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Intensive Nitrification Process Employing Immobilized Nitrifiers on Polyester Carriers in Closed-System Aquaria

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ABSTRACT

Freshwater autotrophic nitrifying bacteria cultivated and fixed on polyester tablets were used as concentrated nitrifiers for rapid start-up of ammonia and nitrite removal in closed-system aquaria. Study on the present process has led to the following conclusions. In wastewater with blank carriers, the carriers acted as supporting media for immobilizing the intrinsic nitrifiers which were able to accelerate moderately the natural nitrification. The natural nitrification, however, showed moderate ammonia-nitrite transformation, but non-significant nitrite-nitrate transformation. The present intensive artificial nitrification process could remove rapidly the ammonia and nitrite to nearly 100 per cent starting from the beginning and continued throughout 40 days experiment. It could rapidly accomplish the ammonia-nitrite-nitrate nitrifying process successfully. The inexpensive materials used and the simple method so developed has made this a cost effective nitrogenous toxic compounds removal process which is very important for wastewater treatment as well as in the aquaculture.
Key words: artificial nitrification, immobilized nitrifying bacteria, intrinsic nitrifiers, polyester carrier.

INTRODUCTION

Water quality in an aquarium is unavoidably worsened by accumulated feed wastes and fish's excreta. The uneaten feed and feces contribute to the organic matter load of the system. Decomposition of organic matter in the system leads to increased growth of microorganisms, total ammonia nitrogen (TAN), nitrite and nitrate. The nitrogen compounds accumulated in aquaria above a certain concentration not only cause eutrophication, but also toxic to aquatic animals (Crab *et al.*, 2007).

Monitoring of feed consumption to prevent over feeding is an important management tool for reducing nitrogen problems. Removing feed waste, excreta, dead fish, and other organic materials that may decay and produce the nitrogenous toxic compounds are also necessary. To separate these materials from water, physical unit operation by sedimentation or mechanical filtration is commonly used (van Rijn, 1996). However, nitrogen compounds such as ammonia, nitrite and nitrate cannot be directly removed by the filtration. To keep the concentration of nitrogen compounds to a safe level, diluting of polluted

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water is necessary which consumes large amounts of water. Chemical unit process employed in aquaculture wastewater treatment is used in conjunction with physical unit operations and biological processes. The inherent disadvantage of most chemical unit processes is that they are additive processes where chemical substances are hazardous to the culture organism (Crab *et al*., 2007).

Biological processes are one of the most important treatments with respect to aquaculture wastewater treatment, and the major process is nitrification. Various aspects have been studied in the removal of ammonium compounds in wastewater treatment; however, immobilization is preferable due to its many advantages. The system also suits nitrifying bacteria that need quite a long time in multiplication, immobilizing them will enhance their cell density (Cassidy *et al*., 1996).

In the 1990s the hybrid system consisting of biomass support systems (BSS, cell immobilization system) immersed in activated sludge reactors (cell suspension system) was commonly used. BSS consist of immersing various types of support media in an activated sludge reactor to favor the growth of fixed bacteria (Mara and Horan, 2003). BSS systems should allow a reduction in the aeration tank volume following the introduction of biomass support to meet a certain objective, and thus an increase in the treatment system stability and performance. The main advantages of these systems are improved nitrification (Rusten and Neu, 1999).

Several materials were used for immobilizing the nitrifying bacteria which were shown to improve nitrogen removal rate in freshwater. Antonina *et al*. (1997) studied the use of polyester fiber in comparing with macro-porous cellulose fiber and found that polyester fiber had higher oxygen pass through rate and resulted in higher nitrification rate.

To accelerate the removing of these toxic compounds, dense autotrophic nitrifiers fixed on polyester carrier as mobile support media is developed. The objectives of this study were to find out a suitable environment for intensive nitrification, appropriate nitrifying agents and tools and to determine efficiency of immobilized nitrifying bacteria on polyester carriers.

