A direct comparison of the performance of the seaweed biofilters, Asparagopsis armata and Ulva rigida

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A direct comparison of the performance of the seaweed biofilters, *Asparagopsis armata* and *Ulva rigida*

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Abstract The tetrasporophyte of *Asparagopsis armata* has been previously established as a novel seaweed biofilter for integrated land-based mariculture. The species growth and biofiltration rates were much higher than the values described in the literature for *Ulva* spp., the most common seaweed biofilter. However, a validation of the advantage of one species over the other requires a study of the performances of these two species in the same system at the same time. In this work, we compared the biofiltration performance and biomass yield of *A. armata* and *Ulva rigida* cultivated in the effluents of a fish farm in southern Portugal. Comparisons were performed at different water renewal rates and in two seasons of the year. The maximum total ammonia nitrogen (TAN) removal rates were similar for both species in December (2.7 and 2.8 g TAN m^{-2} day^{-1} for *U. rigida* and *A. armata*, respectively) and higher for *A. armata* (6.5 g TAN m^{-2} day^{-1}) than for *U. rigida* (5.1 g TAN m^{-2} day^{-1}) in May. Higher differences were observed when estimating the nitrogen biofiltration through the organic nitrogen yield (N yield) of the biomass produced, particularly in May. This estimate is directly related with the biomass yield and the N content in the tissue which were always higher for *A. armata* than for *U. rigida*. In December, the maximum biomass yields were 71 g dry weight (DW) m^{-2} day^{-1} for *A. armata* and 44 g DW m^{-2} day^{-1} for *U. rigida*, while in May, the yield of *A. armata* was 125 g DW m^{-2} day^{-1} and of *U. rigida* was 73 g DW m^{-2} day^{-1}. This study confirmed that *A. armata* is indeed a more efficient biofilter than *U. rigida*. To the best of our knowledge, the production rates reported here are the highest ever reported for macroalgae cultivated in tanks.

Keywords *Asparagopsis armata* · Seaweed biofilter · Integrated aquaculture · *Ulva rigida*

Introduction

Research on seaweed biofilters for treating effluents from mariculture practices started in the mid-1970s (Langton et al. 1977; Ryther et al. 1975) and continued in the 1980s with a few studies (e.g., De Busk et al. 1986; McDonald 1987). It was in the 1990s that this research field gained a renewed and increased interest (see the reviews by Chopin et al. 2001; Neori et al. 2004; Troell et al. 2003). Species of *Ulva* were soon identified as ideal candidates for filtering fish effluents due to their capacity to rapidly absorb and metabolize nitrogen, their high growth rates, their low epiphytism susceptibility, and their worldwide distribution (Jimenez del Rio et al. 1996; Mata and Santos 2003; Msuya and Neori 2002; Neori et al. 2000). However, seaweed biofiltration of fish farm effluents was not widely adopted by the aquaculture industry, and except for Asia, *Ulva* is not grown commercially because it has a low market value. The future development of this technology may depend on increasing the added value of the produced seaweed. Research efforts should focus on the cultivation of economic valuable seaweeds so that nutrient biofiltration may be identified by the aquaculture industry as an economic self-sustainable and environmental-friendly technology.

Asparagopsis armata Harvey is a rich source of halogenated organic compounds (McConnell and Fenical

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1977) with remarkable antibacterial and antifungal activity (Pesando and Caram 1984; Salvador et al. 2007). As such, the species is currently being explored commercially in France for the production of natural preservatives in cosmetic formulations. We have established its mass production culture in an integrated land-based mariculture. The tetrasporophyte of *A. armata* showed exceptional high growth and biofiltration rates (Schuenhoff et al. 2006).

A comparison with literature data on *Ulva* spp. biofilter indicated that the tetrasporophyte of *A. armata* may be a better biofilter (Schuenhoff et al. 2006). However, validation of the advantage of one species over the other requires a study of the two species' performances in the same system at the same time. Factors such as tank design, tank depth, available light (stocking density, tank transparency), nutrients, and water turnover rates may have major effects on seaweed growth and nutrient uptake. The present study assesses the performance of *A. armata* and *Ulva rigida* C. Agardh as biofilters of fish farm effluents at the same time and under the same culture conditions. Comparisons were performed at different water renewal rates and in two seasons of the year testing the effects on the biomass yield and total ammonia nitrogen (TAN) removal rates of both species.

