Determination of rate and order of denitrification of nitrified effluent using activated sludge

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Denitrification studies of synthetic nitrified effluent were performed in laboratory scale batch activated sludge process to examine the effect of absence of organic carbon on denitrification. The orders of the reaction under two conditions of organic carbon content were determined and relevant rate constants were evaluated from the experimental data. During the study, maximum nitrate removal of 92% and 54% was achieved in case of denitrification using methanol and without using methanol respectively under a nitrate to biomass concentration ratio of 0.1. Zero order reaction kinetics was observed to be the best fit for all the studies. The maximum specific denitrification rates were observed to be 11.93±5.13 and 9.46±1.56 mg nitrate/g MLSS/h respectively in presence and absence of methanol. The maximum denitrification rate was attained, when methanol was added @ 1.9 times of nitrate concentration under a nitrate to biomass ratio of 0.2. In presence of sufficient biomass in a reactor, limited supply of readily available organic carbon facilitates endogenous metabolism and addition of external carbon may be reduced, thus economizing the process.

Keywords: Denitrification, nitrified effluent, organic carbon, order of reaction, specific denitrification rate.

Introduction

Nitrogen in form of nitrates when present in water causes serious environmental hazards including eutrophication. Nitrates when converted to nitrosamines and nitrosamides can be carcinogenic[1]. The presence of nitrate in wastewater is mostly because of its higher concentration in some selected industrial effluents. Wastewater from industries producing fertilizers, pharmaceuticals, explosives and nitrogenous compounds including pectin and metal finishing industries contain nitrate[2]. Nitrate concentration in wastewater can be economically lowered using biological denitrification[3], which involves anaerobic reduction of nitrate to nitrogen gas via nitrite. This conversion is generally facilitated by heterotrophic bacteria, which includes organisms of the genera *Pseudomonas, Micrococcus, Achromobacter, Spirillum, Bacillus, Hyphomicrobium* and *Paracoccus*[4,5].

Denitrification generally requires the presence of organic carbon as electron donor which has to be supplied externally if not present and that would incur extra cost. The ratio of organic carbon to nitrate nitrogen is often kept between 5 to 10 for efficient denitrification[6]. External carbon, when absent, endogenous degradation has been hypothesized to take place for meeting the carbon requirement[7]. Being cost effective, internal carbon obtained from endogenous respiration can be preferred to other external carbon sources[8]. For efficient and economic removal of nitrate from wastewater, the process is to be designed with proper knowledge about kinetics especially rate and order of the reactions. Methanol is the most preferred carbon source as it has maximum denitrification rate[8]. Mokhayeri *et al.*[9] observed the denitrification rates with acetate and ethanol to be higher than that obtained using methanol. Recently, a trend has been developed to perform denitrification without external addition of organic carbon to minimize the cost. It is confirmed that the reaction when utilizes endogenous decay as the sole organic carbon source, has slower reaction rates, but the rate is not described quantitatively.

Several studies showed the reaction to be of either zero order[8,10,11] or first order[12,13]. The denitrification rate, considered to be of zero order with respect to nitrate-nitrogen is influenced by presence of organic substances. It is observed that the reaction kinetics is of half-order with respect to nitrate-concentration in attached biomass culture[14]. A batch denitrification study was conducted by Moore and Schroeder[12] assuming first order kinetics using methanol as organic carbon. The process was optimized in terms of maxi-

mum nitrate removal and minimum methanol requirement at a minimum residence time of 5.6 days. In order to obtain a very low nitrogen concentration, Monod's kinetics can be applied that yields a saturation constant of 0.1 mg NO_3^--N/L.

Beccari et al.[10] developed denitrification kinetics based on Monod type equations assuming both nitrate and carbon as substrates. According to them, nitrate reduction follows zero order kinetics in presence of adequate carbon. Glass and Silverstein[11] also developed denitrification kinetics assuming both the reduction reactions to be of zero order with an initial nitrate concentration of 2700 mg/L and acetate as organic carbon source. There lies the controversy in confirming the order of denitrification reaction for nitrate concentration. All these studies were conducted using sufficient organic carbon content in the feed. A complete evaluation of the process kinetics is found to be largely missing, for wastewaters having low carbon content. Moreover, the extent of denitrification without addition of external organic carbon for wastewater characterized by low C:N ratio and its corresponding rate and order is also not investigated.

