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Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks

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Abstract The effects of 16 different combinations of nutrient load and agitation on yield, nutrient uptake and proximate chemical composition of the seaweed *Ulva lactuca* cultured in tanks were evaluated. Intensive fishpond outflow passed through seaweed tanks at four nutrient loading levels and four water agitation combinations of water exchange, bottom aeration and frequently changing water levels (an accelerated tide regime). Specific results from these outdoor experiments were examined further under controlled conditions in laboratory experiments. Agitation treatments affected the performance of *U. lactuca* only under TAN ($NH_3 + NH_4^+$) load levels below 4 g N m^{-2} day^{-1}; biofiltration of TAN was the parameter most affected. Biomass yields at each of the four nutrient loading levels were not significantly different between the agitation treatments. Protein content increased significantly with increasing nutrient loading. The agitation treatments had a slight effect on seaweed protein content only at the lowest nutrient loading levels. There were no significant differences in dissolved oxygen concentration, pH, and temperature among the agitation treatments at all nutrient loading levels. Under laboratory conditions, growth rates, protein content, and photosynthetic and biomass yield of the seaweed were affected by water velocity under low nutrient concentrations. It is concluded that the effect of air agitation under the conditions of these experiments was not directly related to photosynthesis, excess dissolved oxygen, or carbon limitation, but to the diffusion of macro nutrients from the water to the seaweed. Therefore, once nutrient concentrations are high enough (above about 4 μM of TAN with the other nutrients in their corresponding proportions), aeration per se is not essential for effective growth and biofiltration by seaweeds.

Keywords Seaweed biofiltration · Water movement · Agitation treatment · Fishpond effluents · Nutrient uptake · Yield

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Introduction

Seaweeds remove nutrients from effluent water in mariculture operations. Such so-called biofilters have been applied successfully in Israel, the USA and elsewhere (Neori et al. 1996; reviewed in Neori et al. 2004). At the same time, the seaweed biomass created can be sold to feed mills and pet shops or used to feed marine herbivores such as gastropods and echinoderms (Shpigel et al. 1999; Boarder and Shpigel 2001; Evans and Langdon 2000; Troell et al. 2006).

In modern seaweed ponds, bottom aeration is often used to stir the suspended seaweed (Huguenin 1976; reviewed in Neori et al. 2004). This successful approach to seaweed cultivation was advanced by Ryther and co-workers in the 1970s and described later by Huguenin (1976), Lapointe and Ryther (1978, 1979), Bidwell et al. (1985), DeBusk et al. (1986), Bird (1989) and Craigie and Shacklock (1995). This technology uses bottom aeration to agitate suspended seaweed in tanks and ponds as nutrient-rich water passes through them. It has been proposed (reviewed in Neori et al. 2004) that bubble-generated vertical movement of an optimally stocked seaweed pond exposes each algal frond

to optimal light (Vergara et al. 1998). The turbulence generated by the aeration also thins the diffusive boundary layer (DBL) around the frond surfaces, speeding the inflow of nutrients to the fronds and removal of excess oxygen from them, a feature that has been shown to minimize photorespiratory losses in the red alga *Porphyra yezoensis* (Gao et al. 1992).

Aeration has accounted for much of the running cost in pond seaweed cultivation since the early 1970s when the first experiments were conducted (Huguenin 1976), and today is even more significant due to the rise in electricity prices. In addition, use of aeration requires costly infrastructure to supply electrical power and drive the air. These limitations hamper the development of seaweed ponds, seaweed biofilters, and the cost-effective commercial application of this technology in less developed countries.

The present study was undertaken to clarify the issue of aeration and define conditions under which it can be dispensed with. It was hypothesized, based on preliminary observations, that the main effect of agitation by air was on nutrient diffusion across the DBL and therefore, the requirement for aeration could be reduced using high nutrient concentrations in the seaweed tanks.

Materials and methods

Experimental design—outdoor experiments

Outdoor tanks (DOLAV, Israel), installed as described in Vandermeulen (1989), with surface areas of 1 m^2 and water depth of 0.6 m (600 L water volume) were used to culture the seaweed *Ulva lactuca* (identified twice, in Vandermeulen and Gordin 1990 and in Israel et al. 1995) obtained from existing tank cultures. Plastic pipes (5 cm diameter, 50 cm long) were installed as outflow at the top of each tank. Netting material (mesh 5 mm) was placed on the tank outflow to prevent loss of the seaweed from the tank. Seaweed stocking density was 1 kg m^{-2}. The tanks and outflow pipes were brushed clean whenever necessary. The length of each experiment (2–5 weeks) was determined by the availability of suitable fishpond effluents. Four treatments were compared in each experiment in triplicate as follows:

No aeration (N): *Ulva lactuca* was cultured without any kind of aeration in the outdoor tanks. Water movement occurred through water exchange (34 volumes day^{-1} for all tanks), which had to be high to agitate the water, wash out dissolved organic matter, and increase the frequency of the simulated tide (tidal simulation treatment below).

Tide (tidal simulation, S): Simulation of the rise and fall of the tide in the sea was provided using a simple siphon. Outflow U-shaped pipes were placed vertically, extending inside the tanks from the top of the water down to 10 cm above the floor and outside down to the drain below the bottom. Water entered the tanks through taps and filled the tanks. On rising above the outlet pipes, water siphoned out of the tanks, dropping the level of the water and the suspended seaweed in them to 10 from 60 cm. When the lower parts of the inside pipes were exposed, air broke the siphon, the outflow stopped and water level started to rise again. While the seaweed fronds remained suspended in the water all the time, the rise and fall of the water level induced water movement that stirred the seaweed more than in treatment 1. It took 30 min to refill the tanks and 18 min to empty them, yielding a much higher frequency of tidal exchange than in nature, i.e., twice per day.

