SATEM-2019 Special Issue

J. Indian Chem. Soc.,
Vol. 97, April 2020, pp. 671-678

Simultaneous nitrification and denitrification in a moving bed hybrid bioreactor

Roumi Bhattacharya* and Debabrata Mazumder

Civil Engineering Department, Indian Institute of Engineering Science and Technology, Shibpur, Howrah-711 103, West Bengal, India

E-mail: roumi110993@gmail.com, debabrata@civil.iiests.ac.in

Manuscript received online 26 December 2019, accepted 26 March 2020

Laboratory scale experiment was conducted in a moving bed hybrid bioreactor (MBHBR) under batch mode for removal of nitrogen from ammonium rich synthetic wastewater via nitrification. It was observed from experimental data that even in absence of organic carbon in the aerated completely mixed reactor, denitrification took place simultaneously with nitrification. It may be hypothesized that thick biofilms provided necessary conditions for denitrification in deeper regions, where byproducts of nitrification occurring at the outer layer of biofilm served as the substrates of denitrification. The study was conducted on varying ammonium nitrogen concentration between 500 mg/L to 1000 mg/L. Maximum removal of ammonium nitrogen was observed to be 89%, corresponding to which nitrate and nitrite concentration produced as a result of nitrification was found to decrease by 69 and 82% thus confirming simultaneous nitrification and denitrification in MBHBR.

Keywords: Ammonium nitrogen, denitrification, SND, moving bed hybrid bioreactor, synthetic wastewater.

Introduction

Nitrification of ammonium nitrogen results in the formation of unstable nitrite and stable nitrate in wastewater which may impose other environmental problems. Thus, denitrification is essential for complete removal of nitrogen. The processes of nitrification and denitrification require different environmental conditions and if two separate regions facilitating the processes can be created in a single reactor system, simultaneous nitrification and denitrification (SND) will lead to complete removal of nitrogen[1]. Biofilms having thickness more than that through which dissolve oxygen can diffuse, create anoxic conditions in the deeper layers, supporting denitrification. But the absence of organic carbon in wastewater having low C:N ratio possess a challenge for denitrification to occur. In the absence of any organic carbon, endogenous decay of biomass present in the reactor may also contribute to organic carbon requirement[2].

The process of nitrification involves conversion of ammonium to nitrite followed by nitrate. Experimental analysis reveals initial increase of nitrite concentration followed by its decrease; and gradual increase in nitrate concentration. However, during denitrification step, nitrtate gets reduced to nitrite which is further reduced to nitrogen gas. Thus, nitrate concentration can be observed to decrease whereas, nitrite concentration would increase initially and then reduce[3]. Hence, this trend of nitrite concentration confirms denitrification. A study conducted by Meng *et al.*[4] for studying SND in a non-woven hybrid bioreactor showed that flocculent sludge played a major role for both nitrification and denitrification in hybrid processes although attached biomass was mainly responsible for denitrification. Complete ammonium nitrogen removal paired with 36% nitrate removal was observed in the study with COD:NH_4^+-N varying between 5 to 8. In contrary to that Downing and Neremberg[3] exhibited SND in hybrid membrane reactors where they hypothesized low BOD concentration would facilitate the growth of heterotrophic denitrifiers in the suspended form. In this study, BOD:NH_4^+-N was kept around 8. A study conducted by Li *et al.*[5] showed that higher total nitrogen removal was achieved in the airlift MBR with hybrid biomass (63.1%) than that with suspended growth biomass (39.4%) via SND, which makes the process promising.

Treatment of ammonium nitrogen containing wastewater in an aerated MBHBR operated in batch mode by the present authors showed that around 89% removal could be achieved over a batch period of 5 days. With the theoretical concept of variation of nitrite profile over time, this paper aims at understanding the feasibility of denitrification in deep biofilms of the aerated nitrifying MBHBR. The novelty of the present work lies in the fact that study was conducted with low carbon and high nitrogen containing wastewater without the aid of any external organic carbon source. The rate of denitrification is also quantified using Monod's kinetics.

