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A novel three-stage seaweed (*Ulva lactuca*) biofilter design for integrated mariculture

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Abstract

Seaweed biofilters have proven their usefulness in the treatment of fishpond effluents. However, their performance poses a dilemma: TAN (Total Ammonia N) uptake rate – and with it seaweed yield and protein content – is inversely proportional to TAN uptake efficiency. The ideal for a seaweed biofilter performance would be a high uptake rate together with high uptake efficiency. The novel three-stage seaweed biofilter design described here has solved this dilemma. The design used the finding that the performance of seaweed ponds depended on the flux of TAN through them, and that therefore effluents with reduced TAN concentration could provide the seaweed with a high TAN flux if the water flow increased proportionally. Effluents from a seabream fishpond were passed through a series of three successively smaller (25, 12.5 and 6.25 m^2, respectively) air-agitated *Ulva lactuca* ponds. The diminished inflow TAN concentrations to the second and third ponds of the biofilter system were compensated for by the increased water exchange rates, inversely proportional to their sizes. The biofilter performance was evaluated under several TAN loads. TAN was efficiently removed (85–90%), at a high areal rate (up to 2.9 g N m^{-2} d^{-1}) while producing high protein *U. lactuca* (up to 44% dw) in all three stages, although with mediocre yields (up to 189 g fresh m^{-2} d^{-1}). Performance of each seaweed biofilter pond correlated not with TAN concentration, but with areal TAN loads. The novel three-stage design provides significant functional and economic improvements in seaweed biofiltration of intensive fishpond water.

Introduction

Effective and economical treatment of fishculture effluents is crucial, in view of the worldwide environmental concerns facing the sustainable expansion of this industry (Naylor et al., 2000). Fishculture effluents typically contain 60–70% of the ingested nitrogen (Porter et al., 1987), whose treatment by bacterial biofiltration can be prohibitively costly (Losordo, 1998). Marketable biofilters – shellfish and algae – provide a sustainable techhnology, removing the nutrients and at the same time adding to the income and the diversity of the fishfarm (Shpigel et al., 1993). The environmental and economic viability of the main modern approaches to nutrient

removal by filter feeders and algae in integrated mariculture have been recently assessed (Chopin et al., 2001; Troell et al., 2003). The approaches involving seaweed biofiltration, primarily with *Ulva* and *Gracilaria*, were identified as promising, in that they treat water from land-based fishponds to quality levels that allow re-circulation or release to the sea.

Large-scale quantitative seaweed biofiltration studies focusing on the integration of seabream, abalone and *Ulva lactuca*/*Gracilaria* spp. have been done in Israel (Neori et al., 1998, 2000), while in Chile, salmonid culture effluents were filtered by using the agarophyte *Gracilaria chilensis* (Buschmann et al., 1996). These studies have allowed the design of commercial integrated farms (Neori et al., 2001). However, the optimization of seaweed biofilters requires a choice, which can be understood by considering what is required from a seaweed biofilter. A good biofilter should remove a large fraction of the pollutant it handles (such as total ammonia N (TAN, i.e. NH_3^+ & NH_4^+). It should also remove the pollutant at a high areal rate (such as g TAN m^{-2} d^{-1}), and produce a high yield of seaweed, which contains a high percentage of protein.

Unfortunately, this is not possible in a 1-stage seaweed biofilter region. An *Ulva lactuca* biofilter has to be N- starved to strip TAN efficiently, yet starved algae grow slowly, contain little protein and take up ammonia at a low areal rate. This is a crucial fact, because the lower the areal TAN uptake rate, the larger the biofilter area required to treat a given output of nitrogen. Furthermore, the low-protein seaweed yield from the starved biofilters is of low commercial value (e.g. poor abalone food, Shpigel et al., 1999). In contrast, *U. lactuca* biofilters that receive a high load of nutrients (>4 g TAN m^{-2} d^{-1}) take up TAN at a high rate and produce a high yield of high protein biomass. However, their TAN uptake efficiency is low, removing only half or less of the inflow TAN (Ryther et al., 1975; Cohen & Neori, 1991; Neori et al., 1991). The dilemma, then, is that TAN uptake rate (and with it yield and protein content) is inversely proportional to TAN uptake efficiency.