Tide and aeration (SA): The tidal simulation (S) was combined with aeration (A) and water exchange to form a combined tidal simulation–aeration (SA) culture regime. Water motion was generated by water exchange, tidal simulation by water level change, and bottom aeration through thin perforated pipes. This experiment was aimed at testing whether the addition of aeration to the tidal simulation would change the performance of the seaweed biofilter compared with the tidal simulation alone.

Aeration (A): The water was agitated by bottom aeration and water exchange, which is a standard regime used in culturing seaweed in tanks and ponds (Vandermeulen 1989).

Nutrient load levels

Each tank received 20.4 m^3 day^{-1} (0.85 m^3 h^{-1}) of fishpond effluent. The four levels of TAN ($NH_3 + NH_4^+$) loading were defined as "very high" (38 g N m^{-2} day^{-1}, ~134 μM), "high" (7 g N m^{-2} day^{-1}, ~26 μM), "low" (4 g N m^{-2} day^{-1}, ~20 μM) and "very low" (1 g N m^{-2} day^{-1}, ~7 μM). The lower three nutrient load levels were achieved by dilution of the fishpond effluent with clean seawater in an intermediate mixing tank. The low nutrient load level equals the optimum ammonia-N load level in Cohen and Neori (1991) and Neori et al. (2004).

Indoor experiments—experimental design

Indoor culture room experiments were undertaken to examine the influence of water velocity on seaweed growth, photosynthesis, and nutrient uptake under controlled conditions.

Whole thalli of *U. lactuca* that were healthy and large enough to be attached were selected and pre-acclimated in aerated low nutrient seawater indoors for 24 h. For nutrient biofiltration and growth experiments, the thalli were attached

to plastic-coated stainless steel rings with a diameter of 15.6 cm and made of two intertwined wires; the thalli were inserted between the two wires. The steel wire used was flexible enough that, if bent, the rings would return to their original shape. This was necessary for insertion of the rings into culture flasks (3 L, 17 cm diameter, Fernbach). Approximately 60 thalli (11 g, Ni et al. 1999) 8.5 cm in length were used in each treatment, 20 being attached to each of three rings, which were inserted into three separate flasks. Two flasks with no seaweed were used to check nutrient mineralization resulting from bacterial breakdown of fish-excreted, dissolved organic matter.

For the 6-h photosynthesis experiments, 16 thalli were attached to glass slabs previously fixed to transparent plastic rectangular 5-L containers, 8 thalli for each nutrient treatment. The process of attaching the thalli took about 10 min.

Water movement was generated by a custom-made shaker (Ari J. Levi, Israel) at speeds of 0, 40, 50, and 70 revolutions min^{-1} that produced velocities of 0, 1.5, 3.7, and 27.5 cm s^{-1} respectively. The velocities were measured using a small styrofoam particle to determine the time needed for one revolution in the flask (Phang et al. 2000). One velocity was studied in each experimental run. In the 2-week-long growth experiments indoors, water velocities studied were 0, 1.5 and 27.5 cm s^{-1}.

Effluent of an intensive fishpond was used as the nutrient source. Concentrations of 136±21 μM TAN, 79±20 μM phosphate, and 1,055±338 μM ToxN (nitrate + nitrite) were defined for purposes of the indoor experiments as "high concentration," while 31±6 μM TAN, 20±6 μM phosphate, and 271±107 μM ToxN (one part fishpond effluent in three parts clean seawater) was considered as "low concentration." This ratio was reached after conducting preliminary experiments to decide the desired low concentration. The high ToxN levels relative to TAN were the result of the nitrification biofilter that operated in the fishpond that provided water for the experiments. Light (~80 μmol photons m^{-2} s^{-1}) was supplied by four fluorescence bulbs (Osram L 36 W/10) suspended above the flasks. Nutrient concentrations in the effluent differed slightly from day to day.

All indoor experiments were conducted at 25 ±1°C.

Sample collection and analysis

In the outdoor experiments, water samples for nutrient analysis were collected twice a week at 08:00 and 14:00 h at the inflow and outflow of each tank using 250-mL sampling bottles. The water was then filtered through GF/C Whatman filters. In the laboratory experiments, 30 mL of water was taken using syringes at 30-min intervals over 4 h. Nutrient analysis for TAN, ToxN, and phosphate was done with a Technicon Autoanalyser II (Krom et al. 1985). Biofiltration rates and efficiencies were calculated from the difference in nutrient concentrations between the inflow and respective outflow according to Cohen and Neori (1991).

Seaweed growth rate and biomass yield

In the outdoor experiments, the seaweed was harvested once a week using a converted washing machine as a centrifuge (Vandermeulen and Gordin 1990) for 3 min to remove surface water. The seaweed was then weighed and 1 kg was removed for restocking of the culture tanks. In the indoor experiments, the seaweed was shaken, blotted and weighed. Specific growth rate (SGR, %) was calculated as:

$$\mathrm{SGR} = 100 \times [\ln (w_t/w_o)]/t$$

where w_o is the initial biomass and w_t is the biomass after t culture days. Biomass yield (fresh weight) was calculated as the difference between initial and final weights and expressed in units of g m^{-2} day^{-1}, based on the areas of the culture vessels, i.e., 1 m^2 for tanks and 0.0199 m^2 for the Fernbach flasks. In order to compare the results with the outdoor experiments, biomass yield of the indoor experiments was also expressed per unit area of the culture vessel (Smith and Walker 1980).

