Environment Protection Engineering

Vol. 43 2017 No. 1

DOI: 10.5277/epe170106

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KEY FACTORS CONTRIBUTING TO SIMULTANEOUS NITRIFICATION-DENITRIFICATION IN A BIOLOGICAL AERATED FILTER SYSTEM USING OYSTER SHELL MEDIUM

Factors contributing to nitrogen removal in a biological aerated filter (BAF) using oyster shell medium have been investigated. The system was operated in parallel with a bio-ball filter. Both filters were fed with a synthetic domestic wastewater containing approximately 25 mg N/dm^3 of total nitrogen (TN). The COD of wastewater was 200 mg O_2/dm^3. The sizes and dissolved oxygen (DO) of the voids within both filters were measured. Results indicated that the oyster shell system performed better with a nitrogen removal of 64.3%. The two systems exhibited a similar COD removal efficiency of approximated 80%. The oyster shell filter showed higher degree of variability in both sizes and DO levels of its void spaces. The condition provided a favorable environment for nitrogen removal through simultaneous nitrification and denitrification (SND). The release of carbonates from oyster shells were minimal, as judged from mass balance analysis of the system using calcium. It is concluded that the function of a SND reactor can be enhanced by using non-uniform filter media such as oyster shells. On the other hand, alkalinity is not a major concern when treating wastewaters with moderate ammonia concentration, such as that of domestic wastewaters.

1. INTRODUCTION

Due to ever-growing discharge of nutrients from point and non-point sources, eutrophication has become a global concern for the health of ecosystems in both inland and coastal waters. To minimize the impacts, nutrient removal has become a common practice in the treatment of wastewater from domestic, as well as industrial sources. Biological processes using microorganisms are currently the most cost-effective technology in wastewater treatment. Nitrogen has several oxidation states which provide

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a variety of pathways for its transformation and cycling in the ecosystem. They also offer plenty opportunities for process design in removing nitrogen from wastewaters.

In a conventional aerobic-anaerobic/anoxic design of nitrification-denitrification, the reduced species of nitrogen are converted to higher oxidation states through reactions carried out by nitrifying bacteria including the *Nitrosomonas* sp. and *Nitrobacter* sp.:

$$NH_4^+ + 1.5O_2 \xrightarrow{\textit{Nitrosomonas}\ \text{sp.}} NO_2^- + H_2O + 2H^+ \tag{1}$$

$$NO_2^- + 0.5O_2 \xrightarrow{\textit{Nitrobacter}\ \text{sp.}} NO_3^- \tag{2}$$

A complete nitrification is achieved upon combining the two reactions:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+ \tag{3}$$

According to Eq. (3), hydrogen ions are produced during nitrification. Therefore, an adequate supply of alkalinity is essential for a nitrifying system in maintaining a suitable range of pH for the microorganisms. In a second stage of denitrification, the oxidized species of nitrogen are reduced to molecular nitrogen by denitrifying microorganisms under anaerobic/anoxic conditions:

$$2NO_3^- + 10H^+ + 10e^- \xrightarrow{\text{denitrifiers}} N_2 + 2OH^- + 4H_2O \tag{4}$$

Organic carbon is used as an electron donor in the process. A lack of organic carbon in nitrified effluents is frequently a constraint to the efficiency of such an aerobic-anaerobic process.

Nitrification is usually the limiting process in a two-step biological nitrogen removal due to low growth rate of nitrifying bacteria [1]. Dissolved oxygen (DO) is critical for nitrification. The optimal DO for nitrification covers a range between 0.3 and 4.0 mg O_2/dm^3 depending on system designs [2]. Species composition of nitrifying microorganisms and the size of bio-flocs have been projected as the major causes for the differences in optimal DO. Nitrifying microorganisms are also sensitive to pH. A suitable pH for nitrification lies in a range of 7.5–9.0 [3]. Therefore, it is crucial that a wastewater contains adequate alkalinity for pH buffering during nitrification.

