Periphyton For Biofiltration And Fish Feeding in An Integrated Multi-Trophic Aquaculture System: A Case Study in The Gulf of Aqaba

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Received: September 09 2019

Published: September 18, 2019

Abstract

The environmental footprint of mariculture is a major obstacle towards expansion of this agro-industry. Additional economic constraints are the high costs of effluent treatment and expensive, non-sustainable ingredients in aquafeeds. Both Ulva and periphyton reveal great potential in nitrogen removal in mariculture effluent. To overcome the relatively minor uptake of nitrate by Ulva, a periphyton-based biofilter can be used either as a stand-alone biofiltration unit or as a polishing unit in an integrated Ulva-periphyton biofilter. The biomass produced is rich in protein and is suitable for feeding of marine fish, served fresh on the plastic net substrate or included in pelleted aquafeeds to replace fishmeal.

Keywords: Aquaculture; IMTA; Nutrient removal; Periphyton; Ulva

Introduction

Excess nutrients in aquaculture effluent increase environmental footprint and may limit the expansion of this industry. While protein content in commercial feed pellets may reach 70% of dry biomass, fish retain only about 20-30% of feed nitrogen while the remainder is released into the water as dissolved and organic N. Treatment of the dissolved toxic ammonia by bacterial nitrification is efficient but results in a nitrate-rich effluent, and subsequent denitrification has yet to limit nitrate accumulation in recirculating aquaculture systems (RAS) [1]. Moreover, in these dissimilatory metabolisms' expensive nitrogen in aquafeeds is discharged to the atmosphere as N_2 upon its assimilation into edible biomass. The positive role of photosynthetic organisms in nutrient assimilation and fish nutrition has been known for years [2]. Amongst other plant-based biofilters for water purification, periphyton has been suggested as a relatively inexpensive method [3]. This microbial mat develops naturally on submerged surfaces in the presence of light and nutrients. The diverse microbiota in periphyton (primary photoautotrophs) exhibit various metabolisms for nutrient utilisation [4]. Periphyton also reveals high potential in fish nutrition and may significantly reduce the use of commercial pelleted aquafeeds [5]. Currently, periphyton aquaculture is established mainly in freshwater

extensive fishponds for farming of carp [6], tilapia [7], and catfish [8]. The development of periphyton on artificial surfaces in the ponds is facilitated by the addition of organic fertilizers [9]. In recent years, a periphyton-based biofilter for mariculture effluent was developed and integrated in a land-based, semi-intensive, integrated multi-trophic aquaculture system (IMTA) located at the National Center for Mariculture (NCM) in the Gulf of Aqaba (Eilat, Israel). The conceptual model of this periphyton biofilter (Figure 1) was innovative, as instead of co-culture of periphyton and fish in the same pond, the periphyton biofilter was separated from the primary fish culture. This spatial separation is similar to other biofilters in IMTA with extractive seaweeds of *Ulva* sp. [10] and *Gracilaria* sp. [11] and allowed examination of periphyton performance under various conditions with minimal interference (e.g., grazing of the produced biomass). A later development included the combination of a downstream periphyton biofilter with an upstream Ulva biofilter. The potential of the periphytic biomass in feeding of common marine cultured fish, the omnivorous marbled spinefoot (*Siganus rivulatus*) and carnivorous sea bream (*Sparus aurata*), was also examined. This manuscript presents primary results from the case study of periphyton in IMTA.

Figure 1: A diagram of periphyton in IMTA systems. Periphyton was integrated in IMTA either as sole biofilters or in combination with macroalgae (*Ulva fasciata*) for biofiltration of fishponds effluent and for feeding marine fish.

