RESEARCH ARTICLE

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Treatment of effluent from shrimp farms using watermeal (*Wolffia arrhiza*)

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ABSTRACT: Watermeal (*Wollfia arrhiza*) is a small aquatic plant which we have used to treat water from black tiger shrimp ponds. The relationship between watermeal biomass and treatment length, the changes in water quality parameters, and N-balance were evaluated for the treatment of black tiger shrimp farm effluent in low-salinity areas. A biomass of 12 g of watermeal per litre of shrimp farm effluent and a treatment period of 30 days were found to provide the best conditions for the growth of watermeal, and the quality of the treated effluent in terms of biological oxygen demands, suspended solids, total phosphorus, nitrate, total ammonia nitrogen, and total Kjeldahl nitrogen. The pH and salinity were similar for each level of biomass. The watermeal biomasses of 4–12 g per litre of effluent were suitable for watermeal survival over time. Since watermeal can fix nitrogen from the atmosphere, it can grow very well in effluent containing a low level of nitrogen, maintaining the N-balance.

KEYWORDS: biomass, *Penaeus monodon*, shrimp farm, water plant, water quality

INTRODUCTION

Penaeus monodon or black tiger shrimp is an economic species cultured in several tropical countries, including Thailand, yielding a high annual revenue. Because of this, *P. monodon* culture has been expanded from along the shoreline to brackish, and then to low-salinity areas. In 1985, the area devoted to *P. monodon* culture in Thailand was about 408 km^2 and had increased to 744 km^2 by 2004[1,2]. Raising *P. monodon* in low-salinity areas has been increasing because this species of shrimp belongs to the Euryhaline group, which can survive well in variable salinity ranging from 5–30 ppt, or even in almost pure fresh water[3]. Normally, a salinity of 0–10 ppt hinders the growth of disease-causing bacteria that infect freshwater and seawater animals. Accordingly, raising *P. monodon* in low-salinity water can reduce the risk of infection by *Vibrio harveyi*[4], especially the type with luminescent characteristics[5].

As a result of the expansion of *P. monodon* culture into low-salinity areas, developments have been made in husbandry methods. The traditional method, the natural one, has given way to modern techniques which, without a proper management system, can cause many environmental problems. These environmental problems include poor water quality and quantity, as well as a variety of pollutants in the water that are the result of substances used at each stage of shrimp production, preparation and cleaning of the ponds, rearing and harvesting of shrimp[6]. In each of these steps, the water can be contaminated with organic substances, discarded feed, soil sediment, and plankton. Wastewater from shrimp ponds is released into canals on the farm and/or public water sources where aquatic plants are found in abundance[5]. It is possible that the aquatic plants could absorb organic substances from the wastewater allowing farmers to recycle the water. Raising *P. monodon* in a water-recirculatory system is a way to lower the cost of production, lower the risk of infections, and possibly increase the farmers' income.

For all of these reasons, the idea of studying the treatment of effluent from *P. monodon* ponds by using watermeal (*Wolffia arrhiza* (L.) Wimm) emerged. The plant grows in natural water sources. Apart from rapid growth and reproduction, watermeal provides high nutrition, especially protein, so it can be used to make animal feed, and is possibly even suitable for human consumption.

MATERIALS AND METHODS

The quality of the effluent, the biomass of the watermeal, the nitrogen-balance (N-balance) of the effluent, and the watermeal were assessed in a 5 x 3 factorial arrangement with 4 replications in a completely randomized design, with the first factor being the watermeal biomasses of 0, 0.2, 0.4, 0.6, and 0.8 kg/50 l of effluent and the second factor being the experimental time period of 10, 20, and 30 days. The data were subjected to an analysis of variance and the comparisons among means were made using Duncan's new multiple range test of the Statistical Analysis System (SAS version 6.12)[7].

The ponds were located at the Pichet Farm, Bansang District, Prachinburi Province. A closed zero-water exchange system was used for the ponds in the low-salinity area. The experiment was carried out from December 2005 to April 2006. Effluent totalling 1,100 l was randomly collected after the shrimp harvest. The water quality was analysed at the Faculty of Public Health, Mahidol University, Bangkok prior to the start of the experiment. Watermeal was obtained from local markets located at Muang District, Chaiyaphoom Province. This was washed, planted in clean water for 7 days, sampled randomly, and analysed for total Kjeldahl nitrogen (TKN) prior to the start of the experiment.

