Indian Journal of Chemical Technology
Vol. 29, July 2022, pp. 429-435

Effect of C/N ratio, temperature, and *p*H on the removal of ammonia-nitrogen from wastewater using inverse fluidized bed biofilm reactor

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Received 02 February 2022; accepted 18 May 2022

Experiments have been carried out in an inverse fluidized bed biofilm reactor (IFBBR) to study the effects of carbon to nitrogen (C/N) ratio, temperature (T), and *p*H on the removal of ammonia-nitrogen (NH_4^+-N) from wastewater using mixed microbial-culture. IFBBR is operated at settled bed volume to reactor volume ratio (V_b/V_r) of 0.380, superficial gas velocity (U_g) of 0.0085 m/s, and liquid recirculation velocity (U_l) of 0.0021 m/s. Three initial NH_4^+-N concentrations (40, 100, and 200 mg/L) are considered here. Effects of different parameters are studied for wastewater treatment by varying C/N ratio from 0.0 to 2.5, T from 26 to 34°C, and *p*H from 7.5 to 9.0. Maximum NH_4^+-N removal is observed at C/N ratio of 0.0, T of 30°C, and *p*H of 8.3. NH_4^+-N removal is found to decrease with increase in C/N ratio. Both NO_3^--N formation and nitrification percentage are found to decrease with increase in C/N ratio and initial NH_4^+-N concentration. Fractional Factorial Design analysis is used to predict the NH_4^+-N removal. Average absolute percent deviation is found to be 11.75 implying the proposed correlation is in good agreement with experimental values. Kinetic constants are found to be higher than the values reported in literatures.

Keywords: Ammonia-nitrogen, Carbon to nitrogen ratio, Fractional factorial design, Inverse fluidization, Nitrification, Wastewater treatment

Beyond regulated limits, aquatic systems contaminated with NH_4^+-N causes eutrophication, deteriorates taste and odour, reduces dissolved oxygen (DO) level, complicates the chlorination process, damages human nervous system[1], and is toxic to aquatic organisms[2]. This pollutant can be removed physically, chemically, physico-chemically, or biologically[3]. Use of biological treatment of wastewater containing NH_4^+-N is advantageous due to ease of operation, no addition of chemicals, less volume of regenerated sludge, low operational expenses, and high efficiency[1,4]. The two-step biological conversion of NH_4^+-N first to unstable nitrite-nitrogen (NO_2^--N) by *Nitrosomonas* and then to less harmful nitrate-nitrogen (NO_3^--N) by *Nitrobacter* is known as nitrification[3,5]. The stability of nitrification depends on several factors such as T; *p*H; DO level; salinity; presence of toxicants, heavy metals, and organic substrate[6]; and concentrations of NH_4^+-N, NO_2^--N, and NO_3^--N[4].

The nitrifying bacteria become more active and reproduce rapidly at increasing temperatures between 5 and 40°C with zero nitrification rates being observed above 40°C and below 5°C[7]. Autotrophic aerobic ammonia-oxidizing bacteria responsible for nitrification are mesophilic in nature which grow well in this temperature range. Nitrification rate was reported to be maximum in the temperature range between 30 and 35°C[8]. Devi and Setty have studied the removal of NH_4^+-N in the temperature range between 24 and 39°C and observed the maximum NH_4^+-N removal to be occurring at 30°C[3]. Nitrification process is *p*H sensitive. Autotrophic aerobic ammonia-oxidizing bacteria responsible for nitrification are neutrophilic in nature which has higher growth rates in the *p*H range of 7.5 to 8.5[9] and can remain active in the range of 5 to 8.5. But for laboratory work, the optimum *p*H range was reported to be 8.1 to 8.5[7]. As the *p*H of wastewater is dependent on alkalinity, substances such as sodium bicarbonate or magnesium hydroxide are used to maintain the required *p*H ranges. Initial alkalinity and initial concentration of NH_4^+-N decide the amount of alkaline substances to be used[10].