The processes of nitrification and denitrification can be carried out in separate reactors, such as those of many suspended growth systems. They can also be achieved using a single reactor through a sequence of alternating aerobic and anaerobic operations in systems like sequential batch reactors. A major challenge to the separated system design is the maintenance of adequate organic carbon in the nitrified effluents for subsequent denitrification [4]. In addressing this and other issues in biological denitrification, a number of novel process have been proposed in the past decades [5, 6].

Simultaneous nitrification and denitrification (SND) represents a set of biological nitrogen-removing processes where nitrification and denitrification occur concurrently in a single reactor. The advantages of such processes include low cost, simplified system configuration, and ease of operation. SND is achieved primarily through the creation of various oxidation states in a single reactor suitable separately for nitrification and denitrification. In attached growth systems such as biological aerated filters (BAFs), simultaneous aerobic and anaerobic/anoxic conditions can occur in either micro- or macro--scales. In a micro-environment, oxygen gradient occurs within the biofilms attached to the surface of growth media [7]. Oxygen gradient can also occur in the macro-environment of a non-uniform filter bed between voids of different sizes, shapes, and hydraulic conditions. Therefore, a non-uniform filter medium offers more favorable conditions for SND [8]. Alkalinity is released during denitrification. It is readily available for nitrification in a SND system. Therefore, biological removal of nitrogen through SND consumes less alkalinity, and the requirements for external supply of alkalinity is minimized.

Oyster shell is a major waste of oyster production. In Taiwan, the estimated 160 000 metric tons of wasted oyster shells generated each year present substantial burdens to the waste disposal of many fishery communities. Oyster shells have been demonstrated to be excellent biological carriers for BAF systems [9, 10]. The rough surface of oyster shells provides excellent sites for the attachment and growth of microorganisms. Since oyster shells are made up of approximately 98% calcium carbonate [11], they have also been credited for improving ammonia removal in BAF systems through the supply of alkalinity and pH buffering [9]. Our previous studies have also demonstrated that BAFs using oyster shells offer better nitrogen removal as compared with systems using commercial media or other natural materials [10, 12].

While the micro-environment theory of SND is well established, the effect of macro-scale oxygen gradient within the filter beds of BAF system has not been studied in detail. The contribution can be particularly significant in the highly non-uniform oyster shell medium. A major purpose of this study was to examine the variability of void sizes and DO levels within oyster shell filters, and to assess the relative importance of these features in determining the SND efficiency of BAF reactors. A second purpose of this study was to quantify the alkalinity release by oyster shells, and its contribution to pH buffering of the system. The study should provide better insights on the processes of SND in attached growth systems.

2. MATERIALS AND METHODS

System setup. The lab scale system consisted of an oyster shell reactor, a bio-ball reactor, a wastewater storage tank, a wastewater supply system, and an aeration system (Fig. 1). The reactor tanks were cut out of a clear plexiglass tube. Each tank had a height of 100 cm and a diameter of 20.3 cm. Four columns of sampling holes were drilled with equal spacing around the circumferences of the tanks. The holes in each column were separated by a height of 10 cm. The sampling holes were sealed using silicone gel. An outlet was made for each tank at a height of 80 cm giving an effective tank volume of 25.9 L. The tanks were filled respectively with oyster shells and bio-balls to a height of 64 cm, resulting in a packing ratio of 80%. Wastewater was fed through PVC tubes to the bottoms of the tanks. Air was provided using a direct current aeration pump and delivered to the filters through diffusers fixed on the bottoms of the tanks.

