Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioresource Technology 88 (2003) 107–113
BIORESOURCE TECHNOLOGY

Nitrogen removal from wastewater using a double-biofilm reactor with a continuous-flow method

Yuan-Lynn Hsieh [a,*], Szu-Kung Tseng [a], Yu-Jie Chang [b]

[a] Graduate Institute of Environmental Engineering, National Taiwan University, 71 Chou-Shan Road, Taipei 106, Taiwan, ROC
[b] Department of Environmental Engineering, Tung Nan Institute of Technology, 92 Wan-Fu Hamlet, Shen-Kun Village, Taipei 222, Taiwan, ROC

Received 17 July 2002; received in revised form 1 November 2002; accepted 7 November 2002

Abstract

Wastewater microorganisms of nitrification and denitrification were cultivated to compose two biofilm modules, termed the permeable support bioreactor (PSB) and the membrane feeding substrate bioreactor (MFSB). PSB and MFSB were combined in a single tank to develop a double-biofilm reactor, which was used to treat nitrogen contaminants in wastewater. With a membrane supplement of substrates (O_2 and CH_3OH), the D.O. and COD levels were at a low value in the bulk solution thus inhibitive effects between nitrification and denitrification were minimized. Simultaneous nitrification/denitrification was conducted in the reactor and the double-biofilm reactor achieved high nitrification and denitrification efficiency, of 96.5% and 82%, respectively.

Keywords: Nitrification; Denitrification; Biofilm; PSB; MFSB; Polyvinyl alcohol

1. Introduction

Advanced wastewater treatment processes for nitrogen removal are usually separated into two biological stages, namely—aerobic nitrification and anaerobic denitrification. The operating requirements differ significantly between these two. For example, nitrification tanks need a bulk solution with high D.O. but without organic carbon, while denitrification tanks require an anaerobic environment and an organic carbon source (Benfield and Randall, 1980). Consequently, in the two-stage de-nitrogen system, residual D.O. in the nitrification tank generally inhibits the subsequent denitrification process while additional organic carbon in the denitrification tank either increases the effluent COD level or influences nitrification if the water is recirculated. The interference from substrates between nitrification and denitrification tanks usually reduces nitrogen elimination efficiency during advanced wastewater treatment.

Two modules introduced here probably could improve the traditional method for treating nitrogen contaminants. First, a silicone membrane fed oxygen to a biofilm, a system named the permeable support bioreactor (PSB). Second, a silicone membrane fed methanol to a biofilm, called the membrane feeding substrate bioreactor (MFSB). In earlier work, combining PSB and MFSB in a single tank was noted to develop a simultaneous nitrification/denitrification system (Chang and Tseng, 1999). However, the autotrophic nitrifying microorganisms had a low growth rate and were very sensitive to environmental conditions such as pH, D.O. (Campos et al., 1999), and organic matters, immobilization of the nitrifiers with polyvinyl alcohol (PVA) was introduced by Hsieh et al. (2002). The PVA-immobilized bacteria on the tube allowed the formation of biofilm with high biomass concentration and could endure a long time of operation without sloughing off. This module was investigated by Hsieh et al. (2002) to achieve a 95% ammonium removal rate and was named PSB in this paper. The MFSB module in this work was adopted from Chang and Tseng (1998), and it had several advantages: (1) achieving a high denitrification rate ($4.5\ g\,N\,m^{-2}\,d^{-1}$) and (2) substantially reducing residual COD level ($\leqslant 50\ mg\,l^{-1}$) in denitrification.

In this work, PSB and MFSB were combined in a single reactor that conducted simultaneous nitrification/denitrification, and the major substrates, O_2 and carbon

* Corresponding author. Fax: +886-2-23637854.
E-mail address: r7541101@ms.cc.ntu.edu.tw (Y.-L. Hsieh).

0960-8524/03/$ - see front matter
PII: S0960-8524(02)00274-2

source were supplied by silicone tube rather than by direct addition. The purpose of this study was to evaluate the optimal operating conditions for the double-biofilm reactor treating nitrogen contaminants in wastewater at a bench-scale continuous-flow method.