Protein, phosphorus and ash contents of the seaweed

Analysis was carried out using 150-g seaweed samples taken from harvested seaweed that had been rinsed with fresh water. Samples were oven-dried after washing at 60°C for 24 h and then dried at 105°C to constant weight. Protein contents were analyzed using the Kjeldahl digestion method according to Blaedel and Meloche (1957) and multiplied by 6.25 to reflect the N content in protein (i.e., 16%; Msuya 2004). For total P content, seaweed samples were pulverized and 500 mg was mixed with 9 mL 65% nitric acid and 2 mL HCl in 120 mL Teflon PFA digestion vessels. Samples and blanks were prepared for analysis by microwave-assisted digestion (10 min at 500 W and another 10 min at 580 W). Liquid residues were supplemented with deionized water to a final volume of 25 mL. Analyses were conducted on portions of the solutions and compared with certified standards by inductively coupled plasma atomic emission spectrometry (ICP-AES, Spectro Analytical Instruments, Germany), equipped with cross-flow nebulizers (precision: <2%; accuracy: <5%). Dry matter was calculated by weight loss after drying at 105°C for 24 h, and ash content was calculated from weight remaining after incineration of the dry samples for 24 h at 550°C in a muffle furnace.

Basic parameters

Oxygen concentration and temperature of the water in the tanks were measured (OxyGuard Handy Mk III, Denmark)

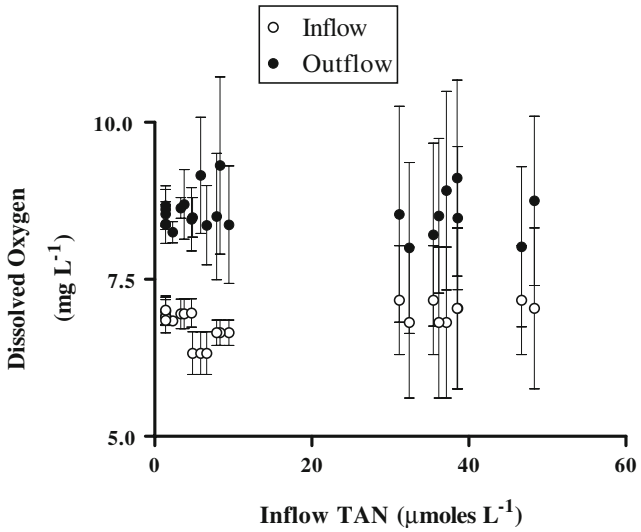


Fig. 1 Averages of dissolved oxygen concentration (mg L^{-1}; mean ± SD) of the water vs. TAN load level (μmoles L^{-1}) for all treatments pooled together under all TAN loading levels

along with pH (HANNA Instruments, Canada, model no. HI 8424) twice a week at 08:30 and 14:00 h. Water flow was measured daily using 5-L beakers and adjusted when necessary. Biochemical oxygen demand (BOD_5) of the water was measured every 5 days with an OxiTop (IS12) 12-position automatic BOD system.

Photosynthetic performance

The photosynthesis of the seaweed was measured in indoor experiments by a pulse amplitude-modulated (PAM) fluorometer (Walz, Germany) as described in Beer et al. (1998). Effective quantum yields of photosystem II (Y) were measured at eight different irradiances in eight thalli for both low and high nutrient concentrations, each thallus being measured three times. Y was measured while subjecting the seaweed to 60-min intervals of no motion alternating with 60-min intervals of shaking at a designated velocity of 1.5, 3.7 or 27 cm s^{-1} for 6 h. Y was calculated as:

$$Y = (Fm' - F)/Fm'$$

where Fm′ is the maximum fluorescence yield when reaction centres are closed (i.e., reduced) and F is the fluorescence yield at a given irradiance. The first set of readings was taken with no water motion immediately after adding nutrients, and the shaker was turned on. The second set of readings was taken at the end of the first 60-min shaking interval, followed by a third set of readings after a second 60-min interval of no motion. This reading regime was repeated several times as necessary. The process was repeated with each of the eight thalli at low and high nutrient concentrations at the three designated water velocities; the fluorescence data measured at the velocity of 27 cm s^{-1} were erratic.

Data analysis

Data were analyzed by ANOVA to determine the statistically significant effect of the different treatments. The Pearson correlation coefficient and regression analyses were used to test relationships between variables that were assumed to have direct effects on each other from the biotic and abiotic variables measured. Data that were measured as percentages that were out of the range of 30–70% were arcsine transformed to ensure normality before statistical tests were performed. The data were analyzed using SPSS and XLSTAT and plotted using GraphPad Prism 3.

Results

Temperature of the inflow water during the entire experimental period varied between 21 and 25°C, and the outflow temperature remained within a degree of these values in all treatments. There was no significant variability in temperature across all sampling points in each experiment at any time ($P>0.05$). The temperature variation throughout the day was less than 3°C on any day.

