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Treatment of wastewater from shrimp farms using a combination of fish, photosynthetic bacteria, and vegetation

Wen Luo[a], Xianyu Deng[b], Wentao Zeng[a,*], Daheng Zheng[a]

[a]*School of Life Sciences, Shaoxing University, No. 900 South City Road, Shaoxing, Zhejiang Province 312000, P.R. China*
Tel. +86 0575 88342955; email: zengwt2010@gmail.com
[b]*School of Life Sciences, Hunan University of Science and Technology, Taoyuan Road, Xiangtan, Hunan Province 411201, P.R. China*

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ABSTRACT

An innovative system combining fish, photosynthetic bacteria, and vegetable cultivation was used to treat the wastewater discharged from the shrimp farms. The experiment was divided into three phases, starting with photosynthetic bacteria *Rhodopseudomonas palustris* which are added to shrimp aquaculture wastewater at concentrations of 0.5×10^6, 1.0×10^6, 2.0×10^6, 4.0×10^6, and 8.0×10^6 colony-forming units (CFU)/mL (treatment 1, 2 3, 4, and 5, respectively) for 4 days to determine the optimal bacteria level. In the second phase, silver carp (*Hypophthalmichthys molitrix*) were introduced at the biomass levels of 0.25, 0.50, 0.75, 1.00, and 1.25 kg/m^3 (treatment 6, 7, 8, 9, 10, respectively) for 7 days. In phase 3, *Ipomoea aquatica*, *Oenanthe javanica*, *Lactuca sativa*, and *Brassica pekinensis* were introduced and cultivated (treatment 11, 12, 13, 14, respectively) for 7 days. The wastewater values of TP, TN, COD$_{Cr}$, NH$_4$, NO$_3$, and NO$_2$ were analyzed after each phase of treatment. The results showed that the wastewater quality changed drastically after the completion of all wastewater treatments. The removal rates of TP, TN, COD$_{Cr}$, NH$_4$, NO$_3$, and NO$_2$ increased with increasing photosynthetic bacterial concentrations in Phase I. The removal rates of TP, TN, COD$_{Cr}$, and NO$_3$ increased with increasing fish biomass, while the removal rates of NH$_4$ and NO$_2$ decreased in Phase II. The removal rates of TP, TN, NO$_3$, and NO$_2$ were the highest for treatment 11, while the removal rate of COD$_{Cr}$ reached the highest value in treatment 12, and NH$_4$ in treatment 14. *I. aquatica* showed the best removal of nutrients from among the four vegetables in Phase III. It is suggested that a combination photosynthetic bacteria (4.0×10^6 CFU/mL)–fish (0.75 kg/m^3 silver carp)–vegetable (1.00 kg/m^2 *Ipomoea aquatica*) system could be a practical system for nutrient recycling in shrimp aquaculture wastewater on a larger scale.

Keywords: Fish; Photosynthetic bacteria; Vegetable system; Removal rate; Shrimp farm wastewater

1. Introduction

Intensive shrimp aquaculture, especially of *Penaeus vannamei*, is developing at a very fast pace in response to the worldwide demand. The wastewater discharged from shrimp farms contains a large amount of nitrogenous (ammonia, nitrite, and nitrate) and phosphorous compounds, most of which is directly discharged without treatment into water bodies. This

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*Corresponding author.

phenomenon in turn is the root cause for the development of eutrophication and algal blooms, contributing to one of the greatest environmental problems in the world today [1].

Physical, chemical, and biological methods, and their combinations, have been used to treat the wastewater from shrimp aquaculture, with biological treatment considered the most feasible approach for permitting water reuse [2,3]. However, the current biological methods used are often unable to achieve satisfactory results. Presently, combinations of several biological treatment systems are being examined [4,10–15].

Photosynthetic bacteria—anoxygenic phototrophs that grow only under anaerobic or low oxygen tension environments—can be used as the driving force behind nutrient conversion [5]. Many photosynthetic bacteria have been successfully isolated and identified in the environment [6]. Recent studies have shown that photosynthetic bacteria have the capacity to control the organic pollutants in wastewater, so are widely used to augment the water quality in intensive aquaculture. The application of photosynthetic bacteria to wastewater treatment is an attractive alternative, since the microorganisms are naturally present in nature [7–9].