The shrimp pond effluent was placed into 20 black plastic containers (50 l/container). Varying weights of watermeal (0, 0.2, 0.4, 0.6, and 0.8 kg) were placed in the containers for each treatment (4 replications) and reared in an experimental house for 30 days.

Effluent from each treatment was sampled at 10.00 a.m. at the start of the experiment and after

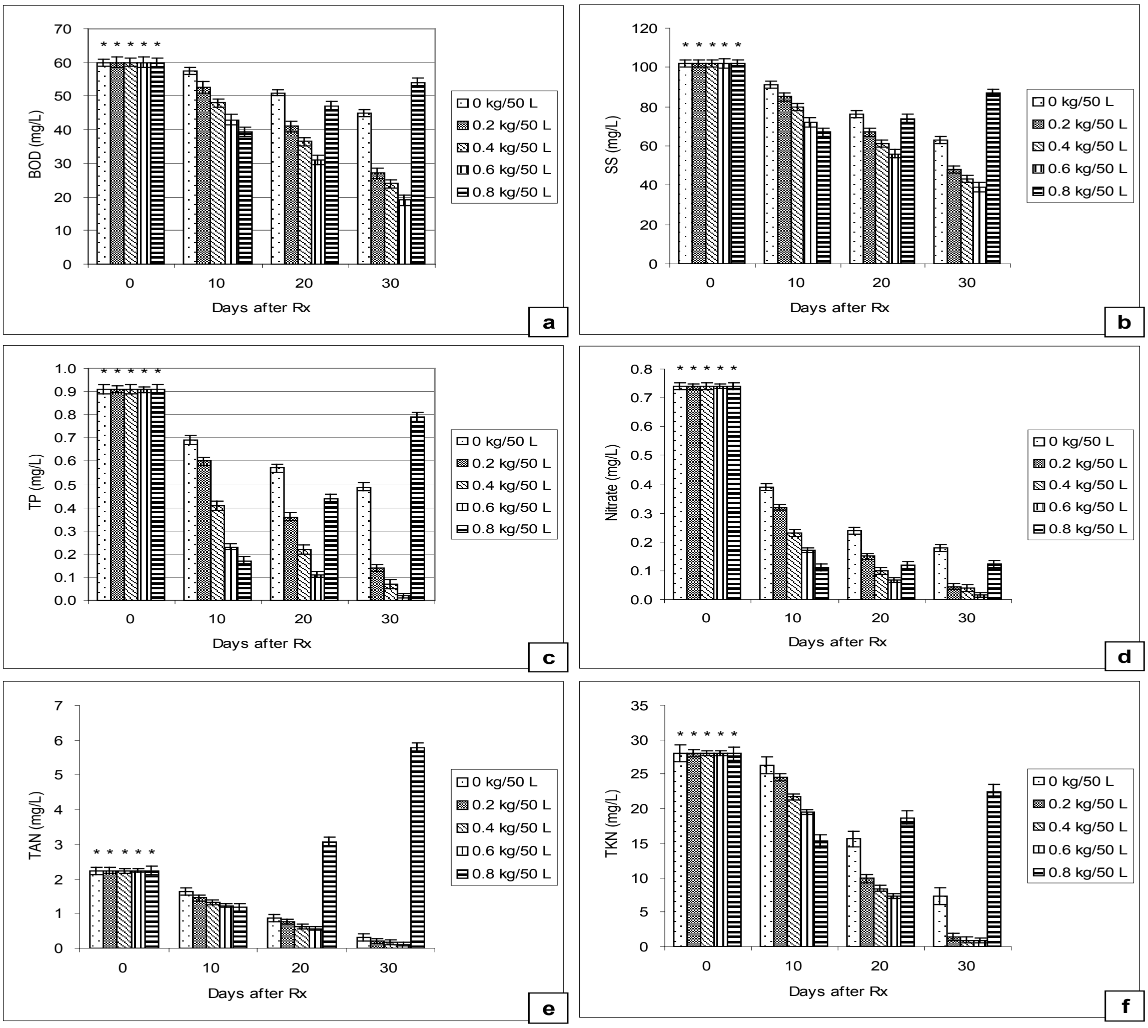

Fig. 1 BOD (a), SS (b), TP (c), NO_3^- (d), TAN (e) and TKN (f) at different watermeal biomasses and periods of treatment. * indicates $p<0.05$ *vs* other periods of treatment.