MATERIALS AND METHODS

Monitoring of water quality changes in aquaria

Three sets of 120-litres aquaria (40x75x40 cm) in triplicate were prepared. Set 1 was used as control where only aeration through air-stone was provided without any filtration. In set 2 each tank was installed with two corner-box filters (7.5×7.5×18.5 cm) using synthetic wool. Water was air-driven uplifted to the filters. Aquaria in set 3 tanks were installed with two under-gravel filters (29×71 cm). Water was circulated by air-stone uplift tubes. Ninety litres of tap water was filled up in each tank. The system was kept running for 24 hours prior to the experiment. Goldfish (*Carassius auratus*) of 15 grams in average were acclimatized for one week in a large tank equipped with air pump and fed on processed feed pellet. Thirty goldfish were then transferred to each of nine tanks and started feeding 5 grams of pellet once a day.

Changes in temperature, pH, oxygen contents, turbidity, chemical oxygen demand and nitrogen concentrations in the water of all tanks were recorded. Turbidity was measured at 600nm (OD_{600}) with a spectrophotometer (Shimazu UV-240). Chemical oxygen demand (COD) was obtained by using potassium dichromate as oxidizing agent (APHA, 1992). Ammonium-N (NH_4-N) concentration was determined by Berthelot's Reaction (Merck's Spectroquant® 14752). Nitrite-Nitrogen (NO_2-N) concentration was analyzed by Griess' Reaction (Merck's Spectroquant® 14776). Nitrate-Nitrogen (NO_3-N) concentration was evaluated by brucine method (APHA, 1992).

Immobilization of nitrifying bacteria on polyester carrier

The carrier was made from polyester strands (Figure 1B) with a diameter of 30 µm and formed into a tablet-shape sized of 1×1×0.2 cm (Figure 1A). Activated sludge collected from the sand gravel at the bottom of the aquaria was used as the source of the nitrifiers for preparation of inoculum. Polyester tablets was put in a 20-litre container for half of its capacity and soaked with ammonium broth (Table 1) which was the synthetic medium for nitrifying bacteria. Sufficient quantity of the inoculum was then added and

Table 1 Ammonium broth media composition.

Composition	Ammonium broth media (mg)
NH_4Cl	378 (100 mgN)
Na_2HPO_4	22.9
$NaHCO_3$	2000
Inorganic salts	
NaCl	30
KCl	14
$CaCl_2$	14
$MgSO_4.7H_2O$	10
Tap water	1 litre