Material and methods

Experimentation took place in an integrated fish/seaweed cultivation system established at Aquamarim, a fish farm in southern Portugal, previously described in Schuenhoff et al. (2006). Twelve cylindrical white (transparency ~70%) polyethylene tanks (Allibert Buckhorn C1100; 110-L capacity, 0.23-m^2 surface area) were supplied with 150 µm filtered fish pond effluent. Six tanks were stocked with *A. armata* and the other six tanks with *U. rigida* at previously established biomass densities that optimize biomass yield in these tanks. *A. armata* was stocked at 5×*g* centrifuged fresh weight (FW) L^{-1} (Schuenhoff et al. 2006) and *U. rigida* at 4 g FW L^{-1} (unpublished data).

The experiments were performed in December and May to compare growth and biofiltration performance during a low and high temperature and irradiance time of the year. During 1 week, the tanks were subjected to different nutrient fluxes which covered a range from very low water exchange rates to the highest that was possible in this system (between 0.1 and 4 vol h^{-1}). In order to prevent individual variations between tanks, before the experiment, the biomass of each species was stocked at a similar biomass density in one tank of 1.4 m^3 and supplied with the fish effluent water. At the end of the experimental weeks, the biomass yield in each tank was calculated from the equation $Y(\mathrm{g\,DW\,m^{-2}\,day^{-1}}) = (N_t - N_0)/t/(\mathrm{DW/FW})/A$, where N_t is the final fresh weight, N_0 the initial fresh weight, t the number of days, DW/FW the dry weight/centrifuged fresh weight ratio, and A the area covered by the tank in m^2. Triplicate seaweed samples of each tank (10 g FW) were taken at the beginning and end of the experiments and oven-dried (48 h; 60°C) both for DW/FW ratio determination and for tissue N content analysis using an elemental analyser (Flash EA 1112, ThermoFinnigan).

Water samples for TAN analysis were taken from the inflow and the outflow of each individual seaweed tank. These samples were collected every 2 days of each experimental week and at three different times of the day (0600, 1300, and 1600/1800 hours) to estimate the daily biofiltration performance of each species. For TAN concentration analysis, duplicate water samples were filtered (0.25 µm; CF Whatman) into acid-washed vials and taken immediately to the laboratory for analysis on a loop-flow analyser (uMAC-1000 multiparametric, Systea, Anagni, Italy) using the standard indophenol blue procedure described in Grasshoff (1983). The inflow and outflow TAN fluxes (µmol L^{-1}h^{-1}) were calculated as the product between the TAN concentration (µmol L^{-1}) of the incoming and outgoing water, respectively, and the water renewal rates (number of volumes per hour) of each individual tank. Biofiltration in each tank was calculated as the difference between incoming and outgoing TAN fluxes. Daily TAN removal was estimated considering that the 0600-hour samples were representative of the whole night hours (15 h in December and 10 h in May). During the light hours, biofiltration was calculated considering the average between the values obtained at 1300 and 1600 hours in December and 1300 and 1800 hours in May and the number of light hours (9 h in December and 14 h in May). Biofiltration was also estimated through the daily N yield of the produced biomass which was calculated by multiplying the biomass yield by the N content of the seaweed tissue. Michaelis–Menten type curves were fitted to plots of TAN flux versus both TAN removal and biomass yield data using least square nonlinear regression. Maximum TAN removal ($V_{\max}$) and half saturation constants (K_s) were estimated.

The temperature and the pH of the fish effluent water were regularly monitored using a portable pH probe (YSI model 63, USA). A Li-190SA Quantum Sensor connected to a Li-1000 Data Logger (both LI-COR Inc., USA) monitored open air photon flux density (PFD) during the whole experimental periods.

Results

The daily averages of PFD and fish effluent temperature were fairly constant throughout each experimental week. The total daily PFD was four times higher in May than in December (around 510 and 170 mol photons m^{-2}, respectively). Maximum light intensity values recorded at 1300 hours were 960 µmol photons m^{-2} s^{-1} in December and 1940 µmol photons m^{-2} s^{-1} in May. The water temperature in the fish effluent had absolute maxima/minima of 24/19°C in May, with a weekly average of 21.5±1.3°C. In December, the daily variation of fishpond temperature was less pronounced, and the weekly average was 14.2± 0.5°C. The average pH of the fish effluent, measured at midday, was 7.34±0.12 in December and 7.48±0.03 in May. The TAN concentration of the fish farm effluent was relatively constant along each experimental week and was about twofold higher in May (52.5±8.6 µmol L^{-1}) than in December (26.0±3.2 µmol L^{-1}). The range of water renewals tested resulted in a range of TAN fluxes that varied from 4 to 135 µmol $L^{-1}h^{-1}$ in December and from 1 to 250 µmol $L^{-1}h^{-1}$ in May.