2. Methods

2.1. The double-biofilm reactor

The part of the reactor that conducted nitrification, PSB, comprised of a 6-m-long silicone membrane tube (outer diam. = 2.5 mm, inner diam. = 1.5 mm) which was wrapped in PVA-immobilized nitrifying biofilm, while in the tube with the wrapping being wound around a 4-pillar acrylic base PVA performed as an immobilizing agent to strengthen the biofilm and keep it from sloughing off (Chen and Lin, 1994). The method of immobilization was described in our previous work (Hsieh et al., 2002). Another part of the reactor, MFSB, conducting the denitrification, was adopted from Chang and Tseng (1998). This module used the same silicone membrane tube as PSB, but without a PVA coating. Furthermore, the lumen was filled with CH_3OH solution instead of O_2 for the MFSB module.

PSB and MFSB were initially acclimatized in separate 1.5-l tanks with independent substrate nutrition until they stabilized on both biofilms. Since the biofilm on PSB tube was artificially constructed using PVA immobilized with nitrifiers, the cultivation time was reduced to less than a week. Meanwhile, denitrifying bacteria spontaneously formed a biofilm layer on the surface of the MFSB tube after three-weeks of culturing. Fig. 1 illustrates how PSB and MFSB were arranged in a 3-l-tank reactor. The O_2 for nitrification was supplied by bottled gas, while the carbon source for denitrification was bottled in a 1-l CH_3OH solution reservoir, which was renewed daily to ensure a steady concentration gradient through the tube membrane. The reactor was maintained at 30 °C via a water bath controller for maximum microorganism activity (Dawson and Murphy, 1973; Fdz-Polanco et al., 1994) throughout the experiments. Table 1 lists the summary of circumstances in each experimental run with the double-biofilm reactor.

2.2. Synthetic wastewater

Synthetic wastewater containing $(NH_4)_2SO_4$ (118–707 mg l^{-1}) and other nutritious substrates, including KH_2PO_4 (8.5 mg l^{-1}), K_2HPO_4 (21.8 mg l^{-1}), $Na_2HPO_4 \cdot 7H_2O$ (33.4 mg l^{-1}), $CaCl_2 \cdot 2H_2O$ (27.5 mg l^{-1}), $MgSO_4 \cdot 7H_2O$ (22.5 mg l^{-1}), $FeCl_3 \cdot 7H_2O$ (0.25 mg l^{-1}), and $NaHCO_3$ (500 mg l^{-1}), was used to cultivate the biofilm in a continuous stirred tank reactor (CSTR) method.

2.3. Analytical methods

All of the effluent samples were filtered by 0.22 μm teflon microfilter before collection, in order to minimize suspended solids (SS) or microorganisms in the liquid sample. The pH and D.O. were measured by portable electrode (WTW Microprocessor pH95, Germany and HANNA HI9142 DO meter, Italy, respectively), and nitrate, nitrite were measured with an ion chromatograph analyzer (Alltech ERIS™ 1000 Autosuppressor, USA). Standard methods 4500, 5220 (APHA, 1995) were followed for measuring ammonium and COD, respectively, in the liquid phase.

3. Results and discussion

3.1. Determination of tube side substrate concentration

The bottled CH_3OH, which was circulated in the MFSB module, supplied organic carbon during the denitrification process. Low CH_3OH concentration

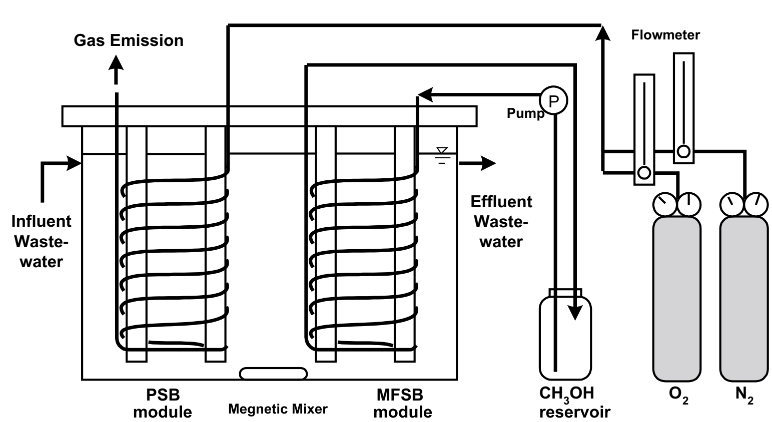

Fig. 1. Schematic diagram of the double-biofilm reactor (combined-mode).

