Optimization of yield and biofiltering efficiencies of *Ulva rigida* C. Ag. cultivated with *Sparus aurata* L. waste waters*

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SUMMARY: *Ulva rigida* C. Ag. was cultivated in 750 l tanks at different densities and flow rates with waste waters from *Sparus aurata* cultivation systems, to estimate the conditions for optimizing biofiltering efficiency and yield. To maximize both characteristics: i) seaweed density should be adjusted to 2.7 g FW l⁻¹ (1.2 kg m⁻³) and ii) *NH₄*-nitrogen flow should be (at least) 150 mmol m⁻² d⁻¹. Such conditions will yield 27 g DW m⁻² d⁻¹ with uptake efficiencies of 76 % of the daily input in our system at 12 volumes d⁻¹ (110 mmol m⁻² d⁻¹) at seaweed density; fish biomass ratio of 1:4. *NH₄* removal efficiencies would probably be increased if fish were fed as early as possible in the morning to provide maximum *NH₄* concentrations during the light period, as uptake efficiencies are higher than in the dark.

**Keywords:** *Ulva rigida*, *Sparus aurata*, biofiltering, ammonia uptake, aquaculture

INTRODUCTION

Waste waters resulting from intensive fish cultivation systems contain large amounts of dissolved metabolites, mostly ammonia, generated from excretory products and bacterial ammonification of organic matter (Krom et al., 1985a; Porter et al., 1987; Krom and Van Run, 1989). Ammonia can increase to toxic levels, up to 450 µM, in fishponds with low or intermediate water exchange rates (Porter et al., 1987; Krom et al., 1989), producing
hypereutrophic conditions due to the fertilizing effects on marine phytoplankton (Krom et al., 1985a; 1985b; Neori et al., 1989). From the point of view of minimum environmental impact, recycling or recirculation systems of aquaculture would be ideal, as there would be only reduced discharges into open water bodies. The development of such systems requires the removal of solid compounds and dissolved metabolites contained in outflowing water. Solids can be easily removed by filtration or other mechanical processes but removal of dissolved metabolites requires more complex and expensive processes.

The use of seaweeds as biofilters to remove dissolved nitrogen from fish pond effluent has been reported previously (De Boer and Ryther, 1977; Fralick, 1979). Species of the genus Gracilaria and Ulva have been tested, showing high growth rates and high ammonia removal efficiencies (Harlin et al., 1979).

Ulva species not only show a higher N-removal capacity than Gracilaria but also a higher resistance to epiphytes. Ammonia removal efficiencies of up to 85%, independently of light or temperature fluctuations, were obtained with Ulva lactuca L., cultivated with effluents of the marine fish Sparus aurata at water exchange rates of 8 volumes per day and NH₄⁺ concentrations of 10-20 µmol l⁻¹ h⁻¹ (van der Meulen and Gordin, 1990). Sustained Ulva yields over 30 g DW m⁻² d⁻¹ have been reported by several authors (Vandermeulen and Gordin, 1990; Neori et al., 1991).

Seaweed stocking densities, water exchange (NH₄⁺-nitrogen flow through tanks) and fish:seaweed biomass rates are key factors affecting growth rate, yield and biofiltering efficiency. Those variables are usually not clearly stated in the literature. Different optimum densities for maximum yield in tank cultures have been given for different species (Ulva = 0.8 kg FW m⁻², Lapointe and Tenore, 1981; Gracilaria = 2.3 kg FW m⁻², Lapointe and Ryther, 1978). However, higher stocking densities than those given for maximal yield in Ulva have been recently reported for maximal ammonium uptake and tissue nitrogen content (Neori et al., 1991).

The present study was conducted to determine the interaction between NH₄⁺ flow, seaweed density and seaweed:fish biomass ratio in a co-culture system, to maximize biofiltering efficiencies and seaweed yield.

MATERIAL AND METHODS

Plant material and culture conditions

Plants of Ulva rigida collected from the east coast of the island of Gran Canaria (Canary Islands, Spain) were precultivated for one month under continuous wastewater flow. Necrotic or epiphytized plants were removed during this period. Preculture and experiments were conducted under greenhouse conditions in 750 l (1.75 m³) semicircular tanks 80 cm deep. Algae were grown suspended in the water column with the aid of air diffusers located at the bottom of the tank.

Fishpond effluent was pumped from a 1 m³ reservoir tank (washout of sediments every two weeks) connected to the output of six 1 m³ tanks with approximately 12 kg of Sparus aurata in each. The water exchange rate was 8-10 vol d⁻¹ per seaweed tank. Water passed through seaweed tanks was directly released to the sea.

Noon average PFD during experiments was 710 ± 80 µmol m⁻² s⁻¹, with a daily water temperature fluctuation of 25.6 °C (at noon) to 19.9 °C (during the night). Differences in temperature between tanks did not exceed 0.5 °C in each experimental set.