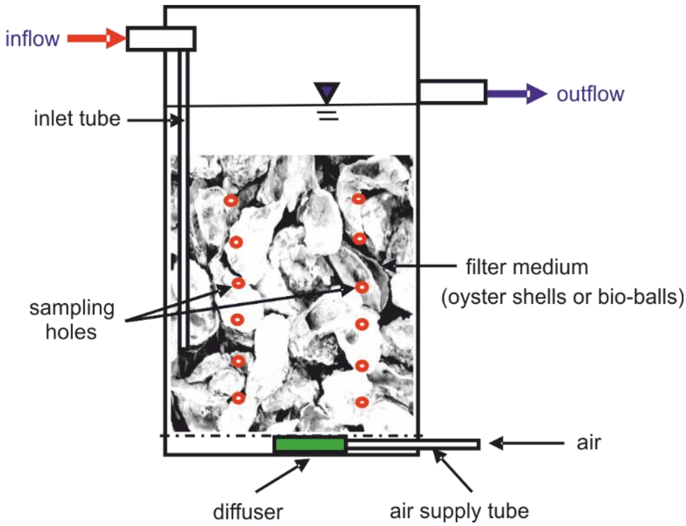

Fig. 1. System layout for the lab scale biological aerated filters

Filter media. Oyster shells (Fig. 2, left) were randomly picked from a pile of wasted oyster shells. The heights of individual shells were measured and recorded. The shells were washed and soaked in tap water for several days before use. The oyster shells chosen ranged from 6 to 17 cm in size with a mean of 8.1 cm. The surface areas of individual oyster shells were calculated from the heights using equations proposed by Morales-Alamo [13]:

- right valve

– outer surface

$$\log Y = -0.012 + 1.086\log X \tag{5}$$

– inner surface

$$\log Y = 0.093 + 0.967\log X \tag{6}$$

- left valve

– outer surface

$$\log Y = 0.153 + 1.004\log X \tag{7}$$

– inner surface

$$\log Y = 0.085 + 0.994\log X \tag{8}$$

where X and Y are the height and surface area of an oyster shell in cm and cm^2, respectively. The specific surface area of 38.3 m^2/m^3 was obtained for the filter bed by dividing the total surface area of the oyster shells by the packed volume of the filter bed.

Fig. 2. Filter media: oyster shells (left), and bio-balls (right)

Each bio-ball (HC-4, Aqua-systems, Taiwan, Fig. 2, right) measured 40 mm in diameter. The specific surface area of the packed bio-ball filter was 450 m^2/m^3 as provided by the supplier. The void volume of the filter was calculated from geometrical dimensions of the filter bed and those of the bio-balls. A diameter of 1.7 cm was obtained for the voids within the bio-ball filter by assuming uniform spherical void spaces. The average void size of the oyster shell filter was calculated from measurements of 23 randomly chosen voids. The measurements were made through sampling holes using a thin stainless ruler before the tank was filled with wastewater. An average void size of 5.5±2.7 cm was obtained.

Synthetic wastewater. Synthetic domestic wastewater was prepared according to Yoo et al. [8]. The formula gave the total nitrogen (TN) of approximately 25 mg N/dm^3, and chemical oxygen demand (COD) of approximately 200 mg O_2/dm^3. Each batch was analyzed for major wastewater quality constituents right after preparation.

System start-up. Wastewater was fed continuously to the reactors at a hydraulic retention time (HRT) of 6 h. Both reactors were seeded with activated sludge from the final clarifier of a local wastewater treatment plant. The systems were operated continuously for a period of approximately 2 months for the biofilms to develop fully. The systems were then operated under DO levels of approximately 1.0, 2.0, 3.0, 3.5, 4.0, and 4.5 mg O_2/dm^3 for a period of 2 week each.

Determination of optimal DO. In each test cycle, the systems were monitored closely one day prior to the experiment to ensure stable DO levels in the reactors. The systems were then sampled and removal efficiency of COD and TN determined. An optimal DO was determined for each of the two reactors based on a maximum TN removal efficiency.

Long-term performance test. Both reactors were operated continuously for a period of 70 days under optimal DO levels. Effluent samples were taken every 3 days. Monitored wastewater quality parameters were temperature, pH, COD, TN, and calcium level. Temperature and pH were measured using a pH meter (EcoMet P25, Esteck, South Korea). COD samples were tested using COD digestion vials (HACH, USA). TN and Ca^{2+} were analyzed using a TN/TOC analyzer (Lachat Instruments IL530, USA), and an ion chromatography system (Metrohm IC-761, Switzerland), respectively.