10, 20 and 30 days. Water quality was analysed for biological oxygen demand (BOD), suspended solids (SS), pH, salinity, total phosphorus (TP), nitrate (NO_3^-), total ammonia nitrogen (TAN), and TKN using standard methods[8]. In each replication, effluent was kept in one litre plastic bottles, cooled in an icebox, and sent to the laboratory. At the end of the experiment, watermeal in each replication was filtered using a net for about 5 min. The biomasses were obtained after the effluent was sampled and analysed for TKN using the AOAC method[9].

The N-balance was studied from the total nitrogen in the system containing the TKN value in the effluent and the watermeal (before and after the experiment), TKN losses due to volatilization, transformation and collected water samples, and N fixation from the atmosphere by the watermeal.

RESULTS AND DISCUSSION

Biological oxygen demand

The values of BOD at the starting biomasses of the watermeal when different treatment times were applied were different ($p<0.05$) (Fig. 1a). The watermeal biomass of 0.6 kg/50 l and the treatment time of 30 days gave the highest BOD removal; the residual BOD was 19 mg/l. The BOD of the effluent decreased over time in the 0 to 0.6 kg/50 l treatments. This might be because the watermeal adsorbed organic substances from the effluent for its growth resulting in a decreasing demand for the digestion of organic substances[10]. However, at the watermeal biomass level of 0.8 kg/50 l, the BOD dropped by 34.5% after 10 days. At days 20 and 30 it was 21.7% and 10% less than the day 0 value for this treatment, respectively. At this level, the watermeal died, and most likely led to the high level of organic substances in the water.

Suspended solids

The levels of SS in the various watermeal biomass treatments were different when different treatment times were applied ($p<0.05$) (Fig. 1b). The highest reduction in SS was obtained when a watermeal biomass of 0.6 kg/50 l and a treatment time of 30 days were employed. This resulted in an SS of 39 mg/l from an initial SS of 102 mg/l. Naturally, even without the watermeal, SS was decreasing over time. However, with the presence of the plant, SS decreased at a faster rate. The SS in the form of organic matter might be decomposed by microorganisms[6] and turned into nutrients for the watermeal. As with the findings for the BOD levels, in the watermeal treatment of 0.8 kg/50 l, the SS increased. The reasons for this were the same as for the BOD.

pH

There was no significant difference ($p>0.05$) between the various watermeal biomass treatments and the length of treatment time. It was also found that in experimental units with 0.2–0.8 kg of watermeal, the watermeal was still adapting to its environment and its growth may have been in a lag phase. Therefore, its photosynthesis was incomplete and some of the watermeal died, resulting in an accumulation of CO_2, NO_3^-, SO_4^{2-}, PO_4^{2-} and TAN[11]. Although TAN caused an increase in pH[12], other compounds suspended in the treated effluent caused it to decrease. As a consequence, the treated effluent was in a slightly basic state.

Salinity

As the biomass and treatment period increased, the salinity of the treated effluent increased to 5.2–5.9 ppt from an initial salinity of 5.1 ppt ($p<0.05$). The maximum salinity was 5.9 ppt when a watermeal biomass of 0.6 or 0.8 kg/50 l and treatment time of 30 days were used. This was likely partly due to water evaporation and some water being used for the growth of the watermeal.

Total phosphorus

A comparison between various starting biomasses of watermeal and various treatment times showed differences in the TP of each biomass level and treatment time ($p<0.05$) (Fig. 1c). The lowest reduction in TP was from an initial TP level of 0.91 mg/l dropping to 0.79 mg/l when the watermeal biomass of 0.8 kg/50 l and treatment time of 30 days were applied, whereas the highest reduction of TP left only 0.02 mg/l of TP remaining when a watermeal biomass treatment of 0.6 kg/50 l and a treatment time of 30 days were used. This might be because the watermeal used phosphorus in the form of orthophosphate for its growth[13,14]. This result was similar to that of the BOD.