Experimental design

Three sets of experiments (1, 2 and 3, Table 1) each in triplicate were conducted during a two week period to estimate the effects of: i) stocking densities (1, 2 and 4 g FW⁻¹ l⁻¹) and ii) wastewater exchange rates (4, 8 and 12 vol d⁻¹) on: i) NH₄⁺-removal efficiency (N-efficiency) and ii) seaweed yield. Two control experiments were carried out simultaneously to estimate i) yield in tanks under continuous flow of seawater with weekly pulse-feeding (exp.t 4) and ii) NH₄⁺ losses of aerated control tanks (exp. 5). In experiment 4, water supply was turned off once a week and tanks were supplied with ammonium chloride and sodium orthophosphate to a final concentration of 2.0 mM NH₄⁺ and 0.15 mM PO₄³⁻ (following the concentrations used in tank cultures of Ulva lactuca by DeBusk et al., 1986). After 24 h, the water supply was turned on and adjusted to a flow rate of 8 vol d⁻¹.

Inflow and outflow of NH₄⁺ were measured as follows: i) every three hours (24 h period) the day after algae were re-stocked to their initial density and, ii) at 21:00 (daily PFD), temperature and pH values were taken simultaneously.
TABLE 1.- Effect of stocking density and NH₄⁺ flow on ammonia removal efficiencies, growth rate and yield of Ulva rigida.

<table>
<thead>
<tr>
<th>Exp. n°</th>
<th>Esch. rate (d⁻¹)</th>
<th>Density (kg FW · m⁻²)</th>
<th>NH₄⁺ inflow (mmol m⁻² d⁻¹) ± SE</th>
<th>NH₄⁺ outflow (mmol m⁻² d⁻¹) ± SE</th>
<th>NUR (%) ± SE</th>
<th>NUE (%) ± SE</th>
<th>Growth rate (g FW m⁻² d⁻¹) ± SE</th>
<th>Yield (g DW m⁻² d⁻¹) ± SE</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>48.2±3.4</td>
<td>19.3±1.4</td>
<td>28.9±2.0</td>
<td>7.2±0.3</td>
<td>40.9±2.8</td>
<td>85.0</td>
<td>92.9</td>
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<td>2</td>
<td>4</td>
<td>48.2±3.4</td>
<td>19.3±1.4</td>
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<tr>
<td>3</td>
<td>4</td>
<td>51.4±2.0</td>
<td>20.6±0.8</td>
<td>30.8±1.3</td>
<td>3.4±0.3</td>
<td>47.9±1.9</td>
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<td>8</td>
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<td>40.0±1.7</td>
<td>58.3±2.5</td>
<td>29.8±2.4</td>
<td>68.7±2.4</td>
<td>96.6</td>
<td>78.4</td>
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<td>107.4±3.0</td>
<td>43.0±1.2</td>
<td>64.6±1.9</td>
<td>20.9±1.4</td>
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<td>7</td>
<td>12</td>
<td>154.6±3.9</td>
<td>68.5±1.6</td>
<td>86.1±2.3</td>
<td>44.7±1.5</td>
<td>110.2±2.8</td>
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<td>157.7±2.6</td>
<td>69.8±0.9</td>
<td>88.8±1.5</td>
<td>37.5±1.0</td>
<td>120.7±2.3</td>
<td>76.6</td>
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L* = light period; D* = dark period; ± = % of the total NH₄⁺ (100%) which enters the system during the respective periods.

Harvesting and growth rate determination

*Ulva rigida* plants were harvested weekly, spun at 2,800 rpm in a domestic clothes centrifuge (Miele WZ268) for 30 s and re-stocked to the initial density. Specific growth rates (µ) were calculated according to the equation (µ = 100 ln(W/W₀)/t) by Delia and Deboer (1978), where W₀ = initial fresh weight; W = final fresh weight; and t = time (days). A sample of 10 g was removed and dried at 80 °C to constant weight to obtain the wet weight/dry weight ratios. Yields were calculated according to the equation (Y = (Nₑ⁻N₀)/t + (DW/FW) / A) by Deboer and Rytter (1977), where N₀ = initial fresh weight, Nₑ = final fresh weight and A = area in m².

Analytical measurements

Ammonia analysis was carried out using a Flow Injector Analyzer (Fluorastar 5010 Analyzer, Tecator, Sweden), through the gas diffusion method (Ronnestad and Knutson, 1991). PFD was measured with a LI-COR LI-1000 Datalogger using a spherical quantum sensor to estimate the decrease of PFD into the tanks, at half depth (40 cm) and at the bottom (80 cm). A plane quantum sensor was used to measure the irradiation just above the surface of the tanks. The pH and temperature values were measured with a HI 9025 microcomputer pH meter (Hanna Instruments).

