Korean J. Chem. Eng., **22**(3), 393-398 (2005)

Nitrogen Compounds Removal in a Packed Bed External Loop Airlift Bioreactor

Siriwan Silapakul, Sorawit Powtongsook* and Prasert Pavasant†

Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand
*Marine Biotechnology Research Unit (at Chulalongkorn University),
National Center of Genetic Engineering and Biotechnology, Bangkok, Thailand
(*Received 29 April 2004 • accepted 2 February 2005*)

Abstract−A packed bed external loop airlift bioreactor (PBELAB) was proposed as an alternative treatment system for wastewater containing ammonia and nitrate compounds. The 60L PBELAB consisted of aeration and non-aeration zones, both of which were packed with plastic bioballs to enhance the surface area for the attachment of bacteria. The system was able to achieve complete removal of all nitrogen compounds with simultaneous nitrification and denitrification, i.e., ammonia was decomposed in the aeration zone and nitrate was biodegraded in the non-aeration zone. At normal operation, the nitrification rate obtained from the system was in the range of 0.14-0.87 gNH_3-N/m^2d and the denitrification rate was 0.04 gNO_3-N/m^2d. The factors found to have great influence on the system included dissolved oxygen concentration and biofilm thickness. In addition, PBELAB was proven to perform well under nitrate shock load condition.

Key words: Nitrification, Denitrification, Ammonia, Nitrate, Wastewater

INTRODUCTION

During marine culture such as shrimp cultivation, the seawater in a shrimp pond accumulates several unwanted components such as uneaten feed, feces and metabolic wastes. Some of the degradation products are toxic to the shrimp and may also cause stress and mortality through disease and oxygen depletion. Hence, periodical or partial exchanges of seawater in the pond are necessary to maintain high quality medium for further growth of the shrimp. However, the exchange of spent seawater, which is usually required in large quantity, often leads to environmental problems in the discharge area, particularly the deterioration of a natural seawater system. Closed culture systems are, therefore, presented as a foreseeable alternative for the conventional flow-through pond which holds much promise for profitable and sustainable shrimp culture. Closed systems require that spent culture seawater be treated for reuse and, hence, a proper water treatment is needed in addition to the culture pond. The objective of the treatment of the spent seawater is to prevent the accumulation of metabolic wastes, particularly nitrogen compounds, e.g. ammonia, nitrite, and nitrate in seawater through nitrification and denitrification processes. Examples of this treatment system include the work of Menasveta et al. [2001] who employed a combination of an aerated fixed film biological filter and a nitrogen purged filter for the removal of nitrogen contaminants in shrimp farming. However, the separation of nitrification and denitrification units required that a large area must be available for the installation of the treatment system, and this often led to an extra need for other facilities such as energy and maintenance. Other reports on nitrification and denitrification are available and have been reviewed by Hargreaves [1998].

This work proposes a novel nitrification and denitrification apparatus based on the airlift reactor configuration. The airlift system provides both aerated and unaerated compartments which serve as the nitrification and denitrification in the same unit, respectively. The operation of this airlift system requires relatively low energy input when compared to other systems as there is no need for mechanical circulating devices, which makes the system attractive as an alternative treatment process. The performance of this novel airlift bioreactor in treating a synthetic wastewater containing ammonia and nitrates is described in this article.

MATERIALS AND METHODS

1. Preparation of Immobilized Nitrifiers/Denitrifiers

Unidentified microorganisms both for nitrifying and denitrifying mechanisms were immobilized on plastic bioballs. The immobilization was achieved simply by immersing the plastic bioballs in a shrimp culture pond for 2 weeks.

2. Preparation of Nitrification/Denitrification Experimental Setup

Seawater was prepared with a salinity of 30 g/L, and 8.7 mg NH_4-N/L of ammonium chloride (NH_4Cl) or 10 mg NO_3-N/L of potassium nitrate (KNO_3) was added as initial nitrogen components. Calcium carbonate ($CaCO_3$) at 100-120 mg/L was added as inorganic carbon for nitrifying bacteria and methanol (95% v/v of CH_3OH) was employed as an organic carbon source for the denitrifying bacteria [Menasveta et al., 2001]. The C/N ratio of the prepared seawater was controlled at 2.