Nitrate

When different watermeal biomasses and treatment times were used, the NO_3^- levels were also different ($p<0.05$) (Fig. 1d). After the experiments were completed, the remaining NO_3^- levels ranged from 0.015–0.39 mg/l, which was less than the initial NO_3^- value of 0.74 mg/l. The highest reduction of NO_3^- was found for the watermeal biomass treatment of 0.6 kg/50 l and the treatment time of 30 days, leaving a remaining NO_3^- level of 0.015 mg/l, whereas the lowest reduction of NO_3^- was found for the watermeal biomass treatment of 0 kg/50 l and the treatment time of 10 days, leaving a remaining NO_3^- level of 0.39 mg/l. This might be because the watermeal absorbed NO_3^- for its growth[14,15]. However, for the treatments with a watermeal biomass of 0.8 kg/50 l, the NO_3^- value increased because the watermeal biomass was too dense and some of the plants died. The watermeal was decomposed by bacteria, which in turn resulted in nitrification, adding NO_3^- to the effluent.

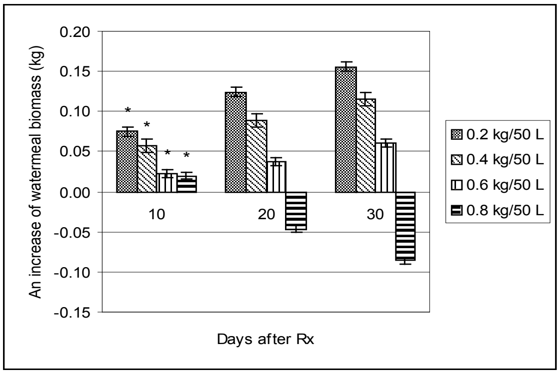

Fig. 2 Watermeal's biomass at different starting biomasses and periods of treatment.
* indicates $p<0.05$ *vs* other periods of treatment.

Total ammonia nitrogen

When different starting biomasses of watermeal and treatment times were applied, the concentrations of TAN were different ($p<0.05$) (Fig. 1e). When the watermeal biomass treatment of 0.6 kg/50 l and a treatment time of 30 days were used, the lowest TAN, 0.11 mg/l, was achieved. From the initial concentration of TAN, which was 2.24 mg/l, the TAN tended to decrease as the biomass and treatment time increased. This might be because the higher the biomass, the higher the adsorption of TAN from the water[15]. However, when a watermeal biomass of 0.8 kg/50 l was used, TAN tended to increase as the treatment time increased, by up to 5.79 mg/l. This might be because of the death of some watermeal plants.