The aim of the present work is to examine whether denitrification is possible in absence of organic carbon in the wastewater. An attempt has been made to determine the order and establish the kinetics of the reaction. The present study thus compares the rate and order of the reactions using synthetic nitrified effluents with and without addition of external organic carbon.

Experimental

The synthetic nitrified effluent was collected from a biological nitrifying Activated Sludge Reactor, which is under operation in the Environmental Engineering Laboratory of the Civil Engineering Department, IIEST, Shibpur. Several laboratory scale batch denitrification studies were conducted using a glass measuring cylinder with a working volume of 1 L. Synthetic nitrified effluent having initial NO_3^--N concentration in the range of (50–250) mg/L was used, under nitrate to biomass ratio of 0.1 and 0.2 and at pH of 7.5–8.0. 20 number of batches were conducted with following initial substrate conditions as represented in Fig. 1. Comparison of denitrification rates was done with respect to methanol as organic carbon source that showed highest reaction rates. Methanol consumption (in mg) per mg NO_3^--N reduced to nitrogen gas is theoretically estimated to be 1.91[7], although

in practice, the value is observed to be much higher. In this study, this ratio was cd for comparison of reaction rates.
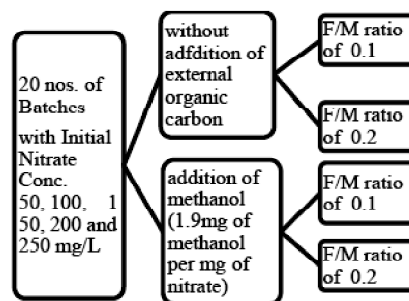

Fig 1. Batch studies with various initial NO_3^--N substrate conditions.

Nitrate, nitrite, DO and COD concentrations for all batches were monitored periodically following analytical methods as mentioned in APHA (1998)[15]. The kinetic relationship for n-th order reactions was approached in order to determine the rate and order of denitrification reactions.

$$C^{1-n} = C_0^{1-n} + (n-1) \, k.t \qquad (1)$$

where, C = concentration of the substrate at time t, C_0 = initial concentration of the substrate initially, n = order of the reaction and k = rate of the reaction.

The experimental data obtained for concentration (C) of substrates for various batch studies were processed as per eq. (1). Thereafter, the values of C^{1-n} are plotted with respect to t in order to determine the rate of denitrification. Slope of the mean line gives the rate of denitrification reaction in each case. Various values for n starting with 0 were trailed in the eq. (1) to yield the best regression coefficient. The rate of reaction was determined from the equation that showed maximum value of regression coefficient.

Results and discussion

Time course study was performed for investigating and comparing the change of nitrate and nitrite concentration over time. Considering nitrate removal, it was observed that significant nitrate was not removed in absence of organic carbon. The studies performed with minimum biomass concentration showed a minimum nitrate removal around 10%. In the studies conducted using methanol and nitrate to biomass ratio of 0.1, maximum removal of around 92% was achieved. Maximum nitrate removal obtained during the study conducted under similar conditions but without any external

organic carbon source was 54%. Nitrate removal efficiency under lower biomass content (viz. nitrate:biomass ratio 0.2) varied drastically with maximum removal of 86% and 17% respectively for studies with and without organic carbon. This indicates the necessity of organic carbon for denitrification as well as the dependency of nitrate removal on organic carbon content. A typical trend of nitrate and nitrite profile with respect to time for studies without organic carbon and with methanol under nitrate to biomass concentration ratio of 0.1 is shown in Fig. 2 through Fig. 5 respectively. Comparing the trend of nitrate degradation without organic carbon to that of studies, conducted with carbon, it can be concluded that complete nitrite conversion did not take place.
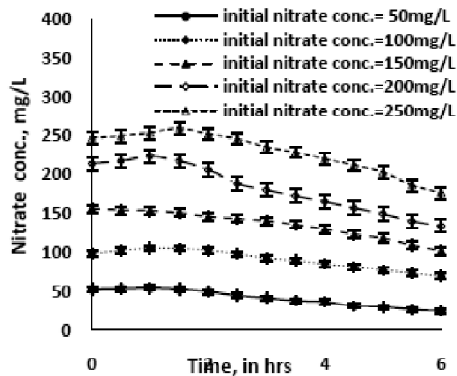