Fish and vegetable production can be connected in a recirculating water system designed to achieve a high degree of efficiency in water use for food production. Such systems have an additional advantage of functional and technological simplicity [10]. Recently, filter-feeding fish were stocked to control microalgae. This method of stocking filter-feeding fish was successfully used to improve the quality of water in Lake Taihu, especially to control noxious the cyanobacteria [11]. Studies have reported that hydroponics grown in artificially cultivated wetlands improve the quality of wastewater by absorbing the nitrogenous and phosphorous compounds [2,3,12–14]. However, the hydrophytes growing vary in their capacity to absorb water. Innovative combined fish-vegetable systems use the nutrient-rich by-products of the fish culture as a direct input for vegetable production, constantly recycling the water [15–17]. The production of a valuable secondary crop would not only improve the quality of water but also garner additional income to farmers.

An economically feasible alternative for removing the nutrients which are produced by intensive aquaculture is the use of a combination of animals, plants, and photosynthetic bacteria. The objective of the present study was to evaluate the efficacy of such a combination in an aquaculture wastewater treatment system.

2. Materials and methods

2.1. Combination of photosynthetic bacterium, fish, and vegetables

To obtain an optimal combination of fish, photosynthetic bacteria, and vegetables, the experiment was performed in three phases (Fig. 1). Fifteen experimental tanks (6 m^2 surface area, 1.5 m depth) were filled with the wastewater drawn from the *P. vannamei* shrimp aquaculture belonging to the Shaoxing Shuijiang Aquaculture Company, Shaoxing, China. Strains from the photosynthetic bacteria *Rhodopseudomonas palustris* (Shanghai Sijiqin Microbial Preparation Inc., Shanghai, China) were added to the water in the experimental tanks according to the manufacturer's instructions.

Phase I: Five treatments were conducted at final bacterial concentrations (which is the result of growth to equilibrium) of 0.5×10^6, 1.0×10^6, 2.0×10^6, 4.0×10^6, and 8.0×10^6 CFU/mL for treatments 1 to 5, respectively. Each treatment was conducted in triplicate. At the beginning of Phase I, the hydraulic retention time (HRT) was for a duration of 4 days. At the end of Phase I, the water quality was measured, and the optimal bacterial concentration was selected for the next phase of the experiment.

Phase II: the optimal bacteria concentration was added to water in the experimental tanks containing age and weight-matched (wet weight 70 ± 5 g) silver carp *Hypophthalmichthys molitrix*. The biomass of silver carp in the five treatments ( ) was set to 0.25, 0.50, 0.75, 1.00, and 1.25 kg/m^3 for treatments 6–10, respectively. The experiment was conducted in triplicate. The sex ratio of fish was 1:1. The number of microalgae was calculated everyday. At the end of Phase II, the water quality values were measured, and the fish biomass producing the highest water quality was used in Phase III of the experiment.

Phase III: 12 experimental tanks, treatment 11–14 in triplicate, were used for growing the vegetables. Individual plants (10 ± 2 g) of water spinach *Ipomoea aquatica*, water dropwort *Oenanthe javanica,* lettuce *Lactuca sativa,* and celery cabbage *Brassica pekinensis* (treatments 11–14, respectively) were tied to foam planks placed on the water surface at a density of 1.00 kg/m^2. After 7 days, the water quality was analyzed. Quality of the influent (initial) water before fish culture in Phase II and before vegetable cultivation in Phase III was used as the control.