Table 1
Summary of experimental circumstances of each run

Items	Experimental run number				
	1	2	3	4	5
PSB O_2 Partial pressure	1	0–1	1	1	1
MFSB carbon source (mg CH_3OH l^{-1})	5–30	20	20	20	20
NH_4^+-loading rate (g N m^{-2} d^{-1})	1.91	3.83	2–11.5	3.83	3.45
Water temp. (°C)	30	30	30	30	30
Influent COD (mg l^{-1})	0	0	0	0–1500	0
Annotation	CH_3OH test	P_{O_2} test	Loading test	COD test	Separated-mode reactor test

could cause insufficient denitrification (McCarty et al., 1969), while high CH_3OH concentration created a risk of CH_3OH breakthrough of the biofilm and contamination of the bulk solution in the reactor (Chang and Tseng, 1998). During run 1, CH_3OH concentrations ranging from 5 to 30 g l^{-1} were applied in the MFSB module at a flow rate of 30 ml min^{-1}, and other variables were left unchanged, as described on Table 1. The performance of the reactor stabilized after two weeks of running. Fig. 2 illustrates the results that denitrification efficiency increased markedly with increasing CH_3OH concentration, from 24.3% to 79%. Compared to the maximum concentration of CH_3OH solution in the tube side (30 g CH_3OH l^{-1} or 45,000 mg COD l^{-1}), contamination of leaching organic carbon was extremely low at a value of 19.6 mg COD l^{-1} (Fig. 2c). Since denitrification occurred, it inferred that most of the organic carbon penetrated through the tube membrane was consumed by the biofilm of denitrifiers outside the MFSB tube. In Fig. 2a, the efficiency of nitrification by PSB maintained from 93.7% to 98.3%, showing that the leaching of organic carbon from MFSB did not significantly disturb nitrification on PSB in the reactor. Although the D.O. value decreased to the level of 0.8 mg l^{-1} with increasing bulk organic carbon, it did not disrupt nitrification reaction, either. Since nitrifying bacteria (the biofilm) on PSB took O_2 from the tube side rather than the bulk phase and the D.O. gradient was higher in the bottom of the biofilm than that on the surface, nitrification efficiency was hardly influenced by low bulk D.O. Consequently, the optimal concentration of bottled CH_3OH was set at the level of 20 g l^{-1} to ensure the denitrification efficiency of over 75.5%.

Oxygen is an essential substrate for aerobic nitrification but inhibits anaerobic denitrification while it is present in a suspended system (Bitton, 1994). The biofilm, however, creates an anoxic region in the deep layer, and could resist higher bulk D.O. (Brower and Barford, 1997). To determine the O_2 requirements of the reactor, gases of various O_2 partial pressures (P_{O_2} ranging from 0 to 1) were produced by blending O_2 and N_2 in the tube side of the PSB at a flow rate of 36 ml min^{-1}. Other