Autotrophic microorganisms under aerobic conditions derive energy from inorganic carbon rather than organic carbon sources for their growth[11]. In biofilm systems, the presence of organic carbon affects the nitrification process. Organic carbon favours the growth of heterotrophic microorganisms and successfully competes with autotrophic microorganisms for oxygen[6]. Also, increase in organic carbon content accelerates the sludge production and inhibits the growth of autotrophic microorganisms which will decrease the nitrification process[11]. It was observed for a fluidized bed reactor that, the addition of sucrose (organic carbon) inhibited both the ammonium oxidation and nitrite oxidation processes and the nitrification efficiency obtained was 94%[12]. For multi-species microorganisms present in biofilms in an aerated filter, it was observed that both the ammonia- and nitrite-oxidizers decreased with an increase in C/N ratio while at C/N ratio of 1.5 no nitrification was observed[13]. For a fixed film bio-filter, it was observed that the addition of sucrose having a C/N ratio of 1 or 2, reduced the total NH_4^+-N removal rate by 70% as compared to a nitrification process with C/N ratio of 0[14]. It was observed for a fluidized sand filter that, the nitrification rate reduced to about 60 to 70% for a substrate concentration of 10 mg total NH_4^+-N per liter when the C/N ratio was increased from 0 to 3[15]. For low density polypropylene (PP) particles in a three-phase fluidized bed bioreactor, the optimum sucrose concentration was found to be 7 g/L at which 92.8% NH_4^+-N removal was observed[3,16].

Different types of bioreactors have been reported for the removal of NH_4^+-N from wastewater such as sequencing batch biofilm reactor[17], single high ammonia removal over nitrite reactor[18], fluidized bed biofilm reactor (FBBR)[19-21], and IFBBR[3,22]. However, the use of IFBBR in treating wastewater containing NH_4^+-N is observed to be very little.

In three-phase FBBRs, the solid density is greater than the liquid density and the fluidization of solids is in the upward direction by the upward flow of both liquid and gas phases. But in the IFBBRs, the solid density is less than the liquid density and the fluidization of solids is in the downward direction by upward flow of gas with counter current downward flow of liquid[23]. Some of the major advantages of IFBBRs are higher gas-phase holdup (ε_g), higher heat and mass transfer rates, lower bed pressure drop, higher volumetric efficiency, absence of clogging & channeling, less power consumption, formation of faster & uniform biofilm thickness, better contact among phases, and greater biodegradation effect[24,25].

It was first reported that, the nitrification of a high-strength NH_4^+-N wastewater (250 - 2000 mgNH_4^+-N/L) was experimented in an inverse turbulent bed reactor operated at 30 to 35°C, *p*H of 7.2, and U_g of 0.006 m/s in which complete removal of NH_4^+-N was observed with nitrification of 68 to 100%[22]. In a three-phase bioreactor operated at an air flow rate of 2 lpm, *p*H of 7, temperature of 30°C, and V_b/V_r ratio of 0.00334 for an initial NH_4^+-N concentration of 100 ppm, 92.8% removal of NH_4^+-N was reported with nitrification of 81.04%[3]. These investigations, though useful, lack several aspects such as narrow range of operating parameters of C/N ratio, T, and *p*H; less V_r; absence of air distributor; small sized solids; and no provision for removal of suspended biomass. Modeling and kinetic studies are also missing in these investigations.

In this study, the effect of C/N ratio, T, and *p*H on the removal of NH_4^+-N from wastewater has been investigated using an IFBBR for different initial NH_4^+-N concentrations. Fractional Factorial Design method has been used to develop model equation for the amount of NH_4^+-N removed (NH_4^+-N_R). Kinetic constants were calculated for the basic Mickaelis-Menten expression.

Experimental Section

Reactor setup

An IFBBR (Fig. 1) having height to diameter ratio of 10:1 and total working volume of 10 L was used in this study[26]. Air was introduced at the bottom of the column through a combination of air-sparger and air-distributor plate while water was fed counter-currently from the top. Temperature controlled water tank was provided from which wastewater was pumped into the column under recirculated mode. A height-adjustable discharge pipe was used to maintain the liquid level inside the column. Spherical PP particles having density of 920 kg/m^3 and average diameter of 5.63 mm were used as biomass support particles.