Both species showed an asymptotic increase of biomass yield with TAN flux (Fig. 1). In December, *A. armata* maximum biomass yield was 71 g DW m^{-2} day^{-1}, whereas *U. rigida* was 44 g DW m^{-2} day^{-1} when supplied with the maximum TAN fluxes tested (weekly average of 100 µmol $L^{-1}h^{-1}$). Both maximum yield values were near the estimated V_{max} of the curves (72 and 52 g DW m^{-2} day^{-1}, respectively; Table 1). The *U. rigida* N content at the time of stocking was 4.98±0.05% DW, and the *A. armata* was 5.90±0.05% DW. At the time of harvesting, the N content slightly increased, with an average of 5.25±0.18% DW for *U. rigida* and 6.04±0.07% DW for *A. armata*.

In May, the biomass yield of both species was significantly higher than it was in December. At mean maximum TAN fluxes of around 200 µmol $L^{-1}h^{-1}$, the biomass yield of *A. armata* was near saturation values, growing 125 g DW m^{-2} day^{-1}, whereas *U. rigida* yield was saturated at much lower TAN fluxes (73 µmol TAN L^{-1} h^{-1}; Fig. 1). The N content of both species was lower in May than it was in December. *U. rigida* had an N content of 4.17±0.03% when it was stocked and *A. armata* had 5.56±0.13%. At the time of harvest, *U. rigida* N content increased in all experimental TAN fluxes to 4.8±0.19%, except at the lowest flux where it decreased to 3.28±0.01%. *A. armata* N content increased to 5.62±0.19%.

In both *U. rigida* and *A. armata*, the two biofiltration estimates, TAN removal and N yield, showed asymptotic responses to TAN fluxes. A direct comparison of the weekly estimates of biofiltration is presented in Fig. 2 and maximum values are shown in Table 1. The *A. armata* biofilter was generally more efficient than *U. rigida* in both seasons and at all TAN fluxes tested. The biofiltration estimated by the N yield integrative approach was always higher than the TAN removal approach, except for *U. rigida* in May (Fig. 2) where some biomass may have been lost through the outflow mesh. In December, the maximum biofiltration rates were similar for both species (Table 1), except in the case of the estimates through the N yield approach which showed that the biofiltration of *A. armata* was 55% higher than *U. rigida*. In May, the biofiltration of *A. armata* was always higher than *U. rigida*. *A. armata* TAN biofiltration was 27% higher than *U. rigida*, and the N yield biofiltration was more than twofold higher (Table 1).

Discussion

The direct comparison between the performance of *A. armata* and *U. rigida* as biofilters for integrated, inland

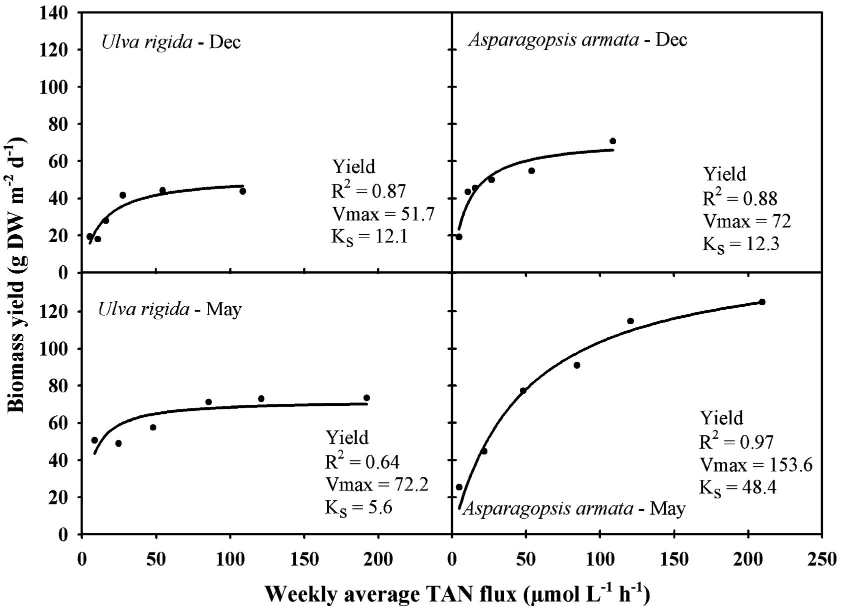

Fig. 1 Effects of TAN flux (weekly average) on the biomass yield of *A. armata* and *U. rigida*. The coefficient of determination of curves (R^2), the maximum biomass yield (V_{max}), and the half saturation constants (K_s) are presented