Marine Periphyton Biofilter for Mariculture Effluent

As a sub-class of photosynthetic-based biofilters, periphyton is capable of removing inorganic nutrients from aquaculture waste with minimal aeration costs. The separation of the periphyton biofilter from primary culture units allowed the study of periphyton performance and characteristics under various conditions, towards improving biomass production and its capacity to remove nutrients. The nutrient uptake capacity of freshwater periphyton was studied previously [12] but knowledge was still required for marine periphyton. For this purpose, a marine periphyton biofiltration system was established at NCM. The periphyton system, as described elsewhere [13], comprised 12 tanks that were fed with nutrient-enriched effluent of a semi-intensive mullet production system. Biomass development in these biofilters occurs naturally on the submerged artificial plastic nets, requiring no prior inoculation. The production rate of periphyton is rapid during summer, reaching specific growth rate of 27% d⁻¹, or 4g ash-free dry weight (AFDW) m⁻² d⁻¹, and slower in other seasons of the year, up to only 7.5% d⁻¹ in winter. While in traditional aquaculture ponds substrate density for periphyton development is at a 1:1 ratio to pond surface area [14], separating the periphyton biofilter allows doubling and even tripling of biomass production yields by increasing substrate density respectively [15]. Hence, if the aim is production of periphyton as edible biomass, such a management tool is highly recommended. Ammonia removal by periphyton (as total ammonium nitrogen - TAN) is efficient and rapid, up to rate of 3.8g TAN m⁻² d⁻¹, but highly affected by both biomass yield and effluent retention time in the biofilter. According to an empirical model [16] it can be estimated that in order to remove 70% of TAN in fishpond effluent at a daily rate of 1.4g TAN m⁻² d⁻¹, a rapid streaming of nutrients is required when biomass yield is relatively high (for ~100g AFDW biomass at a retention time of only 1h). Such efficiency can also be achieved when biomass yield is only a third (~30g AFDW), for example, in

the first week of periphyton development, but it requires a five-fold slower streaming of nutrients.

The Combination of Seaweed *Ulva Spp* and Periphyton for Mariculture Effluent

Although dissolved ammonia is the main catabolic product of fish culture, formation of nitrate (NO₃-N) by microbial activity (i.e., nitrification) may lead to relatively similar proportions of these N forms in effluent. Discharge of nitrate in effluent can damage sensitive coastal ecosystems via eutrophication (Howarth and Marino, 2006). In RAS, rapid nitrification leads to accumulation of nitrate (van Rijn, 1996) which harms fish growth and feed intake [16], even in systems with a denitrification treatment (van Rijn, 2013, 1996). Moreover, denitrification is dependent on the availability of organic carbon which, in some cases, is added artificially to the denitrification compartment in the form of methanol, starch, etc. In most seaweeds, ammonia uptake is more rapid than that of the oxidized N forms of nitrate and nitrite (NO₂-N). Metabolism of the latter requires energy investment [17] for activation of the endogenous enzymes nitrate- and nitrite reductase which reduce the oxidized N forms to ammonia toward assimilation. Thus, removal of NO₃-N by seaweeds will occur only when ammonia is nearly depleted (Syrett, 1981). Previous studies at NCM confirmed negligible uptake of NO₃-N by *Ulva lactuca* when fed with fishpond effluent comprising both TAN and NO₃-N (Neori, 1996). In contrast, autotrophic and heterotrophic organisms in periphyton enable metabolization of nitrogen in its various organic and inorganic forms. Nitrification, denitrification and annamox have been reported in periphyton [18]. In order to improve effluent biofiltration by means of total N removal, a novel integrated two-step biofilter with *Ulva* and periphyton was designed at NCM. Similar to a previous two-step biofilter with only *Ulva* sp. in both stages (Neori, 1996), the modified system integrated the periphyton biofilter downstream with an *Ulva fasciata* biofilter upstream. The *Ulva* would remove much of the ammonia in fishpond effluent, followed by polishing