Dissolved oxygen (DO) concentration in the inflow water averaged about 7 mg L^{-1} and outflow water DO during the day averaged 8–9 mg L^{-1} in all the experiments and treatments (Fig. 1). Passing through the seaweed increased DO concentration in all treatments under very high (ANOVA, $P<0.05$), high, low, and very low nutrient loading levels ($P<0.001$). However, DO levels in the outflow of the tanks were similar in all the agitation treatments under each specific nutrient loading experiment

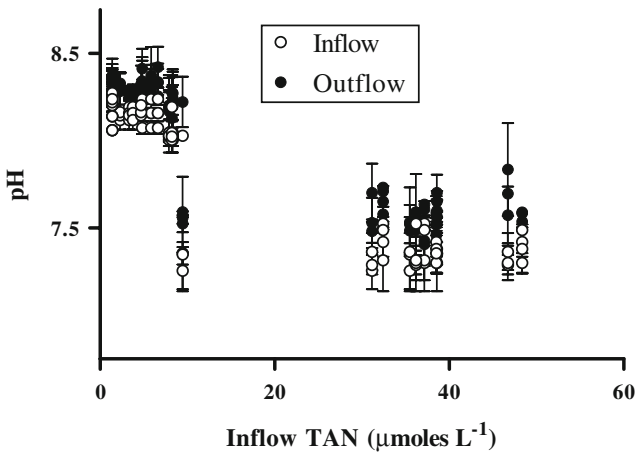


Fig. 2 Averages of pH values in the water vs. TAN load level (μmoles L^{-1}; mean ± SD) for all treatments pooled together under all TAN loading levels

Table 1 BOD_5 values (mg L^{-1}; mean ± SD) in tanks stocked with the seaweed *Ulva lactuca*, cultured under four nutrient loading levels and four agitation regimes

Treatment	Nutrient load level (g N m^{-2} day^{-1})			
	Very low (1.4)(n=4)	Low (3.68±1.00) (n=4)	High (7.31±1.61) (n=4)	Very high (38.32±5.84) (n=6)
Inflow	7.0±0.3	6.0±0.1	8.0±0.0	9.0±0.0
Outflow				
N	7.0±0.8	4.0±1.3	8.4±0.6	8.0±2.6
S	6.0±0.0	4.0±1.1	7.4±0.6	9.0±1.7
SA	7.0±0.6	7.0±0.4	7.8±0.4	8.3±1.2
A	8.0±0.8	6.0±0.9	8.2±1.1	9.0±1.0

Agitation regimes: *N* water exchange only, *S* tidal simulation, *SA* a combination of tidal simulation and aeration, and *A* aeration only

(P>0.05). DO concentration of the water increased by 1.8 to 2.7 mg L^{-1} between the inflow and outflow under the four nutrient loading levels. DO concentrations in the outflow in all experiments and treatments were independent of TAN loading level (Fig. 1). The variability in DO levels in the inflow water from day to day was smaller in the lower TAN loading levels, where most of the inflow consisted of dilution water that came directly from the temperature-stable sea.

As the water passed through the tanks, seaweed photosynthesis significantly raised the pH in the outflow water in all treatments under each individual nutrient-loading experiment; however, as with DO, the values in all four treatments within each nutrient-loading experiment were similar (Fig. 2; P>0.05). The seaweed raised the pH of the water by 0.1 to 0.4 pH units, which is a known effect of photosynthetic activity. The pH levels in the outflow were highest in the afternoon (mostly above pH 7.5 compared with ≤pH 7.5 in the morning). There was a higher pH variation under the very high TAN loading level compared to the lower TAN loading levels. As with DO, the variability in pH levels in the inflow water from day to day was smaller in the lower TAN loading levels.

Biochemical oxygen demand measured as BOD_5 (Table 1) was low (between 4 and 9 mg L^{-1}) at the inflow and outflow of the respective treatments, under all nutrient loading levels and all agitation regimes. BOD levels did not differ statistically between the inflow and the outflow of all the treatments and among the outflows of all the treatments (P>0.05).

Biomass yield of *Ulva lactuca* in the outdoor tanks depended on nutrient load far more than on the agitation treatment. Biomass yield (Table 2) was higher under the two highest nutrient loading levels and lower under the remaining ones. However, agitation treatments under each individual nutrient loading level had no influence on seaweed biomass yield. Still, the ANOVA P values between treatments (not shown) were smaller at the two lower nutrient loading levels than at the two higher ones, suggesting the effect of agitation type on the biomass yield was increasing as the seaweed was becoming nutrient-limited.

As water velocity increased in the indoor experiments to 27.5 from 1.5 cm s^{-1}, biomass yield increased by about 2.5× under low nutrient concentration (ANOVA, P=0.003) and by about 1.8× under high nutrient concentration (ANOVA, P=0.002) (Table 3). Biomass yield correlated

Table 2 Effect of agitation regime and nutrient loading level on biomass yield (g m^{-2} day^{-1}; mean ± SD) in the seaweed *Ulva lactuca* cultured in tanks

Treatment	Nutrient load level (g N m^{-2} day^{-1})			
	Very low (1.4) (n=4)	Low (3.68±1.00) (n=6)	High (7.31±1.61) (n=8)	Very high (38.32±5.84) (n=10)
N	147±48a	132±20a	365±119c	279±148b
S	134±26a	112±13a	311±111c	243±170b
SA	189±28a	122±26a	284±88c	263±151b
A	209±61a	168±16a	376±86c	296±158b

Different letters indicate groups that are statistically different at P<0.05 in a two-way ANOVA
Treatments: *N* water exchange only, *S* tidal simulation, *SA* a combination of tidal simulation and aeration, and *A* aeration only