2.2. Water quality analysis

Influent (before treatment in every phase) and final (at the end of each phase) water samples were filtered

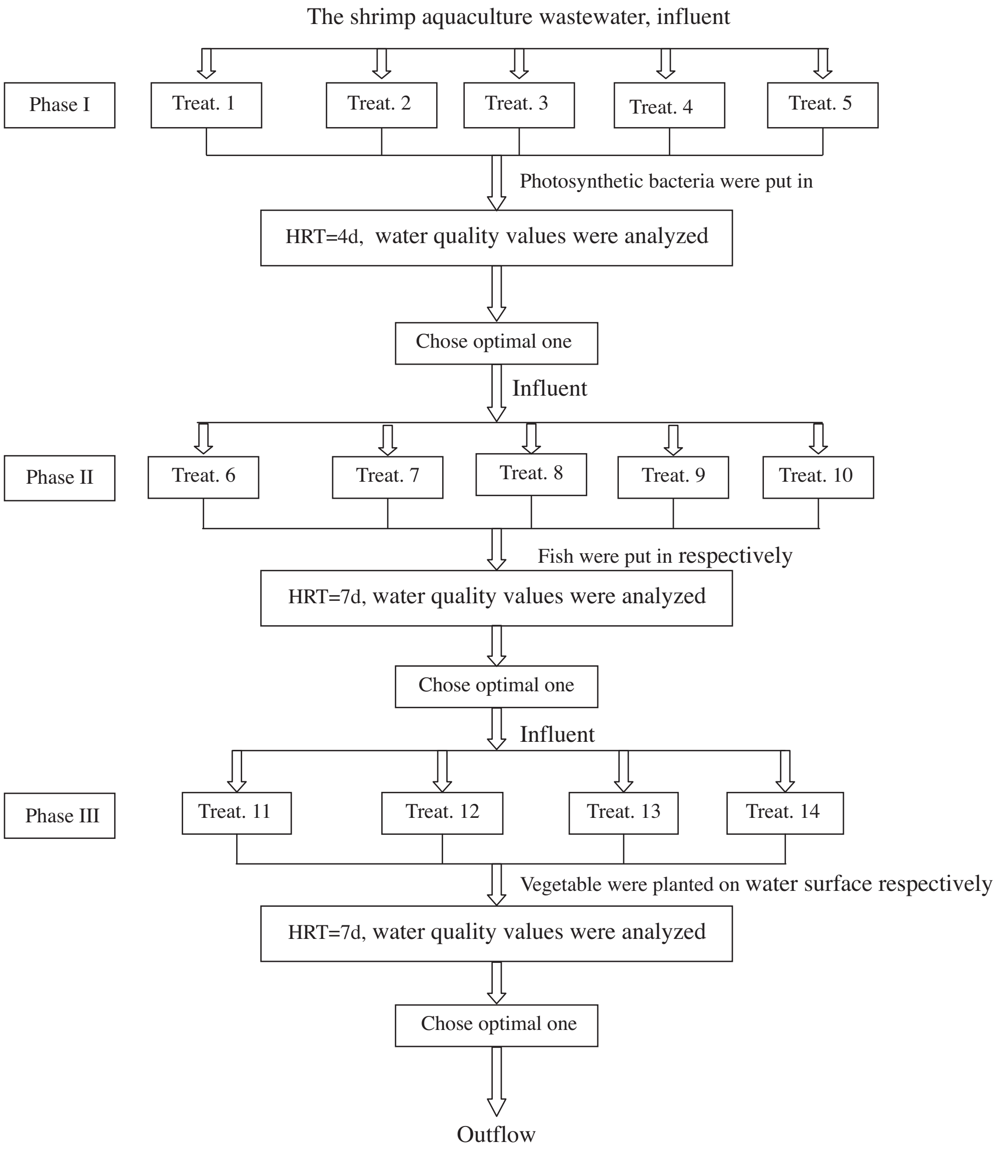

Fig. 1. Experimental scheme.

and analyzed for chemical oxygen demand (COD_{Cr}) by COD DETERMINATOR (DR1010 COD, HACH, USA), total phosphorus (TP) by ammonium molybdate spectrophotometric method, total nitrogen (TN) by alkaline potassium persulfate digestion-UV spectrophotometry, nitrate (NO_3) by spectrophotometry with phenol disulfonic acid, nitrite N (NO_2) by nitrite-spectrophotometry, and total ammoniacal-N (NH_4) by Nessler's reagent spectrophotometry [18,19]. The number of microalgae was calculated using a counting frame [20].