Materials and methods

Several batch studies were conducted in the Environmental Engineering Laboratory of the Civil Engineering Department, IIEST, Shibpur using a laboratory scale MBHBR with a working volume of 15.6 L. Synthetic wastewater characterized by high ammonium nitrogen concentration (500–1000 mg NH_4^+N/L) and no organic carbon was used in the study. Biomass concentration in the reactor was kept around 3000 mg/L using 25% filling ratio for attached biomass growth. In order to investigate the feasibility of denitrification, acclimatized heterotrophic biomass was used along with autotrophic nitrifiers in the ratio of 1:6 since the growth rate of denitrifying biomass is six times faster than nitrifiers. Necessary parameters for the study were monitored periodically following analytical methods mentioned in APHA Standard Methods[6].

(A) Preparation of synthetic feed:

Since the present study was investigating the occurrence of denitrification in a nitrifying MBHBR, synthetic feed was prepared varying initial ammonium concentration. The nitrate and nitrite concentration in the present study is the result of nitrification process. The synthetic feed was prepared with ammonium nitrogen concentration of 10000 mg/L, which was further added with $NaHCO_3$ and KH_2PO_4 as inorganic carbon and phosphorous source respectively. The pH of the reactor was maintained at 7.8±0.15 throughout the study.

(B) Analytical method:

Parameters like pH, COD, nitrogen species (NH_4^+-N, NO_3^-N, NO_2^-N) were monitored regularly as per the procedure mentioned in APHA Standard Methods[6] respective details of which are listed in Table 1. The attached biomass in the carriers was measured according to the procedure followed by Herbert *et al.*[7] using Folin-Ciocalteu reagent. Minor modifications regarding the amount of alkali copper reagent to be added were undertaken for colour development of protein.

Table 1. Analytical procedures followed for various parameters

Sl. No.	Parameters	Procedures followed	References
1.	pH	Electrometric method	4500-H Part B
2.	TSS	Dried at 103–105°C	2540 Part D
3.	NO_2^--N	Colorimetric method	4500-NO_2^- Part B
4.	NO_3^--N	UV spectrophotometric Screening method	4500-NO_3^- Part B
5.	COD	Closed reflux, titrimetric method	5220 Part C

(C) Experimental setup:

An innovative lab scale reactor is designed and fabricated as MBBR, operated in hybrid mode. The reactor is made of acrylic fibre having thickness 4 mm. The reactor has a vertical height of 63 cm and a diameter of 20 cm, which makes a height to diameter ratio of 3.15. An innovative coarse bubble aeration system is designed for the supply of air in the reactor, which comprises of a long tube with four open nozzles with perforations. The flow rate may be regulated using a pinchcock before the entry of air from pump to maintain specific dissolved oxygen within the reactor. Aeration system is set on the top of the reactor by means of air pump. The media used for attached biomass are cylindrical octahedral polypropylene bio-carrier with fins on projected outside with a specific surface area of 8.84 cm^2/cm^3.

Results and discussion

Treatment of ammonium nitrogen containing wastewater in an aerated MBHBR operated in batch mode by the present authors showed that around 89% removal can be achieved over a batch period of 5 days. During the study period, analysis of nitrate and nitrite concentration in the reactor reveals an interesting aspect. The profiles of nitrite and nitrate concentration are shown in Fig. 1 and Fig. 2 respectively.

As observed in Fig. 1, nitrite concentration for all the batches was increasing initially. The reason may be attributed to nitrification causing conversion of ammonium to nitrite. Consequent to this phase, there was gradual decrease due to further conversion of nitrite to nitrates. After a certain residence time, nitrite concentration in each batch was observed to increase, the reason for which may be assumed