Table 3 Biomass yields (fw, g m^{-2} day^{-1}; mean ± SD) of the seaweed *Ulva lactuca* cultured in flasks under laboratory conditions and subjected to different nutrient concentrations and water velocities

Water velocity (cm s^{-1})	Yield (g m^{-2} day^{-1}) (n=9)	
	Low nutrient concentration	High nutrient concentration
0	NA	NA
1.5	10.6±5.2	25.3±3.3
27.49	29.6±1.0	53.1±6.2

NA The seaweed started to disintegrate after 7 days

(Pearson correlation) significantly with water velocity under both high (R=0.948, P<0.001) and low (R=0.941, P<0.01) nutrient concentrations, which explained 89–90% of the results (R^2=0.899 and 0.885, respectively).

Contents of phosphorus, dry matter, and ash of the algal biomass were not affected (ANOVA, P>0.05) by the agitation treatment under each individual nutrient loading level (Table 4). The contents of protein and phosphorus decreased and ash content and N:P ratio increased with decrease in nutrient loading level, while dry matter content remained about the same (Table 4). The variability in protein content between agitation treatments was insignificant (Table 5) except for the very low nutrient loading level and even then only during the first weeks, when each set of weekly results was analyzed separately (protein content fell as the experiment progressed, Table 4).

Difference in protein content of the seaweed in the indoor experiments between the low and high nutrient treatments depended on water velocity (Table 6). There was a 5% difference (31 vs. 26%, respectively) in favor of the high over the low nutrient concentration at 0 cm s^{-1} velocity, 2% at the low water velocity and no difference at the high velocity. The difference in phosphorus content also depended on water velocity (Table 6). There was a 0.1% difference (0.3 vs. 0.2%, respectively) in favor of the high over the low nutrient concentration at 0 cm s^{-1} velocity, 0.02% at the low water velocity and 0 at the high velocity. The other composition parameters were hardly affected by water velocity and nutrient concentration. The parameters of dry matter and ash contents, being independent of N and thus expected to be less influenced by N and P, did not differ (Table 6). N:P ratios responded to the interaction of nutrient levels and water velocities. At 0 cm s^{-1} velocity and at the low water velocity, N:P ratios differed to a large extent between the high and the low nutrient concentrations, while no difference was observed at the high water velocity (Table 6).

Table 4 Protein, phosphorus, dry matter, and ash contents (% in DW; mean ± SD) and N:P ratios of the seaweed *Ulva lactuca* cultured under different nutrient loading levels and agitation regimes

Nutrient load level/agitation treatment	Chemical parameter				
	Protein (% DW)	Phosphorus (% DW)	Ash (% DW)	Dry matter (% DW)	N:P molar ratio
Very low (n=4)					
N	17.9±9.1	0.19±0.04	26.1±1.1	13.7±0.8	55
S	18.4±9.4	0.17±0.02	25.2±2.2	13.8±0.6	56
SA	16.3±8.8	0.15±0.04	27.4±0.5	12.3±1.3	52
A	14.2±9.6	0.19±0.05	28.1±0.2	12.2±0.6	60
Low (n=6)					
N	37.0±9.4	0.24±0.06	19.7±1.0	13.4±0.2	33
S	36.8±8.8	0.24±0.09	20.8±1.8	14.4±0.2	48
SA	36.3±9.6	0.25±0.11	20.7±2.9	12.7±0.9	45
A	36.6±9.1	0.23±0.08	19.2±1.8	13.0±0.4	26
High (n=8)					
N	35.6±2.9	0.38±0.05	18.1±4.6	9.6±0.4	33
S	35.1±4.6	0.41±0.04	19.5±5.2	9.9±0.8	31
SA	36.3±2.5	0.42±0.06	18.9±3.0	10.2±0.3	31
A	37.0±3.0	0.43±0.05	17.1±3.1	10.1±1.0	31
Very high (n=10)					
N	41.8±2.1	0.76±0.25	17.0±1.0	12.3±1.7	23
S	44.1±3.1	0.75±0.17	15.7±1.2	12.0±1.8	25
SA	44.3±2.7	0.63±0.10	16.3±2.6	12.7±0.9	26
A	43.6±3.0	0.62±0.09	16.0±2.2	12.7±1.2	26

See Table 1 for nutrient load values

Agitation treatments: *N* water exchange only, *S* tidal simulation, *SA* a combination of tidal simulation and aeration, and *A* aeration only

Table 5 ANOVA *P* values by week[a] and experiment for protein content (dw) of *Ulva lactuca* cultured under different nutrient loading levels

Nutrient load level	ANOVA *P* value				
	Week 1	Week 2	Week 3	Week 4	Week 5
Very high	0.29	0.26	0.83	0.47	0.21
High	0.12	0.38	0.19	0.14	–
Low	0.99	0.59	0.35	–	–
Very low	0.026	0.028	–	–	–

Protein percentages were arc-sine transformed before analysis

[a] The experiments were conducted for 5 weeks for very high, 4 weeks for high, 3 weeks for low, and 2 weeks for very low nutrient loading level