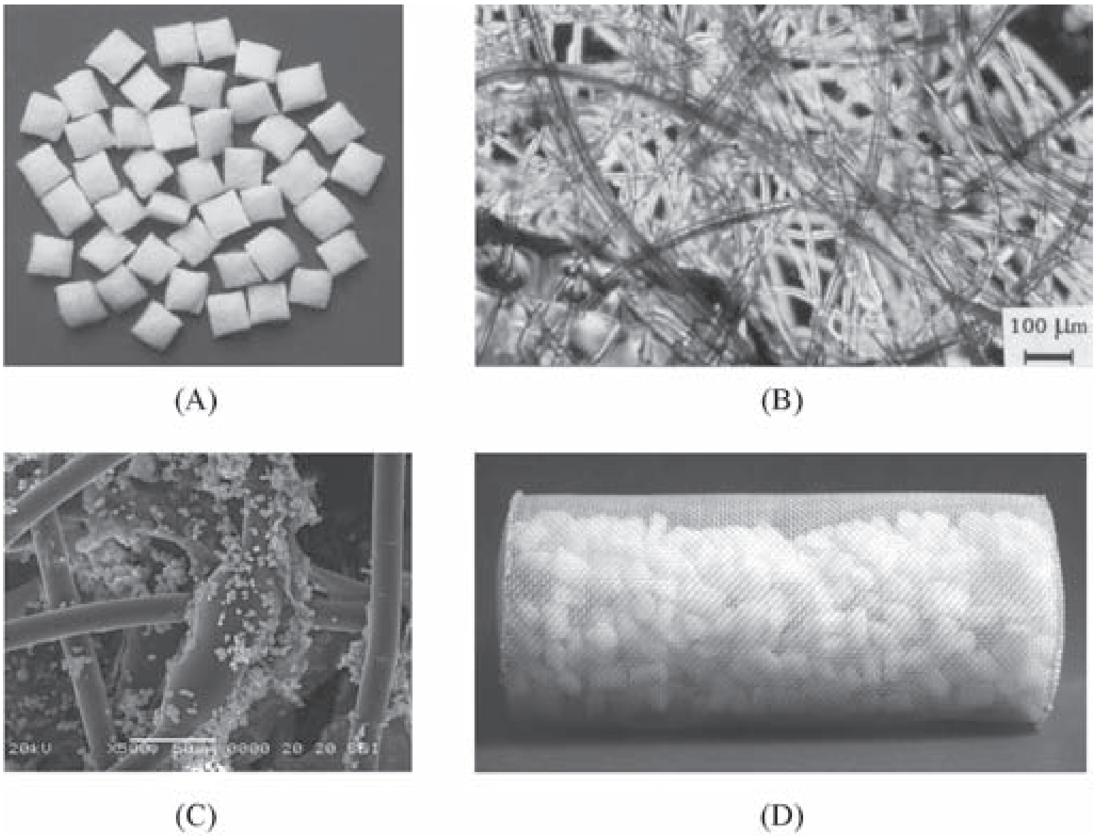

Figure 1 (A) Polyester tablets with size of 1cm x 1cm x 0.2cm.
(B) Light micrograph of unseeded polyester strands in a tablet (100x).
(C) Scanning electron micrograph of nitrifying bacteria deposited in a tablet (500x).
(D) Cylindrical nylon-net bag used as the container for polyester tablets.

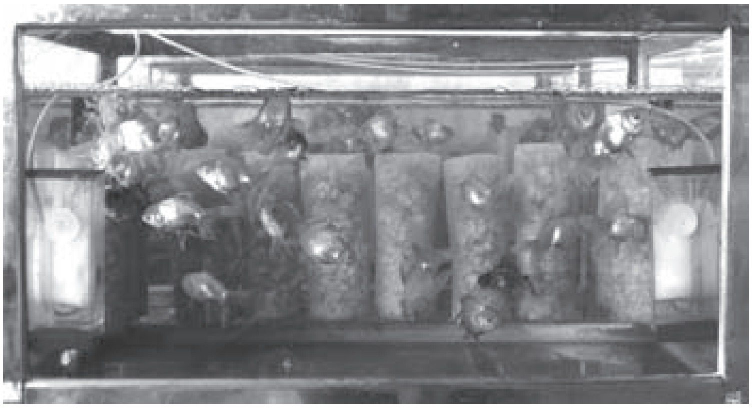

Figure 2 Glass tank installed with corner-box filters and polyester carriers in nylon-net bags.

mixed and filled up the container with ammonium broth until all tablets were covered. Batch cultivation was carried out under aerobic condition. Experiment was conducted at room temperature (about 30°C). During the cultivation, medium broth was sampled intermittently to examine ammonia, nitrite and nitrate concentrations. The spent ammonia solution was replaced with a new medium till it reached the daily equilibrium cycle and the system was maintained for several days. The nitrifying bacteria fixed polyester tablets were then ready for using after confirmation by observation under light microscope (Figure 1C). The number of nitrifying bacteria deposited in a tablet was counted to be an average of 2×10^8 cells.

Various amounts of seeded carriers prepared earlier were used to nitrify the ammonia in synthetic wastewater with various concentrations of NH_4-N for 24 hours of batch operation. Data were collected and processed to determine the appropriate volume ratio of immobilized bacteria to wastewater (Table 2, 3). The synthetic wastewater was prepared by diluting ammonium broth to get 1 to 10 mg/l of NH_4-N.

Intensive nitrification process

Nine corner-box filtration (type 2) tanks were used in this experiment. Three of them were installed with immobilized nitrifying bacteria fixed polyester carriers bags (Figure 1D) containing predetermined volume ratio of carriers to wastewater (Figure 2). Another three were installed with unseeded polyester carrier bag as secondary control. The last three units were left blank for primary control. Each tank was filled up with 90 litres of tap water. The system was kept running for one day without feeding. Thirty goldfish were then transferred to each tank and 5 grams of processed food pellet was fed once a day. Observations and measurements of temperature, pH, turbidity, oxygen contents, chemical oxygen demand, ammonia, nitrite and nitrate were carried out daily throughout 40 days.

