate	0.2
Ferric chloride	0.02
Calcium chloride	0.2
Magnesium sulfate	0.22

Table 2 | Composition of synthetic wastewater containing high concentrations of ammonium nitrogen (Feed 2)

Constituents	Amount (in g/L)
Sodium bicarbonate	90.06
Ammonium sulfate	47.17
Potassium dihydrogen phosphate	9.067
Ferric chloride	0.02
Calcium chloride	0.2
Magnesium sulfate	0.22

7.8–8.5. During the study, the pH was therefore maintained at 8.1 ± 0.2 . The variation in initial ammonium nitrogen concentrations in the batch nitrification study is shown in Table 3.

The parameters monitored during the experiment were analysed according to the procedure mentioned in the American Public Health Association (APHA) *Standard Methods* (Eaton *et al.* 2005), respective references of which are listed in Table 4.

Kinetics of batch nitrification

The experimental data were used to determine the concerned kinetic coefficients from Monod's kinetic approach. The specific growth rate constant for nitrifying organisms can be evaluated from Monod's Equation (Metcalf & Eddy 2014):

$$\mu = \mu_{\max} \frac{N}{K_N + N} \quad (1)$$

where μ_{\max} = maximum specific growth rate constant per day; N = ammonium nitrogen concentration in the solution at any time (mg/L); and K_N = half saturation constant for ammonium nitrogen oxidation (mg/L).

The rate of nitrifying biomass growth (r_g) can now be written as:

$$r_g = \frac{dx}{dt} = -Y \cdot r_{su} \quad (2)$$

Table 3 | Initial ammonium nitrogen concentrations in the batch nitrification study

Batch no.	Initial ammonium nitrogen concentration (mg/L)
1	200
2	235
3	272
4	310
5	355
6	407

Table 4 | Reference of procedures followed for analysis of various parameters

SI no.	Parameters	Procedures followed	References
1	pH	Electrometric method	4500-H part B
2	Total suspended solids	Dried at 103–105 °C	2540 part D
3	COD	Closed reflux, titrimetric method	5220 part C
4	Ammonium nitrogen	Ammonium selective electrode method	4500-N
5	Nitrate nitrogen	UV spectrophotometric screening method	4500-NO ₃ ⁻ part B
6	Nitrite nitrogen	Colorimetric method	4500-NO ₂ ⁻ part B
7	Alkalinity	Titration method	2320 part B

where Y = biomass yield coefficient = mg/L biomass produced per mg/L substrate utilised; r_g = rate of nitrifying biomass growth; and r_{su} = rate of substrate concentration change due to utilisation.

$$\text{Therefore, } \mu X = \frac{(N_0 - N)}{\theta} Y \quad (3)$$

where X = biomass concentration of the nitrifiers and N_0 = initial ammonium nitrogen concentration.

$$\text{Or } \mu_{\max} \frac{N}{K_N + N} X = \frac{(N_0 - N)}{\theta} Y \quad (4)$$

$$\text{Or } \frac{\mu_{\max}}{Y} \frac{N}{K_N + N} = \frac{(N_0 - N)}{\theta \cdot X} \quad (5)$$

Replacing $\frac{\mu_{\max}}{Y}$ with k (where k = specific substrate utilisation rate in mg/L).

$$\text{Or } \frac{(N_0 - N)}{\theta \cdot X} = \frac{k \cdot N}{K_N + N}$$

$$\text{Or } \frac{\theta X}{(N_0 - N)} = \frac{K_N}{k} \cdot \frac{1}{N} + \frac{1}{k} \quad (6)$$

By plotting a graph with $\frac{\theta X}{(N_0 - N)}$ in the Y axis and $\frac{1}{N}$ in the X axis, two important kinetic parameters k and K_N are determined from the slope and intercept. The value of k is obtained from the y intercept, whereas K_N is obtained from the slope of the best-fit line. In accordance with ammonium nitrogen utilisation, nitrifying biomass increases along with endogenous decay, which is proportional to biomass present in the reactor.

$$\text{Therefore, the net biomass growth} = Y \cdot \frac{\mu_{\max} N X}{K_N + N} - k_d X,$$

where k_d = endogenous decay constant per day

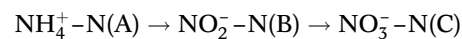
$$\text{Or } \frac{(X' - X)}{\theta X} = \frac{N_0 - N}{\theta X} Y - k_d \quad (7)$$

where X' = final biomass over a time interval θ .