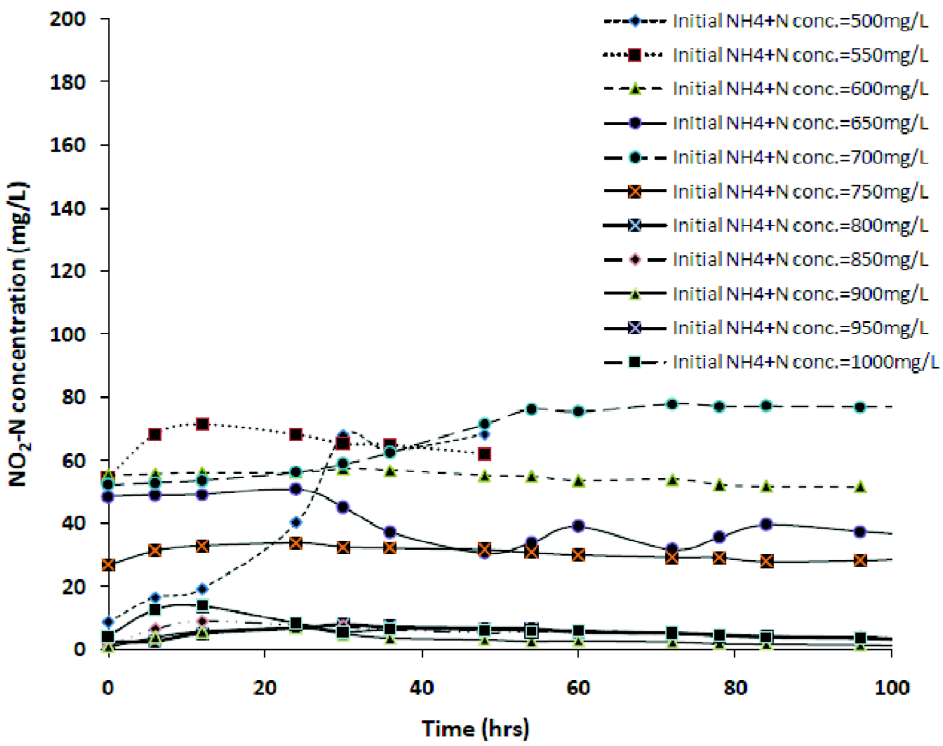

Fig. 1. Nitrite profile over the time course of the study.

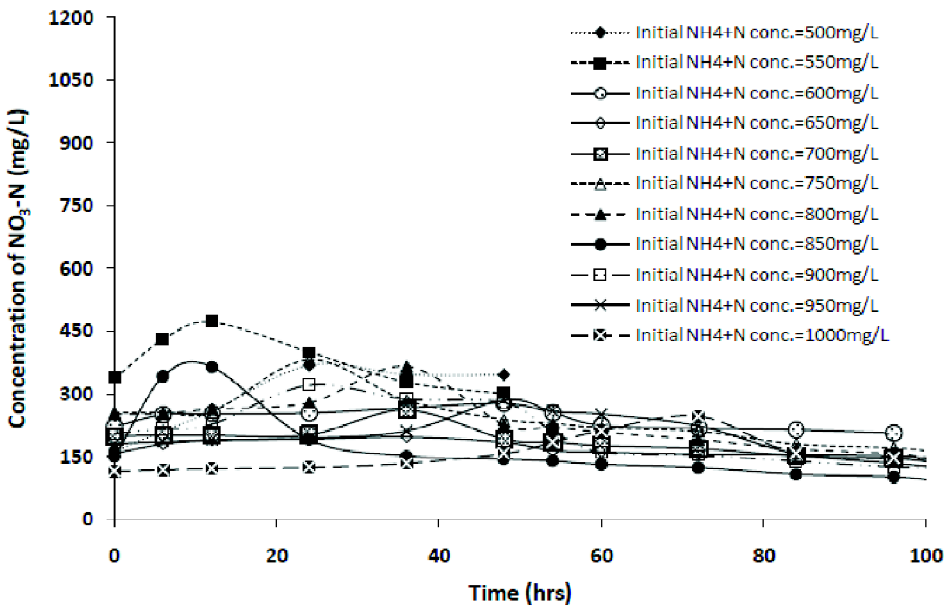

Fig. 2. Nitrate profile over the time course of the study.

as an indication of denitrification as nitrates are converted to nitrites. The time of occurrence of this phenomenon, though, is observed to vary in different batches due to the effect of influencing factors including accumulated nitrite concentration, biomass, pH, biofilm thickness, dissolved oxygen (DO) penetrated in the biofilm or flocs.

At the terminating phase of several batches nitrite concentration is observed to decrease which indicates further reduction of nitrites. This is the most acceptable and obvious explanation for the trend observed for nitrite profile. The reactor when operated as simply nitrifying MBHBR, produced nitrite and nitrate as by product. If the reactor could be facilitated to undergo SND, nitrate and nitrite concentration would also decrease thereby progressing in a complete nitrogen removal prospect. The maximum nitrite removal in the batch corresponding to the maximum ammonium nitrogen removal of 89% was obtained around 82% which was quite satisfactory for denitrification without any aid of external organic carbon.