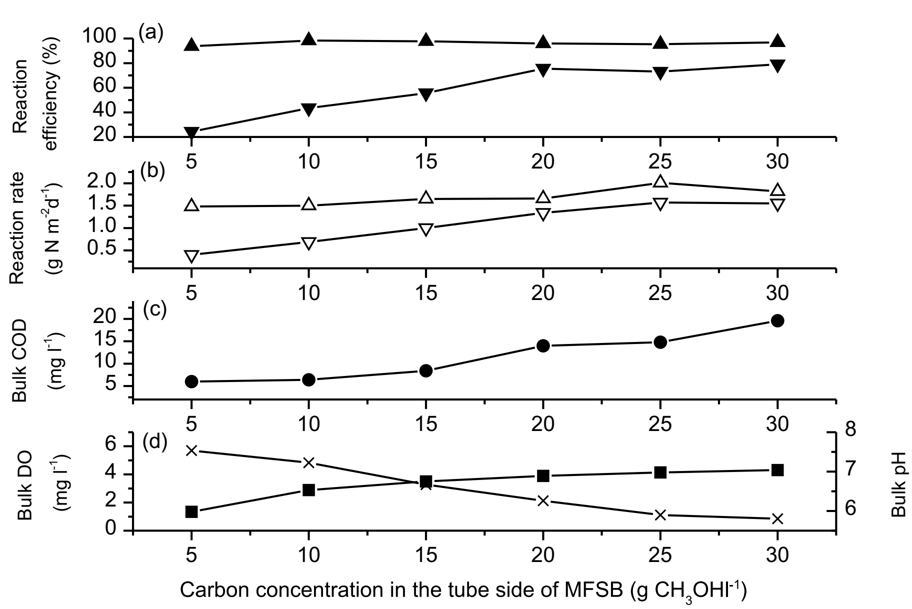

Fig. 2. Determination of optimal CH_3OH concentration in MFSB tube: (a) (▲), nitrification efficiency, (▼), denitrification efficiency; (b) (△), nitrification rate, (▽), denitrification rate; (c) (●), bulk COD; (d) (×), bulk D.O., (■), bulk pH.

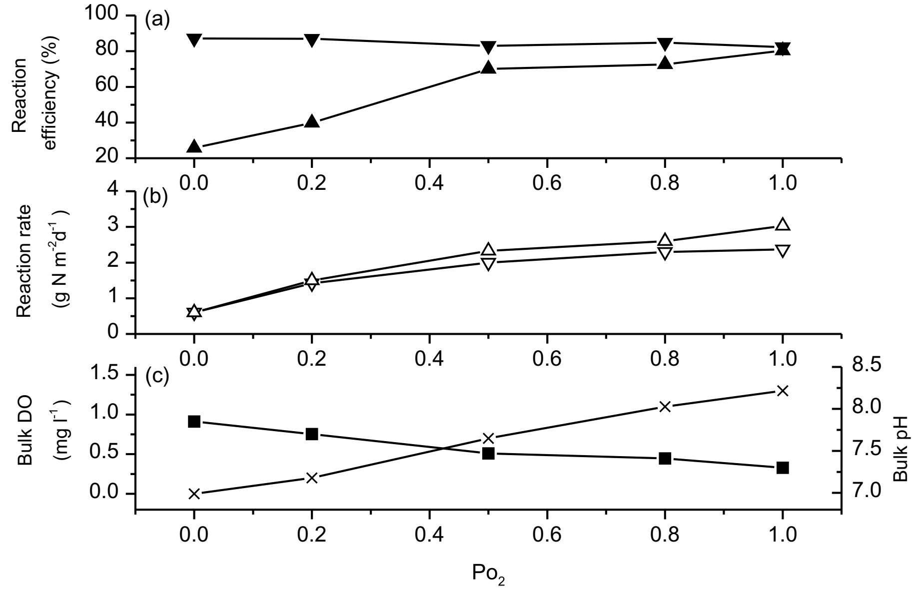

Fig. 3. Determination of optimal O_2 partial pressure (P_{O_2}) in PSB tube: (a) (▲), nitrification efficiency, (▼), denitrification efficiency; (b) (△), nitrification rate, (▽), denitrification rate; (c) (×), bulk D.O., (■), bulk pH.

experimental variables were kept constant as listed in the second run from Table 1. The reactor ran for two weeks for stabilizing its performance. Fig. 3 displays the results. Since O_2 is an essential substrate for nitrifying microorganisms, nitrification efficiency with PSB increased from 25.8% to 80.3% as P_{O_2} increased from 0 (all N_2) to 1 (all O_2). Meanwhile, denitrification efficiency with MFSB slightly decreased from 87.1% to 82.3% (Fig. 3a). Since the tube side oxygen, which diffused outwardly, was largely consumed via nitrification on the PSB biofilm, the bulk solution could be kept in a low D.O. condition, which caused less impact on denitrification. Consequently, Po_2 was found to be set at least the level of 0.5 to maintain nitrification efficiency above 70%.