3. Packed Bed External Loop Airlift Bioreactor (PBELAB)

Nitrification and denitrification in synthetic seawater were performed in a 60 L external loop airlift reactor packed with immobilized nitrified and denitrified plastic bioballs. The schematic diagram of this experimental setup including the picture of bioballs is shown in Fig. 1. The plastic bioball packing had a diameter of 2.5 cm with a surface area of 32 cm^2. The PBELAB was designed as one aerated

†To whom correspondence should be addressed.
E-mail: prasert.p@chula.ac.th

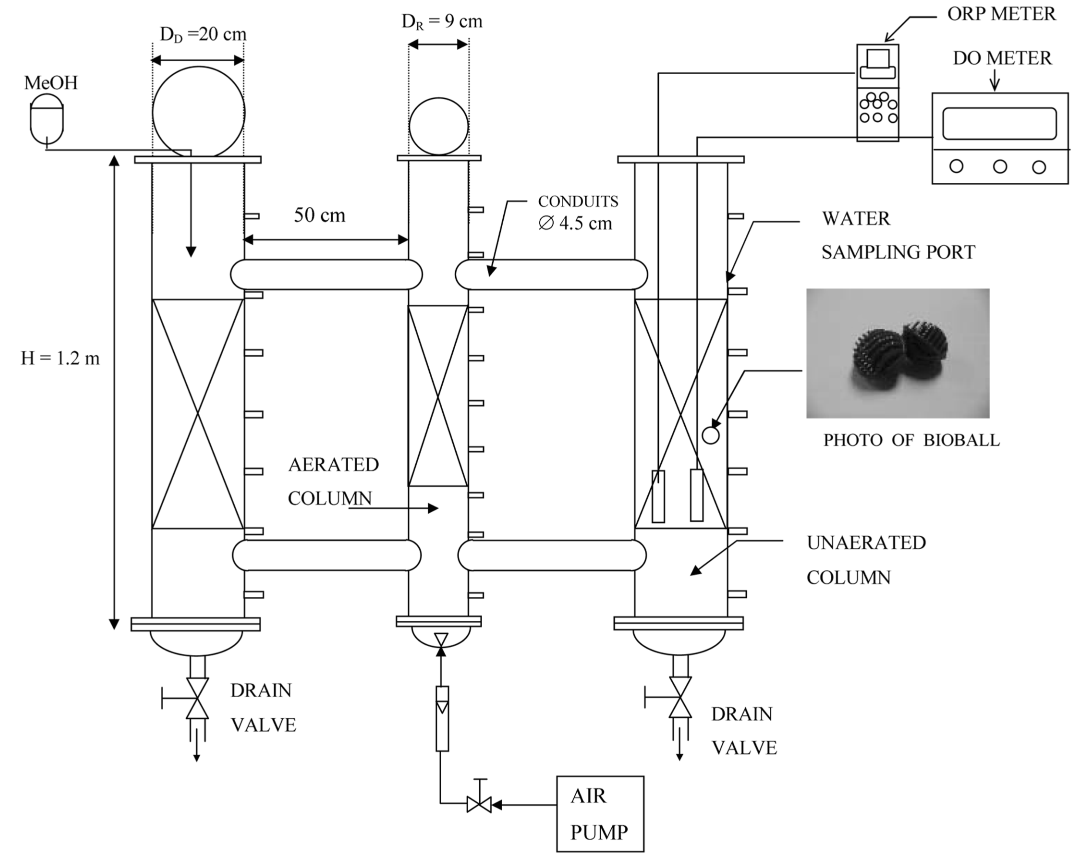

Fig. 1. Experimental setup.