Nitrogen uptake efficiencies (NUE) shows the average reduction (%) in ammonia concentration. Nitrogen uptake rate (NUR) shows the amount of ammonium removed per unit of time. The light period for the calculation of NUE, NUR and NUR per unit of biomass was from sunrise (7:30) to sunset (20:30).

RESULTS

Stocking density

According to the results of several authors for *Ulva* spp. (Lapointe and Tenore, 1981), maximum *Ulva rigida* yields were obtained at an initial stocking density of 2 g FW m⁻² l⁻¹ (0.86 kg FW m⁻³), with declining yields at both lower and higher stocking densities at any water exchange rate (Table 1). Densities of 1, 2 and 4 g FW m⁻² l⁻¹ reduced PFD by 58.3, 75.6 and 95.4% at half depth of the tank (40 cm) and by 62.2, 93.3 and 100% at the bottom of the tank (80 cm), respectively.

As observed from Fig. 1 B, increments in stocking densities can also be related to reductions in inorganic carbon concentrations or high pH, caused by photosynthetic carbon assimilation, specially in low water exchange rate culture tanks.

Growth rate and Yield

Both the growth rate and yield at 12 vol d⁻¹ were twice those observed at 4 vol d⁻¹ at all densities.
Effect of water exchange rates

One of the main effects of the increase in water exchange rates were the increment in the amount of NH$_4^+$ and inorganic carbon provided to the plants per unit of time. Flow rates of 4 and 8 vol d$^{-1}$ produced strong fluctuations in pH at densities of 2 and 4 g FW$^{-1}$ l$^{-1}$, higher water exchange rates (12 vol d$^{-1}$) had a stabilizing effect on pH at all densities (Fig. 1B) and on temperature.

Ammonia levels in the fishpond effluent showed marked daily oscillations, with maximum concentrations during the “dark” period and minimum during the “light” period (Fig. 1A). Daily NH$_4^+$ flow was quite stable during the experimental period. NH$_4^+$ losses due to the system (by water recirculation and aeration of tanks without seaweed) were insignificant (less than 1.5%).

NUE were inversely related to water exchange rates and directly related with stocking density (Table 1). However NUR were directly related to

(Tables 1). However, growth rate and yield at 8 and 12 vol d$^{-1}$ were quite similar. Similar growth rates and yields were obtained between weekly-pulsed cultures with continuous flow of wastewater (Table 1). Disintegration of the plants and decrease in weight were observed in running through seawater tanks (non-fertilized cultures, data not shown).
both flow rate and stocking densities (Table 1). NUE during the light period were higher than during the dark period (Table 1). However, non significant differences in NUE (p > 0.05 ANOVA) were detected between light and dark periods at lowest water exchange rates (4 and 8 vol d⁻¹) (Fig.2). At higher ammonia flows (achieved by higher water exchange rates) NUE were higher during the light period at all densities (p < 0.05 ANOVA) (Fig.2). 

NUE per unit of biomass was inversely related to density, both in dark and light periods (Fig. 3). Nitrogen uptake capacity by Ulva rigida plants in the light period seems to become saturated at NH₄⁺ flow rates of 18.8 µmol NH₄⁺ g FW⁻¹ h⁻¹. However, during the dark period plants are nearly saturated at 11.8 µmol NH₄⁺ g FW⁻¹ h⁻¹ (Fig.3). Increments in yield shows a very high correlation (α = 0.9999) with increments in NUE per unit of biomass per day.

DISCUSSION

Daily ammonia oscillations are due to the fish feeding period (between 9:00 and 14:00 h) which produce a peak of ammonia 7 hours later (Fig. 1A) due to fish excretion and microbial degradation of leftover food. Differences in the timing of peak ammonia levels reported by PORTER et al., (1987) (4.5 hours after feeding) might be related to differences in fish age and feed.

The decrease in growth rate by the increase in density in our cultures is probably due to self-shading according to DUKE et al., (1986); (1989a); LAPointe and TENORE, (1981), but may also depend on carbon limitation according to DEBUSK et al., (1986).

Even though inorganic carbon concentrations in wastewater inflow have been increased by heterotrophic breakdown of dissolved organic substances (KROM and NEORI, 1989) and fish respiration (PIEDRAHITA, 1990) which results in a decrease of the pH, our results indicate that cultures over 2 g FW l⁻¹ (0.86 kg m⁻³) might be carbon limited (Fig. 1B). At 15:00 hours, the rise in pH, especially with lower exchange rate (4 vol d⁻¹) indicate that the buffering capacity of the carbonate system is decreasing, and photosynthesis causes the pH rise. According to VANDERMEULEN and GORDIN (1990), the higher pH values recorded under such conditions would indicate carbon limitation. Moreover, yield doubling (at the three densities), when doubling (8 vol d⁻¹) or tripling (12 vol d⁻¹) exchange rates (Table 1), fits well with similar increases in the amount of carbon provided to the plants per unit of time. The similar pH values of 1 g FW l⁻¹ at 4 water exchanges per day and 4 g FW l⁻¹ at 12 vol d⁻¹ (Fig. 1) shows that photosynthetically assimilable carbon can be maintained at the same levels at four fold higher densities by a three fold increase in flow rate, which in turn produces almost 2.5 times more biomass and 3 times more NUR (Fig.3). However, NUE are lower than at 1 g FW l⁻¹ and 4 vol d⁻¹ (Table 1).