The highest rate of TAN removal per unit area of tank surface was attained under the very high nutrient loading level whereas the lowest rate was found under the very low nutrient loading level (Table 7). In contrast, maximum TAN uptake efficiency (fraction of the N load that was removed) was attained under the very low nutrient loading level while the lowest TAN uptake efficiency was attained under the very high nutrient loading level (Table 7). The biofiltration rates were similar between the agitation treatments (ANOVA, $P>0.05$) under the very high TAN loading level. When a similar experiment was conducted at 1/5th of the TAN loading level (high treatment), the TAN uptake rates dropped by about 50% (Table 7), still with no significant variation between the agitation treatments ($P>0.05$). With TAN loading level cut a further 50% (low treatment), TAN uptake rates per unit area dropped a further 50% (Table 7) with a highly significant variance in the TAN uptake rate between the agitation treatments ($P<0.001$). The biofiltration performance of the seaweed under the very low TAN loading level could not be determined rigorously because the nutrient concentrations in the tank outflows were mostly below the detection limit of the method used in this study. However, a 1-day measurement of biofiltration performance gave highly variable values (Table 7).

The reduction in TAN concentration due to seaweed biofiltration increased as the nutrient load level decreased (Table 7). The effect of the agitation treatment was significant ($P<0.001$) only under the low nutrient loading level. Under the very low TAN loading level, no statistical analysis was possible. There was a weak correlation between nutrient loading levels and uptake rates under high and low TAN loading levels (Pearson correlation analysis, $R\leq0.5$). Nutrient biofiltration rates (Table 7) and thallus protein content (Table 4) were strongly correlated ($P<0.01$) in all treatments and under all nutrient loading levels.

In the indoor experiments, the overall N:P molar ratio in the nutrient supply solutions was 15, close to the Redfield ratio of 16 considered optimum for marine algae. However, only a small fraction of the nitrogen was in the form of TAN, preferred by seaweeds (Wallentinus 1984; Neori 1996). As a result, while TAN was taken up vigorously under all the tested experimental conditions (data not shown), uptake of ToxN and orthophosphate was hardly detectable (data not shown). Unfortunately, irregularities in the TAN data made the small differences between the slopes of the TAN uptake curves statistically indistinguishable (data not shown). No significant changes with time in nutrient concentration were observed in the control flasks without seaweed.

Water velocity in flasks had significant effects on the photosynthetic performance relative to the 0 cm s^{-1} velocity in the indoor experiments for the designated water velocities of 1.5 and 3.7 cm s^{-1} at both nutrient concentrations tested (Fig. 3). There was a uniformly repeated pattern of increased photosynthetic quantum yields during shaking intervals at 1.5 cm s^{-1} water velocity and a decreased quantum yield at 0 cm s^{-1} velocity under both low and high nutrient concentrations (Fig. 3, top). The shaking increased the quantum yield by up to 13% under low nutrient concentration and by up to 15% under high nutrient concentration. Reductions in quantum yield at 0 cm s^{-1} velocity following cessation of shaking varied between 10 and 16% under low nutrient concentration and between 10 and 12% under high nutrient concentration.

Table 6 Chemical composition of *Ulva lactuca* (% dw content; mean ± SD) cultured under laboratory conditions and subjected to different nutrient concentrations at various water velocities, $n=9$

Water velocity (cm s^{-1})	Nutrient concentration	Dry matter (% dw)	Protein (% dw)	Ash (% dw)	Phosphorus (% dw)	N:P molar ratio
0	Low	15±1.0	26±2.4	17±2.7	0.20±0.01	47±2.8
	High	14±0.8	31±0.9	17±0.4	0.30±0.02	37±3.6
1.5	Low	18±1.0	27±3.8	18±1.6	0.16±0.01	59±10.7
	High	16±0.7	29±1.0	19±0.6	0.14±0.02	72±9.0
27.49	Low	15±0.0	31±4.3	18±0.1	0.21±0.04	53±1.7
	High	15±1.4	31±0.1	19±0.7	0.21±0.03	52±6.9

ANOVA tests of the quantum yield data showed highly significant differences between the 1.5 and 3.7 cm s^{-1} velocities and the 0 cm s^{-1} velocity under low ($P<0.001$) and significant differences under high ($P<0.05$) nutrient concentrations. At 3.7 cm s^{-1}, the changes in photosynthetic yield also showed patterns of increase and decrease during the shaking and 0 cm s^{-1} velocity intervals, respectively (Fig. 3, bottom), even though the pattern faded during the last shaking interval.

Agitation regime had a significant effect on thallus morphology in *U. lactuca* under all nutrient loading levels. When quantified under the low nutrient loading level in the outdoor experiments, the average size of the seaweed thalli varied nearly 12× ($P<0.001$) between the treatments with and without aeration. The sizes were 37±15 cm^2 in the combined tidal simulation and aeration treatment, 57±18 cm^2 in the aeration, 82±26 cm^2 in the tidal simulation, and 436±213 cm^2 in the water exchange-only treatment.