The novel system described here was conceived to resolve this dilemma. Cohen & Neori (1991) showed that *Ulva* biofilter performance correlated with TAN areal loads and not with TAN concentration in the inflow. Effluents with a reduced TAN concentration could provide the seaweed with a high TAN load by a proportionally increased water exchange rate. Neori (1991) hypothesised that a series of biofilter ponds, with successive ponds of decreasing size, could resolve the conflict and achieve high and uniform areal TAN uptake rates and high seaweed yields with high protein content, simultaneously with high uptake efficiency of the incoming TAN. The novel three-stage seaweed biofilter pond design was integrated into a pre-commercial pilot scale seabream and abalone production farm at the Israeli National Center for Mariculture in Eilat, Southern Israel. If functioning correctly and loaded with a sufficient amount of nutrients, the performance of the three stages should be similar to each other with respect to areal TAN uptake rate, TAN uptake efficiency, and ultimately seaweed areal yield and protein content. The biofilter effluent should, of course, be of high quality and ready for recycling or discharge. The performance of the entire 3-stage system should be better than anticipated from published results for a 1-stage system of similar size with a similar TAN load.

Materials and methods

System design

The novel 3-stage seaweed culture and biofilter system treated the fishpond effluents in an outdoor integrated shellfish-fish-seaweed culture system (Figure 1), described in detail in Schuenhoff et al. (2003). The system's core was a 40 m^3 fishpond made of a thick plastic foil mounted on a metal frame, stocked with about 500 kg gilthead seabream, and covered with a greenhouse. This fishpond received water from two sources: the effluent of a large abalone / sea urchins culture unit and water recirculated from the seaweed biofilter. The fishpond effluent was first mechanically screened for particles larger than 0.4 mm (Compact Air 2"®, Dorot, Israel) and then split, part of it discharged and the rest biofiltered by the 3-stage seaweed biofilter ponds and recirculated back to the fishpond. The seaweed yield fed the abalone and sea urchins.

The biofilter ponds were 1.4 m wide elongated troughs, of a construction similar to the fishpond, with plastic foil mounted on a metal frame. A central 50 mm (id) pipe along its round bottom (different from the flat bottomed tanks used in Cohen & Neori, 1991) produced a continuous stream of air bubbles to maintain the seaweed in suspension and vertical motion as in Capo et al. (1999). A 5-mm plastic net covered the seaweed pond outflows to avoid washout of the