column (riser) interconnected with two unaerated columns (downcomer). The heights of both aerated/unaerated columns were 1.2 m. The aerated section (riser) of the PBELAB was a cylindrical column with a diameter of 9 cm and a volume of 6 L. Each unaerated column was 20 cm in diameter, resulting in a volume of 27 L and this made a total volume of 60 L for this PBELAB. The cross-sectional area of the unaerated column (A_D) was larger than the aerated (A_R) with a ratio between A_D/A_R of about 9.87. This was to ensure adequate retention time for denitrification which was reported to require approx. 5-10 times longer reaction time than the nitrification reaction [Balderston and Sieburth, 1976]. The aerated and unaerated columns were connected with 50 cm long conduits with a diameter of 4.5 cm. The aerated section was packed with 200 bioballs, whereas each unaerated column was packed with 2000 bioballs. A porous gas sparger for air dispersion in the PBELAB was located at bottom of the aerated column. The airflow rate was determined as a minimum that could induce liquid circulation between aerated and unaerated sections, i.e., approx. 0.66 m^3/h, and was kept constant throughout all experiments in this work. Several experiments were performed during the three month period; the initial conditions for each experiment are summarized in Table 1.

4. Analytical Measurement

Ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N) and nitrate-nitrogen (NO_3-N) were determined by the Strickland and Parson method [1972]. The dissolved oxygen (DO) was measured with a DO meter (Hanna HI 964400), and the oxidation/reduction potential (ORP) was monitored with an ORP meter (Hanna HI 98240).

Table 1. Initial condition for experimental periods carried out in the PBELAB

Period*	Initial condition		Reference symbol
	NH_4-N (mgN/L)	NO_3-N (mgN/L)	
1 (day 1)**	9.67	4.54	P-I
2 (day 9)	10.89	16.11	P-II
3 (day 22)	8.01	0	P-III
4 (day 26)	8.93	0	P-IV
5 (day 39)	12.72	13.15	P-V
6 (day 46)	9.83	11.98	P-VI
7 (day 60)***	0	25.31	P-VII
8 (day 65)	8.70	12.71	P-VIII
9 (day 70)	7.78	20.85	P-IX

*The starting date was provided in parenthesis after the time period.
**The first day of experiment was started with newly inoculated plastic bioballs.
***Only nitrate was provided in this time period.

RESULTS AND DISCUSSION

1. Packed Bed External Loop Airlift Bioreactor: Nitrogen Compound Removal

In this experiment, nitrification and denitrification of synthetic aquacultural seawater were performed in a novel designed packed bed external loop airlift bioreactor (PBELAB). One primary advan-

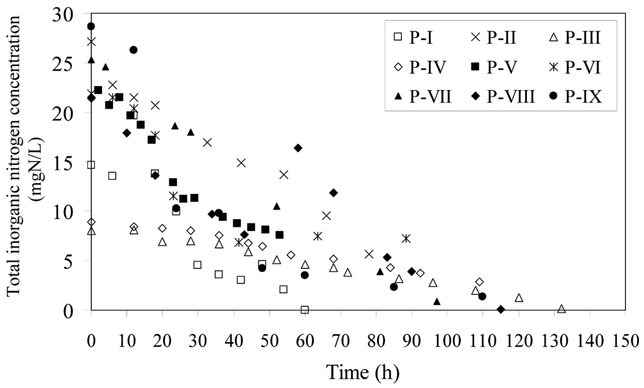

Fig. 2. Total inorganic nitrogen compound concentration profile for all experimental periods (P-I to P-IX).

tage of the airlift system is that it provides both aerated and unaerated compartments, which serve as nitrification and denitrification compartments in the same unit. The recirculation of water was driven from the aeration where water moved up in the riser as the aeration was provided and down through the unaerated downcomer section. In the riser section, the high dissolved oxygen in wastewater was consumed by nitrifying bacteria, where ammonia was oxidized to nitrate. The low dissolved oxygen effluent flowed through downcomer section, ready for nitrate removal by denitrifiers. All experiments were performed at room temperature and the stripping of ammonia was accounted for by a blank experiment in a simple 2 L bubble column. The results (not shown here) demonstrated that the stripping rate of ammonia was quite low at about 3-5% in 200 hours of normal aeration, and this rate was deducted from the total removal rate of ammonia in the calculation for the ammonia degradation rate.