The trend of nitrate profile observed in Fig. 2 supports the explanation given in favour of denitrification. The decrease in nitrite concentration is complementary to the increase in nitrate concentration as observed in the above figures. Thereafter, the conversion of nitrate to nitrite leads to the subsequent decrease in nitrate and increase in nitrite concentration. As DO was never a limiting substrate during the study, SND occurred over nitrate. The comparative rate of formation and decomposition of nitrate and nitrite determines the rate of nitrification and denitrification respectively[8]. Another aspect of denitrification is the requirement of sufficient amount of organic carbon for complete reduction of nitrates and nitrites[9]. In this study, the rate of denitrification is observed to be comparatively low due to the absence of external organic carbon. Heterotrophic denitrification requires organic carbon source because the microorganisms utilize nitrate as terminal hydrogen acceptor and carbon is as similated into the cell[10]. The nature and the amount of the organic substrate used as exogenous carbon source play an important role in the success and cost of denitrification[11]. Methanol is most widely used carbon source[12] followed by internal carbon source obtained from endogenous respiration[13]. With no external organic carbon addition, denitrification rates of suspended biomass and biofilm were obtained as 0.27 and 0.33 mg-NO_3^-N/(g-biomass h), respectively. When using sludge as organ carbon source, the rate of denitrification is generally governed by the amount of organic as well as nitrogenous matter released due to endogenous decay of the biomass[14]. Furthermore, the rate of denitrification is dependent on both nitrate and carbon concentrations.

In general, the ratio of organic carbon to nitrate nitrogen is often kept between 5 and 10 for efficient denitrification[15]. With a view to establish the profile of change in organic nitrogen concentration, COD was also measured for all the samples as shown in Fig. 3. In this case, it can be concluded that since organic carbon to nitrate concentration was far below the required one, denitrification was incomplete and

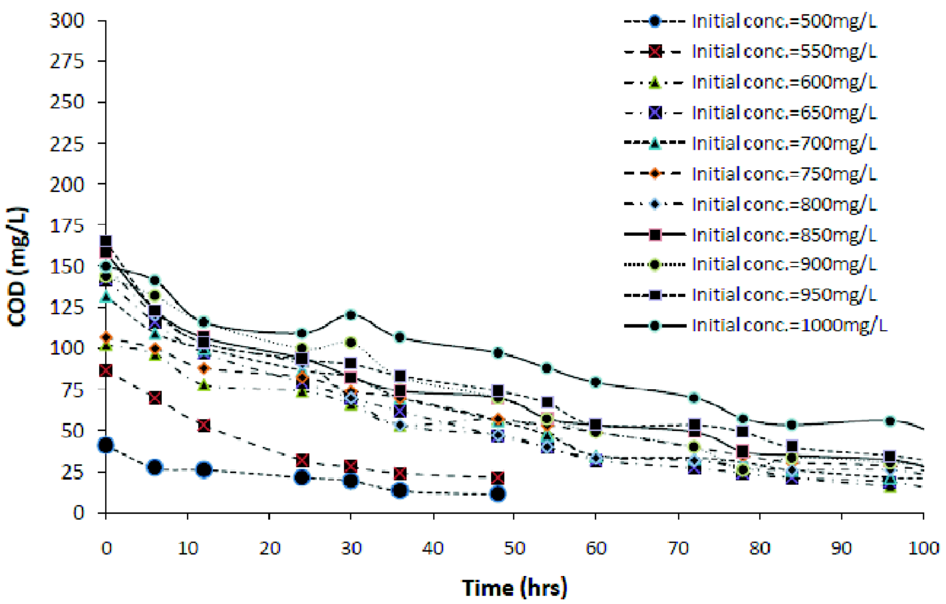

Fig. 3. COD profile over the time course of batch study.

the reaction rate was extremely low. A similar study conducted by Meng *et al.*[4] indicated the occurrence of denitrification although no external carbon source was provided during the study. It is suggested that the sludge might have undergone endogenous denitrification, using the intracellular and/or extracellular biopolymers as carbon sources[16].