Reactor inoculation and operation

All the reagents were prepared using water obtained from Millipore RiOs™ 3 Water Purification Unit. Table 1 shows the composition of the growth medium corresponding to NH_4^+-N concentration of 40 mg/L which was sterilized in an autoclave at 121°C and 15 psi for 15 min[27]. Other higher NH_4^+-N concentrations of 100 and 200 mg/L were prepared as per the recommended composition ratio. Glucose was used as the external source of carbon for maintaining a desired C/N ratio. The inoculum was prepared from the sludge taken from a local steel

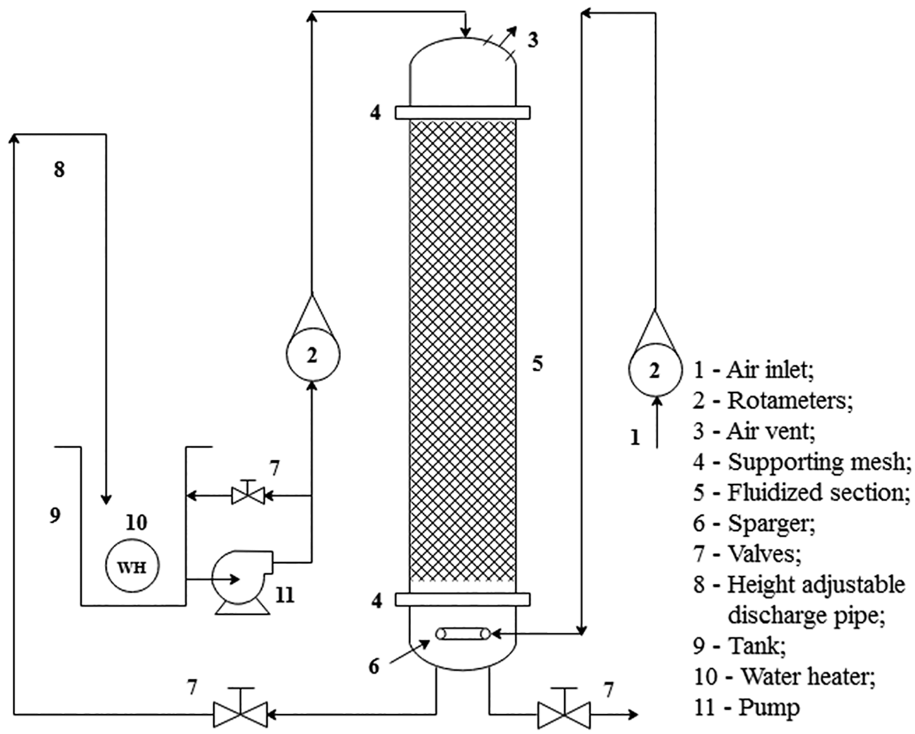

Fig. 1 — Schematic diagram of the IFBBR.

Table 1 — Composition of the growth medium.

Compounds	Composition (mmol/L)
NH_4Cl	2.861
Na_2HPO_4	0.328
$NaHCO_3$	5.572
NaCl	0.352
KCl	0.130
$CaCl_2$	0.087
$MgSO_4.7H_2O$	0.137
$C_6H_{12}O_6$	0.0, 0.556, 1.388

industry's wastewater treatment plant which was acclimatized with the growth medium a number of times in an incubator shaker operated at 30°C and 100 rpm till a steady state growth is achieved[3]. The inoculum thus prepared (1% of the reactor working volume) along with calculated volume of chemically treated PP particles were taken in the reactor and the inoculation of the reactor was done with the liquid in recirculation mode for quicker biofilm formation over the solid particles[28]. IFBBR was operated for two weeks at a NH_4^+-N feeding rate of 40 mg/L/d for the formation of biofilm. During recirculation, suspended biomass (sludge) was removed from the IFBBR by filtration. After the formation of biofilm, all the liquid content was drained keeping only the biofilm laden solid particles. IFBBR was then filled with calculated volume of desired strength of synthetic wastewater and was operated under non-sterile conditions. The reactor was operated at the optimal conditions of V_b/V_r of 0.380 and U_g of 0.0085 m/s with U_l of 0.0021 m/s[26]. The effects of C/N ratio (0 to 2.5), T (26 to 34^{0}C), and *p*H (7.5 to 9) for initial NH_4^+-N concentrations of 40 to 200 mg/L on the removal of NH_4^+-N from wastewater were studied in this work. Between two consecutive experimental runs, a time gap of 48 to 96 h was maintained for the microorganisms to get adapted with the new set of operating conditions. All the experiments were performed in triplicate.