Fig. 4. Nitrite profile for denitrification without external organic carbon and at nitrate to biomass ratio 0.1.

Fig. 2. Nitrate profile for denitrification without external organic carbon and at nitrate to biomass ratio 0.1.

Fig. 5. Nitrite profile for denitrification with methanol and at nitrate to biomass ratio 0.1.
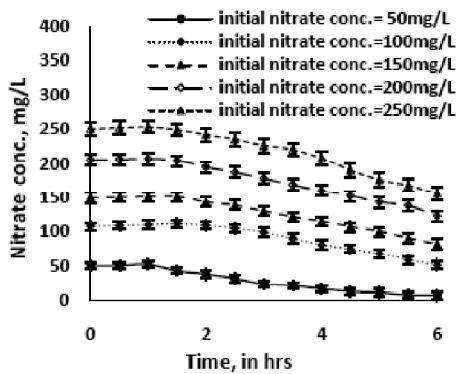

Fig. 3. Nitrate profile for denitrification with methanol and at nitrate to biomass ratio 0.1.

Similar trend in nitrite profile was observed by Peyton *et al.*[13]. It is observed from Fig. 4 and Fig. 5 that nitrite concentration initially decreased during the period along with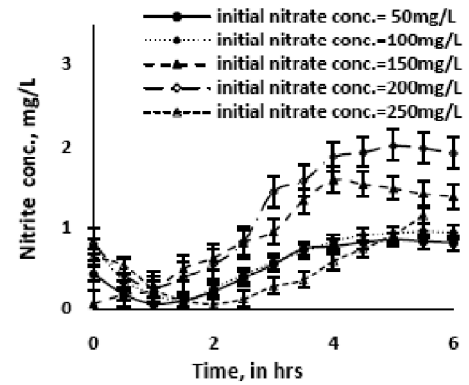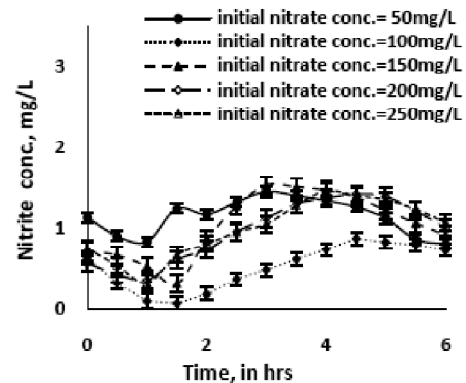 consequent increase in nitrate concentration when DO concentration varied in the range of (1.5–3.0) mg/L, indicating apparent nitrification. Thereafter, with further decrease in DO, nitrate concentration was observed to increase due to conversion of nitrate to nitrite. The build-up of nitrite was observed to take place initially, exhibiting gradual decrease in nitrite reduction. These effects have been demonstrated by Roising and Schroeder[16] and Peyton *et al.*[13]. Under the studies conducted with methanol as external organic carbon source, nitrite concentration decreased during final stage of the batch study due to the conversion of nitrite to nitrogen via intermediate compounds. This phase, however, could not be observed during the batch studies conducted without addition of carbon. The reason may be attributed to the fact that slower denitrification rate could not attain satisfactory transformation of nitrite within the short period of 6 h. In a study conducted by Timmermans and Haute[17], denitrification rate was often observed to be a function of pH and tem-

perature and almost independent on the concentrations of methanol and NO_3^--N. In presence of excess organic carbon, the nitrate nitrogen removal rate did not seem to decrease until the nitrate nitrogen concentration was close to zero[4].