RESULTS AND DISCUSSION

Changes of water quality with merely filtration

During the 40 days observation period, the water temperature in all tanks was fairly stable in the range of 29±1°C with little fluctuation in accordance with room temperature (Figure 3A). The suitable temperature range for growth of nitrifying bacteria and nitrification was found to be 28-35°C (Painter, 1970).

Average pH values of water in all tanks varied in range of 6.2 to 8.5 (Figure 3B). This is the preference pH range for fish culture (Boyd,

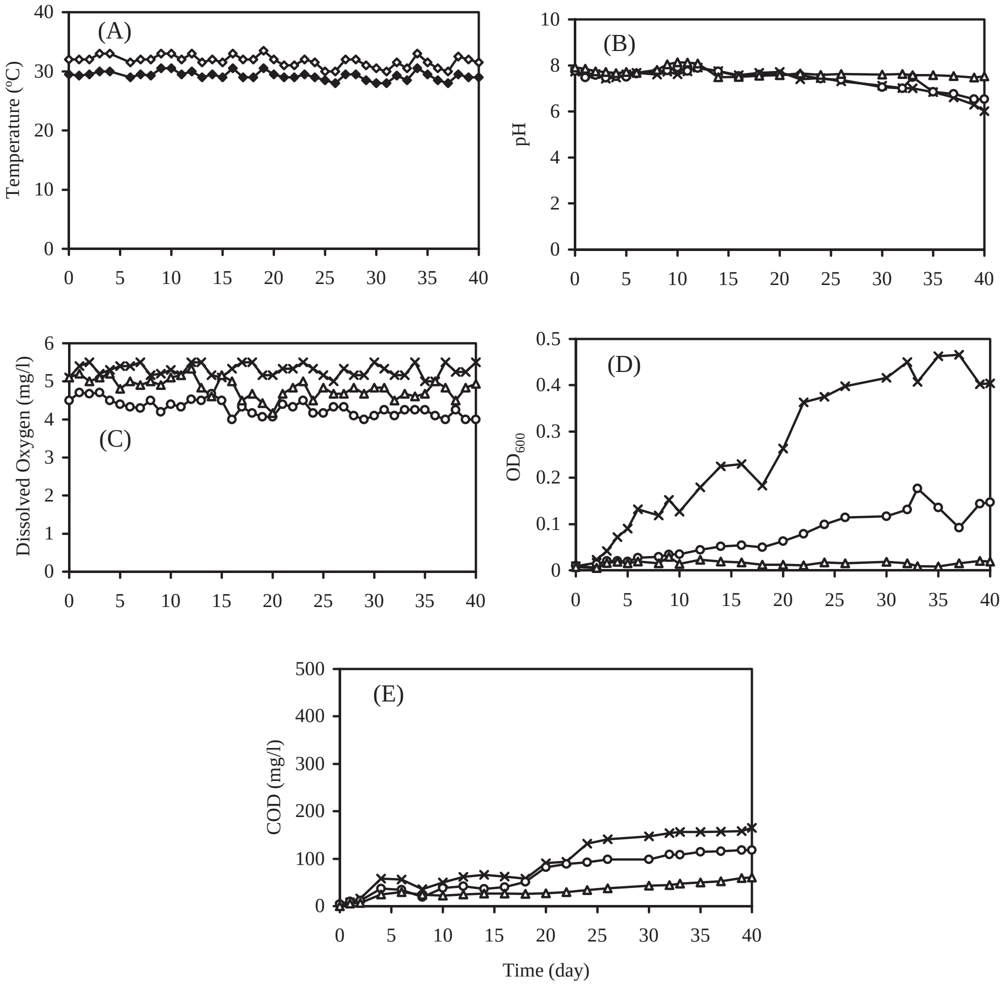

Figure 3 (A) Changes of room temperature (◇) and water temperature in tanks (♦), (B) pH, (C) dissolved oxygen, (D) optical density, and (E) chemical oxygen demand of water in tanks with no filter(X), corner-box filters (O), and under-gravel filters(Δ).