2.3. Statistical analysis

The nutrient removal rates were calculated as $R = (C_0 - C_t)/C_0 \times 100\%$, where R is the removal rate, C_0 is the value of the initial variable (influent water), and C_t is the value after treatment. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc.). One-way ANOVA was performed on treatments at the same phase, after the normality of distribution and homogeneity of variances were confirmed by the Kolmogorov–Smirnov test and Levene's F-test, respectively. Statistically significant differences were determined using Tukey's test (ANOVA). The differences were considered statistically significant at $p < 0.05$.

3. Results

The concentrations of ammonia, nitrate, nitrite, total nitrogen, and total phosphorus in wastewater

Table 1
The removal rate of wastewater nutrients after using different photosynthetic bacteria concentration after 4 days

	Influent value (mg/L)	Treat. 1 removal rate (%)	Treat. 2 removal rate (%)	Treat. 3 removal rate (%)	Treat. 4 removal rate (%)	Treat. 5 removal rate (%)
TP	6.1	36.2 ± 0.84^a	38.3 ± 1.76^a	44.2 ± 1.42^b	54.1 ± 1.32^c	57.1 ± 1.63^c
TN	67	21.5 ± 0.65^a	29.3 ± 0.77^b	32.5 ± 1.07^b	52.5 ± 0.97^d	38.6 ± 1.12^c
COD_{Cr}	508	27.3 ± 0.64^a	26.6 ± 0.73^a	30.7 ± 0.82^b	39.5 ± 0.93^c	37.4 ± 0.96^c
NH_4	0.53	37.1 ± 1.41^a	41.3 ± 1.47^a	55.1 ± 2.12^b	65.3 ± 2.45^c	$60.7 \pm 2.24b^c$
NO_3	18.74	39.8 ± 1.71^a	42.7 ± 1.65^a	53.1 ± 1.36^b	51.7 ± 1.87^b	51.2 ± 1.43^b
NO_2	0.25	10.6 ± 0.67^a	14.2 ± 0.68^b	21.2 ± 1.37^c	27.1 ± 1.65^d	28.6 ± 1.31^d

Values are means ± SD, $n = 3$. Different superscript letters within rows represent significant differences ($p < 0.05$, a < b < c < d).

from shrimp culture are shown in Table 1 (influent values). The values of wastewater quality changed significantly after 4 days of treatment with photosynthetic bacteria. The removal rates of TP, TN, COD_{Cr}, NH_4, NO_3, and NO_2 increased with increasing photosynthetic bacterium concentration. The removal rates of TN, COD_{Cr}, NH_4, and NO_3 reached the highest values in treatment 4, and of TP and NO_2 in treatment 5. There were no significant differences between treatment 4 and treatment 5 ($p > 0.05$).

The wastewater quality parameters showed varying values after 7 days of treatment with silver carp. The removal rates of TP, TN, COD_{Cr}, and NO_3 increased with increasing fish biomass, while the removal rates of NH_4 and NO_2 decreased. The removal rates of TP, TN, and COD_{Cr} were at its highest value in treatment 10. The concentrations of NH_4 and NO_2 increased with increasing fish biomass and reached their highest values in treatment 10 (indicated as minus values of removal rate percent, Table 2). The biomass of microalgae decreased with increasing fish biomass, with the least amount (360 ± 28 cells ind./mL) recorded in treatment 10 after 7 days (Fig. 2).