Table 7 Uptake rate per unit area of TAN biofiltration and biofiltration efficiency (mean ± SD) of the seaweed *Ulva lactuca* cultured under very low, low, high, and very high nutrient loading levels

Treatment	Uptake rate per unit area (g N m^{-2} day^{-1})	Efficiency (%)
TAN load level	Very low[a]	
N	0.4	1.2
S	0.8	59
SA	0.9	66
A	1.2	84
TAN load level	Low	
N	1.2±0.6a	42±9a
S	1.1±0.6a	34±4a
SA	1.6±0.8a	54±14a
A	2.0±1.1a	65±3a
TAN load level	High	
N	2.7±0.8b	37±10b
S	2.9±0.7b	40±10b
SA	3.4±1.0b	49±12b
A	3.7±1.1b	50±14b
TAN load level	Very high	
N	6.4±1.1c	17±3c
S	5.9±1.1c	16±3c
SA	6.4±1.6c	17±3c
A	7.4±1.1c	19±3c

Different letters indicate groups that are statistically different at $P<0.001$ in a two-way ANOVA

See Table 1 for nutrient load level values

Agitation treatments: *N* water exchange only, *S* tidal simulation, *SA* a combination of tidal simulation and aeration, and *A* aeration only

[a] Based on one day's data

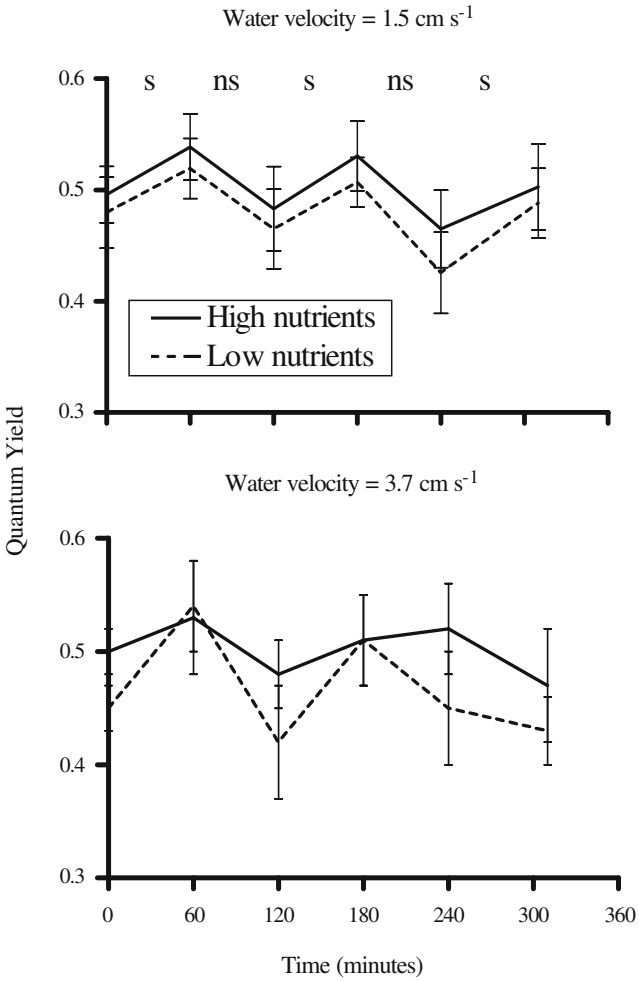


Fig. 3 Effective quantum yields of the seaweed *Ulva lactuca* under high and low nutrient concentrations experiencing 1-h intervals of shaking (*s*) and no motion (*ns*) rhythms at two water velocities: 1.5 cm s^{-1} (*top*) and 3.7 cm s^{-1} (*bottom*). Each *point* is an average of 24 measurements. *Bars* denote standard deviation

Discussion

The benefit of aeration for the *Ulva lactuca* tanks was in accelerating nutrient diffusion under nutrient limitation, most likely by thinning of the DBL around the fronds (Gonen et al. 1993). The other benefits suggested for aeration–increased supply of inorganic carbon, expulsion of excess photosynthetically generated oxygen from the fronds, and moving the fronds vertically through the exponentially decaying light field in the tanks (Vandermeulen and Gordin 1990; Gao et al. 1992; Vergara et al. 1998; Neori et al. 2004)—were not confirmed, at least under the specific conditions of these experiments. Water movement that was generated by other means than aeration achieved the same effect. Of course, the high rate of water exchange in the tanks, which was necessary for the experimental

design, reduced the effect of the algae on carbon and oxygen concentrations. The less effective agitation regimes may lead to photosynthetic carbon limitation and oxygen inhibition at very low water exchange rates.

The relationships between the agitation treatments and the abiotic parameters of DO concentration and pH were weak, ruling out the possibility that oxygen expulsion and CO_2 supply were important contributions of aeration to the functioning of *Ulva lactuca* in the tanks. Apparently, carbon and light were not limiting factors in any of the treatments. Even though the agitation treatments had no effect on DO concentration or pH in these experiments, under lower water exchange rates or with a water source less rich in CO_2 than the effluent of an intensive fishpond, results might have been different.

Biomass yield of the seaweed depended only on nutrient load level. Likewise, protein content was not affected by water agitation treatments within each experiment, except marginally under the most extreme nutrient limitation. Even the minimum water movement allowed seaweed growth under conditions of low nutrient levels reported by Lapointe and Ryther (1979) and Hanisak and Ryther (1984). DeBusk et al. (1986) reported that aeration was not essential for achieving a high biomass yield in *U. lactuca* tanks, while Duke et al. (1986) found that seaweed growth was independent of tanks' N contents above a minimum supply of TAN. In the present work, as in Cohen and Neori (1991) and Neori et al. (1991), biomass yield of low N *U. lactuca* was lower relative to the higher N *U. lactuca*.