A graph is plotted by taking $\frac{(X' - X)}{X \cdot \theta}$ in the Y axis and $\frac{N_0 - N}{X \theta}$ in the X axis to find the values of Y and k_d . The value of k_d is obtained from the intercept of the Y axis and the slope of the graph gives the value of Y .

Determination of overall rates of nitrification reactions

Considering nitrification reaction as a consecutive reaction, the product (nitrite ion, NO_2^-) formed from the first reaction, i.e. oxidation of ammonium, serves as the reactant for the following reaction of nitrite oxidation. The consecutive reaction is:



Assuming first-order kinetics for the above consecutive reactions, the rate equations for the first step can be expressed as:

$$\frac{dC_A}{dt} = -K_A C_A \quad (8)$$

where C_A = concentration of the ammonium nitrogen (mg/L) and K_A = rate constant of nitrite nitrogen formation.

Integrating Equation (8) between the boundary conditions as follows:

(i) $t = 0$, $C_A = C_{A0}$ = initial ammonium nitrogen concentration (mg/L) and

(ii) $t = t$, $C_A = C_A$ = ammonium nitrogen concentration (mg/L) at time t

$$C_A = C_{A0} \cdot e^{-K_A t}$$

$$\text{Or } \ln(C_{A0}/C_A) = K_A t \quad (9)$$

The rate equations for the second step can be expressed as:

$$dC_B/dt = K_A C_A - K_B C_B \quad (10)$$

where C_B = concentration of the nitrite nitrogen (mg/L) and K_B = rate constant of nitrate nitrogen formation.

The value of K_A can be obtained as per Equation (9) from the slope of the graph plotted between $\ln(C_{A0}/C_A)$ and t . The values of dC_B/dt can be calculated as

$$\frac{\text{Initial } C_B - \text{Final } C_B}{\text{Batch Period}} \quad (11)$$

Accordingly, the value of K_B can be estimated from the slope of the graph plotted between dC_B/dt and C_B as expressed in Equation (10).

RESULTS AND DISCUSSION

Acclimatisation of nitrifying biomass

The acclimatisation study of nitrifying microorganisms with the synthetic feed having ammonium nitrogen exclusively was conducted by increasing the volume of nitrogenous feed in a total 100 mL volume as shown in Figure 1. The observed data in respect of ammonium nitrogen and COD

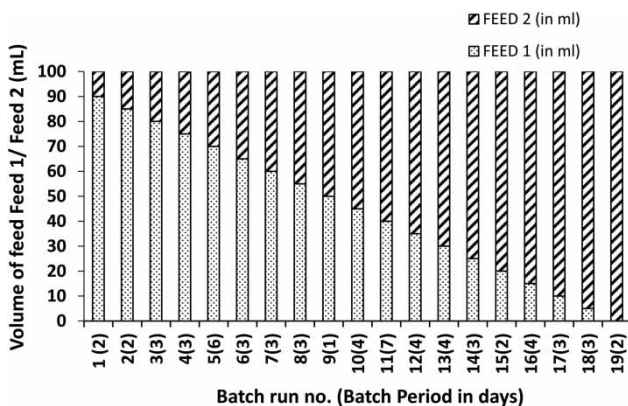


Figure 1 | Composition of the feed given for acclimatisation of nitrifiers.