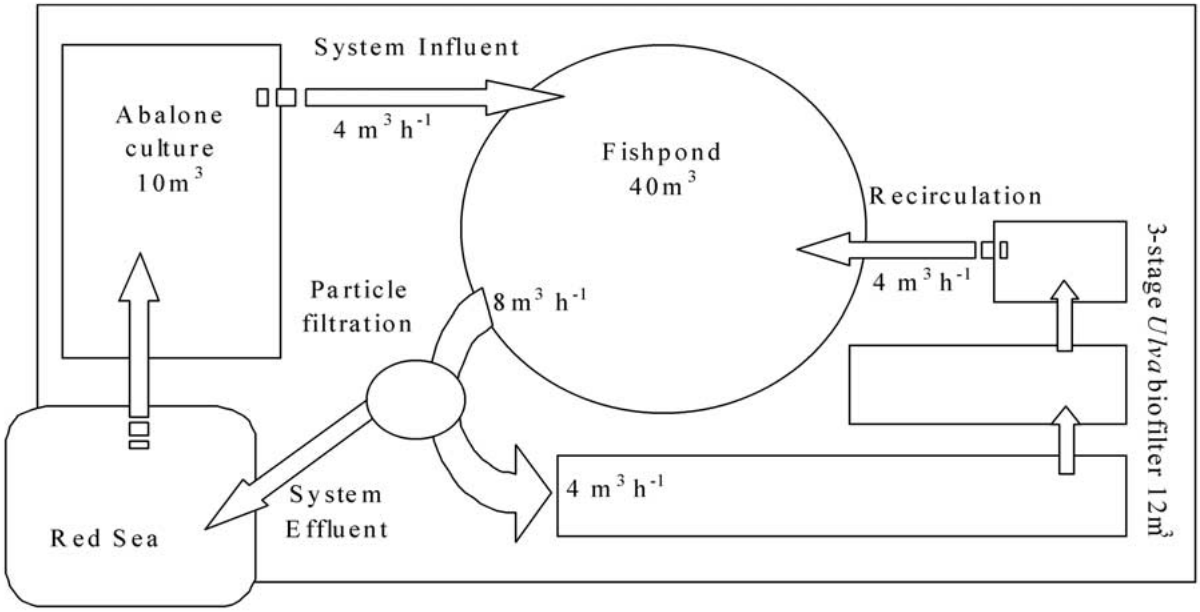


Figure 1. The three-stage integrated system: showing water flow from the sea, through abalone culture tanks to a fishpond, then to a recirculating 3-stage seaweed biofilter or to the sea (water flow varied between experiments; rates here are as in experiment #4).

seaweed. The seaweed ponds were outdoors and were covered by a transparent greenhouse only for the last winter experiment, to conserve heat (Schuenhoff et al., 2003). Seaweed was harvested by removing the outflow nets and draining the water / seaweed suspensions to a net box below pond level. From there, the water was either discharged or pumped back to the biofilter. The harvest was centrifuged to remove excess water, weighed, sampled for chemical analyses, and then part of it returned to the dried and cleaned biofilter ponds as described below and the rest (net yield) transferred as food to the abalone / sea urchin culture unit.

The seaweed biofilter system consisted of three ponds, stocked with *Ulva lactuca.* The ponds (Figure 1) were 18, 9 and 4.5 m long, with surface areas of 25, 12.5 and 6.25 m^2, respectively, and with water volumes of 6.9, 3.43 and 1.7 m^3, respectively. Fishpond effluent water was pumped into the 18 m pond, from where it was drained into the 9 m pond and from there into the 4.5 m pond, from whence it was pumped back to the fishpond. The water flows to each of the three seaweed ponds in a single experiment were identical, since water flowed from one pond to the next. Water flows varied only between entire experiments (Table 1). Since the entire water flow passed on from pond to pond, it follows that in the second seaweed ponds (3.43 m^3), water exchange rate (pond volumes d^{-1}) and the hydraulic load (m^3 m^{-2} d^{-1}) doubled and in the third seaweed pond (1.7 m^3) quadrupled compared with the first seaweed pond (6.9 m^3). It further follows from Table 1 that water exchange rates ranged from about 4.5 volumes d^{-1} in the first pond in experiment #1 to about 72 volumes d^{-1} in the last pond in experiment #2. The high water exchange rates caused some mishaps, reflected in the occasional high variability in the data.

Experimental conditions

The system operated from the autumn of 1999 to the spring of 2001. During this period, four experiments were conducted. Each spanned several weeks of an intensive sampling program (Table 1). During each experiment, the system was exposed to a different average TAN loading rate (concentration of TAN in the water coming out of the fishpond *times* water flow rate). TAN load variability between experiments was needed to test our hypothesis that the three-pond series improves the seaweed biofilter performance with respect to nitrogen removal, seaweed yield and seaweed protein content within a range of TAN loads. In the first experiment, a TAN load of 32 g d^{-1} was provided by 1.3 m^3h^{-1} of fishpond effluents with about 80 μmol L^{-1} of TAN (Table 1). In the second experiment, a TAN load of 135 g per day was provided by 5 m^3h^{-1} of fishpond effluents with about 81 μmol L^{-1} of TAN