These studies were carried out in either insufficient or no carbon condition and as such, the rate of conversion of nitrite was slow. Occurrence of nitrite accumulation could not be concluded thus. Accumulation of nitrite generally depends on the relative rates of nitrate and nitrite reduction. It has been observed that different denitrifying species have variable effects in nitrite accumulation. As an instance, *Alcaligenes* species and *Pseudomonas fluorescens* accumulate nitrite during nitrate reduction whereas, a *Flavobacterium* isolate does not[18]. Accumulation of nitrite in some bacterial species has been shown to interfere with Krebs cycle activity[19] which would have the effect of making nitrite reduction slower than nitrate reduction in a mixed population.

In order to determine the order of the reactions for denitrification without carbon and with methanol, the experimental data were fitted in zero order reactions. In order to obtain better regression coefficients, several other higher values for reaction order (n) were taken for trials. The regression coefficients obtained for various studies are shown in Table 1.

**Table 1.** Regression coefficients for various reaction orders

Batch specifications		Average R^2 values		
Organic carbon	Nitrate: biomass	$n = 0$	$n = 0.1$	$n = 0.2$
Without external	0.1	0.918	0.917	0.916
organic carbon	0.2	0.647	0.645	0.647
With methanol	0.1	0.935	0.935	0.933
	0.2	0.941	0.940	0.939

From Table 1, the regression coefficients for reaction orders 0.1 and 0.2 were quite close to those obtained in case of zero order reactions but decreased gradually for trials with higher order. Highest regression coefficient was obtained in case of trials with zero order. Thus, denitrification reactions can be concluded to follow zero order, which corroborates the results obtained by previous researchers. After being confirmed about the order of denitrification, the rate needs to be determined. In accordance with the equation of zero order reaction, the values of substrate concentration are plot-

ted with respect to time and the slope of the said graph represents the rate of reaction. The rate of denitrification was determined for two nitrate:biomass ratios under both the cases with and without addition of methanol as shown in Fig. 6 to Fig. 9.
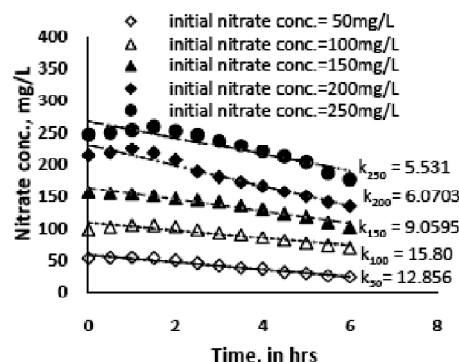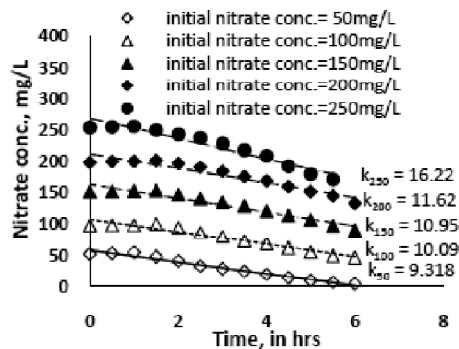

Fig. 6. Determination of rate of denitrification from the study without external organic carbon at nitrate to biomass ratio 0.1.
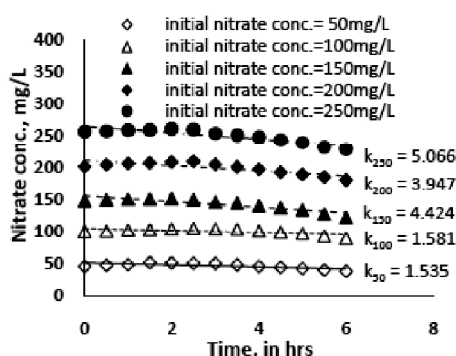
Fig. 7. Determination of rate of denitrification from the study without external organic carbon at nitrate to biomass ratio 0.2.
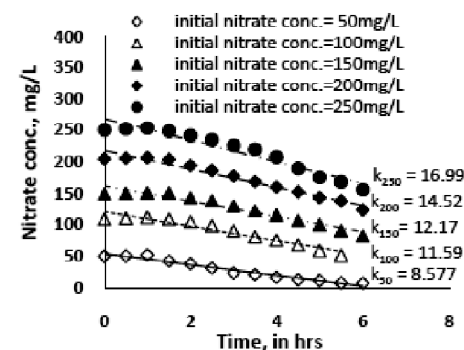
Fig. 8. Determination of rate of denitrification from the study with methanol at nitrate to biomass ratio 0.1.