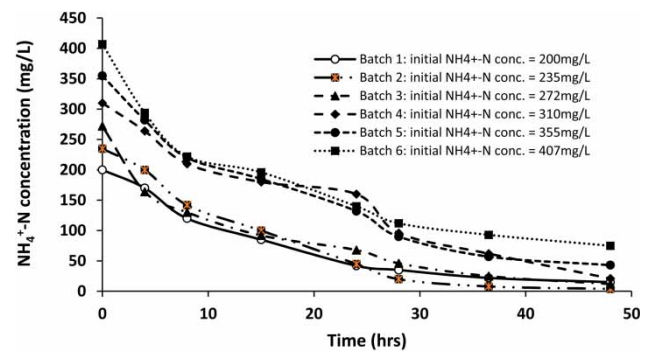


Figure 2 | Ammonium nitrogen profile during batch nitrification study.

during acclimatisation of the autotrophic nitrifiers are plotted in Figure A1 and Figure A2 (in the Appendix), respectively. It was observed that during the acclimatisation study, biomass (mixed liquor suspended solids, MLSS) of the reactor increased from 2,700 mg/L to 3,800 mg/L.

During the 60-day acclimatisation period, the biomass in the reactor was exposed to gradually increasing amounts of ammonium nitrogen and reciprocally decreasing amounts of carbonaceous matter (COD). Because the nitrogenous feed (Feed 2) contained the bicarbonate ion (HCO_3^-), the biomass was adapting to inorganic carbon rather than organic carbon present in the dextrose. When 100% of nitrogenous feed was reached, the biomass present in the batch reactor was only able to uptake inorganic carbon source, which is the major attribute of the nitrifying biomass. At the end of the 60-days acclimatisation period, the biomass available in the reactor was predominantly the nitrifiers. It is obvious from the results in Figures 2 and 3 that uptake of ammonium nitrogen was increasing with a reciprocal decrease in COD utilisation. It was also observed that the nitrifying biomass steadily increased during the acclimatisation study, indicating the ability to oxidise ammonium nitrogen.

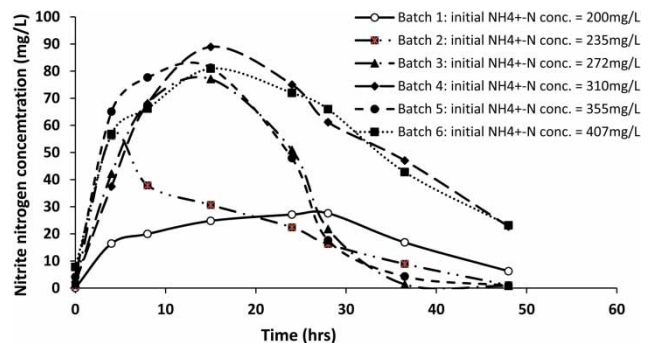


Figure 3 | Nitrite nitrogen profile during batch nitrification study.

Batch nitrification study

The batch nitrification study was performed using synthetic wastewater with ammonium nitrogen concentration of 200–407 mg/L. The results of the batch study revealed that a maximum 98.3% ammonium nitrogen removal was obtained at an initial concentration of 235 mg/L. The minimum removal of 81.57% ammonium nitrogen was attained when the initial ammonium nitrogen concentration was 407 mg/L. Ammonium nitrogen profiles for the various batch experiments are shown in Figure 2. The batch experiments showed that significant removal of ammonium nitrogen occurred within 28 h. After that, there was little change in ammonium nitrogen concentration observed. This can be attributed to the ammonium nitrogen being converted to nitrite nitrogen rapidly, and then nitrite nitrogen accumulating in the system due to a slower formation rate of nitrate nitrogen from nitrite nitrogen oxidation. As a result, conversion of ammonium nitrogen to nitrite nitrogen was hindered in all batch experiments. Considering this fact, a batch period of 28 h can be regarded as the optimum time for attaining significant removal of ammonium nitrogen in the present study.

Nitrite nitrogen profiles for the various batch experiments are shown in Figure 3. In all batch runs, the concentration of the nitrite ion increased up to a peak value and then decreased steadily. This was presumably due to rapid oxidation of ammonium nitrogen at the first stage, which was concomitant with the slow rate of nitrite nitrogen oxidation. This kind of conversion process was continued for up to 5 h, as in the case of ammonium nitrogen concentration ranging between 200 and 235 mg/L. It was extended up to about 15 h for an ammonium nitrogen concentration of 272–407 mg/L. Nitrite nitrogen concentration decreased differently beyond the peak value, depending on available pH, temperature and dissolved oxygen (DO) level.