Determination of void dissolved oxygen. Wastewater samples for DO measurements were drawn from voids within the filter beds by penetrating the silicone gel seals on tank walls using a 10 cm^3 syringe. The sampled water was then injected into a 10 cm^3 round glass cell and measured immediately using an optical DO sensor (ProODO, Yellow Spring Instrument, USA).

Evaluation of alkalinity release by oyster shells. Both reactors are open systems where the alkalinity released by the oyster shell bed cannot be quantified using direct measurements. However, knowing that calcium is released in conjunction with carbonate ions, the input of alkalinity from this source was quantified using mass balance analysis of calcium for the reactor. The potential for pH buffering was evaluated by comparing the pH of system effluent with that of the bio-ball filter.

3. RESULTS AND DISCUSSION

3.1. OPTIMAL DO FOR SND

The removal efficiencies of TN for both reactors under different reactor DO (DO_r) are shown in Fig. 3. Both systems demonstrated an optimal DO_r of 3.0 m O_2/dm^3. Although nitrogen in a biological reactor can be removed via a number of processes, the processes other than a two-step nitrification-denitrification generally require strict environment conditions. Such conditions are not expected to have occurred in substantial scales in open systems such as those of this study. System responses to DO variations further confirm such a notion. In a two-step nitrification-denitrification process, a maximum removal efficiency for nitrogen is reached under a specific DO level when the rate of nitrification is in balance with that of denitrification.

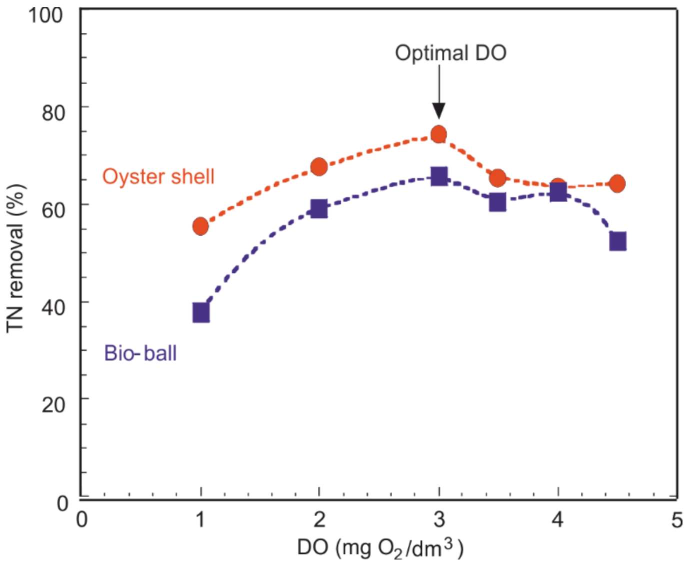

Fig. 3. Determination of optimal DO for the filters

The optimal DO level for attached growth SND systems tends to be higher than that for suspended growth systems. Molecular diffusion within bio-films of the attached growth systems are usually less efficient as compared with that of the biological flocs in suspended growth systems. Attached growth systems can also tolerate a wider range of oxygen fluctuation, possibly due to greater microbial diversity of the biofilms [14–16].

3.2. OXYGEN LEVELS IN VOID SPACES

Dissolved oxygen in 23 void spaces of the oyster shell filter and 24 of the bio-ball filter was measured under reactor DO levels of 3.0, 3.5, and 4.0 mg O_2/dm^3. As shown in Table 1, average DO in the voids of the oyster shell filter was consistently lower than that of the bio-ball filter. The oyster shell filter also exhibited a greater variability in DO, as can be inferred from the higher values of its coefficient of variance (*CV*). Figure 4 illustrates the relationship between sizes (d_p) and DO concentrations of the voids in the oyster shell filter. There is a clear tendency that bigger voids contained higher levels of oxygen due to better hydraulic circulation and smaller specific microbial mass ratio. Therefore, the macro-scale DO gradient in the oyster shell filter provided favorable conditions for nitrogen removal through SND.