The performance of this PBELAB in removing total inorganic nitrogen in the synthetic waste-seawater is demonstrated in Fig. 2. The total inorganic nitrogen compounds including ammonium, nitrite and nitrate during several experiment periods could be satisfactorily removed. At the beginning of each experimental period, ammonia and/or nitrate was added into the synthetic waste-seawater with initial concentrations as indicated in Table 1. The system was operated in a batch mode with respect to the nitrogen compounds, i.e., the system was operated until all nitrogen compounds were removed before a new experimental period was started. However, the packing was not changed between each experimental period, which means that the system was operated with new/fresh biofilm (both nitrifying and denitrifying bacteria) during the first experimental periods and with older biofilm in the later periods. The oxidation reduction potential (ORP) in the downcomer sections was found to be in a range suitable for denitrification reaction, and in this experiment, it ranged from −100 to 80 mV [Balderston and Sieburth, 1976].

Fig. 3 demonstrates the ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen concentrations obtained from the experiment, where Fig. 3A displays the results from the initial period of the experiment and Fig. 3B was the results with 22 days old biofilm. This data set indicates that a complete removal of ammonium-nitrogen was obtained quite rapidly with new biofilm. The rate of nitrification in this case was as much as 0.87 g NH_4-N/m^{2}d. In the later experi-

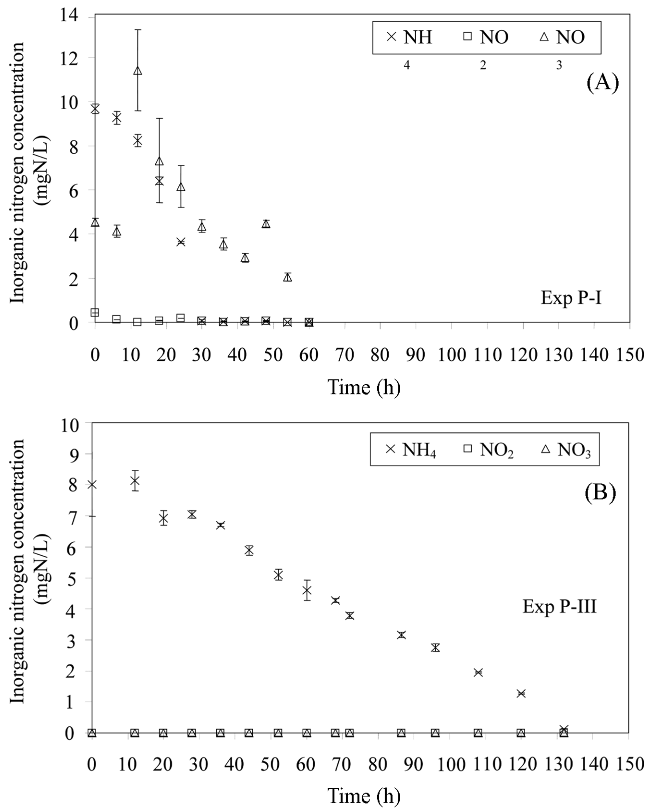

Fig. 3. Ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen concentration profiles during: (A) Experiment P-I; and (B) Experiment P-III.

mental periods, the biofilm grew thicker leading to a drop in the nitrification rate as depicted in Fig. 3B where the nitrification rate was found to be only about 0.14 g NH_4-N/m^{2}d. This drop in nitrification rate was potentially due to the extra mass transfer resistance from excessive growth of nitrifying bacteria on the bioballs in the nitrification section of PBELAB. As the biofilm grew older, its thickness also increased steadily; this excessive growth of biofilm finally caused clogging in the packing, which rendered oxygen transfer between bulk liquid and biofilm difficult. Hence, a lower nitrification rate was obtained. Nevertheless, after this experimental period (22 days), the biofilm growth no longer had great influence on the nitrification rate as the increase in biofilm thickness after this period was much slower than the initial period (No data on biofilm thickness are presented as the measurement of the biofilm thickness was not possible in PBELAB.).