Water velocity affected biomass yields and biofiltration performance of *U. lactuca* under low nutrient concentration in the laboratory experiments. Thanks to the reduced experimental variability, these experiments have allowed identification of a limited, yet significant influence of water velocity on the seaweed performance; possibly a similar effect that may have occurred outdoors was obscured by the higher variability involved with such experiments. Doty (1971) and Glenn and Doty (1992) made similar observations. Parker (1981) showed that the application of simulated current (from 1.5 to 22.5 cm s^{-1}) consistently enhanced growth rates in *Ulva lactuca* under laboratory conditions, except at very low light intensity, when light limitation set in. Light was unlikely a limiting factor in the present study, considering the minimum light levels necessary for seaweed growth as reported by Rosenberg and Ramus (1982) and Duke et al. (1989). They found no photosynthetic limitation of seaweed photosynthesis between 35 and 80 μmol photons m^{-2} s^{-1}, hence the indoor irradiances used in the present study were sufficient for good *Ulva* growth. As might be expected, the biofiltration rates (i.e., grams of N taken up per square meter per day) were higher under higher nutrient loading levels, while the biofiltration efficiencies (i.e., fraction of incoming N taken up) were higher under lower nutrient loading levels (Chopin et al. 2001).

Biochemical oxygen demand (BOD_5) provides a straightforward assay of the concentration of microbially degradable organic matter in water. A large difference between inflow and outflow, as in Neori et al. (2003) (from 40 to less than 1 mg L^{-1}), suggests that nutrient mineralization processes within the tanks efficiently convert nutrients from organic to inorganic forms, a process that can confound the interpretation of nutrient levels in biofilter seaweed tanks (Krom et al. 1995). No consumption of BOD in the seaweed tanks was measured in the present study, thanks to the washing out of the organic matter by the high water exchange rate before bacterial degradation took place. The lack of difference in BOD between the inflow and the outflow of each treatment and between the outflows of all the treatments suggests the above-mentioned dissolved organic matter was washed out, so that nutrient remineralization processes in the tanks were of undetected magnitude and should not interfere with nutrient uptake calculations.

The ability of the seaweed biofilter to store N was affected primarily by nutrient loading level, as in Shpigel et al. (1999) and Neori et al. (2003). The water agitation method influenced protein content only under the very low nutrient loading level, when the seaweed was severely N-limited, as indicated by protein content values that, in this experiment, were half of those in the higher nutrient loading levels. Under these conditions, P limitation was also severe, as indicated by the low biomass P content and high N:P ratios of all agitation treatments in the very low nutrient loading level experiment. The seaweed has also shown accumulation of P under the highest nutrient loading level.

High indoor water velocity raised protein content to the same level in both nutrient concentrations. Apparently, the nutrient limitation that developed under these experimental conditions was not severe enough to allow a full expression of the water velocity effect. Other quality parameters in this study included phosphorus (the high content of which indicates high nutritious value of the seaweed to herbivores) and ash (whose high content similarly indicates low biomass quality). High dry matter content is associated with high growth and photosynthetic rates (Friedlander and Dawes 1985).

Under the controlled conditions of the indoor experiments, water velocity influenced photosynthetic performance of the seaweed by increasing photosynthetic quantum yields. In earlier studies photosynthetic rates of the seaweeds were positively correlated with water velocity

of up to 10 cm s^{-1} (Madsen et al. 2001). Gonen et al. (1993) found up to 50% increase in photosynthetic rates in *Gracilaria conferta* when water velocity was increased from 0 to about 1.5 cm s^{-1}. Dodds (1991) found a 50% photosynthetic increase in *Cladophora glomerata* for a water velocity change from 0 to 8 cm s^{-1}.

Low photosynthetic yields under stagnant water conditions are often attributed to a restriction in diffusion of CO_2 and oxygen through the DBL (Smith and Walker 1980; Madsen and Sand-Jensen 1991). However, since the data in the present study show no influence of agitation regime on seaweed biofilter growth, pH, and DO concentration in the seaweed tanks, it seems unlikely that carbon limitation or oxygen inhibition had anything to do with the differences in seaweed performance between treatments. Since the water motion effect increased as nutrient levels fell in the indoor studies, in cases where water exchange rate was low, it can be proposed that the main effect of water motion on the photosynthetic yield is associated with the rate of nutrient diffusion to the seaweed fronds. Generally, effects of water motion were observed under both low and high nutrient concentrations, but higher effects were found under low than high nutrient concentrations at both water velocities. These results indicate that under low nutrient concentration, the seaweed was already facing some degree of stress from limited nutrients compared to high nutrient concentration.

The results of differences in thalli surface areas may be an indication of mechanical breakage by the stronger agitation, either a result of physical weakness of the fronds or a survival strategy. Seaweeds and other macrophytes under stress express a number of survival strategies, including increase in the ratio of absorptive surface area to volume by breakage (Lobban et al. 1985). This finding is relevant to the food industry, where appearance, color, size and consistency can determine quality and value of different cultured seaweeds (McHugh 2003).

Practical relevance to seaweed biofilter design and operation

The most marked result of these experiments is that agitation influence on the different seaweed performance parameters grew in importance as nutrient load level decreased, i.e., as the seaweeds became nutrient limited. Thus, different approaches to the generation of water movement influenced seaweed performance only under low (below 13 μM of TAN) nutrient concentrations. These results indicate that the role of water agitation in general and aeration in particular in *Ulva* culture is the acceleration of the rate of N and phosphorus diffusion into the seaweeds; it is less related to CO_2 uptake or oxygen expulsion.

An aerated seaweed biofilter can operate effectively with minimal water exchange as long as the nutrient load level is high enough (above about 3 g TAN m^{-2} day^{-1} with the other nutrients in proper proportion to it).

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