Table 1. Water flow, nutrient load and water temperature in each of four experiments (average ± SD)

Experiment:	1	2	3	4
Period:	OCT-DEC 1999	JAN-FEB 2000	SEP-OCT 2000	JAN-MAR 2001
Parameter				
Average TAN concentration at the inflow (μmol L^{-1})	80 ± 17	81 ± 9	51 ± 2	45 ± 7
Average water flow (m^3h^{-1})	1.3 ± 0.1	4.95 ± 0.3	5.1 ± 0.2	4.1 ± 0.1
Average TAN load (g d^{-1})	32 ± 9	135 ± 15	89 ± 9	62 ± 11
Average water temp. (°C)	22 ± 0.45	19 ± 0.2	26 ± 0.9	20 ± 1.5

(Table 1). In the other two experiments, intermediate loads were provided with the same approach (Table 1). Of course, these mass loads (different from areal loads) only applied to the first (and largest) seaweed pond in each experiment, since the TAN concentrations in the water passing on to the second and then to the third seaweed ponds were reduced by seaweed uptake.

Fresh *Ulva lactuca* was stocked in the ponds at a density of 1 kg m^{-2}, as recommended by Neori et al. (1991). The seaweed was harvested weekly or biweekly through 0.5-cm screens. Water was squeezed from the harvest by centrifugation to obtain the drained fresh weight. Unmeasured seemingly small (but possibly larger than anticipated) fractions of the yield were lost in this process. For protein analysis a 150-g sub sample from each harvest of each pond was rinsed with tap water and oven-dried at 60 °C for 24 h. The samples were then dried farther at 105°C to constant weights (% dw content) before Kjeldahl N analysis. To estimate protein content in dw, the Kjeldahl N content was multiplied by 6.25. Twice a week, at 08:00 and 14:00 hours, water samples for nutrient uptake assessment were taken at the inflows and outflows of the ponds. Nutrients were analysed by an automated system (Technicon Autoanalyser II). TAN was analysed by the modification of the Berthelot phenol reaction described by Krom et al. (1985). ToxN (nitrate + nitrite) was analyzed after Solorzano & Sharp (1980). The ToxN data have not been included in the present study, since nitrification in the frequently harvested and dried seaweed pond complex was negligible and ToxN values were low (Schuenhoff et al., 2003). Biochemical oxygen demand (BOD), measure of the content of organic matter in the water, was measured in triplicates by a 12-position automatic system (OxyTop IS12 BOD instrument), which provided BOD values of each sample for 5 days running. Oxygen (DO) concentration and temperature in the water were measured *in situ* by an OxyGuard® Handy Mk III oxygen meter. The pH was measured using a Hanna Instruments HI 8424 meter. Water flow was measured by a fixed electronic induction flow meter and the rate was stored automatically in a computer.

Statistical analyses

ANOVA analyses of the influence of the experimental independent variables on the dependent variables were tested by SPSS version 11.5. The critical value in all tests was $p = 0.05$, unless stated otherwise.

Results

TAN loads and physical & chemical parameters

The concentration of TAN dropped in each successive seaweed pond. In experiment #1 effluents entered the 18 m pond with 80 (± 19) μmol L^{-1} of TAN. Its values at the outflows of the 18 m, 9 m and 4.5 m ponds were 21.5 (± 23) μmol L^{-1}, 15.5 (± 20) μmol L^{-1} and 7 (± 8) μmol L^{-1}, respectively. In experiment #2 effluents entered the 18 m pond with 81 (± 9) μmol L^{-1} of TAN. Its values at the outflows of the 18 m, 9 m and 4.5 m ponds were 37 (±15) μmol L^{-1}, 22.5 (±10) μmol L^{-1} and 12.5 (± 6) μmol L^{-1}, respectively.