1982). Moreover, this pH range was also suitable for nitrification process which was between 7.5 - 8.6 (Chen *et al*., 2006).

On the other hand average dissolved oxygen (DO) concentration in type 1 tank was in the range of 5 to 5.7 mg/l while those of type 2 and type 3 were 4.4 to 5 mg/l and 4 to 4.7 mg/l, respectively (Figure 3C). Alabaster and Lloyd (1980) indicated that the optimum DO for growing fish was 4 mg/l. However, fish could survive in water with 1 – 5 mg/l (Boyd, 1982). DO level that suited nitrification should be above 2 mg/l (Christensen and Harremoes, 1978). In stagnant water ponds whereby oxygen was depleted, TAN tended to accumulate in the system due to insufficient nitrification activity (Grommen *et al*., 2002).

It was therefore clearly seen that variations of temperature, pH and DO were all favorable for fish culture, as well as for nitrification regardless of the type of filter equipped. However, the values of OD_{600} indicated that turbidity in the unfiltered water (type 1) grew more rapidly than the others where the under-gravel filter (type 3) was the least turbid (Figure 3D). This apparent difference in the three setups was not only came from the ability of filtration but also on the capability of biofilm formation. As biofilm formation in the under-gravel filter (type 3) was greatest for its large surface area while no biofilm was observed in the unfiltered water (type 1).

As far as COD level was concerned, the type 3 was the best whereas that of type 1 was the worst (Figure 3E). The lower the levels of COD indicated the better achievement in nitrification (Boongorsrang *et al*., 1982; Wungkobkiat *et al*., 1993). Low COD level would assist high DO concentrations which would in turn favor nitrification.

Changes in NH_4-N, NO_2-N, NO_3-N concentrations of 3 type treatment systems in closed system aquaria were shown in Figure 4A. All of the 3 types showed naturally biological processes of ammonification and nitrification at the beginning with slight differences in duration. NH_4-N concentrations increased exponentially from the beginning in type 1, 2, and 3 and peaked at 26 mg/l (on 16^{th} day), 25 mg/l (on 14^{th} day), and 20 mg/l (on 10^{th} day), respectively (Figure 4A). Average accumulation of ammonia was found to be 2.5 ± 0.5 mg NH_4-N/l.d. Nitrite concentrations in all types were built up insignificantly during the first ten days but climbed up rapidly later on (Figure 4B). Although nitrate was observed in all filter types (Figure 4C), ammonia and nitrite were also accumulated at high levels indicating that the ammonia producing water influences the ammonia-nitrite-nitrate nitrifying process from the beginning.

Removal of organic compounds including nitrogenous materials was clearly better in the system with filtration because it not only physically eliminated suspended materials such as feces, undigested food, and microorganisms, but also indirectly reduced soluble organic and inorganic compounds by biological processes. In both systems with filtration, bacteria might develop on the solid supports and perform the biological functions. It appeared that ammonia and nitrite concentrations were relatively high in both filtration systems. However, the goldfish with the average 15g weight could tolerate NH_4-N and NO_2-N up to 29 mg/l and 72 mg/l, respectively. Boyd (1982) indicated that the ionized ammonium was not toxic to fishes, but the unionized form was found to be very toxic. In general, the unionized ammonia and ionized ammonium were in equilibrium depending on the pH and the