Total Kjeldahl nitrogen

When different starting biomasses of watermeal

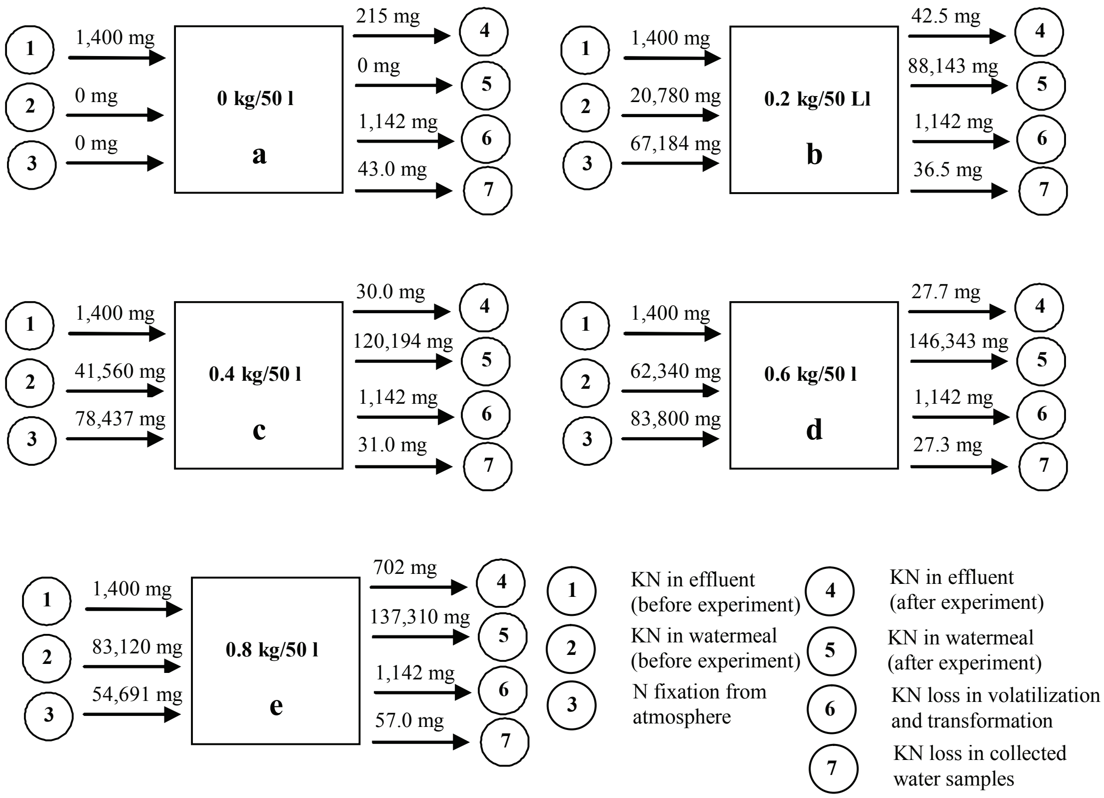

Fig. 3 N-balance at 0 (a), 0.2 (b), 0.4 (c), 0.6 (d), and 0.8 (e) kg biomasses of watermeal.

and treatment times were applied, the TKN levels in the effluent were also different ($p<0.05$) (Fig. 1f). The lowest level of TKN contamination in the effluent was 0.9 mg/l and was obtained from the biomass treatment of 0.6 and a treatment time of 30 days. This might be because the watermeal used N in the form of TAN and NO_3^- for its growth[16], and this resulted in TKN levels of 0.9–24.6 mg/l. The initial value of TKN was 28 mg/l. In contrast, when the watermeal biomass of 0.8 kg/50 l and the treatment times of 20 and 30 days were used, the level of TKN increased, since there was an excess of watermeal that led to its decomposition, transforming it into N in the effluent. The reason for this was similar to this phenomenon for TAN and NO_3^- levels.

Biomass

Significant differences ($p<0.05$) in watermeal biomass levels arose as the treatment period lengthened. The initial biomass level of 0.2 kg/50 l and a treatment time of 30 days yielded the highest increase in biomass, about 0.156 kg, whereas the initial biomass level of 0.8 kg/50 l and a treatment time of 30 days showed the greatest decrease in biomass, about -0.086 kg (Fig. 2). This might be because there was a high density of biomass. It was found that the lower the initial amount of biomass, the higher the survival of watermeal, and the higher the initial amount of biomass, the lower the survival of watermeal.

N-balance

The total N in the system was composed of the total N in the effluent before the experiment, which was equal for all experimental units, and the total N in the watermeal before the experiment, which depended on the initial amount of watermeal biomass. It was found that the greater the amount of watermeal biomass, the higher the level of total N (Fig. 3). During the experiment, total N was lost when some effluent was removed for analysis. Also, total N was lost due to the volatilization and transformation of N. However, there was an increase in the total N from the nitrification process of atmospheric nitrogen. This process increased the total N as the biomass increased. As a result of the increases in the total N mentioned, the results showed an increase in the total N released from the system, which was accumulated in the biomass. The results also showed that the total N in the treated effluent at the end of the experiment decreased as the biomass increased whereas the total N in the watermeal increased as the biomass increased except for the biomass level of 0.8 kg/50 l. In this case, the total N in the watermeal decreased when the biomass treatment of 0.8 kg/50 l was used because some of the plants died due to an excessive density of biomass.

CONCLUSIONS

The biomass level of 0.6 kg watermeal/50 l effluent and the treatment time of 30 days showed the best performance in water quality improvement in this experiment. The growth of the watermeal declined when a biomass concentration of 0.8 kg/50 l was employed, because of an excess of the watermeal. N could be fixed from the atmosphere by watermeal for its growth. Thus, watermeal can grow very well in effluent containing a low level of N. The use of watermeal for treating effluent from *P. monodon* ponds is practical and feasible when optimal conditions are met and a suitable biomass concentration and time period is used.

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