Areal TAN load was of course the product of the concentration and the water flow, divided by the surface area of the pond (Table 2). Areal TAN load varied significantly ($p < 0.001$) between the four experiments in a Post-Hoc Tukey test. The load to the first pond averaged 1.25 g N m^{-2} d^{-1} in experiment #1, and then in an increasing order 2.5 g N m^{-2} d^{-1}in experiment #4, 3.5 g N m^{-2} d^{-1} in experiment #3, and the highest load averaged 5.4 g N m^{-2} d^{-1} in

Table 2. TAN load, pH and DO (average ± sd) for each pond in each experiment

Parameter	Experiment: Pond	1	2	3	4
TAN load	Fish	NA	NA	NA	NA
($g\ m^{-2}\ d^{-1}$)	18 m	1.25 ± 0.39	5.4 ± 0.6	3.5 ± 0.35	2.5 ± 0.4
	9 m	0.7 ± 0.7	5.5 ± 1.1	1.4 ± 0.3	1.25 ± 0.9
	4.5 m	1.0 ± 1.15	6.7 ± 1.9	2.3 ± 0.9	1.36 ± 1.4
pH	Fish	7.5 ± 0.1	8.0 ± 0.2	7.4 ± 0.21	7.8 ± 0.2
	18 m	8.8 ± 0.8	8.55 ± 0.24	8.36 ± 0.16	8.30 ± 0.16
	9 m	8.97 ± 0.24	8.57 ± 0.18	8.44 ± 0.19	8.40 ± 0.21
	4.5 m	9.18 ± 0.25	8.6 ± 0.17	8.5 ± 0.19	8.42 ± 0.25
DO ($mg\ L^{-1}$)	Fish	5.9 ± 0.5	7.1 ± 0.3	6.9 ± 0.91	5.9 ± 0.2
	18 m	8.8 ± 0.85	9.47 ± 0.38	8.9 ± 0.70	*
	9 m	9.3 ± 0.97	10.5 ± 0.32	9.2 ± 0.80	*
	4.5 m	9.2 ± 0.95	10.7 ± 0.47	8.9 ± 1.1	*

* = Not measured; NA = not applicable.

experiment #2. TAN load to the first pond obviously influenced the load to the second and third ponds (Table 2). TAN loads were intended to be similar between the three seaweed ponds in each individual experiment. In three of the four experiments, this was achieved, as no significant difference between TAN concentrations in the three ponds was found in a Post Hoc Tukey test. Only in experiment #3, was areal TAN load to the first pond significantly higher than to the other two ponds (Table 2).

The variability in TAN loads was the result of different TAN concentrations in the fishpond effluents and of different water flow rates into the system (Table 1). Different water exchanges can of course affect additional water parameters – temperature, pH and dissolved oxygen concentrations (DO) – as can seasonality. Three of the four experiments took place in winter and only one (#3) in summer (Table 1). Effects between experiments that could be attributed to season only were not expected under our conditions, however (Israel et al., 1995) and were not found (see below).

The values of pH and DO in the three seaweed ponds were significantly higher than in the fishpond (Table 2), a natural consequence of photosynthesis that has been already described in Schuenhoff et al. (2003). Usually, pH and DO levels rose progressively from the first to the third pond (Table 2). The pH in the last seaweed pond was therefore as much as 2 pH units higher than in the fishpond effluents (Table 2). Naturally, these pH differences between the seaweed biofilter effluents and the fishpond effluents dropped when water flow rates were increased. Thus, pH in the water flowing out of the biofilter was 1.5 to 2 units above the fishpond water in the first experiment (water flow of 1.3 $m^3\ h^{-1}$), whereas in the second experiment (water flow of 5 $m^3\ h^{-1}$) the pH difference amounted to less than 1 unit (Table 2). Oxygen concentration in the fishpond effluents was raised by two to three mg l^{-1} by the time the water had been treated by the seaweed ponds (Table 2).