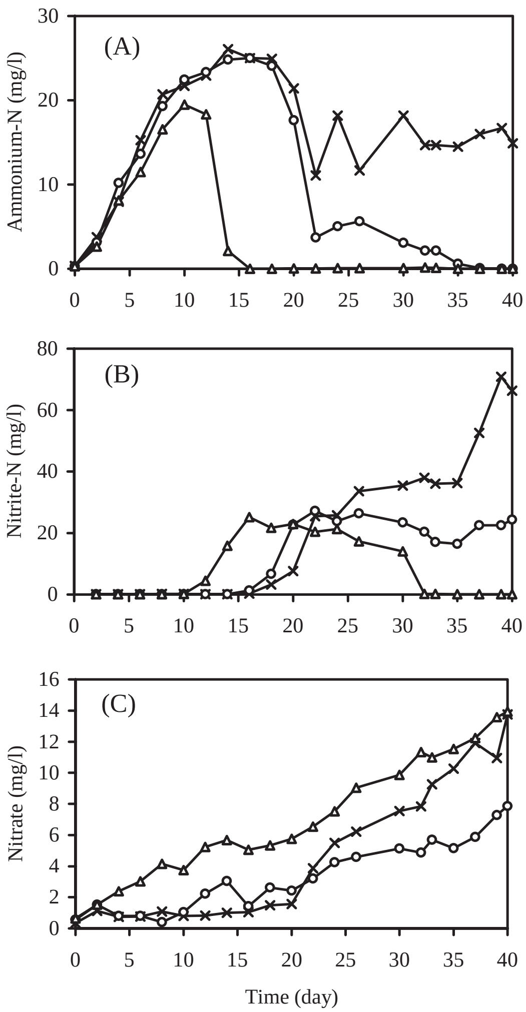

Figure 4 (A) Changes of concentrations of NH_4-N, (B) NO_2-N, and (C) NO_3-N of water in tanks with no filter(x), corner-box filters(O), and under-gravel filters(Δ).

temperature of the water. In the present study, the water with pH 7.8, temperature 29°C, and total NH_4-N 29 mg/l might had a concentration of unionized ammonia at 1.5 mg/l. Hence, the goldfish with average 15 g weight in this experiment might tolerate unionized ammonia up to and possibly beyond 1.5 mg/l. Though the susceptibility of different species varied, in general, exposure levels of unionized ammonia higher than 0.1 mg/l for extended periods could cause chronic thickening of gills, and higher levels might result in death (Scott, 1996). The toxic concentrations of ammonia for short-term exposure were between 0.6 and 2 mg/l of unionized ammonia for most species, and the 96-h LC_{50} value of unionized ammonia to fish range from 0.4 to 3.1 mg/l was reported (Boyd, 1982).

Intensive nitrification process

Preliminary experiments revealed that the appropriate volume ratio of nitrifying bacteria fixed carriers to wastewater was found to be 3 per cent.

During 40 days of the study, there were no significant changes in temperature (Figure 5A) and pH (Figure 5B) as observed earlier. It was clearly shown that the system designed was rather stable. The amounts of DO in water of all tanks varied in a very narrow range of 5 ± 0.5 mg/l (Figure 5C) which was optimal for fish culture (Alabaster and Lloyd, 1980). Therefore, this intensive nitrification process could be conducted