3.2. Ammonium loading test

From the previous experiment runs, the operational conditions were optimized when P_{O_2} and bottled CH_3OH concentration were 1 (all O_2) and 20 $g\,l^{-1}$, respectively. Run 3 investigated the nitrogen elimination ability of the reactor. Inflow ammonium loading ranging from 2 to 11.5 $g\,N\,m^{-2}\,d^{-1}$ was applied in the reactor, while other experimental parameters were kept constant as described on Table 1. The results display on Fig. 4. Both nitrification and denitrification efficiency decreased with increasing loading from 96.5% to 38.3% and 82% to 69% (Fig. 4a) respectively, which could have resulted from limited surface area of the biofilm causing insufficient reaction site. Besides, the nitrification and denitrification rates were found to reach the peak values of 5.0 and 3.7 $g\,N\,m^{-2}\,d^{-1}$ (Fig. 4b), respectively, indicating the nitrification and denitrification abilities of PSB and MFSB in the reactor. The nitrification and denitrification rates, however, decreased at the highest ammonium loading, and the interpretation might be that as the ammonium inflow rate exceeded the rate of ammonium consumption, ammonium accumulated and some part of it transformed into free ammonia, which was toxic to most microorganisms (Liu and Capdeville, 1994). Therefore, the nitrification and denitrification rates decreased at the highest loading rate.

3.3. Impact of inflow wastewater containing COD

In previous runs, synthetic wastewater was free of COD hence the only carbon source in the reactor came from the tube side of MFSB. From observation on run 1, the leaching organic carbon (19.6 mg COD l^{-1}) did not significantly influence the performance of the biofilm on the PSB side. However, wastewater usually contains organic matters. In run 4, COD from 0 to 1500 $mg\,l^{-1}$ was added to the synthetic wastewater to examine its impact on the reactor, while other experimental variables were kept unchanged, as shown in Table 1. During this run, an increasing turbidity in the bulk solution was observed following the appearance of inflow COD. The increase in turbidity was related to the growth of microorganisms suspended in the reactor, since the reactor was an open system. These suspended microorganisms would adhere on both PSB and MFSB biofilm; therefore they thickened the biofilm and blocked the transportation of substrates from outside such as ammonium or nitrate. In PSB, the distribution of substrate was supposed to be lacking in ammonium at the bottom layer of

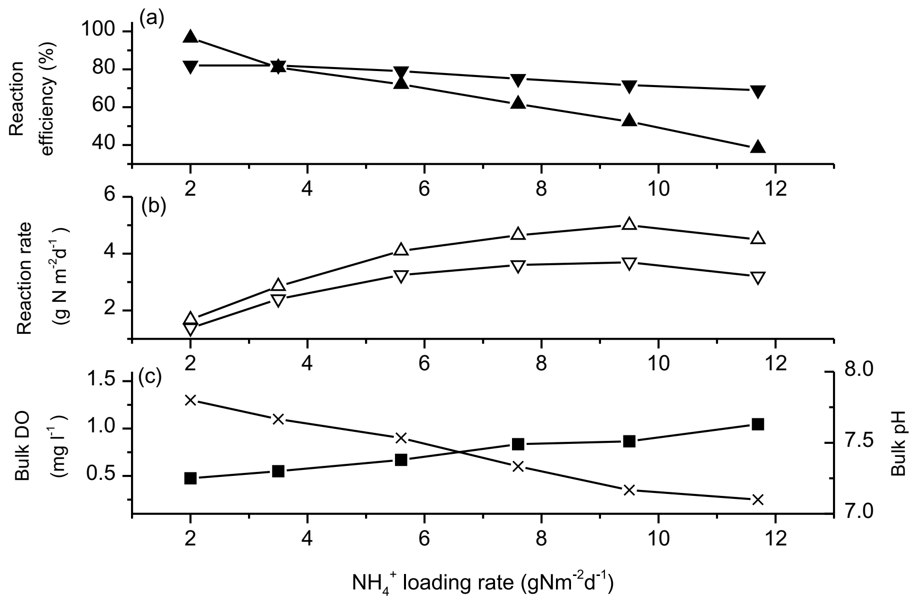

Fig. 4. Effect of NH_4^+ loading on the reactor: (a) (▲), nitrification efficiency, (▼), denitrification efficiency; (b) (△), nitrification rate, (▽), denitrification rate; (c) (×), bulk D.O., (■), bulk pH.