of the TAN-depleted but nitrate-rich effluents from this biofilter by the downstream periphyton. Such a combination is also expected to reduce costs of effluent treatment by not requiring aeration in the periphyton unit. The combination of Ulva and periphyton resulted in higher removal of both nitrogen and phosphorus as compared to their removal by a single unit comprising only Ulva or periphyton, and also when compared to a combined Ulva-Ulva biofilter. Overall, 76% of the nitrogen in effluent (97% of the ammonia and 67% of the nitrate) can be removed by the combination of Ulva and periphyton, with daily uptake rate of nearly $2\text{ g N m}^{-2}\text{ d}^{-1}$ [19]. In contrast to Ulva, periphyton does not show N preference, exhibiting flexible shifts between TAN and $\text{NO}_3\text{-N}$ metabolism. Moreover, rapid and efficient removal of $\text{NO}_3\text{-N}$ by periphyton (up to $1.4\text{ g N m}^{-2}\text{ d}^{-1}$ or removal of 63% of $\text{NO}_3\text{-N}$) may occur in TAN-rich effluent ten-fold higher than that required for Ulva's transfer to $\text{NO}_3\text{-N}$ metabolism [20]. Since the specific uptake rate of TAN (in $\text{g N gram}^{-1}\text{ DW d}^{-1}$) by periphyton is relatively similar to that of Ulva and much faster in the case of $\text{NO}_3\text{-N}$, the combined biofiltration module is very promising for mariculture effluent. Performance can be further improved by enhancing biomass production in the periphyton unit by increasing substrate density.

Biochemical and Community Composition in Periphyton for Mariculture Effluent

Periphyton is a complex community that includes representatives of various taxonomic groups from different trophic levels including detritus, micro- and macroalgae, bacteria, fungi, protozoa, zooplankton and small invertebrates [21]. Variation and dynamics in periphyton community composition are driven by various factors such as substrate submersion time [22], the type of substrate [23], seasonal changes, light intensity [24], and the available nutrients [25]. Changes in community composition also affect periphyton's biochemical composition and hence its nutritional value. For example, diatoms are rich in lipids and proteins [26] and their being served as supplemental feed on an artificial substrate improved production of post-larval shrimps [27]. The study at NCM revealed seasonal shifts in the community composition in periphyton, with cyanobacteria dominating in seasons of warmer temperature (summer and spring) and diatoms in winter and autumn. To induce diatom dominance over that of cyanobacteria in the warmer seasons, enrichment of fishpond effluent with silica is required. Moreover, effluent enrichment with silica at a ratio of 5:1 between Si: N is useful in postponing the development of filamentous macroalgae on the net substrate, thereby preventing shading of the submerged periphyton by a filamentous algal mat on the water surface that would cause a crash of its biota. Silica enrichment also affects the biochemical composition of periphyton by increasing its lipid content up to tenfold higher compared to cyanobacteria-dominated periphyton. Protein level in marine periphyton from the biofilter in different seasons may vary between 29 – 40% of DW, with higher protein levels when cyanobacteria dominate the microbiota. Overall, protein content in marine periphyton from IMTA is somewhat higher than that measured in freshwater periphyton [28] or other studied marine periphyton [29], and comparable to measured

levels in IMTA-Ulva (36% of DW; [30]). However, the proportion of ash in periphyton may reach higher levels, up to 36% of DW, when diatoms dominate the periphyton. This may question the use of such biomass for fish feeding, due to lower digestibility. Metagenomics analyses of periphyton in mariculture effluent (using high through-put sequencing) revealed eleven bacterial phyla, with Firmicutes (53%), Proteobacteria (17%) and Bacteroidetes (13%) dominating the bacterial community. Periphytic autotrophs consisted of cyanobacteria, diatoms, and chlorophytes. Among eight identified members of diatoms, genus *Cylindrotheca* was found to be the first diatom to colonise the substrate, followed by the appearance of members of genera *Navicula* and *Amphora*. Other eukaryotes in the periphyton included Chlorophytes (from two genus taxa), Dinoflagellates, Ciliates (from two order taxa), Amoeboae, Nematodes and Copepods, as well as several Fungi.

