Algal Research 34 (2018) 235–243

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Combinations of *Ulva* and periphyton as biofilters for both ammonia and nitrate in mariculture fishpond effluents

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ARTICLE INFO

Keywords:
Biofilter
Mariculture
Nutrient composition
Periphyton
Ulva

ABSTRACT

Periphyton-based biofilters for aquaculture effluent possess multiple advantages, in water oxygenation, CO_2 reduction and production of useful biomass. The performance of a marine periphyton biofilter, in terms of uptake rate and efficiency in removing different forms of nitrogen, was investigated. A periphyton biofilter was paired with an upstream macroalgae biofilter stocked with *Ulva* to expose the periphyton to ammonia-depleted but nitrate-rich effluent. Three trials compared the removal of total ammonia-N (TAN), nitrate (NO_3-N), total N and phosphorus. The biofiltration and growth performance of (1) the periphyton downstream to the *Ulva* tank, (2) periphyton alone and (3) *Ulva* alone were compared. Biofiltration performance was evaluated at different areal loads of TAN and NO_3-N.

Periphyton growth performance did not depend on the effluent nutrient composition or on N loads, and yielded between 7.3 and 10.6 g dry weight $m^{-2} d^{-1}$. While the *Ulva* preferred uptake of TAN over NO_3-N, the periphyton showed no preference between them, demonstrating flexible shifts between TAN and NO_3-N uptake. TAN uptake rate by the periphyton was not influenced by the effluent composition. However, periphyton NO_3-N uptake rate and efficiency rose about fivefold, up to 1.4 g NO_3-N $m^{-2} d^{-1}$ and 63%, respectively, upon depletion of TAN areal load below 0.18 g N $m^{-2} d^{-1}$ (< 0.3 mg L^{-1}) by the *Ulva* pre-treatment. Normalizing nutrient uptake rate to biomass revealed similar uptake rate of TAN and phosphorus by periphyton and *Ulva*, while the periphyton took up nitrate much faster.

By removing up to 76% of the total nitrogen, with specific removal efficiency of 97% of the TAN and of 67% of the NO_3-N, the novel dual *Ulva*-periphyton biofilter revealed a synergistic potential in treatment of nutrient-rich mariculture effluents.

1. Introduction

Microbial technologies for treatment of aquaculture waste are often constrained by high energy and concomitant capital costs [1,2]. Integrated multi-trophic aquaculture (IMTA) systems overcome some of these constraints by using photosynthetic biofilters which recycle nutrients that would otherwise be wasted, turning them into usable plant biomass. Land-based IMTA systems for mariculture have been extensively studied and developed at the National Center for Mariculture (NCM) in Eilat (Red Sea, Israel). The macroalgae *Ulva* sp. and *Gracilaria* spp. have mostly been used to treat effluent from the fish mariculture facilities [3–5]. Macroalgae harvested from the biofilters can feed additional commercially valuable marine animals such as fish [6], abalone and sea urchins [7], as well as terrestrial animals such as ruminants [8].

However, the macroalgae-based biofilters usually take up ammonia (total ammonia N, NH_3 and NH_4^+, or TAN) much more rapidly than the oxidized N forms of nitrate (NO_3-N) and nitrite (NO_2-N) that require higher energy investment [9,10], and will resort to NO_3 removal only when TAN is nearly depleted [11]. Perference of TAN over NO_3-N was also indicated in outdoor facilities at NCM with *Ulva lactuca* biofilter for intensive fish farming by Neori [12].

Although ammonia is the main catabolic product, effluents from aquaculture also carry nitrate formed by microbial nitrification. While efficient removal of nitrate is less critical for fish health, its discharge can damage sensitive coastal ecosystems [13]. In recirculating aquaculture systems nitrate accumulates over time [14] and may harm fish growth and feed intake [15]. The combination of denitrifying biofilters with algal biofilters can be complex and costly for the farmer [1]. Hence the desirability of setting up treatment systems designed to effectively remove all N forms.

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https://doi.org/10.1016/j.algal.2018.08.002
Received 4 December 2017; Received in revised form 28 July 2018; Accepted 2 August 2018

The diverse community of organisms - algae, fungi, bacteria, and protozoa - that develop on submerged substrates and constitute the periphyton can remove various nutrients from the water column including phosphorus and both N forms, TAN and NO_3-N [16,17]. Removal of both ammonia and nitrate was also documented in a bioflock-based system for shrimp production with bacterial assimilation and nitrification processes for ammonia removal and denitrification for nitrate removal [18]. Lately, periphyton systems for nitrogen removal have been indicated to require half the land of a wetland treatment at less capital costs [19]. In addition, similarly to macroalgae, periphyton can have a secondary use as food for fish, crustaceans and other cultured organisms [20]. For example, in *Tilapia* culture, periphyton in the ponds reduced the use of commercial pellets by nearly 50% [21].

The current study evaluated biofiltration performance of marine periphyton when exposed to enriched fishpond effluent under different N compositions and loads. Periphyton was integrated downstream to an *Ulva* biofilter to investigate its potential in removing the dissolved inorganic nitrogen forms TAN, NO_3-N, total nitrogen (TN), and phosphorus (PO_4-P) from mariculture effluents in comparison to a similar periphyton biofilter that received the enriched effluent directly from the fishponds. The biofiltration performance of the dual *Ulva*-periphyton was also studied.