Table 1

DO concentration in voids of the filters under various reactor DO_r

Medium type	Void size [cm]			Void DO [mg O_2/dm^3]								
				DO_r = 2.5 mg O_2/dm^3			DO_r = 3.0 mg O_2/dm^3			DO_r = 3.5 mg O_2/dm^3		
	Mean	*SD*	*CV*	Mean	*SD*	*CV*	Mean	*SD*	*CV*	*Mean*	*SD*	*CV*
Oyster shell	5.5	27	0.49	1.9	0.54	0.28	2.1	0.66	0.31	2.8	0.68	0.24
Bio-ball	1.7	NA	NA	2.7	0.18	0.07	2.6	0.25	0.10	3.6	0.26	0.07

SD – standard deviation, *CV* – coefficient of variance, NA = not applicable

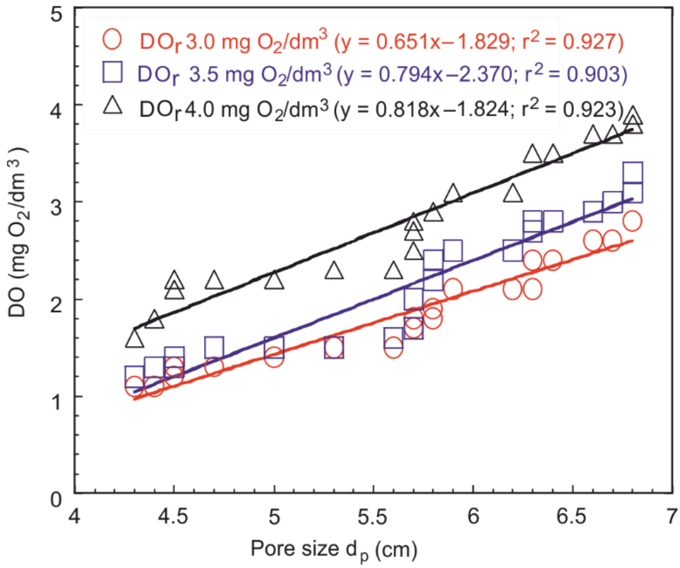

Fig. 4. Relationships between void size and DO concentration in the oyster shell filter under various levels of reactor DO (DO_r)

3.3. LONG-TERM SYSTEM PERFORMANCE

In a long-term test, both systems were operated for 70 days under a HRT of 6 h and an optimal DO of 3.0 mg O_2/dm^3. Concentrations of COD and TN were monitored at a frequency of every 3 days. The results are shown in Fig. 5. Average TN concentration of the influent was 26.5±0.9 mg N/dm^3. Effluent TN concentrations were 9.4±0.5 and 10.6±0.7 mg N/dm^3 for the oyster shell filter and the bio-ball filter, respectively.

TN removal efficiencies were 64.3±2.4% and 60.0±2.5% ($p < 0.01$, $n = 24$), respectively. The oyster shell filter performed better with statistical significance than the bio-ball filter in term of nitrogen removal.