Nitrate nitrogen profiles for the various batch experiments are shown in Figure 4. In all the batch runs, the concentration of the nitrate ion gradually increased up to a peak value, which is expected as per nitrification reaction as stated earlier. The rate of nitrate nitrogen conversion is considerable up to about 28 h and it decreased to some extent in all the cases except at an initial ammonium nitrogen concentration of 200 mg/L. This was mainly due to the accumulation of nitrite nitrogen in the system, which might have caused some inhibition.

COD concentration profiles for the various batch experiments are shown in Figure A3 (in the Appendix). It is noteworthy that COD concentration rapidly decreased

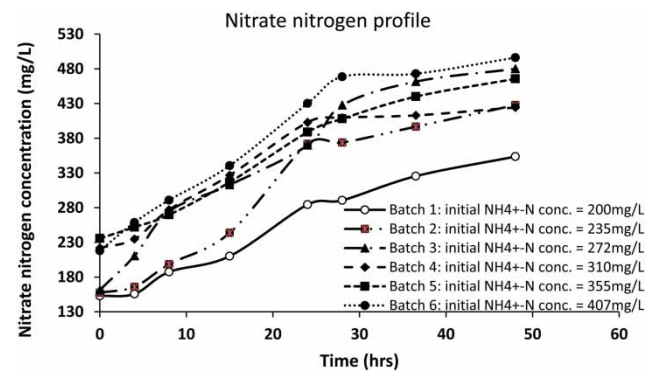


Figure 4 | Nitrate nitrogen profile during batch nitrification study.

within a short period in all the batch operations. In fact, this COD represented the nitrogenous oxygen demand, which is commensurate with the concentration of ammonium nitrogen and nitrite nitrogen. Because oxidation of ammonium nitrogen to nitrite nitrogen occurred with a fast rate, COD contribution of ammonium nitrogen also decreased rapidly at the first stage. Thereafter, although nitrite nitrogen was accumulated to some extent, no considerable COD reduction was observed. This is perhaps due to fact that COD contribution of nitrite nitrogen is one-third of that for ammonium nitrogen.

The profiles of initial and final biomass concentration for the various batch experiments are shown in Figure A4 (in the Appendix). It is noteworthy that MLSS concentration remained consistent throughout the batch period for all initial ammonium nitrogen concentrations. There was marginal growth in biomass concentration, especially due to slow growth rate of nitrifiers. Moreover, the biomass was subjected to endogenous decay, leading to a low amount of net growth. Because there was no decrease in the overall biomass concentration, it can be assumed that the microbial growth was not inhibited.

During the batch nitrification study, the concentration of DO was kept at 4.0 ± 0.4 mg/L. There is disagreement among the previous researchers regarding bulk optimum DO concentration, although, in general, a minimum concentration of 1.0 to 2.0 mg/L needs to be maintained to avoid inhibition in nitrification (Wild *et al.* 1971). It was observed that a DO concentration above 2.0 mg/L did not influence the growth rate constant of *Nitrosomonas* species (Knowles *et al.* 1965). In the present study, initial ammonium nitrogen concentration was varied and it was assumed that enough DO was available throughout the experiment as adequate aeration was provided.

Determination of kinetics of ammonium nitrogen oxidation

The experimental data obtained from the batch study were analysed using Monod's approach as mentioned in Equations (6) and (7). For K_N and k , the values of initial and final ammonium nitrogen concentration (N_0 and N) as well as θ and X were used to calculate $\frac{\theta X}{(N_0 - N)}$ and $\frac{1}{N}$. Similarly, to find out Y and k_d , the values of initial and final ammonium nitrogen concentration (N_0 and N), as well as θ , X and X' , were used to calculate $\frac{(X' - X)}{X \cdot \theta}$ and $\frac{N_0 - N}{X\theta}$. Graphical plots for determination of K_N and k and also Y and k_d are shown in Figures 5 and 6, respectively.