2. Materials and methods

2.1. System design

Three experiments (referred to here as trials 1, 2 and 3) were carried out at the National Center for Mariculture (NCM) located in Eilat (Red Sea, Israel), lasting 2–3 weeks each. The experimental design (Fig. 1) included two biofilters, one periphyton-only and the other dual *Ulva*-periphyton. The dual *Ulva*-periphyton biofilter (UP) included two tanks in a series: an aerated one stocked with *Ulva* sp. (probably *U. lactuca*) [22] from an integrated multi-trophic system at NCM, and a non-aerated tank downstream (UPP) stocked with white flexible nets as substrate for periphyton development. The periphyton-only biofilter tank was stocked with the same substrate for periphyton development as the UPP tanks. This design allowed the evaluation of each of the units separately as well as of the dual biofilter as one integrated biofilter.

The wastewater feed to the biofilters originated from three semi-intensive fishponds (40 m^3 each), stocked with grey mullet (*Mugil cephalus*) at a density of 10–15 kg m^{-3}. The fish were fed a feed containing 36% protein at a rate of 2% body weight d^{-1}. Effluent from the fish ponds flowed to a settling pond, where the organic particles sank. The clarified effluent flowed from the settling pond to the experimental biofilters.

The maintained conditions in the different trials (Table 1) resulted in effluent retention time of 6 h in the periphyton-only tank and in each of the *Ulva* and subsequent periphyton tanks in the dual biofilter (a total of 12 h in the UP treatment) during trials 1 and 2; while in the third trial, the doubled flow-rate halved the retention times. In trial 2, areal loads of TAN and of NO_3 were half of that in other trials while maintaining the nearly 1:1 ratio between these N forms.

The effluent leaving the settling pond had a background concentration (mean value) of 0.81 mg L^{-1} (± 0.07) TAN, 0.07 (± 0.02) mg L^{-1} nitrite-nitrogen (NO_2^--N), 0.82 (± 0.05) mg L^{-1} nitrate-nitrogen (NO_3^--N) and 0.78 (± 0.04) mg L^{-1} phosphate-phosphorus (PO_4^--P). In order to expose the biofilters to relatively constant nutrient load along biomass development and measurements, as well as to ensure that the *Ulva* and periphyton were not nutrient limited,

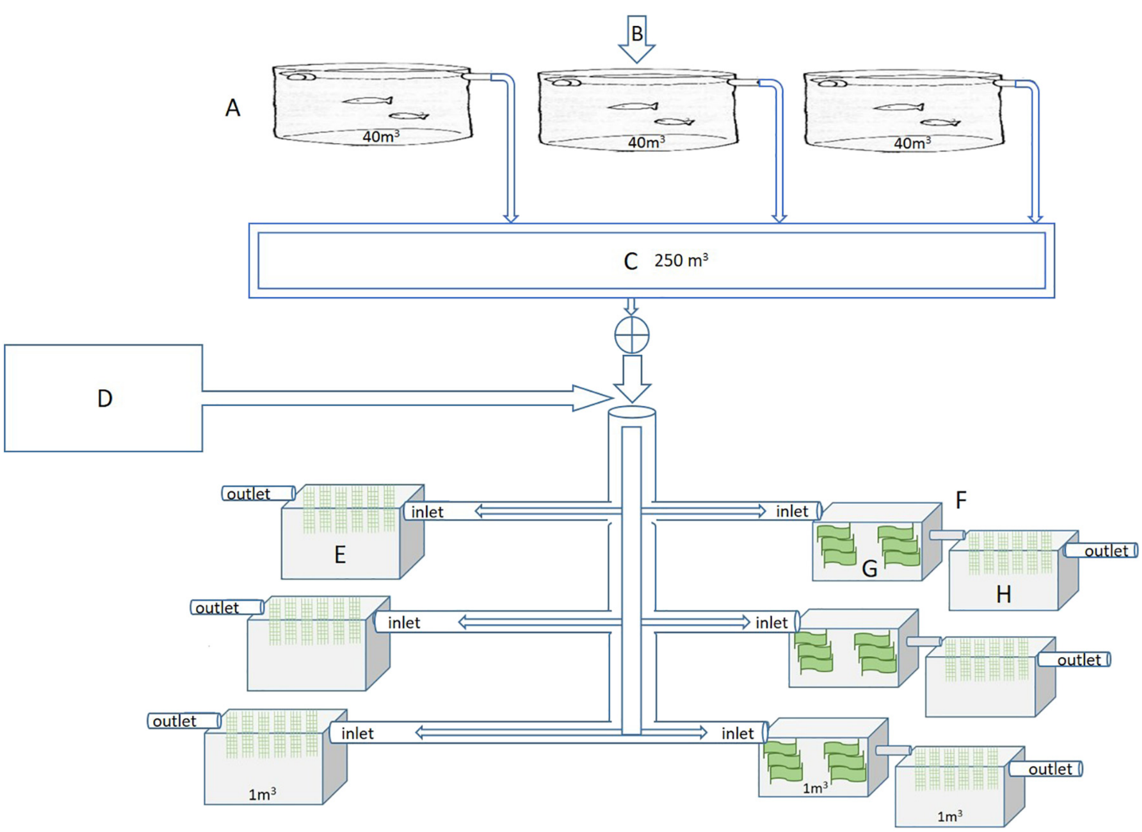

Fig. 1. Diagram of the experimental system showing the three treatments. The system consists three fish culture tanks (A), supplied with fresh Red Sea water (B) at daily exchange rate of 50%. Fishponds effluents were transferred to a 250 m^3 sedimentation pond (C) and portion of the upper water from this pond were enriched with nutrients from a stock tank (D) following transfer of the enriched effluent to the different biofilters. Biofilters included a periphyton-only biofilter (E) and a combined *Ulva*-periphyton biofilter (F, also referred as UP) that consisted an *Ulva*-only biofilter (G) as 1st stage in the combined biofilter, and a downstream periphyton biofilter (H, also referred as UPP).