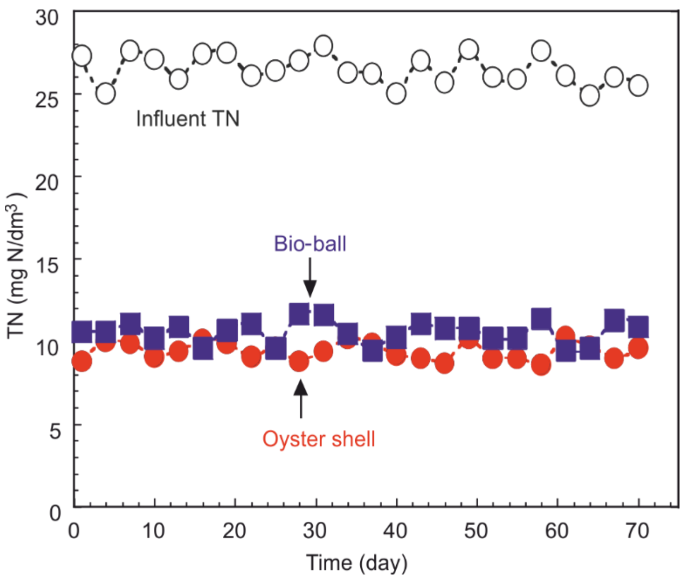

Fig. 5. Total nitrogen concentration in treated effluent of the BAF systems

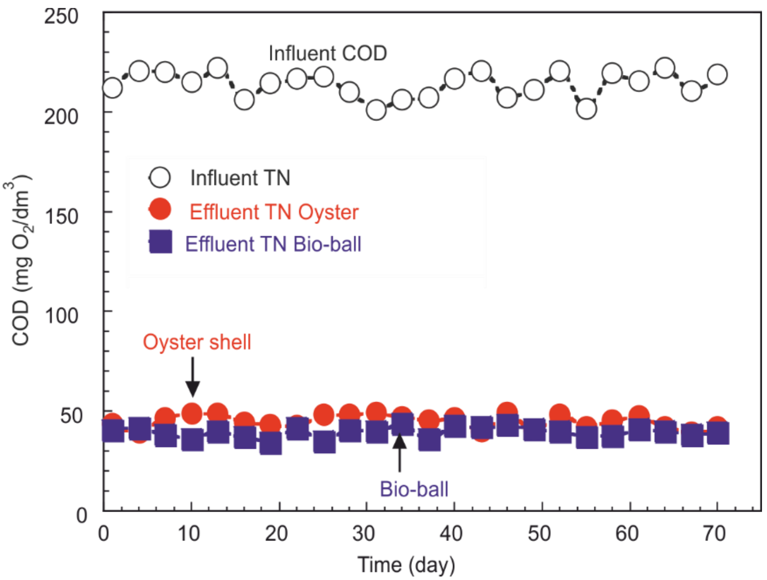

Fig. 6. COD concentration in the influent and treated effluent of the BAF systems

Concentrations of COD in the effluents of both systems are shown in Fig. 6. Average concentrations were 45.1±3.4 and 39.6±2.5 mg O_2/dm^3 for the oyster shell system and the bio-ball system, respectively. Average removal efficiencies were 78.9±1.8% and 81.5±1.3% ($p < 0.01$, $n = 24$), respectively.