Among the various kinetic constants, the maximum growth rate (μ_{max}) and half velocity constant (K_N) are completely dependent on characteristics of the biomass as well as the environment to which they are exposed (Poduska & Andrews 1975). From Figure 5, the values of half velocity constant (K_N) and maximum specific NH_4^+ -N utilisation rate (k) were estimated to be 12.95 mg/L and 0.17 per day, respectively. Similarly, from Figure 6 the values of the

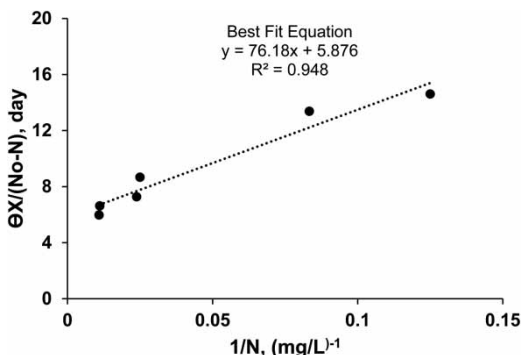


Figure 5 | Graphical plot for determination of k and K_N .

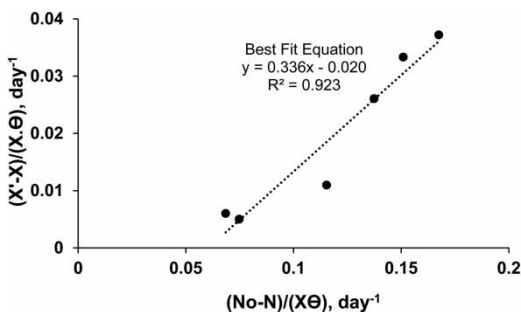


Figure 6 | Graphical plot for determination of Y and k_d .

yield coefficient (Y) and endogenous decay constant (k_d) were calculated as 0.336 mg/mg and 0.020 per day, respectively. Hence, the maximum growth rate (μ_{max}) of the nitrifiers can be calculated as 0.057 per day. The maximum growth rate for nitrifying organisms was reported as 0.25, 0.1224 and 0.45 per day by Charley *et al.* (1980), Chudoba *et al.* (1985) and Choubert *et al.* (2008), respectively. The value of μ_{max} is therefore about four, two and eight times more than those reported by Charley *et al.* (1980), Chudoba *et al.* (1985) and Choubert *et al.* (2008), respectively. The discrepancy in the value of μ_{max} is obviously due to dissimilar experimental conditions. The extreme deviation in the value of K_s (here K_N) has also been observed for 0.72 mg/L as reported by Chudoba *et al.* (1985).

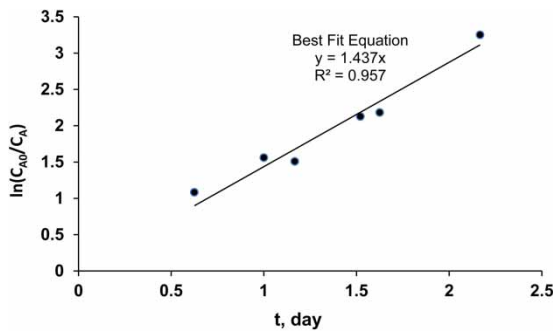
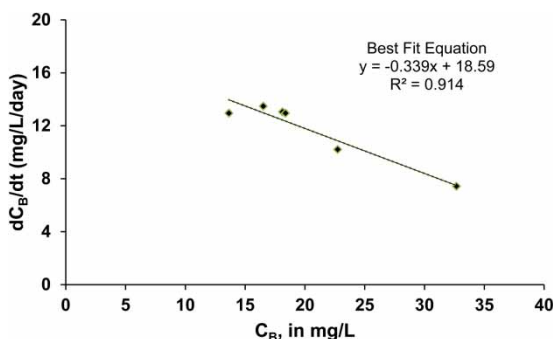
The value of the yield coefficient is observed to be about six times that reported by Charley *et al.* (1980). This disagreement in the result may be attributed to the fact that the ranges of ammonium nitrogen concentration in the two studies are totally different. The endogenous decay coefficient (k_d), determined in the present study, also does not match that reported by Choubert *et al.* (2008). Fillos *et al.* (2000) measured the decay rate of nitrifiers as 0.04 per day at 20 °C, which is about twice that obtained in the present study. This discrepancy is due to dissimilar experimental conditions, as well as the input ammonium nitrogen concentrations. This may also be due to higher biomass concentration taken in the present study compared to that reported by Choubert *et al.* (2008). A comparison of various kinetic coefficients for nitrification evaluated by earlier researchers under different conditions is presented in Table 5.