Four common vegetables characterized by high productivity and nutrient absorbing capacity were selected to investigate their suitability in the combination system. The wastewater quality changed drastically after 7 days of cultivation. The removal rates of TP, TN, NO_3, and NO_2 were the greatest for treatment 11. The removal rate of COD_{Cr} reached the highest

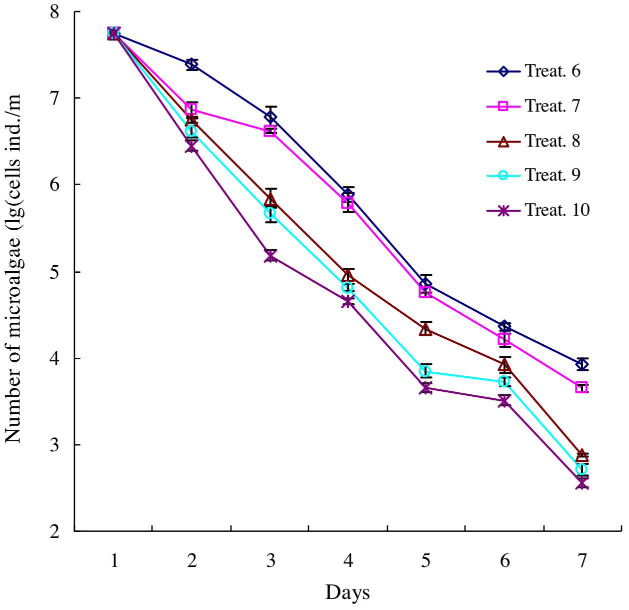

Fig. 2. The changes of microalgae number with different fish biomass in Phase II.

Table 2
The removal rate of wastewater nutrients after using different fish biomass after 7 days

	Influent value (mg/L)	Treat. 6 removal rate (%)	Treat. 7 removal rate (%)	Treat. 8 removal rate (%)	Treat. 9 removal rate (%)	Treat. 10 removal rate (%)
TP	2.8	25.1 ± 0.46^{a}	28.7 ± 1.12^{b}	34.6 ± 1.09^{c}	38.2 ± 1.43^{d}	42.3 ± 1.37^{e}
TN	31.8	18.7 ± 0.67^{a}	25.1 ± 0.57^{b}	30.2 ± 1.13^{c}	35.5 ± 1.86^{d}	40.2 ± 1.72^{e}
COD_{cr}	307	8.5 ± 0.74^{a}	13.6 ± 0.43^{b}	20.3 ± 0.71^{c}	29.5 ± 0.63^{d}	30.4 ± 0.42^{d}
NH_4	0.18	-7.3 ± 0.32^{a}	-11.2 ± 1.07^{b}	-13.7 ± 1.14^{b}	-18.3 ± 1.68^{c}	-20.3 ± 1.52^{c}
NO_3	9.05	17.4 ± 1.24^{a}	22.6 ± 2.18^{b}	27.3 ± 1.79^{c}	31.4 ± 2.47^{d}	28.5 ± 2.13^{cd}
NO_2	0.18	-4.8 ± 0.18^{a}	-7.2 ± 0.52^{b}	-10.1 ± 0.74^{c}	-14.2 ± 1.27^{d}	-18.2 ± 1.38^{e}

Values are means ± SD, $n = 3$. Different superscript letters within rows represent significant differences ($p < 0.05$, a < b < c < d < e).

value in treatment 12 and NH_4 in treatment 14 (Table 3). The result showed that *I. aquatica* had the best removal of nutrients among the four vegetables.

4. Discussion

Many photosynthetic bacteria have been isolated that grow, not only when subject to anaerobic photosynthesis, but also by using the oxygen, nitrate, trimethylamine N-oxide (TMAO), or dimethylsulfoxide (DMSO) as terminal electron acceptors [21]. They are widely used in aquaculture and play an important role in controlling organic pollution [6]. *R. palustris* is a photosynthetic bacterium that is acknowledged to be one of the most metabolically versatile bacteria ever described [7]. It grows by absorbing the carbon dioxide, but can increase the biomass by degrading organic compounds [7,9]. When oxygen is present, *R. palustris* generates energy by respiration and by degrading a variety of carbon-containing compounds, including sugars, lignin monomers, and methanol [6].

In the present study, the removal of nutrients increased with an increase in the concentration of *R. palustris*. However, excessive levels of photosynthetic bacteria would increase the cost without significantly improving the removal efficiency. Our results showed that 4.0×10^6 CFU/mL of photosynthetic bacteria may be the optimal concentration.