Analytical measurement

Concentrations of NH_4^+-N and NO_3^-N in the treated wastewater were measured by UV/VIS Spectrophotometer (JASCOV-530) at 640 nm and at 220 & 275 nm respectively[29]. Nitrification behaviour of the IFBBR at different operating conditions was measured in terms of nitrification percentage using Equation 1.

$$\% \, Nitrification = \frac{Concentration\ of\ NO_3^- - N\ formed\ at\ the\ end\ of\ experimental\ run}{Initial\ NH_4^+ - N\ concentration} \times 100 \quad \ldots (1)$$

Experimental design

2^{6-2} Fractional Factorial Design method[30] was chosen to develop the correlation for NH_4^+-N_R by considering six different parameters which are mentioned in Table 2. The correlation was developed based on ANOVA results obtained from Design Expert® v. 13 software which is of the form as presented in Equation 2.

$$NH_4^+\text{-}N_R = a_1 + a_2 (A) + a_3 (B) + a_4 (C) + a_5 (D) + a_6 (E) + a_7 (F) + a_8 (AB) + a_9 (AC) + a_{10} (AD) + a_{11} (AE) + a_{12} (AF) + a_{13} (BD) + a_{14} (CD) \quad \ldots(2)$$

where, a_1 to a_{14} are the interaction coefficients while A to F are the process parameters. Kinetic constants were calculated for the basic Mickaelis-Menten expression (Equation 3)[31]:

$$-r = \frac{k\,[S]}{[S] + K_S} \quad \ldots (3)$$

where, r = substrate reaction rate, h^{-1}; k = maximum substrate reaction rate, h^{-1}; [S] = substrate concentration, mg/L; and K_S = half-velocity constant, mg/L. Kinetic constants k and K_S are determined by plotting the experimental data of (1/-r) against (1/[S]).

Table 2 — Parameters and their range for 2^{6-2} Fractional Factorial Design.

Parameters	Unit	Low value	High value
A = Initial NH_4^+-N concentration	mg/L	40	200
B = U_g	m/s	0.0064	0.0127
C = V_b/V_r	-	0.228	0.445
D = C/N	-	0	2.5
E = T	^{0}C	26	34
F = *p*H	-	7.5	9.0

Results and Discussion

Effect of C/N ratio on the removal of NH_4^+-N

C/N ratios were varied as 0.0, 1.0, and 2.5 for initial NH_4^+-N concentrations of 40, 100, and 200 mg/L. While other parameters were maintained at V_b/V_r ratio of 0.380, U_g of 0.0085 m/s, U_l of 0.0021 m/s, T of 30°C, and *p*H of 8.3. Figure 2a shows the removal of NH_4^+-N under different C/N ratios. It is observed that 100% NH_4^+-N removal occurred within 8 to 32 h