Regarding the pathway of denitrification, either aerobic or anoxic process could have taken place the reactor with high DO concentrations that support aerobic nitrification. Aerobic denitrification follows quite a similar pathway as that of anoxic denitrification and thus, it is experimentally challenging to identify the process from its intermediate by-products without gene analysis. Since the intermediate products formed and degraded are identical in both cases, their trends during the reaction period is similar[17]. Denitrification is observed to occur almost exclusively under facultative anaerobic or microaerophilic conditions[18]. Aerobic denitrification requires an optimal C/N ratio of 9–10 which is higher than anoxic denitrification[19,20]. With low C/N ratio, the reaction rate falls drastically and higher organic carbon concentration confirms faster aerobic denitrification[21]. During the present study, practically no external organic carbon was used and low denitrification rates was achieved by carbon obtained through endogenous degradation. This suggests the preference of anoxic denitrification over aerobic process. According to Chen *et al.*[17] aerobic denitrification could be confirmed to occur over anoxic denitrification when there is steady decrease in pH. Since, available organic carbon concentration is one of the major rate limiting parameters for aerobic denitrification and in this case, it was almost negligible, denitrification could be assumed to have followed anaerobic pathway.

Since nitrification was autotrophic, conventional anoxic denitrification in aerobic hybrid reactors requires anoxic zones within the system where DO will not be able to penetrate. Now, two possibilities of formation of such zones are larger flocs and thicker biofilms. For SND to be feasible in hybrid reactors, co-existence of both aerobic and anoxic zones within the flocculent sludge or the biofilm is necessary along with the presence of an optimal dissolved oxygen (DO) concentration in the mixed liquor[4]. The ratio of suspended to attached biomass varies according to numerous factors including reactor conditions[22]. The location of heterotrophic biomass can be established by controlling detention time in the reactor. When detention time is kept less than 3 h, attached biomass will comprise of both autotrophic and heterotrophic organisms[23]. The present analysis of the data for biomass was done considering the cumulative suspended and attached biomass concentration as the occurrence of nitrification and denitrification was not distinctively specified to occur in attached and suspended form. Paul *et al.*[24] observed that autotrophs are predominantly in biofilm components and in that study, 40% of total microorganisms comprised of nitrifiers. In the present study, the ratio of attached to suspended biomass concentration was low and ranged between 0.85 and 1.2. In addition, during the end of each batch, it was observed that the ratio increased, though at a narrow interval. In certain cases, the ratio can be as high as 5–13[22].

The study of denitrification is observed to follow Monod's kinetics from previous literatures. In order to quantify the process of denitrification undergone in the present study, the rates of nitrate utilisation must be specified. Now, for complete denitrification, nitrite kinetics should also be determined but from Fig. 2, it is inferred that nitrite degradation did not achieve steady state. The process is thus not yet complete in such low organic carbon concentration. Accordingly, nitrate utilisation kinetics in the light of Monod's theory has been established to analyse the rate of reaction. The various kinetic coefficients related to this theory are determined as shown in Fig. 4 and Fig. 5. The magnitudes of half saturation constant (K_s), specific substrate utilisation rate (k), endogenous decay constant (k_d) and yield coefficient (Y) were determined as 25.02 mg/L, 0.117/d, 0.002/d and 0.039 mg VSS/mg substrate, respectively.

The present authors had previously undertaken denitrification studies in batch activated sludge reactor to compare the reaction kinetics with and without using organic carbon. The organic carbon added externally was methanol and in case of studies without the aid of external carbon source, organic carbon was supplied from endogenous decay. A comparison of the kinetic parameters obtained during these studies is presented in Table 2 although the present study was not aimed for denitrification in the nitrifying MBHBR and reactor conditions were made favorable primarily for nitrification.

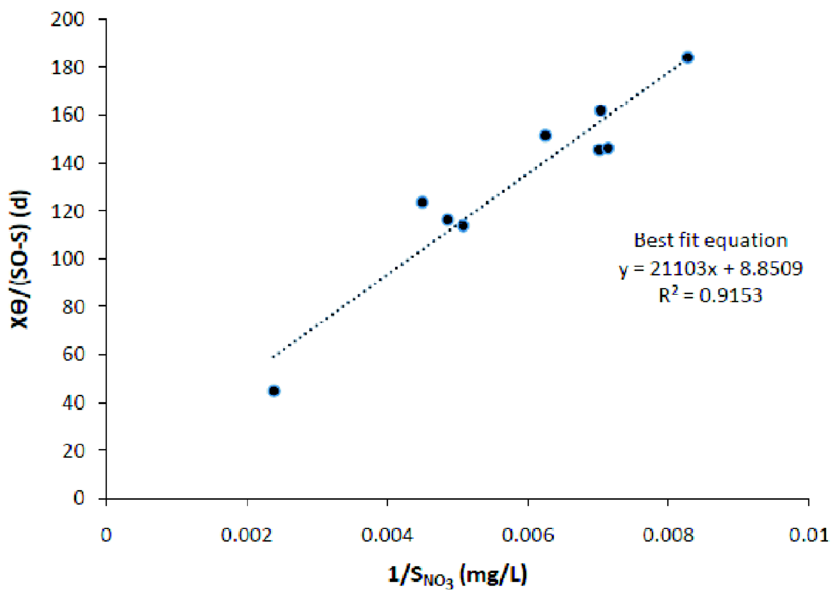

Fig. 4. Determination of K_s and k.