Fig. 9. Determination of rate of denitrification from the study with methanol at nitrate to biomass ratio 0.2

The Specific Rates of denitrification for all the batch runs as shown in Fig. 6 to Fig. 9 have been calculated by dividing their respective biomass concentration. Thus, the Mean Specific Rates of denitrification under four distinct operating conditions are estimated by simple averaging of all the respective values for any operating condition. The mean specific denitrification rates (*k*) considering zero order kinetics for the four batches are presented in Table 2.

Table 2. Mean specific denitrification rates under various batches

Batch specifications		*k* [(mg/L nitrate)/
Organic carbon	Nitrate:biomass	(g/L MLSS)/h]
Without external	0.1	9.46±1.56
organic carbon	0.2	5.09±1.04
With Methanol	0.1	11.93±5.13
	0.2	10.21±0.9

It is evident from Table 2 that biomass concentration has a significant effect on the rate of denitrification, exhibiting about 50% increase in its value, when the biomass concentration was enhanced about two times. Moreover, in presence of methanol there is a marginal increase in the rate of denitrification, when biomass concentration was increased two times. It is also to observe that addition of methanol in presence of high biomass concentration (i.e. nitrate:biomass ratio of 0.1) has no tangible effect on the rate of denitrification. However, in case of low biomass concentration (i.e. nitrate:biomass ratio 0.2) the rate of denitrification has been doubled, when methanol was added. Past literatures reveal that denitrification rate varies with biomass (as organic carbon content) according to the following equation:

$$R = k_e 10^{(F/K_b)} . 10^{K_{t,B}(t-20)}$$

where, $F = F/M$ ratio, $10^{K_{t,B}(t-20)}$ = temperature correction and K_b = constant expressed as mg BOD_5/mg VSS.h)

Higher denitrification rate was observed for the batches conducted with methanol and comparatively higher biomass concentration. Thus, methanol as well as biomass concentration are observed to have directly influence on denitrification rate. In a study conducted by Fernández-Nava *et al.*[3], a maximum denitrification rate of 30.4 mg NO_3^--N/g VSS/h was obtained at 7 h, using methanol as carbon source in the ratio of 3.4. The rate of denitrification was greater than that obtained in the present study probably due to higher carbon content in the treatment system. From the results of the study conducted by Mokhayeri *et al.*[9], specific denitrification rate using methanol at a methanol to carbon source of 4.45 was obtained as 10.1±2.5 mg NO_3^--N/g VSS/h which is similar to those obtained in the present study using lesser organic carbon. The reason behind this is temperature effect, as the present study was carried out at a temperature much higher than that reported by the past researchers. It is to note that increasing F/M decreases the efficiency of nitrogen removal. Considering endogenous decay as the sole carbon source of denitrification, the rate was previously estimated as 0.2–0.3 mg N/g VSS.h at 20^oC[8]. Therefore, the present study showed significantly higher rate of denitrification even in absence of methanol as organic carbon source.

Conclusions

The denitrification studies conducted with and without methanol shows that nitrate removal can be carried out using activated sludge. Comparative analysis for the two cases shows that denitrification is possible without using external organic carbon when biomass would be utilized as carbon source through endogenous decay. However, the rate of denitrification in that case would be slow. In order to increase the rate, sufficient biomass must be present in the reactor. Thus, these parameters could be optimised to bring about efficient as well as economic denitrification in activated sludge reactors.

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