Periphyton for Feeding of Cultured Marine Fish

Periphyton is an important food of invertebrates, tadpoles, and fish in their natural aquatic habitats [31]. In aquaculture, increase of primary production in the ponds is achieved by the addition of artificial substrate for periphyton development and fertilization with organic waste prior to introduction of fish. The shift from phytoplankton-based to periphyton-based production is a primary advantage towards increasing system efficiency by means of energy and nutrient transfer. Moreover, compared to harvesting of small planktonic algae from the water column, attached periphyton can be grazed more efficiently by the cultured fish [32]. This technology has been common in tropical Africa and Asia for many years as a traditional way of enhancing fisheries in coastal lagoons without adding artificial feeds [33]. Periphyton has been used successfully to augment production of carp [34], tilapia [35] and catfish [36] in freshwater ponds. When used in fishponds for tilapia culture, commercial feed in fish diet was reduced by 40% while not harming growth performance and maintaining high water quality [37]. A few studies of herbivorous fish culture in brackish water indicated periphyton to support production of grey mullet (*Mugil cephalus*) and golden grey mullet [38]. Promising results in terms of growth, survival and production were also observed using periphyton in the culture of penaeid shrimp in brackish water [39]. While IMTA-Ulva lactuca and *Gracilaria* spp. have been indicated as nutritious feed in culture of abalone [40], sea urchins [41] and fish [42], the potential of IMTA-periphyton is to some extent unknown. To overcome this gap, a series of nutrition trials were performed at NCM. In the first trial, fresh periphyton was harvested routinely from the IMTA biofilter and served fresh, on the solid net substrate, to rabbitfish (*Siganus rivulatus*) fingerlings. Supplemented fresh periphyton in diet of *S. rivulatus* in its early stages of life (between 3–25g body weight) allowed a total discarding of fishmeal from pelleted feed as well as reducing the share of pelleted feed in fish diet by 40% (calculated as AFDW). The periphyton-supplemented diet did not affect growth performance of the fingerlings, i.e., specific growth rate (SGR), feed conversion ratio (FCR), their protein efficiency ratio (PER) or survival rates. Moreover, protein content in *S. rivulatus* fed with the supplementary fresh periphyton was somewhat higher than that in the pellets diet (51 vs. 49% of DW). In the case of

carnivorous fish like sea bream (*Sparus aurata*), fingerlings are somewhat incapable of grazing periphyton directly from the solid substrate [43-55]. To overcome this, periphyton can be dried, powdered and incorporated into the pellets. This allowed reducing fishmeal content in the pellets by half, to only 125g per 1kg feed, when compensating with periphyton meal at the rate of 250g kg⁻¹ of feed. The inclusion of periphyton meal into pellets is assumed to be efficient for production of *S. aurata* over a longer period than in the case of *S. rivulatus*, as the former gained between 80111g of body weight under this diet regime (starting from body weight of ~10g), with SGR between 1.8–2% d⁻¹, which is comparable to other trials with *S. aurata* using plant-based protein to reduce fishmeal in pelleted feeds [56-61].

Acknowledgements

The research at NCM was supported by Israel Ministry of Agriculture and Rural Development Grant No. 8940193-16 and the Bi-National Agriculture Research and Development (BARD) grant No. IS 4995 17R. The author would like to thank to all researchers, students and technicians at NCM and elsewhere for their contribution: Prof. M. Shpigel, Dr. A. Neori, Dr. A. Milstein, Prof. S. Harpaz, Prof. M. Verdegem, Dr. S.E. Boxman, A. Levy, R. Barkan, B. Sachar, M. Masasa, K. Buisman, G. Savonitto, L. Breyll, D. Ben-Ezra, V. Odinetsov, H. Chernova and A. Zalmanzon. The author would also like to thank Mikhal Ben Shaprut for making useful suggestions to the MS.

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