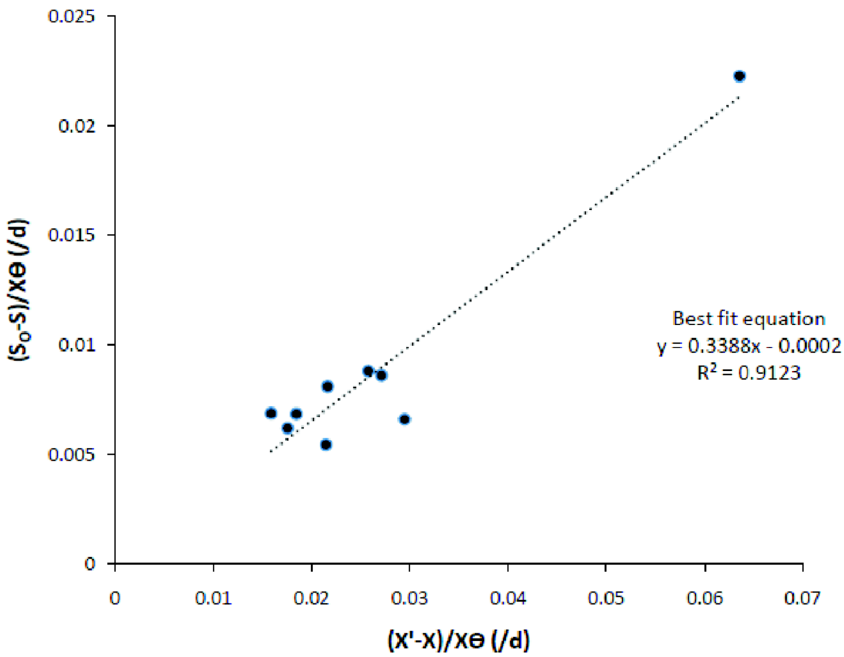

Fig. 5. Determination of k_d and Y.

From Table 2, it can be observed that although special attention was not given for denitrification, the kinetics parameters obtained were quite comparable. It can be concluded that denitrification was incomplete and its rate was low. However, denitrification is feasible in this type of reactor undergoing nitrification thereby facilitating SND. As compared with the results obtained from suspended growth reactors, the magnitudes of the parameters were observed to be low. In comparison with suspended process, hybrid reactor was supposed to yield higher values of the coefficients that indi-

Table 2. Comparison of kinetic parameters

Parameter	Present study	Denitrification without external carbon	Denitrification with methanol (1.9 times NO_3)
Reactor type	Hybrid	Suspended	Suspended
Max. initial NO_3 (mg/L)	340	250	250
Min. initial NO_3 (mg/L)	110	50	50
K_s (mg/L)	25.02	4.68	11.69
k (/d)	0.117	0.28	0.312
Y (mg VSS/mg NO_3)	0.039	0.121	0.143
k_d (/d)	0.002	0.069	0.102

cating better removal. However, it has to be kept in mind that the process was not designed to operate as an SND and sufficient environmental conditions of denitrification had not been provided. It merely shows the feasibility of the process and that it could be operated optimally if requisite condition in terms of organic carbon and DO is provided. The kinetic parameters, specifically, the specific growth rate is observed to influence the formation of attached biomass colonies. This characteristic of formation of biofilms can be manipulated to form slow growing organisms in the biofilm and heterotrophic biomass in the reactor[25]. The results obtained from the present study could not be compared with previous literature because the kinetic studies carried out earlier did not account nitrate nitrogen as the limiting substrate. No parameters had been previously determined based on nitrate concentration. In respect of endogenous decay constant, it was observed to be quite low as compared to the previous studies. Carucci *et al.*[26] observed that using endogenous carbon, decay rate was found to be 0.07/d whereas it was 0.25/d for acetate when complete denitrification was achieved.