In all experiments, no accumulation of nitrite and nitrate was found. This indicated that denitrification was quite rapid in PBELAB. The actual rates at which nitrite and nitrate removal took place could not be determined from the experimental data in Fig. 3 because these two components were intermediates in nitrification/denitrification reactions and it was not possible to measure their exact time profiles. However, experimental results suggested that the nitrate degradation increased with biofilm age, i.e., nitrate removal rate at the later experiment period occurred more rapidly than that from initial runs. It is also possible that the thick biofilm in the riser led to a low dissolved oxygen level in the riser, which helped reduce the dissolved oxygen in the downcomer section of the PBELAB. This, as a result,

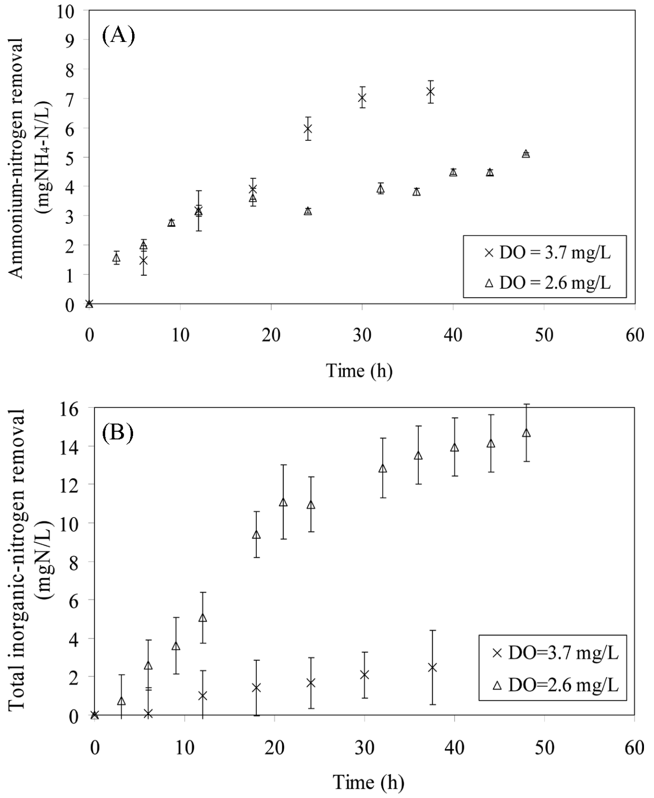

Fig. 4. Effect of dissolved oxygen concentration in riser on the performance of PBELAB: (A) ammonia nitrogen removal, and (B) total inorganic nitrogen removal.

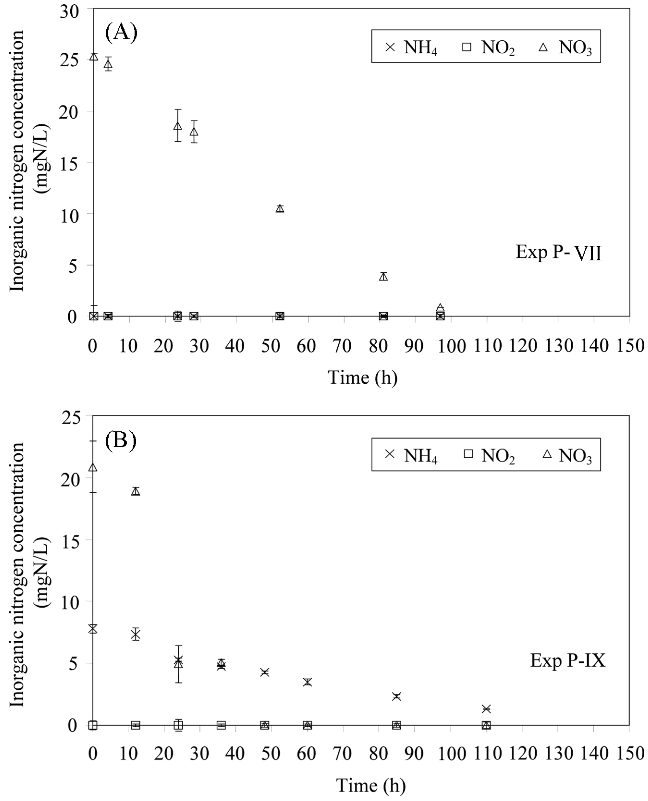

Fig. 5. Ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen concentration profiles during: (A) Experiment P-VII; and (B) Experiment P-IX.

facilitated the growth of the denitrifying bacteria leading to a rapid decomposition of nitrate.