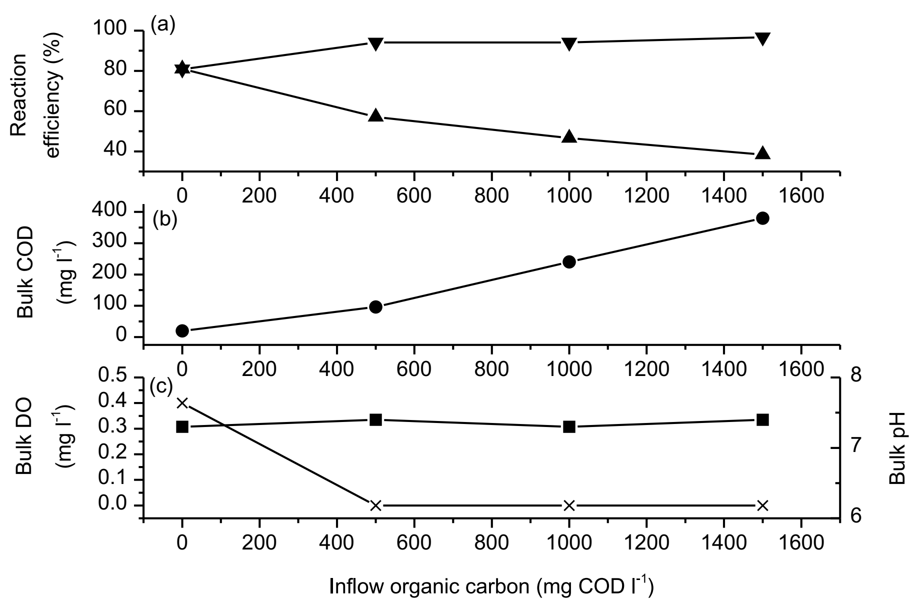

Fig. 5. Impact of inflow wastewater containing COD on the reactor: (a) (▲), nitrification efficiency, (▼), denitrification efficiency; (b) (●), bulk COD; (c) (×), bulk D.O., (■), bulk pH.

the biofilm and lacking in D.O. at the surface layer, thus the nitrification efficiency decreased from 81.1% to 42.6% (Fig. 5a) with increasing inflow COD. Conversely, denitrification efficiency by MFSB increased from 80.8% to 96.7% as the inflow of COD into the reactor increased. The reason was that both organic carbon and nitrate appeared on the surface layer of the MFSB biofilm thus enhancing denitrification in the reactor. Since there was lack in D.O. in the bulk solution, excess organic carbon with effluent concentration ranging from 19.6 to 380 mg/l (Fig. 5b) was left residual. Clearly, the reactor was unsuitable for treating wastewater that contained organic matters.

3.4. Advantages of the combined-mode reactor as compared to separated-mode reactor

The advantages of combining PSB and MFSB in a single reactor rather than separating the two can be demonstrated by the following experiment. The

Table 2
Results of combined-mode reactor and separated-mode reactor

Items	Combined-mode reactor	Separated-mode reactor	
		PSB	MFSB
Nitrification efficiency (%)	82	77.7	–
Denitrification efficiency (%)	80	–	77.8
Nitrification rate ($g\,N\,m^{-2}\,d^{-1}$)	3.0	2.86	–
Denitrification rate ($g\,N\,m^{-2}\,d^{-1}$)	2.4	–	2.28
Bulk pH	7.3	6.58	7.54
Bulk D.O.	0.4	1.1	0.13

experimental devices were all the same as the set-up on Fig. 1 except that the PSB and MFSB module were separated into two 1.5-l tanks for nitrification and denitrification. The total volume of the two tanks, 3 l, was equal to that of the combined-mode reactor. The two reactors began running simultaneously and under identical circumstances as shown on Table 1, run 5. Table 2 lists the results of two reactors and reveals that the combined-mode reactor performed better than the separated-mode reactor in both nitrification and denitrification efficiency, by 4.6% and 2.5%, respectively. Combining PSB and MFSB in a single reactor was proven not to be disadvantageous in dealing with nitrogen contaminants compared to the two-stage system. Given the same reaction time for PSB or MFSB modules in the two reactors, the combined-mode reactor required only 8 h of HRT in a single reactor to conduct nitrification and denitrification but 16 h was needed in the separated-mode reactor. Therefore, combining PSB and MFSB in a single reactor achieved savings in reactor volume and reaction time. For second reason, the pH value in the combined-mode reactor was more stable for microorganisms of nitrification and denitrification than in the separated-mode reactor. Since nitrification consumes alkalinity and reduces pH value in the water while denitrification does the contrary (Sharma and Ahlert, 1977; Matêjü and Cižinská, 1992), the pH value in the PSB tank of the separated-mode dropped to 6.58 (Table 2), which was not in the proper range for nitrification. However, in the combined-mode reactor, compensation of alkalinity by denitrification could avoid the drop of pH value and maintain it within a stable range for nitrification and denitrification reactions.