The absolute differences in both pH and oxygen between the seaweed ponds themselves were, however small. The dependence of pH and DO in each pond, on experiment #, pond position and TAN load to each pond (3-w ANOVA) was insignificant, except for the interaction of the effects of TAN load and experiment number on DO ($p = 0.003$).

Passage through the fishpond raised the biochemical oxygen demand (BOD_5) of the water from undetectable levels in the inflow water to nearly 40 mg L^{-1} in the fishpond outflow in the low TAN load experiment (#1), yet after leaving the seaweed ponds, the BOD_5 of the water in this experiment dropped back to undetectable levels (Figure 2). In the high TAN load experiment (#2), the BOD_5 in the inflow to the fishpond was 2 mg L^{-1} because of a higher animal biomass in the invertebrates unit. Nevertheless, because of a higher rate of water exchange, the BOD_5 value in the fishpond effluent in this experiment was lower (12 mg L^{-1}) than in the first experiment (Figure 3). Yet, in spite of the lower residence time of the water, even in this experiment the biofilter system reduced the BOD_5 value by 63%, to 4.5 mg L^{-1}.

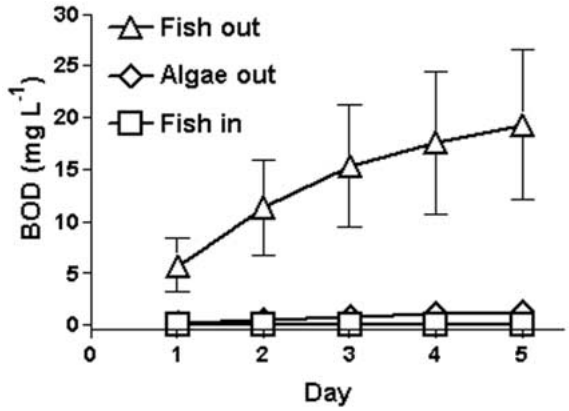


Figure 2. Biochemical oxygen demand (BOD_1-5 average values ± SD; n = 9) in the water at different parts of the integrated system, low TAN load experiment (Experiment #1), as a function of days of BOD incubation.

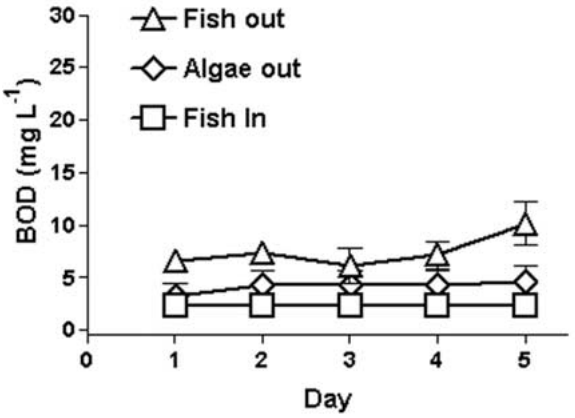


Figure 3. Biochemical oxygen demand (BOD_1-5 average ± SD; n = 6) as in Figure 2, high TAN load experiment (experiment #2), as a function of days of BOD incubation.

Table 3. A) Seaweed yield in each harvest of each pond in the four experiments (average ± SD); B) Protein content – analyses made on each of the harvested biomasses from each pond (average ± SD)

Experiment:	1	2	3	4
A.	Fresh yield (g $m^{-2}d^{-1}$)			
Pond				
18 m	62 ± 21	189 ± 13	181 ± 48	121 ± 11
9 m	84 ± 12	160 ± 26	129 ± 19	104 ± 32
4.5 m	61 ± 30	180 ± 15	129 ± 31	125 ± 34
Total	70 ± 23	176 ± 22	146 ± 40	117 ± 27
B.	Protein content (% in dw)			
Pond				
18 m	36 ± 2.1	44 ± 3.5	38 ± 0.7	34 ± 2.7
9 m	29 ± 5.1	43 ± 2.9	36 ± 2.4	34 ± 4.7
4.5 m	19 ± 12	42 ± 2.3	35 ± 2.3	35 ± 5.7
Total	32	43	37	34

