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Bioremediation efficiency of *Gracilaria verrucosa* for an integrated multi-trophic aquaculture system with *Pseudosciaena crocea* in Xiangshan harbor, China

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ABSTRACT

The red algae, *Gracilaria verrucosa*, was co-cultivated with the fish *Pseudosciaena crocea* in an integrated multi-trophic aquaculture (IMTA) system for nearly two months in summer at coastal waters of Xiangshan harbor in the East China Sea. The nutrient reduction efficiency of ammonium and phosphorus was determined. Prior to the *G. verrucosa* co-cultured, the eutrophication index (*E*) values of cage areas ranged from 4.2 to 32.0, indicating serious eutrophication conditions, and the high ratio of N/P and relative high P concentration indicated a nitrogen surplus. During the co-culture period, *G. verrucosa* showed efficiently at removing inorganic nitrogen (IN) and inorganic phosphate (IP), and maintained a more stable dissolved oxygen (DO) and chlorophyll a (Chl *a*) level in the IMTA system. The maximum reduction efficiency of PO_4–P, NO_2–N, NH_4–N and NO_3–N was 58%, 48%, 61% and 47%, respectively. Based on the DIN balance in the IMTA system, the optimal co-cultivation proportion of *P. crocea* to *G. verrucosa* in this area was 1 cage, 144.95 m^2 or 1 kg, 7.27 kg, respectively. Results of the present study indicated that environmental advantage could be achieved by integrating the red alga *G. verrucosa* co-cultured with fish at the open coastal waters in the East China Sea.

1. Introduction

Intensive mariculture of fish, shrimp, shellfish or the other economic aquatic animals not only results in an increase of nutrient concentrations in coastal waters, but also leads to changes of the dissolved inorganic nutrient structures and sedimentary environments (Neori et al., 2004; Victor et al., 2002). In an integrated multi-trophic aquaculture system (IMTA), seaweed can achieve high assimilative capacity to nutrients (Krom, 1986). With solar energy and the excess nutrients (particularly C, N and P), seaweed photosynthesizes new biomass. The operation recreates a proper balance among mini-ecosystem, seaweed autotrophy counters fish (shellfish or shrimp) and microbial heterotrophy, not only with respect to nutrients but also with respect to oxygen, pH and CO_2 (Hirata et al., 1994; Rai et al., 2000). Seaweed biofilters can thus, in one step, greatly reduce the overall environmental impacts of fish culture and stabilize the cultural environments (Schuenhoff et al., 2003). Studies on integrated systems of seaweed co-cultured with other economic aquatic animals have been achieved a rapid development in the past several decades. But most studies were confined to the experimental system in the laboratory (Carmona et al., 2006; Hernández et al., 2002; Yang et al., 2006), small-scale pond experimental co-culture systems (Hernández et al., 2006; Metaxa et al., 2006; Porrello et al., 2003a,b; Xia et al., 2004), and circulating water treatment systems (Matos et al., 2006; Neori et al., 2003). However, to our knowledge, few studies are available on the seaweed co-cultured in an IMTA system for the bioremediation in the open sea (Fei, 2004).

The genus *Gracilaria* (Rhodophyta) offer both high bioremediation efficiency and commercial value in established markets, such as agar–agar, human consumption, and feed (Chopin et al., 2001; Fei, 2004; Neori et al., 2004). *Gracilaria lemaneiformis* could remove most nutrients from the experimental co-culture system, and in the field cultivation trials, the mean uptake rates of N and P by *G. lemaneiformis* were estimated at 10.64 and 0.38 μmol g^{-1} dry weight h^{-1}, respectively (Zhou et al., 2006). Troell et al. (1997) reported that 1 ha *Gracilaria chilensis* cultivated close to fish cages, had the potential to remove at least 5% of dissolved inorganic nitrogen (DIN) and 27% of released dissolved inorganic phosphorous (DIP) released from the fish farm. In the *Epinephelus awoara*/*Gracilaria lichenoides* and *Litopenaeus vannamei*/*G. lichenoides* co-culture ponds, the chemical oxygen demand (COD), DIN, DIP and chlorophyll a (Chl *a*) concentrations were significantly lower than that in the control (Xu et al., 2008). Up to now, *Gracilaria* species studied in IMTA systems for the bioremediation were confined to *G. lichenoides*, *G. lemaneiformis*, and *G. chilensis*, but few documents on the bioremediation efficiency of

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Gracilaria verrucosa was available, especially in the real field (Buschmann et al., 1996; Huo et al., 2010; 2011; Mao et al., 2009; Troell et al., 1997; Xu et al., 2008; Yang et al., 2006; Zhou et al., 2006)

Xiangshan harbor in Zhejiang province of China is one of the most important aquaculture bases in the southeastern coast. In 2005, the number of fish cages in the whole bay has increased to 58,854 ones (Ningbo Ocean and Fishery Bureau, 2006). Thousands of fish cages in the shallow waters resulted poor water exchange, and made the residual feed and fish excretion easily deposit into the sediment. Most sea areas of Xiangshan harbor appeared severe eutrophication (Shu et al., 2004). *G. verrucosa* is the main raw material for processing agar, and also can be used as abalone feed, or the ideal materials for thermal conversion of bio-energy (Xu and He, 2006). The purpose of the present study was to evaluate the bioremediation efficiency and the optimal proportion of *G. verrucosa* co-cultured with *Pseudosciaena crocea* in an IMTA system in Xiangshan harbor. The results of the present paper could provide references for the reduction of mariculture self-pollution and popularization of IMTA systems.