Combinations of biological treatments can reduce the water requirements for producing quality protein and vegetable products [15,16]. Innovative fish–vegetable co-existence systems use the nutrient by-products of fish culture as direct input for vegetable production, constantly recycling the water [10]. To decrease the biomass of microalgae in aquaculture wastewater, filter-feeding fish are often used. The silver carp is a filter-feeding fish widely cultured in China [11] that feeds largely on phytoplankton, but also on zooplankton and detritus. Theoretically, the more silver carp in a water body, the more microalgae will be consumed. However, stocking silver carp into water containing microalgae bloom does not always produce the desired effect. Metabolic products of the silver carp, such NH_4 and NO_2, gradually increase in water. These compounds can lead to the death of fish and a deteriorating water quality. Therefore, the fish biomass in aquaculture wastewater should be taken into account. Silver carp at 0.75 kg/m^3 water was found to have the optimal density in this study.

The potential for the recovery of nitrate and phosphate was introduced with vegetable cultivation, which also produced a second crop [17,22–24]. Maximum nitrate and phosphate uptake rates require the

Table 3
The removal rate of wastewater nutrients by using different vegetable after 7 days

	Influent value (mg/L)	Treat. 11 removal rate (%)	Treat. 12 removal rate (%)	Treat. 13 removal rate (%)	Treat. 14 removal rate (%)
TP	1.8	78.4 ± 5.84^{a}	72.7 ± 4.76^{b}	64.2 ± 5.46^{c}	68.1 ± 6.22^{bc}
TN	22.2	81.5 ± 7.62^{a}	80.7 ± 7.79^{a}	65.5 ± 6.04^{b}	62.5 ± 7.37^{b}
COD_{Cr}	245	67.4 ± 5.64^{a}	71.3 ± 6.53^{b}	57.3 ± 4.38^{c}	59.5 ± 7.82^{c}
NH_4	0.20	62.1 ± 6.41^{a}	51.3 ± 4.47^{b}	55.5 ± 5.13^{b}	62.8 ± 6.46^{a}
NO_3	6.58	57.8 ± 6.74^{a}	52.8 ± 7.63^{b}	48.1 ± 4.31^{c}	45.7 ± 5.82^{d}
NO_2	0.20	55.6 ± 6.61^{a}	53.1 ± 6.63^{a}	51.3 ± 5.28^{b}	47.5 ± 3.61^{c}

Values are means ± SD, $n = 3$. Different superscript letters within rows represent significant differences ($p < 0.05$, $a < b < c < d$).

use of plants with high nutrient absorbing capacities such as water spinach, water dropwort, lettuce, and celery cabbage [25,26]. A large leaf surface area provides a site for sufficient photosynthesis. The four species used are common vegetables in China. In this study, *I. aquatica* was the most effective. *I. aquatica* can absorb a large amount of nitrogen and phosphorus in water, grows rapidly because of its long hollow stems [25], and is suitable for farming in the specific environment.

A combination of fish, photosynthetic bacteria, and vegetable culture provides a high-efficiency water use in the production of quality food compared to previous fish/vegetable co-culture systems [10]. The photosynthetic bacterium *R. palustris* added to this system may degrade a variety of carbon containing compounds, converting them to nutrients utilized by algae to provide the diet for fish. Further work should be conducted to optimize the production of fish or vegetables while maintaining this functional balance.

The values of wastewater quality of TP, TN, COD_{Cr}, NH_4, NO_3, and NO_2 decreased rapidly from 6.1, 67, 508, 0.53, 18.74, 0.25 mg/L to 0.39, 4.11, 79.87, 0.07, 2.78, and 0.09 mg/L, respectively, through a combination of photosynthetic bacteria (4.0×10^6 CFU/mL), fish ($0.75\,kg/m^3$ silver carp), and vegetable ($1.00\,kg/m^2$ *I. aquatica*) in the culture system. It is suggested that a fish, photosynthetic bacterium, and vegetable system could be applied to remove the nutrients in recycling wastewater from the shrimp aquaculture on a larger scale.

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