NUE are similar to those described by VANDERMEULEN and GORDIN (1990) and COHEN and NEORI (1991) for Ulva lactuca. The higher yields and NUR obtained by COHEN and NEORI (1991) at similar ammonia fluxes probably depends on the higher (almost double) irradiation in their experiments. Regarding the variable to maximize, efforts to maximize NUE and NUR values in biofiltering experiments resemble growth rate and yield values applied to production. Considerations based on only NUE will lead to wrong conclusions on its application as a biofilter (as growth rate on biomass production) (Figs. 2 and 3).

The maximum yields obtained at 2 g FW l⁻¹ (0.86 kg FW m⁻³) at any exchange rate, are in accordance with the optimum density to maximize production in Ulva described by LAPointe and TENORE (1981) and RYTHER et al.,(1984). The absence of correlation between yield and NUR might be explained by: i) a higher excretion of organic matter promted by the stress (HELEBRAND, 1974) of highly dense cultures, or ii) a lower C:N ratio of the biomass at highy
dense cultures (as reported by Duke et al., 1989b; Vandermeulen and Gordin, 1990; Cohen and Neori, 1991). From these results, and in accordance with Duke et al. (1986; 1989a) it seems evident that ammonium uptake by Ulva is less limited by light than growth. However, the conclusion is just the opposite when analysing the data per unit of biomass in light and dark periods (discussed below).

Contrary to results obtained by Vandermeulen and Gordin (1990) (same NUE during day and night periods) our data shows differences in NUE during light and dark periods (Table 1). These apparent contradictory results might be explained by the low ammonia flow in the experiments of those authors. In agreement with the data reported by Cohen and Neori (1991) with Ulva lactuca, differences in NUE during light and dark periods are shown at high ammonia fluxes.

Higher NUR during dark period at low water exchange rates (4 vol d⁻¹) (Fig. 2) might be explained by the lower NH₄⁺ fluxes during the light period. Higher NUR in light at the highest water exchange rates (12 vol d⁻¹) at all densities (Fig. 2) can be explained by the increase in ammonia (achieved by higher water exchange rates) and inorganic carbon flows. Similarities of NUR (and NUR per unit of biomass) in the dark at 12 and 8 vol d⁻¹ (Figs. 2 and 3) indicate that dark cultures are N-saturated at 8 exchange rates (107 mmol m⁻² d⁻¹), but not light cultures at any density. From Fig. 3, it seems that N-saturating flow rate in light lies around 18.4 μmol NH₄⁺ g FW⁻¹ h⁻¹. As the N-saturating threshold flow per unit of biomass varies significantly between the three densities (higher uptakes in plants grown at lower densities = lower self-shading) (Fig. 3), clearly shows that light has a strong influence in N-efficiency of the plant, masked by opposite efficiencies of the system (Table 1).

Harlin et al. (1979) used Gracilaria sp to remove the ammonium produced by the fish Fundulus heteroclitus, removing lightly more ammonium (30 - 54 mmol kg⁻¹ FW d⁻¹) than produced by an equal biomass of fish. Similar results were reported by Haglund and Pedersen (1993) in a co-culture system of Gracilaria teniustiophila Zang et Xia with rainbow trout. Daily NH₄⁺ uptake rates of 40 mmol kg⁻¹ FW d⁻¹ were obtained at a seaweed:fish biomass ratio of 1:1. Our results, indicate that at least two times higher (91.6 mmol kg⁻¹ FW d⁻¹) uptake rate efficiencies than that of Gracilaria teniustiophila can be obtained at four times higher fish concentration.

To maximize biofiltration efficiency of the wastewater from Sparus aurata hatcheries and seaweed yield, the fish should be fed as early in the morning as possible (in order to provide maximum NH₄⁺ concentrations during the light period), the density of seaweeds should be adjusted to 2.7 g FW⁻¹ (1.2 kg FW m⁻²) and water exchange rates should be (at least) 12 vol d⁻¹ (150 mmol m⁻² d⁻¹). Such conditions (at a seaweed:fish biomass ratio of :1:4 in our experimental system), will yield 27 g DW m⁻² d⁻¹ and a daily ammonia uptake rate of 110 mmol m⁻² d⁻¹ (76 % of the daily input at 12 vol d⁻¹).

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