Seaweed biofilter performance – seaweed harvest and protein content

The variability in areal seaweed yield per experiment and biomass protein content (Table 3) were both associated significantly (ANOVA, $p < 0.01$) with the TAN load, as expected (Cohen & Neori, 1991; Neori et al., 1991). The lowest yield was 70 g m^{-2} d^{-1} (25 kg m^{-2} y^{-1}) in experiment #1, obtained with a low TAN load. The yields were progressively higher under higher TAN loads and reached 176 g m^{-2} d^{-1} (64 kg m^{-2} y^{-1}) at the highest TAN load of 135 g N d^{-1} in experiment #2. A 3-way ANOVA of the entire yield data set (3 ponds, harvested several times in each of 4 experiments) for its dependence on experiment #, pond position and TAN load did not show any significant effect showed very insignificant effects for all three parameters. In a 2-way ANOVA the effect of pond position and TAN load on the yield with the entire data set, 48 samples, pond position effect was insignificant ($p = 0.8$) while the effect of TAN load was highly significant ($p < 0.0001$). But in 2-way ANOVA analyses made individually on the yield data from each experiment for the effects of pond position and TAN load, there were no significant effects except for the significant effect on yield of TAN load ($p = 0.044$) in experiment #4. It can be concluded, therefore, that the factor that influenced the yield was the difference in TAN load between experiments.

The picture is about the same with protein content in dw of the yields (Table 3). Protein content was tightly associated with TAN load between experiments, but not with pond position in each experiment. Protein content in dw averaged 32% in the low-load experiment #1 and reached 43% in the high load experiment #2. In the detailed data set (not shown), protein content of the seaweed harvested from the last pond in experiment #1 (low TAN load) gradually dropped, from 35% in the first week to only 9% in the fourth week, indicating a severe N-limitation. Of course, such a temporal variability is reflected in the high standard deviation value in the protein content data of the last pond (4.5 m) in experiment #1 (Table 3). Still, in a 3-way ANOVA of the entire protein content data set (3 ponds, harvested several times in each of 4 experiments) as a function of experiment #, pond

Table 4. A) Nitrogen uptake rate in harvested protein (average ± SD); B) Nitrogen uptake efficiency (%, relative to TAN load) in harvested protein form

Experiment:	1	2	3	4
A. Pond	* Nitrogen uptake rates as protein N g $m^{-2}d^{-1}$			
18 m	0.50 ± 0.20	1.39 ± 0.07	1.32 ± 0.36	0.81 ± 0.02
9 m	0.48 ± 0.06	1.32 ± 0.28	0.89 ± 0.12	0.69 ± 0.12
4.5 m	0.28 ± 0.22	1.35 ± 0.06	0.88 ± 0.15	0.84 ± 0.18
Overall (weighted)	0.47	1.37	1.13	1.78
B. Pond	Nitrogen in harvested protein: uptake efficiency (% of TAN load to each pond)			
18 m	40	24	37	33
9 m	69	24	65	55
4.5 m	28	20	37	62
Overall**	64	44	56	55

* Yield · % dw · % Kjeldahl N (based on dw).
** Based on entire protein-N harvest from the system (three ponds), divided by average TAN inflow to the system.

Table 5. A) Rate of TAN uptake by seaweed biofilters in four experiments, based on analyses of dissolved TAN (average ± SD). B) TAN uptake efficiency

Experiment:	1	2	3	4
A. Pond	Areal TAN uptake rate, g $m^{-2}d^{-1}$			
18 m	0.9 ± 0.3	2.7 ± 0.6	2.9 ± 0.3	1.9 ± 0.3
9 m	0.19 ± 0.2	2.1 ± 0.4	0.20 ± 0.4	0.57 ± 0.3
4.5 m	0.50 ± 0.9	2.2 ± 2.2	1.1 ± 0.4	0.44 ± 0.3
Overall (weighted by pond size)	0.62	2.5	1.9	1.3
B. Pond	TAN uptake efficiency (% of TAN load to each pond)			
18 m	72	50	81	76
9 m	27	38	15	46
4.5 m	50	33	47	32
Overall*	90	86	91.5	91