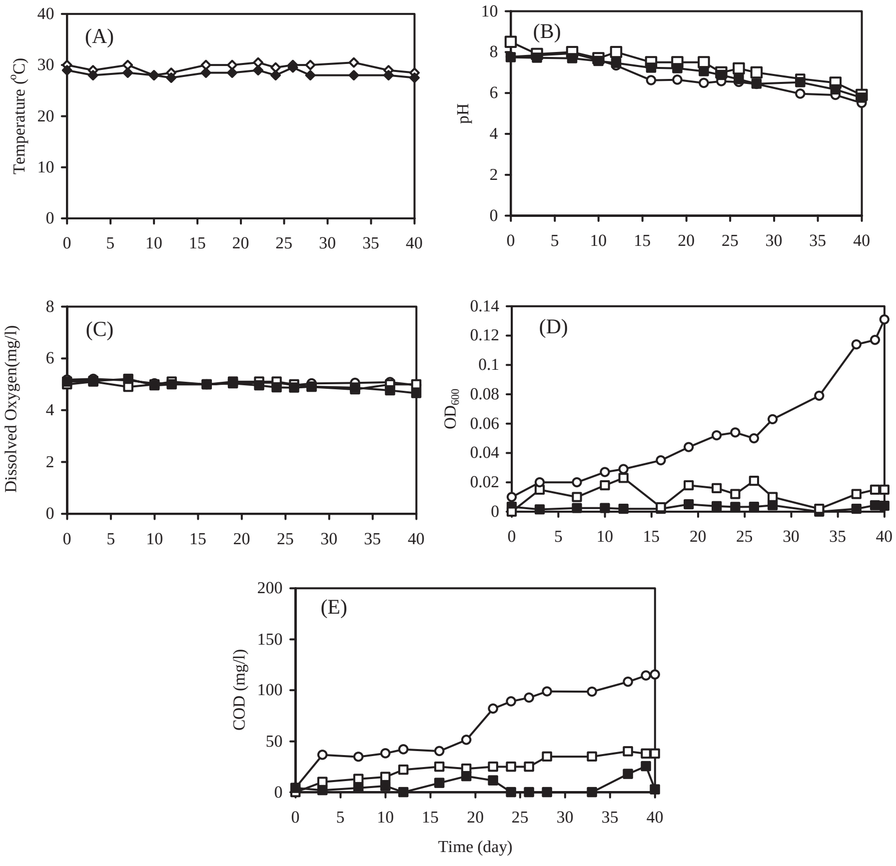

Figure 5 (A) Changes of room temperature (◇) and water temperature in tanks (♦), (B) pH, (C) dissolved oxygen, (D) optical density, and (E) chemical oxygen demand of water in tanks with no polyester carrier(O), unseeded polyester carrier(□),and seeded polyester carrier(■).

in situ for aquaculture.

During the course of experiment, turbidity of water in the tanks without filters (primary control) grew rapidly from 0 to 0.13 OD units while those with unseeded carriers (secondary control) fluctuated in a narrow range from 0 to 0.02 OD units. It indicated that merely corner-box filters could remove most of the suspended solids. However, further improvement was observed in the tanks with seeded carriers as water was very clear with maximum OD at only 0.0043 in the 28th day. These were confirmed by the COD values in water of all corresponding tanks (Figure 5E). Aquaria without carrier showed the trend of increasing of both turbidity and CODs from the beginning to the end of this experiment. On the other hand tanks with carrier (both the unseeded and seeded) showed better water quality. The experiment revealed that polyester carrier could not only directly remove nitrogenous toxic compounds but also decreased turbidity and organic compounds. This was probably due to entrapment of solid wastes in the carrier, as well as colonization of native heterotrophic bacteria that accelerated the organic compound decomposition.

Figure 6A shows the variation of NH_4-N concentrations of the three systems. The NH_4-N concentrations of water in primary control tanks increased quickly from the beginning to the peak at 25 mg/l on the 11th day and fell to around 4 mg/l from the 22nd day onwards. Similar pattern was observed in the tanks with unseeded carriers (secondary control) but the rate was half that of the previous ones with maximum at 12.5 mg/l on the 11th day as well, and fell down faster to the 0 on 20th day. The most prominent NH_4-N concentration profile was that of aquaria with seeded carriers. There was almost no significant existence of NH_4-N through out 40 days of the experiment.

Regarding accumulation of NO_2-N, all tanks showed no significant concentrations from the beginning to the 11th day (Figure 6B). NO_2-N concentrations of both of controls grew rapidly on 11th day which corresponded to the fall of NH_4-N. However, the total NO_2-N concentration of that secondary control was obviously lower. This is probably due to transformation of ammonia to nitrite occurred naturally with the intrinsic nitrifying bacteria. The presence of unseeded carriers in secondary control tanks might act as supporting media for immobilizing the intrinsic nitrifying bacteria. The most prominent NO_2-N