2. Effect of Dissolved Oxygen Concentration on the Performance of PBELAB

PBELAB was found to perform well in decomposing ammonia with a high dissolved oxygen level in the riser. Fig. 4A shows the influence of dissolved oxygen concentration on the ammonia removal rate. High riser dissolved oxygen was obtained by increasing the aeration rate in the riser section of PBELAB, and the results suggested that a 46% increase in dissolved oxygen could increase ammonia removal rate by as much as 75%. However, high dissolved oxygen in the riser often posed problems with the anaerobic denitrification in the downcomer as it meant more time was required in the downcomer section for the reduction of dissolved oxygen to the level appropriate for denitrification. Hence, it can be seen in Fig. 4B that a higher level of dissolved oxygen resulted in a lower removal rate of total inorganic nitrogen. This was because ammonia was converted to nitrate which was not further decomposed properly, and hence, an accumulation of nitrate in the system was observed. In other words, a high aeration rate in the riser could lead to a better ammonia removal rate but not the total nitrogen removal rate as the dissolved oxygen in the downcomer would not be adequately low for a proper decomposition of nitrate.

3. Nitrate Shockload

Nitrate was often found to be completely removed and no nitrate was detected at all in most of the experimental runs. Additional experiments were performed to investigate the performance of the system in terms of nitrate removal. This was to determine the rate at which nitrate was removed and to examine the stability of the system under nitrate shockload condition. For this, two sets of experiments were carried out, and the results are demonstrated in Fig. 5. During Experiments P-VII (Fig. 5A) and P-IX (Fig. 5B), the PBELAB was operated with high level of nitrate (approx. 25 and 21 mg NO_3-N/L, respectively), and the results illustrated that the system could perform satisfactorily. No ammonia was added to the system at the start of Experiment P-VII, which enabled the determination of the nitrate removal rate which was at 0.04 g NO_3-N/m^2d. This indicated that the performance of PBELAB was rather stable with an adequate level of nitrate removal rate. In Experiment IX, on the other hand, both ammonia and nitrate at a high loading rate were added into the system. The system still could cope with this condition and all nitrogen compounds were totally decomposed within 110 hours of operation.

Note that the area for the denitrification in PBELAB was much greater than that for nitrification. Hence, although the specific removal rate for nitrate seemed to be relatively low compared to that of ammonia, the total nitrate removal rate was found to correspond well with the removal rate for ammonia, i.e., the total nitrate removal rate was approx. 0.51 g NO_3-N/d, whereas the total ammonia removal rate was 0.09-0.56 g NH_4-N/d.

4. Comparative Evaluation of Nitrification and Denitrification Performance

Table 2 summarizes the ammonia removal rate from various nitrification systems compared to the rate obtained from PBELAB. It

Table 2. Ammonium-nitrogen removal rate from various treatment systems

Source	NR* (gNH_4-N/m^2d)	Reference
Tricking filter	0.149	Arbiv and van Rijn [1995]
	0.1-0.2	Lakang and Kleppe [2000]
	0.15-0.43	van Rijn and Rivera [1990]
	0.4-1.4	Knosche [1994]
	0.24-0.55	Kamstra et al. [1998]
	0.28-0.69	Nihof and Bovendeur [1990]
	0.4-0.8	Nihof [1995]
	0.6-0.73	Bovendeur et al. [1990]
	0.75	Otte and Rosenthal [1979]
	0.94-3.92	Greiner and Timmons [1998]
Submerge biofilter	0.056	Reyes and Lawson [1996]
	0.59	Davis and Arnold [1998]
	0.43	Wickins [1985]
	0.13-0.57	Greiner and Timmons [1998]
Bead filter (propeller washed)	0.33-0.45	Sastry et al. [1999]
Rotating biofilter contactor	0.257	Reyes and Lawson [1996]
Biodrum	0.4-1.6	Wortman and Wheaton [1991]
Sequencing batch reactor	1.86	Zhu and Chen [1999]
Fluidized bed biofilter	0.21-0.27	Skolstrup et al. [1998]
Moving bed bioreactor	0.59-0.75	Tal et al. [2003]
Airlift bioreactor	**0.14-0.87**	**This work**