4. Conclusions

Owing to the features of PSB and MFSB, they were combined in a single tank to process with simultaneous nitrification/denitrification. Experiments on a bench-scale reactor yielded the following conclusions:

(1) Using MFSB to conduct denitrification of wastewater solved the problem of recontamination by organic carbon, and minimized the substrate influences on PSB as a nitrification module. The bulk D.O. resulting from PSB leaching was small and it hardly reduced denitrification of MFSB.
(2) Treating COD-containing wastewater with the double-biofilm reactor is not recommended.
(3) Combined-mode reactor had several advantages over the separated-mode. (a) Given the same reactor volume, adopting the combined-mode reactor halved the reaction time. (b) Self-compensation of alkalinity in combined-mode reactor possibly attributed to maintaining pH value in a stable range.
(4) The nitrification and denitrification rates can be easily enhanced with additional length of silicone tube in the reactor.

Acknowledgements

The authors would like to thank the National Science Council of the Republic of China for financially supporting this research under contract no. NSC 89-2211-E-002-014.

References

APHA, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, DC.

Benfield, L.D., Randall, C.W., 1980. Active sludge and its process modification. In: Biological Process Design for Wastewater Treatment. Prentice-Hall, New Jersey, pp. 218–255.

Bitton, G., 1994. Role of microorganisms in biogeochemical cycles. In: Wastewater Microbiology. John Wiley & Sons, New York, p. 58.

Brower, J.B., Barford, C.C., 1997. Biological fixed-film systems (a review). Wat. Env. Res. 69, 487–499.

Campos, J.L., Garrido-Fernández, J.M., Méndez, R., Lema, J.M., 1999. Nitrification at high ammonia loading rates in an activated sludge unit. Bioresource Technol. 68, 141–148.

Chang, Y.J., Tseng, S.K., 1998. A new method for carbon addition in an anoxic denitrification bioreactor. Biotechnol. Tech. 12, 367–371.

Chang, Y.J., Tseng, S.K., 1999. A novel double-membrane system for simultaneous nitrification and denitrification in a single tank. Lett. Appl. Microbiol. 28, 453–456.

Chen, K.C., Lin, Y.F., 1994. Immobilization of microorganisms with phosphorylated polyvinyl alcohol (PVA) gel. Enzyme Microb. Technol. 16, 79–83.

Dawson, R.N., Murphy, K.L., 1973. Factors affecting biological denitrification of waste water. In: Jenkins, S.H. (Ed.), Advance in Water Pollution Research. Pergamon Press, Oxford, p. 671.

Fdz-Polanco, F., Villaverde, S., Garcia, P.A., 1994. Temperature effect on nitrifying bacteria activity in biofilters: activation and free ammonia inhibition. Wat. Sci. Tech. 30 (11), 121–130.

Hsieh, Y.L., Tseng, S.K., Chang, Y.J., 2002. Nitrification using polyvinyl alcohol-immobilized nitrifying biofilm on an O_2-enriching membrane. Biotechnol. Lett. 24, 315–319.

Liu, Y., Capdeville, B., 1994. Some observation on free ammonia inhibition to *Nitrobacter* in nitrifying biofilm reactor. Biotechnol. Lett. 16, 309–314.

Matêjü, V., Cižinská, S., 1992. Biological Water Denitrification—A Review. Enzyme Microb. Technol. 14, 170–183.

McCarty, P.L., Beck, L., Amant, P., 1969. Biological denitrification of wastewater by addition of organic materials. In: Proc. 24th Ind. Waste Conf., Purdue University, pp. 1271–1285.

Sharma, B., Ahlert, R.C., 1977. Nitrification and nitrogen removal. Wat. Res. 11, 897–925.