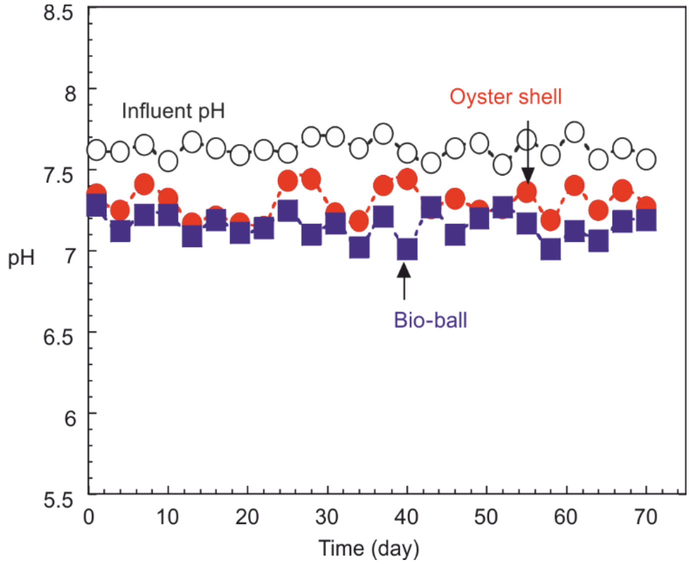

Fig. 7. pH in the influent and effluent of the BAF systems

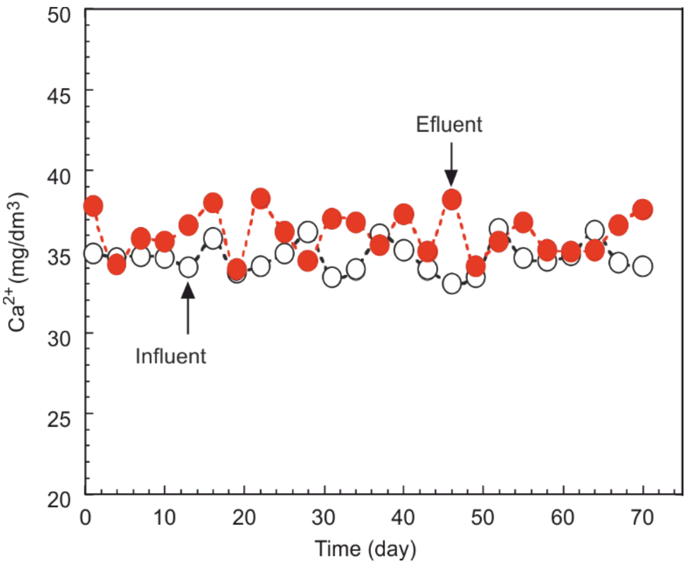

Fig. 8. Calcium in the influent and effluent of the oyster shell filter

It appears that the bio-ball filter provided a more uniform environment favored aerobic removal of organic matters. Organic matters play contradictory roles in SND reactors [17]. Although organic carbon is required for heterotrophic denitrification, autotrophic nitrification can be inhibited when they are present in large quantities. Attached growth SND systems are known to produce low organic effluents [18], which leads to the speculation that nitrogen removal in these systems may have been limited by the shortage of organic carbons.

Both filters had lower pH values in the effluent than in the untreated influent (Fig. 7), suggesting that more alkalinity was consumed during nitrification than was produced from denitrification. Results also show that pH of the oyster shell system was consistently higher than that of the bio-ball system (7.63±0.06 vs. 7.30±0.10, $p < 0.01$, $n = 24$), supposedly due to buffering from carbonates released by oyster shells. The effluent of the oyster shell filter contained more calcium than the influent (Fig. 8). Average concentrations were 34.6±1.0 and 36.1±1.4 mg Ca^{2+}/dm^3 ($p < 0.01$, $n = 24$) for the influent and the effluent, respectively. The 1.5 mg Ca^{2+}/dm^3 increase (approximately 4%) of calcium translates to an addition of 3.75 mg/dm^3 in alkalinity (as $CaCO_3$) from oyster shells which is rather insignificant comparing with a typical range of 100–300 mg $CaCO_3/dm^3$ for domestic wastewaters. However, pH buffering by oyster shells can be significant when treating wastewaters with high ammonia concentration.

4. CONCLUSIONS

A macro-environment theory of SND was not well established as compared with the micro-environment theory. Through parallel comparison of two biological filters, it was confirmed that dissolved oxygen in the voids of the oyster shell filter was more variable than that of the uniform bio-ball filter. The oyster shell filter also performed better in nitrogen removal through SND. It is conceivable that the macro-scale DO gradient in the oyster shell filter had a significant contribution to the nitrogen removal of the oyster shell system. Other contributing factors may include the biomass and characteristics of the biofilms, especially their thickness. Consistent with other studies, the optimal DO for our systems were higher than typical values for the suspended growth systems. The lower material transport within biofilms of the attached growth systems was a probable cause of such differences. The pH buffering from oyster shells in the filter is negligible in our tests using synthetic domestic wastewater. However, the effect can be substantial in systems treating high strength wastewaters with elevated ammonia concentrations. Organic matters in the effluents of both reactors were low, suggesting that nitrogen removal in both systems may have limited by the lack of organic carbon. The requirements for organic carbon in SND processes is a subject that warrants more research.

ACKNOWLEDGMENTS

The research project was supported by a grant from the National University of Tainan, Taiwan (Grant No. AB102-215). The authors gratefully acknowledge Wei-Jun Liu, Wen-Kai Hu, and Shi-Xuan Sun for their assistance in carrying out part of the laboratory experiments for this research.

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