*NR=Nitrification Rate.

Table 3. Nitrate-nitrogen removal rate from various treatment systems

Source	DNR* (gNO_3-N/m^2d)	Reference
Fixed film column	0.05	Balderston and Sieburth [1976]
	0.08	Sauthier et al. [1998]
Fluidized bed column	0.38	Arbiv and van Rijn [1995]
Airlift bioreactor	**0.04**	**This work**

*DNR=Denitrification Rate

can be seen that PBELAB offered a reasonable degree of ammonia removal rate. Similarly, Table 3 indicates that the nitrate removal potential of the PBELAB could be compared satisfactorily with the published denitrification rates, i.e., the rate from the PBELAB was in a range similar to that obtained from standard fixed film columns.

Overall, the performance of the PBELAB in treating wastewater containing inorganic nitrogen compounds was comparable with other treatment systems, although it seems, at times, that the efficiency of nitrification/denitrification was not sufficiently high. It should be mentioned, however, that the PBELAB offered both nitrification and denitrification in one step where both of the reactions took place at similar rates with other individual treatment units. In other words, a satisfactory nitrogen compounds removal could be accomplished with one airlift bioreactor setup whilst most systems require a sequence of two reactors.

CONCLUSION

This study demonstrates the possibility of applying the airlift bioreactor as an integrated system for nitrification/denitrification. PBELAB was relatively stable taking into account the active performance with nitrate shock load and it also provided a satisfactory level of degradation rate at normal operation. Despite several significant differences between the nitrification and denitrification reactions, the airlift bioreactor could still be arranged such that these two reactions occur simultaneously. The application of PBELAB to the treatment of actual shrimp pond medium is considered possible as the level of ammonia and nitrate should be considerably lower than those tested in this article. What remains to be examined is the mode of operation as the treatment of actual medium will require that wastewater flow through the PBELAB system. The results from this work provide an insight into how the system performs and illustrate the possibility of extending PBELAB to actual shrimp pond operation. Further development of this system will be one attractive option for the treatment of wastewater containing nitrogen compounds, and this will be useful particularly for the sustainable aquaculture industry.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to the Thailand Research Fund (TRF) and the Ministry of University Affairs Thesis Grants for Graduated Students, Thailand, for their financial supports.

REFERENCES

Arbiv, R. and van Rijn, J., "Performance of a Treatment System for Inorganic Nitrogen Removal in Intensive Aquaculture Systems," *Aquacultural Engineering*, **14**(2), 189 (1995).

Balderston, W. L. and Sieburth, J. M., "Nitrate Removal in Closed-system Aquaculture by Columnar Denitrification," *Applied Environmental Microbiology*, **32**, 808 (1976).

Bovendeur, J., Zwage, A. B., Lobee, B. G. J. and Blom, J. H., "Fixed-film Reactors on Aquaculture Water Recycle System: Effect of Organic Matter Elimination on Nitrification Kinetic," *Water Research*, **24**(2), 207 (1990).

Davis, D. A. and Arnold, C. R., "The Design, Management and Production of a Recirculating Raceway System for the Production of Marine Shrimp," *Aquacultural Engineering*, **17**(3), 193 (1998).

Greiner, A. D. and Timmons, M. B., "Evaluation of the Nitrification Rates of Microbead and Trickling Filters in an Intensive Recirculating Tilapia Production Facility," *Aquacultural Engineering*, **18**(3), 189 (1998).