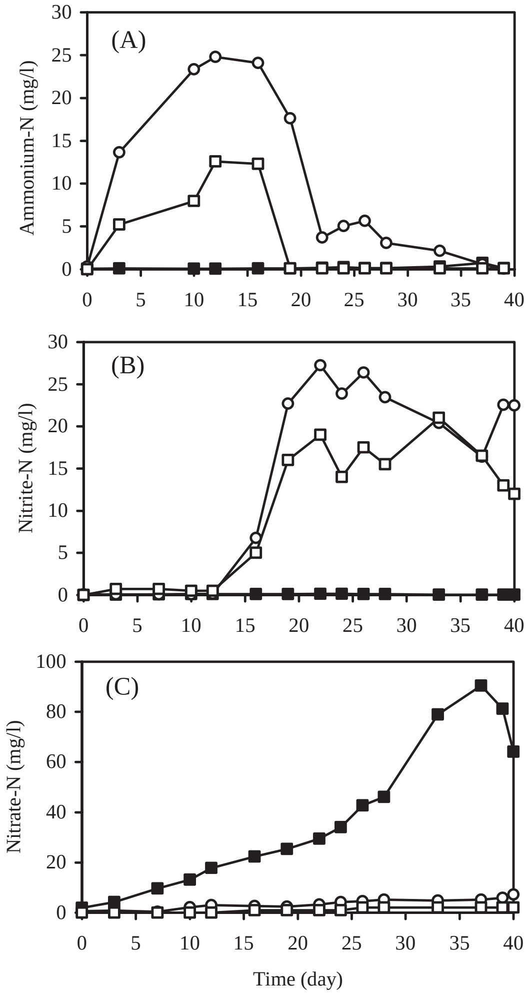

Figure 6 A) Changes of concentrations of NH_4-N, (B) NO_2-N, and (C) NO_3-N of water in tanks with no polyester carrier(O), unseeded polyester carrier(□),and seeded polyester carrier(■).

Table 2 Residual ammonium-nitrogen concentration after 24 hours nitrification with various amounts of seeded polyester carriers.

seeded polyester carriers		(per cent)	Residual ammonium-nitrogen concentration (mg/l)			
			1*	3*	5*	10*
0	without	aeration	1.01	3.02	5.00	9.95
0	with	aeration	0.97	2.78	4.90	9.50
0.5	with	aeration	0.26	1.01	2.35	7.75
1	with	aeration	0.09	0.70	0.83	6.15
3	with	aeration	**0.03**	**0.05**	**0.04**	**0.03**
5	with	aeration	**0.02**	**0.02**	**0.02**	**0.02**

*: initial NH_4-N concentrations (mg/l).
Bold figures: expected level (near zero).

Table 3 Nitrite-nitrogen concentration after 24 hours nitrification with various amounts of seeded polyester carriers.

seeded polyester carriers (per cent)			Nitrite-nitrogen concentration (mg/l)			
			1*	3*	5*	10*
0	without	aeration	0.01	0.00	0.01	0.02
0	with	aeration	0.01	0.00	0.03	0.14
0.5	with	aeration	0.10	0.73	2.30	2.00
1	with	aeration	0.06	0.60	1.90	3.10
3	with	aeration	**0.01**	**0.01**	**0.01**	5.30
5	with	aeration	**0.00**	**0.00**	**0.01**	**0.01**

*: initial NH_4-N concentrations (mg/l).
Bold figures: expected level (near zero)

concentration profile occurred in the tanks with seeded carriers where there was no significant existence of NO_2-N throughout the 40 days of experiment.

As for accumulation of NO_3-N concentrations, only seeded carrier water tanks showed steady increased to the peak at 90 mg/l on the 36th day. This confirmed the effectiveness of the immobilized nitrifying agents on polyester carriers in promoting the conversion of ammonia-nitrite-nitrate nitrification process successfully.

CONCLUSION

Nitrification process occurs naturally by the intrinsic nitrifying bacteria in closed system. To accelerate the process, immobilization of the bacteria on fixed material could be achieved. This study demonstrated the application of polyester carrier as the artificial media for immobilization of bacterial consortium in order to enhanced intensive nitrification process. The polyester tablets were made readily available at relatively low cost. Ammonia and nitrite were almost completely removed assuring the effectiveness of the process. The system rapidly eliminated ammonia and nitrite from the beginning hence improving water quality for an intensive culture of *Carassius auratus* (goldfish) closed-system aquarium, as a case study. It proved to be low cost, easy to apply and able to recycle for use in subsequent aquaculture. In conclusion this happened to be a cost effective treatment process that might be useful for nitrogen rich wastewater and various aquaculture in commercial scale as well.

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