Determination of overall rates of nitrification reactions

Considering nitrification as a consecutive reaction, as illustrated earlier, the rate constants of the two steps of nitrification process are separately evaluated according to Equations (9) and (10). The rate constant for ammonium oxidation is evaluated as 1.44 per day and that for nitrite oxidation is determined to be 0.34 per day, as shown in Figures 7 and 8, respectively. Wong-Chong & Loehr (1975) proved that both the ammonium oxidation rate and the rate constant is affected by pH and temperature and the reaction rate was evaluated as 110 mg nitrogen/L/h at pH 7.5. The values of rate constants for the oxidation of ammonium nitrogen and nitrite nitrogen were not determined previously and therefore the overall rates of nitrification reaction in the present study are not comparable.

Table 5 | Comparison of kinetic parameters of nitrification reaction obtained by earlier researchers

Operating conditions	Initial substrate concentration (mg/L)	Biomass characteristics	μ_{\max} (per day)	K_s (mg/L)	K_d (per day)	Y (mg volatile suspended solids (VSS)/mg substrate)	References
Temp 10–35 °C DO: 38 mg/L	Ammonium nitrogen: 0.5–100 nitrite nitrogen: 0.5–50	Nitrifying organisms	0.25	–	–	0.11	Charley <i>et al.</i> (1980)
Sludge retention time: 20 d Hydraulic retention time: 1 d DO > 2 mg/L	COD: 340 ammonium nitrogen: 100	Nitrifying organisms	0.1224	0.72	–	–	Chudoba <i>et al.</i> (1985)
Sludge retention time: 20 d Temp: 20 and 12 °C pH: 7.1–7.3 DO > 5 m	–	Nitrifying organisms	0.3–0.49	–	0.04	–	Fillos <i>et al.</i> (2000)
Temp: 10 °C DO > 4 mg/L pH: 7–8	Ammonium nitrogen: 15	Nitrifying organisms	0.45	–	0.13	–	Choubert <i>et al.</i> (2008)
pH: 8.1 ± 0.2 Temp: 36 ± 2 °C	Ammonium nitrogen: 200–400	Nitrifying organisms	0.057	12.95	0.02	0.336	Present study

**Figure 7** | Evaluation of rate constant of ammonia oxidation.**Figure 8** | Evaluation of rate constant for nitrite oxidation.

CONCLUSION

The present study confirms that the nitrification kinetics follow both Monod's Equation and the theory of consecutive reactions from the experimental data obtained at a temperature 36 ± 2 °C. The experimental data obtained from the study, when plotted in graphs, showed good correlation ($R^2 = 0.94$ and 0.91) for determining kinetic parameters following Monod's kinetics. With an initial ammonium nitrogen concentration ranging between 200 and 400 mg/L, the following results were obtained: maximum specific growth rate (0.051 per day), half velocity constant (12.95 mg ammonium nitrogen/L), yield coefficient (0.336 mg VSS/mg ammonium nitrogen) and endogenous decay constant (0.02 per day). Considering nitrification as a consecutive equation, the overall rate of ammonium nitrogen and nitrite nitrogen oxidation were evaluated as 1.44 per day and 0.34 per day, respectively, with good correlation ($R^2 = 0.95$ and 0.91 , respectively) of data. This study derived an overall kinetics of nitrification using ammonium nitrogen as a substrate at a temperature 36 ± 2 °C and using activated sludge. It also analysed the nitrification as a two-stage consecutive reaction comprising ammonium nitrogen and nitrite nitrogen oxidation in the first and second step, respectively, in the ammoniacal

nitrogen-enriched synthetic wastewater. This will further aid in designing biological reactors supporting nitrification of wastewater with similar characteristics.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at <https://dx.doi.org/10.2166/wst.2020.080>.

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