Hargreaves, J. A., "Review Nitrogen Biogeochemistry of Aquaculture Ponds," *Aquaculture*, **166**(3-4), 181 (1998).

Kamstra, A., Heul, J. W. and Nihof, M., "Performance and Optimisation of Trickling Filters on Eel Farms," *Aquacultural Engineering*, **17**(3), 175 (1998).

Knosche, R., "An Effective Biofilter Type for Eel Culture in Recirculating Systems," *Aquacultural Engineering*, **13**(1), 71 (1994).

Lakang, O. I. and Kleppe, H., "Efficiency of Nitrification in Trickling Filters Using Different Filter Media," *Aquacultural Engineering*, **21**(3), 181 (2000).

Menasveta, P., Panritdam, T., Sihanonth, P., Powtongsook, S., Chuntapa, B. and Lee, P. G., "Design and Function of Closed, Recirculating Seawater System with Denitrification for the Culture of Black Tiger Shrimp Broodstock," *Aquacultural Engineering*, **25**(1), 35 (2001).

Nijhof, M., "Bacterial Stratification and Hydraulic Loading Effects in a Plug-flow Model for Nitrifying Tricking Filters Applied in Recirculating Fish Culture Systems," *Aquaculture*, **134**(1-2), 49 (1995).

Nijhof, M. and Bovendeur, J., "Fixed Film Nitrification Characteristics in Sea-Water Recirculation Fish Culture Systems," *Aquaculture*, **87**, 133 (1990).

Otte, G. and Rosenthal, H., "Management of a Closed Brackish Water System for High-density Fish Culture by Biological and Chemical Water Treatment," *Aquaculture*, **18**, 169 (1979).

Reyes, A. A. and Lawson, T. B., "Combination of a Bead Filter and Rotating Biological Contactor in a Recirculating Fish Culture System," *Aquacultural Engineering*, **15**(1), 27 (1996).

Sastry, N. B., Reyes, A. A., Rusch, K. A. and Malone, R. F., "Nitrification Performance of a Bubble-washed Bead Filter for Combined Solids Removal and Biological Filtration in a Recirculating Aquaculture System," *Aquacultural Engineering*, **19**(2), 105 (1999).

Sauthier, N., Grasmick, A. and Blancheton, J. P., "Biological Denitrification Applied to a Marine Closed Aquaculture System," *Water Research*, **32**(6), 1932 (1998).

Skolstrup, J., Nielsen, P. H., Frier, J. O. and McLean, E., "Performance Characteristic of Fluidized Bed Biofilters in a Novel Laboratory-scale Recirculation System for Rainbow Trout: Nitrification Rate, Oxygen Consumption and Sludge Collection," *Aquacultural Engineering*, **18**(4), 265 (1998).

Strickland, J. D. H. and Parson, T. R., *A Practice Handbook of Seawater Analysis*, 2nd edn. Fisheries Research Board of Canada Bulletin NO. 167, Ottawa, Canada (1972).

Tal, Y., Watts, E. M., Schreier, S. B., Sowers, K. R. and Schreier, H. J., "Characteristic of the Microbial Community and Nitrogen Transformation Processes Associated with Moving Bed Bioreactors in a Closed Recirculated Mariculture System," *Aquaculture*, **215**(1-4), 187 (2003).

van Rijn, J. and Rivera, G., "Aerobic and Anaerobic Biofiltration in an Aquaculture Unit-nitrite Accumulation as a Result of Nitrification and Denitrification," *Aquacultural Engineering*, **9**, 217 (1990).

Wickins, J. F., "Ammonia Production and Oxidation During the Culture of Marine Prawns and Lobsters in Laboratory Recirculation Systems," *Aquacultural Engineering*, **4**, 155 (1985).

Wortman, B. and Wheaton, F., "Temperature Effects on Biodrum Nitrification," *Aquacultural Engineering*, **10**, 183 (1991).

Zhu, S. and Chen, S., "An Experimental Study on Nitrification Biofilm Performances Using a Series Reactor System," *Aquacultural Engineering*, **20**(4), 245 (1999).