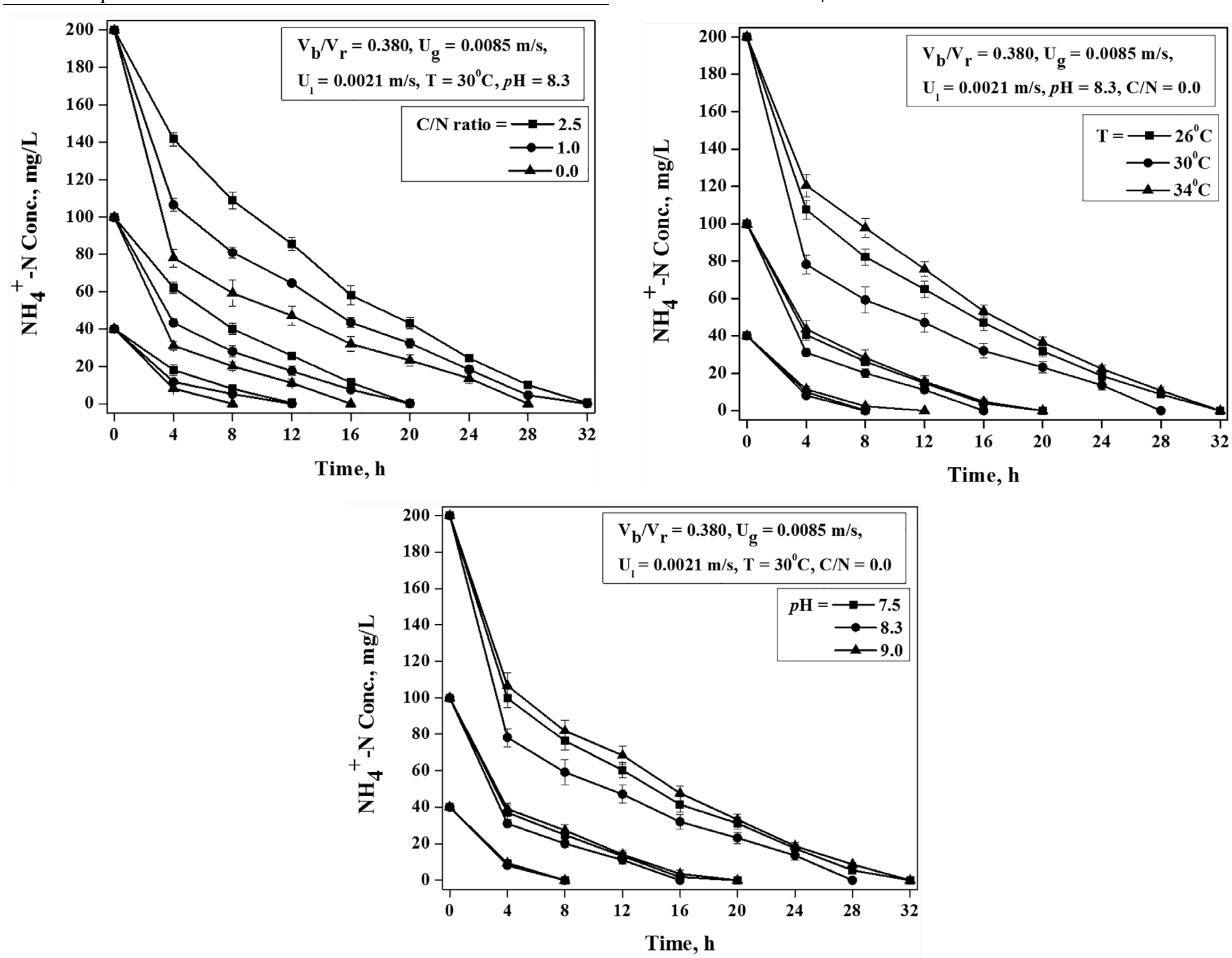

Fig. 2 — (a) Effect of C/N ratios, (b) temperature and (c) *p*H on the removal of NH_4^+-N for different initial NH_4^+-N concentrations.

depending upon initial NH_4^+-N concentrations and C/N ratios. It was also observed that with the increase in C/N ratios from 0.0 to 2.5, the removal of NH_4^+-N decreased for all initial concentrations of NH_4^+-N. This might be due to, in the presence of organic carbon the heterotrophic microorganisms present in biofilm compete with the autotrophic microorganisms for oxygen which inhibits the nitrification process and thereby NH_4^+-N removal decreases[11]. Further it is observed that at higher organic carbon content and at higher initial NH_4^+-N concentrations, higher quantity of sludge is formed which were continuously removed from the reactor by filtration. This sludge production might be enhanced by the presence of organic carbon[12]. With the increase of C/N ratios from 0.0 to 2.5, the NH_4^+-N removal falls by 30.96 to 52.18% for initial NH_4^+-N concentrations of 40 to 200 mg/L respectively. It was reported in literature that, the NH_4^+-N removal reduced by 70% when C/N ratios were increased from 0 to 1 or 2 in a fixed film biofilter[14]. It was also reported in literature that, the NH_4^+-N removal reduced by 60 to 70% for total NH_4^+-N of 10 mg/L when C/N ratio was increased from 0 to 3 in a fluidized bed sand filter[15]. These comparisons suggest the performance of the IFBBR used in this study to be efficient at low C/N ratios. This might be due to the absence of substrate inhibition and chances of control of autotrophic nitrifying microorganisms over heterotrophic microorganisms at low C/N ratios[5]. Therefore, the C/N ratio of 0.0 was considered for studying the effect of T and *p*H on the removal of NH_4^+-N.