2. Materials and methods

2.1. Study area

This study was conducted at the Qiucun town located at the northern bank of Xiangshan harbor (121°36′19 .08 ″~121°37′10 .92″ E, 29°32′37 .68 ″–29°32′20 .4″ N), which is one of the primary cage-aquaculture bases with 5,180 marine cages (Fig. 1). The tidal current at this coastal area was right track half-tide and reciprocating flow with an average tidal range of 2.7–3.3 m and the average flow rate of 0.5–0.6 m/s. The average water depth of this sea area was 10 m. The ranges of salinity, pH and temperature were 25.4 27.5, 7.83 8.30 and 25.7 31.5 °C during the period of experiments, respectively.

24 cages located in the center part of cages were selected for experiments (Fig. 2), of which 6 cages at the west part were designed to co-culture *G. verrucosa* with *Pseudosciaena crocea* and 6 cages at the east part were considered as controls and 12 cages between them was the transitional area. The shape of cage was 3 m × 3 m × 3 m and the mesh size was 2–4.5 cm. Each cage was stocked with 1500 *P. crocea* of 10–15 cm in total length (average weight 41.5 ± 4.3 g) on 1 August 2006 and total initial wet weight was 373.5 kg. Each cage of fish was fed with 30–40 kg fresh raw fish surimi every two days. On polypropylene ropes in every cage, 1.2 kg healthy *G. verrucosa* were divided into 120 clusters (10 ± 0.5 g/cluster) which were nipped at 40–50 cm interval. Two ends of the rope were tied on the opposite sides of the cage and ropes was suspended in the water column about 20–50 cm below the sea surface. The experiment was ended on 15 September 2006 and the duration was 45 days.

2.2. Water samples collection and nutrient concentrations determined

Prior to *G. verrucosa* cultivated on 1 August 2006, water samples were taken at stations which were distributed evenly around the cages and the scope was within 150 m. Fish cages were regarded as the center and sampling stations were located on concentric circles. The radius designed as sampling circles was 20 m, 50 m, 80 m, 100 m, 120 m and 150 m, respectively, and the interval between two sampling sites on the same circle was 15 m. At every sampling station, water samples at 0.5–1 m below the surface were taken using Niskin bottle and a total of 220 water samples were collected.

During the period of experiment, water samples were collected at three sites marked ①, ② and ③ in Fig. 2 at the first day and every two days from August 18 to September 1. Afterwards, water samples were collected every seven days until the end of experiment. The sampling site of ①, ② and ③ was located in the bioremediation area, transitional area and control area, respectively.

On the last day, water samples were collected at 24 sampling stations located along three transects marked A1–A6, B1–B6 and C1–C12, respectively (Fig. 2). Transect A and B were parallel to the direction of tidal current and transect C was perpendicular to the direction of tidal current.

Three water samples were taken at three places surrounding every site during the slack tide time at a depth of 0.5 ~ 1 m below the surface by a 2.5-L Niskin water sampler and following parameters were measured: ammonium–nitrogen (NH_4–N), nitrite–nitrogen (NO_2–N), nitrate–nitrogen (NO_3–N), inorganic phosphate (PO_4–P) and chemical oxygen demand (COD), dissolved oxygen (DO), chlorophyll *a* (Chl *a*) and Secci disk depth (SD). Water samples were immediately transported to the laboratory in cold conditions before analysis. Water samples for NH_4–N, NO_2–N, NO_3–N and PO_4–P measurements were filtered through 0.45 μm GF/C filters and were determined according to protocols of JOGFS (1994). DO concentration was measured by the Winkler method, Chemical oxygen demand

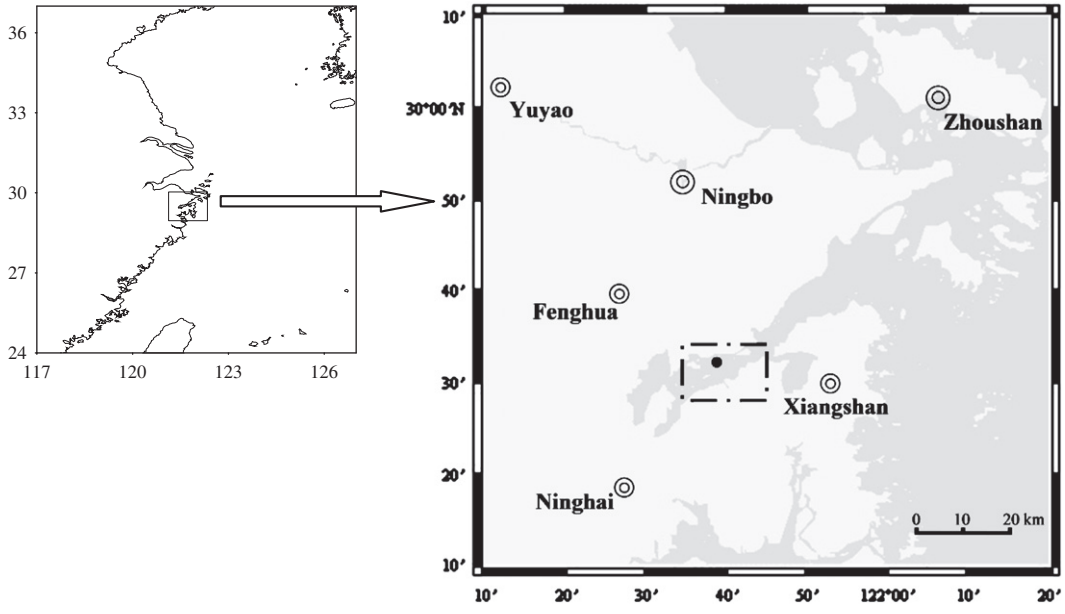

Fig. 1. Map showing the location of Xiangshan harbor and the experimental fish cage area in Xiangshan harbor.