Table 1 Comparison among the cultivation conditions, biofiltration, and biomass yields of *Ulva* spp. and *A. armata* cultivated in flow-through integrated systems

Species	Tank volume (L)	Stocking density (g FW L^{-1})	Mean water temperature (°C)	Maximum water exchange (vol h^{-1})	Maximum biomass yield (g DW $m^{-2}day^{-1}$)	Daily TAN removal (g $m^{-2}day^{-1}$)	N yield (g $m^{-2}day^{-1}$)	Reference
A. armata	110	5	14.2	4.1	71 (72)	2.8 (3.2)	4.2 (4.1)	This study
U. rigida	110	4	14.2	4.1	44 (51.7)	2.7 (2.9)	2.7 (3.2)	This study
A. armata	110	5	21.5	3.8	125 (153)	6.5 (8.2)	7.4 (9.5)	This study
U. rigida	110	4	21.5	3.8	73 (72.2)	5.1 (6.4)	3.6 (3.9)	This study
U. lactuca	600	1.7	20	0.5	55 (60.3)	3.2 (5.6)	2.3	Cohen and Neori 1991; Neori et al. 1991
U. rigida	750	2.5	24	0.5	40 (–)	2 (2.3)	1.8	Jimenez del Rio et al. 1994, 1996
U. rigida	1900	2	22	0.6	48 (47.9)	1.31 (3.1)	1.45	Mata and Santos 2003

Values between parentheses are the estimated maximum biofiltration values (V_{max})

fish/seaweed aquaculture confirmed the hypothesis in Schuenhoff et al. (2006) that *A. armata* is the most efficient biofilter and productive species. The experiments performed here, at the same time and under the same culture conditions, revealed that the differences are lower than what might be concluded by comparing the values reported in the literature (Table 1), highlighting the importance of this approach.

TAN biofiltration and biomass yield values per surface area obtained for *U. rigida* in this cultivation system were the highest ever reported for *Ulva* spp. cultivated in integrated flow-through fish/seaweed aquaculture (1.6- and 1.3-fold higher, respectively; Table 1). The biomass yield values were also higher than the maximum values recorded for *Ulva* growing in raceway ponds (near 40 g DW per square meter per day) in a recirculating system integrated with abalone (Bolton et al. 2009). The remarkable performances obtained for both species in this system are related with the characteristics of the experimental cultivation tanks. These tanks have translucent walls and a smaller volume compared with the tanks used in integrated flow-through aquaculture systems elsewhere (Table 1), increasing the light exposure surface/culture volume ratio. This higher ratio allowed for an ideal *Ulva* stocking density twofold higher than those used elsewhere (Table 1), thus increasing the rates of nutrient biofiltration and the biomass yield per surface area. Regarding *A. armata*, the exceptional performance obtained confirmed the results previously established in Schuenhoff et al. (2006). In winter, both the maximum TAN removal rates and the biomass yields obtained in the present work were higher than those