Effect of T on the removal of NH_4^+-N

To know the effect of T on NH_4^+-N removal for different initial NH_4^+-N concentrations three different temperatures (26, 30, and 34°C) are taken and the IFBBR was operated at V_b/V_r ratio of 0.380, U_g of 0.0085 m/s, and U_l of 0.0021 m/s with C/N ratio of 0.0 and *p*H of 8.3. It is observed from Fig. 2b that the maximum NH_4^+-N removal was occurring at 30°C for all initial NH_4^+-N concentrations. This might be due to higher activity, shortest generation time, and higher nitrification rate for mixed culture microorganisms at this temperature[7,8]. It was also reported in literature that for NH_4^+-N concentration of 100 mg/L the maximum NH_4^+-N removal was occurring at 30°C[3]. For this reason, the T of 30°C was chosen for studying the effect of *p*H on NH_4^+-N removal.

Effect of *p*H on the removal of NH_4^+-N

*p*H values of 7.5, 8.3, and 9.0 were taken to study the effect of *p*H on NH_4^+-N removal for different initial NH_4^+-N concentrations at V_b/V_r ratio of 0.380, U_g of 0.0085 m/s, U_l of 0.0021 m/s with C/N ratio of 0.0 and T of 30°C. It is observed from Fig. 2c that the maximum NH_4^+-N removal was occurring at a *p*H of 8.3 for all initial NH_4^+-N concentrations which might be due to maximum nitrifying bacterial activity and specific growth rates in this *p*H value. It was reported in literature that the maximum NH_4^+-N removal occurs at a *p*H range of 7.5 to 8.5[7]. Also it is observed in this study that for low initial NH_4^+-N concentration of 40 mg/L the *p*H effect is almost negligible.

Effect of C/N ratio on the formation of NO_3^--N and nitrification

Concentration of NO_3^--N were measured for different initial NH_4^+-N concentrations at V_b/V_r ratio of 0.380, U_g of 0.0085 m/s, U_l of 0.0021 m/s, T of 30°C, and *p*H of 8.3 for different C/N ratios of 0.0, 1.0, and 2.5. It is observed from Fig. 3 that the formation of NO_3^--N increases for all C/N ratios and for all initial NH_4^+-N concentrations but with the increase of C/N ratios, the formation of NO_3^--N decreases. This might be due to the inhibitory effect of organic carbon on both ammonium- and nitrite-oxidation[11]. Further it is noticed that with the increase

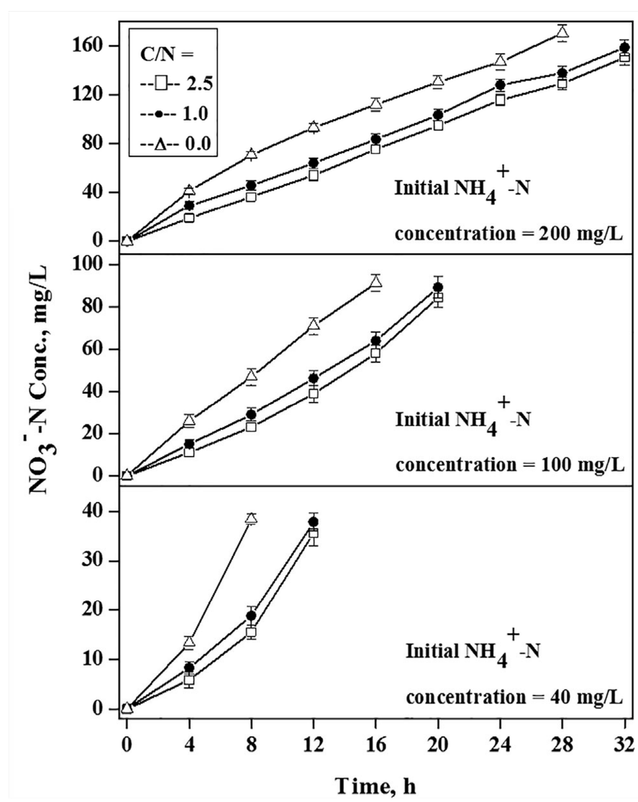

Fig. 3 — Effect of C/N ratios on the formation of NO_3^--N for different initial NH_4^+-N concentrations.