Conclusion

The process of denitrification was observed to take place in a nitrifying aerobic MBHBR, assuming that the biofilm thickness or floc size was suitable for accommodating anoxic zones. Detailed studies regarding the speciation of microorganisms responsible for denitrificationis are yet to be done to confirm their presence in flocs and/or biofilms. It is observed that even if denitrification does occur in nitrifying environment, the rate is quite low in comparison to other reactor systems that had been operated for denitrification. The presence of organic carbon serves as a necessity, which when not present, uses carbon obtained from endogenous decay of the microorganisms. The process seems to be economical as well as convenient and could thus be optimized successfully.

References

1. M. Seifi and M. H. Fazaelipoor, *Applied Mathematical Modelling*, 2012, **36**, 5603.
2. E. E. Metcalf and H. Eddy, "Wastewater engineering: treatment and resource recovery", McGraw Hill Publishers, New York, USA, 2014.
3. L. S. Downing and R. Nerenberg, *Water Science and Technology*, 2007, **55**, 355.
4. F. Meng, Y. Wang, L. N. Huang, J. Li, F. Jiang, S. Li and G. H. Chen, *Biotechnology and Bioengineering*, 2013, **110**, 1903.
5. Y. Z. Li, Y. L. He, D. G. Ohandja, J. Ji, J. F. Li and T. Zhou, *Bioresource Technology*, 2008, **99**, 5867.
6. A. D. Eaton, L. S.Clesceri, E. W. Rice, A. E. Greenberg and M. A. H. A. Franson, APHA: standard methods for the examination of water and wastewater, Centennial Edition, APHA, AWWA, WEF, Washington, DC, 2005.
7. D. Herbert, P. J. Phipps and R. E. Strange, "Chemical analysis of microbial cells", in: Methods in microbiology, Academic Press, 1971, Chap. III.
8. L. Chuand J. Wang, *Chemical Engineering Journal*, 2011, **170**, 220.
9. M. Henze, *Water Science and Technology*, 1991, **23**, 669.
10. R. N. Dawson and K. L. Murphy, *Water Research*, 1972, **6**, 71.
11. L. Foglar and F. Briški, *Process Biochemistry*, 2003, **39**, 95.
12. C. P. L. Grady and H. C. Lim, "Denitrification. Biological Wastewater Treatment", Dekker, New York, 1981, 887.
13. P. Harremoes, Proceedings of the Conference on Nitrogen as a Water Pollutant, Copenhagen, 1975, in: Conference on Nitrogen as a Water Pollutant Pergamon Press, 1977.
14. A. A. Abufayed and E. D. Schroeder, *J. Water Pollution Control Federation*, 1986, 398.
15. E. Klimiuk and M. Aebkowska, "Biotechnology in environ-

mental protection", Wyd. Naukowe PWN, Warszawa, 2003 (in Polish).

16. R. Cui and D. Jahng, *Water Research*, 2004, **38**, 1159.
17. F. Chen, Q. Xia and L. K. Ju, *Appl. Environ. Microbiol*., 2003, **69**, 6715.
18. J. C. A. Marin, A. H. Caravelli and N. E. Zaritzky, *Bioresource Technology*, 2016, **200**, 380.
19. H. K. Huang and S. K. Tseng, *Applied Microbiology and Biotechnology*, 2001, **55**, 90.
20. B. Ji, K. Yang, L. Zhu, Y. Jiang, H. Wang, J. Zhou and H. Zhang, *Biotechnology and Bioprocess Engineering*, 2015, **20**, 643.
21. L. A. Robertson and J. G. Kuenen, *Microbiology*, 1983, **129**, 2847.
22. M. Makowska, M. SpychaBa and R. Mazur, "Removal of Carbon and Nitrogen Compounds in Hybrid Bioreactors", in: Biomass Now-Cultivation and Utilization, Intech Open, 2013.
23. W. A. J. van Benthum, M. C. M. van Loosdrecht and J. J. Heijnen, *Water Science and Technology*, 1997, **36**, 119.
24. E. Paul, D. B. Wolff, J. C. Ochoa and R. H. Ribeiro da Costa, *Water Environment Research*, 2007, **79**, 765.
25. L. Tijhuis, M. C. M. Van Loosdrecht and J. J. Heijnen, *Biotechnology and Bioengineering*, 1994, **44**, 595.
26. A. Carucci, R. Ramadori, S. Rossetti and M. C. Tomei, *Water Research*, 1996, **30**, 51.