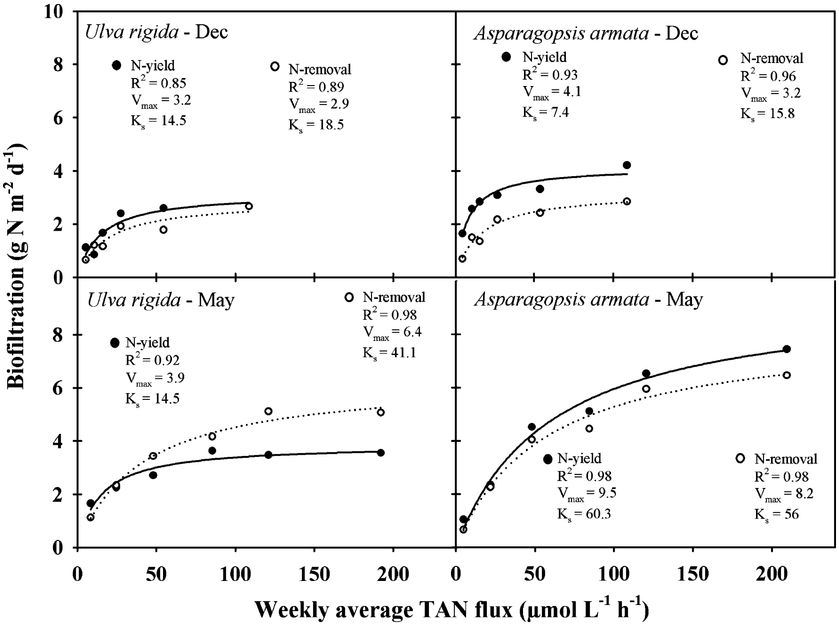

Fig. 2 Effects of TAN flux (weekly average) on biofiltration rates estimated both by TAN removal and N yield (see text). The coefficient of determination of curves (R^2), the maximum biofiltration rates and the maximum N yield (V_{max}), and the half saturation constants (K_s) are presented

obtained by Schuenhoff et al. (2006) due to the higher water renewals used. In flow-through integrated aquaculture systems, it is crucial to use relatively high effluent renewal rates to supply the algae cultures with non-limiting nitrogen and carbon levels. This allows attaining the best performance by species depending almost exclusively on CO_2 from the dissolved inorganic carbon (DIC) pool for their photosynthesis, such as *A. armata* (Mata et al. 2007). *A. armata* in this system needs at least three to four effluent exchanges per hour to maintain daily saturating levels of CO_2 for photosynthesis and biomass production (Mata et al. 2007). *U. rigida* on the other hand, after depleting the CO_2, still has the ability to use HCO_3^- from the DIC pool (Beer 1994). At ~1.5 vol h^{-1}, enough carbon (in both forms) is present in the water to saturate the species' production requirements. This partly explains the different patterns of biomass yield response to the range of water renewals by the two species; from the highest to the lowest water renewals rate, *U. rigida* yield was reduced only by 35%, whereas *A. armata* yield decreased by about 75%.

The two approaches for estimating the effluent TAN biofiltration either based on the TAN removal or on the N yield showed similar patterns, except for *U. rigida* in May when the TAN removal estimates were lower than the N yield estimates. Theoretically, the N yield is a better estimation of biofiltration than the TAN removal derived from the water analysis data because it integrates removal over a week, whereas TAN removal was only assessed in several points in time during that week. However, care must be taken with mechanical fragmentation and washout through the outflow mesh, which may decrease the yield estimate, particularly in the tanks with high water renewal. In fact, this was observed sporadically in our experiments.

This study confirms that *A. armata* is indeed a more efficient TAN biofilter than *U. rigida* and that the biomass yield data are the highest ever reported for open tank cultivated macroalgae (Neori et al. 2004) and microalgae (Chisti 2007). The round-shaped "pom-pom" type morphology of the tetrasporophyte of *A. armata* may also be an advantage for this species as it promotes a constant rollover in the aerated cultures that is not achieved by the thalli of *Ulva*. This increases the frequency of light/dark oscillation, especially in semi-translucent tanks, which is beneficial for photosynthesis (Bidwell et al. 1985; Grobbelaar et al. 1996). Grobbelaar et al. (1996) showed that microalgae productivity can be enhanced as much as six to seven times with increased light/dark frequency, particularly of low-light-acclimated algae, as is the case of *A. armata* within the culture tanks (Mata et al. 2006). The economic feasibility of using such small, translucent tanks in large-scale seaweed cultivation may be questioned. Larger tanks with opaque walls require less hand labor for the maintenance and operational routines (such as the tank cleaning), important to reduce the costs of both effluent biofiltration and biomass production. In such a large-scale system, areal productivity values would probably be closer to the literature values in the case of *U. rigida* (Table 1). In the case of the up-scaling of *A. armata* cultivation, the engineering of larger production tanks should pay special attention to the proper scaling and positioning of the water outflow screens to allow the use of relatively high water renewal rates and prevent the use of artificial CO_2. Both species have high potential to be intensively cultivated in tanks using mariculture effluents as free sources of nutrients and carbon to maximize their production. In fact, the cultivation of both species in the same integrated cultivation system makes sense for southern Portugal where *A. armata* production crashes during summer due to high temperatures (Mata et al. 2006; Schuenhoff et al. 2006). This approach of product diversification will also benefit the farmer. *Ulva* biomass is now gaining a novel interest as a potential source of cell wall polysaccharides (especially ulvan) whose physicochemical and biological properties make them attractive candidates for novel functional and biologically active polymers for the food/feed, pharmaceutical, chemical aquaculture, and agriculture domains (Lahaye and Robic 2007).

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