in initial NH_4^+-N concentrations, the formation of NO_3^--N decreases. These results suggest, the nitrification process is due to the availability of adequate oxygen and electron acceptor[5]. Similar observations have also been reported in literature[20]. Further it is observed from Fig. 4 that with the increase of initial NH_4^+-N concentrations and C/N ratios, the percentage nitrification decreases from 96.2 to 75.15%. This might be due to the fact that the heterotrophic microorganisms compete successfully for oxygen than the autotrophic microorganisms in presence of organic carbon which inhibits the nitrification process. Simultaneously, an increase in C/N ratio might be increasing the de-nitrification in the same reactor which leads to insufficient nitrate production[32].

Fractional Factorial Design for the removal of NH_4^+-N

Based on the ANOVA results, the correlation for NH_4^+-N_R in mg/L is developed (Equation 4). Some parameters are not significant based on their p-values. As there are no previous correlations available in literatures, all the six parameters are used in the proposed correlation to have an idea about the possible combinations of all the six parameters.

$$NH_4^+\text{-}N_R = -2.0067 + (1.0561)(A) - (544.1744)(B) + (10.7954)(C) - (1.7533)(D) + (0.3108)(E) + (0.6359)(F) - (2.6793)(AB) - (0.2874)(AC) - (0.1092)(AD) - (0.0092)(AE) - (0.0235)(AF) - (122.3389)(BD) + (1.3065)(CD) \quad \ldots (4)$$

The R^2 value of 0.9469 obtained from Fig. 5 suggest good agreement between experimental and calculated values of NH_4^+-N_R with an average absolute percent deviation of 11.75. Compared to previous studies by Devi and Setty[3] and Bougard *et al.*[22], the present study is based on a wider operating range of parameters. Thus it can be said that the developed correlation has broader applicability.

Kinetic study

From Fig. 6 the kinetic constants k and K_S were estimated to be 11.668 h^{-1} and 38.463 mg/L respectively. In a three-phase bioreactor for NH_4^+-N concentration of 100 mg/L these constants were reported to be 7.812 h^{-1} and 11.062 mg/L respectively[3]. From this comparison it is clear that the kinetic constants have higher values than the

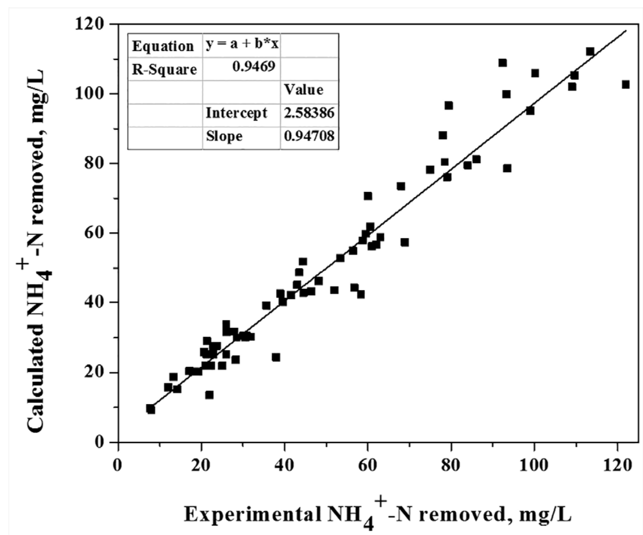

Fig. 5 — Comparison between calculated and experimental values for the amount of NH_4^+-N removed.

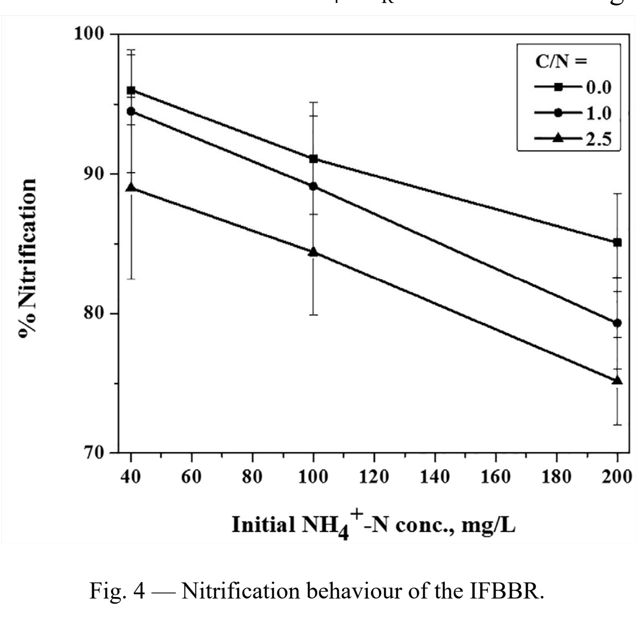

Fig. 4 — Nitrification behaviour of the IFBBR.

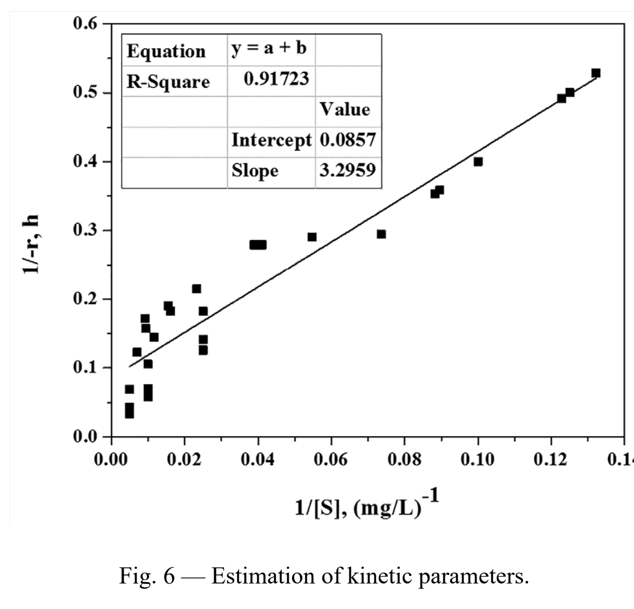

Fig. 6 — Estimation of kinetic parameters.

literature values indicating that the present study using IFBBR provides higher growth rate resulting in higher biodegradation effect.

Conclusion

From the experimental results it is observed that, as C/N ratios were increased from 0.0 to 2.5, the NH_4^+-N removal is found to fall by 30.96 to 52.18% for initial NH_4^+-N concentrations of 40 to 200 mg/L respectively. Faster NH_4^+-N removal is favoured by lower initial NH_4^+-N concentration. As C/N ratios increased from 0.0 to 2.5, complete NH_4^+-N removal time increased from 8 to 12 h for 40 mg/L NH_4^+-N concentration and from 28 to 32 h for 200 mg/L NH_4^+-N concentration. It is further observed that, the complete NH_4^+-N removal time is not affected much at C/N ratio values above 1.0. Optimum operating parameters are found to be as C/N ratio of 0.0, T of 30°C, and *p*H of 8.3. As C/N ratios and initial NH_4^+-N concentrations are increased, the formation of NO_3^--N decreased and the nitrification percentage also decreased from 96.2 to 75.15%. For the prediction of the removal of NH_4^+-N, Fractional Factorial Design analysis is found to be satisfactory as the average absolute percent deviation is found to be low (11.75). Higher kinetic constants obtained in this study indicate the better efficiency of the present IFBBR in terms of higher biodegradation effect. Thus the present IFBBR is found to be effective and environmental friendly for wastewater treatment. Therefore the present IFBBR may be suitably scaled up for large scale wastewater treatment and is well recommended to be applied in all process industries involving generation of liquid effluents.

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