* Based on TAN concentrations in the inflow to the 18 m pond and outflow from the 4.5 m pond.

position and TAN load, highly significant effects of experiment #($p < 0.001$), and TAN load ($p < 0.001$) were found; yet, the effect of pond position on protein content was highly insignificant ($p = 0.276$). Two-way ANOVA analyses were done within experiments, on the protein content data of the harvests from each pond. In experiment #1 (low TAN load) only, both pond position and TAN load had significant effects on protein content ($p = 0.01$ and $p = 0.03$, respectively). In experiment #4, the next in order with respect to TAN load, TAN load had a highly significant effect on protein content in ($p = 0.001$) but there was no effect of pond position. In experiments #3 and #2 (medium TAN load and high TAN load, respectively), the effect of TAN load on protein content was already insignificant ($p = 0.08$ and $p = 0.11$ in the two experiments, respectively). The effects of pond position and of any interaction thereof in experiments #2 and #3, which received the highest TAN loads, were highly insignificant.

Seaweed biofilter performance – TAN removal based on analyses of TAN and of protein harvest

Nitrogen uptake rate by seaweed protein, or protein N uptake (Table 4) depended significantly ($p < 0.0001$) on whole experiment average TAN load, in a Post-Hoc Tukey test. The uptake rate varied from 0.47 g m^{-2} d^{-1} in the first experiment to 1.37 g m^{-2} d^{-1} in the second experiment. There was no significant effect of pond position. However, the variability between ponds in each experiment was high (Table 4), as can be expected from a variable that is calculated by several independently measured variables (fresh yield, dw content of the fresh yield and protein content in dw). In a 3-way ANOVA analysis with the entire data set (48 samples), the rate of protein N uptake was influenced significantly only by experiment number ($p = 0.01$), and not by pond position or by TAN load.

In a 2-way ANOVA of the entire data set of protein N uptake rates, the effect of TAN load was highly significant (P< 0.001) while the effect of pond position was highly insignificant ($p > 0.4$). This effect depended on the difference in TAN load between experiments, as in 2-way ANOVA analyses with the data from each experiment individually, the rate of protein N uptake was not influenced significantly by either pond position or TAN load.

TAN areal uptake rates, calculated by analyses of TAN, increased fourfold between the low (#1) and the high (#2) TAN load experiments (Table 5). In a 3-way ANOVA with the entire data set for the effects of experiment number, pond position and TAN load on TAN removal rate, the first two effects were highly insignificant ($p > 0.5$), while the effect of TAN load was nearly significant ($p = 0.053$).

Nitrogen uptake efficiency

Nitrogen uptake efficiency is the fraction of incoming nitrogen taken up by the biofilter. This was calculated using two approaches, one with the data of N content in the seaweed harvest and the other using difference in TAN concentration between inflow and outflow of the biofilter, both divided by the relevant (time wise) TAN load to the biofilter. The results were highly variable, as could be expected for variables that incorporate the errors of several independently measured variables. TAN uptake efficiency was usually higher when calculated from TAN concentrations than from protein data. Qualitatively, TAN uptake efficiency in the seaweed ponds generally varied inversely with TAN load (Tables 4 and 5) as expected (Cohen & Neori, 1991). Calculated from N content of the harvested seaweed, the N uptake efficiency ranged from 20% in the 4.5 m pond in experiment #2 (high TAN load) to 69% in the 9 m pond in experiment #1 (Table 4). Yet, because of the high variability in the values, the protein N uptake efficiency was independent of both experiment number and pond position in 2-way ANOVA analyses. The N uptake efficiency values calculated by TAN analyses ranged from 15% in the 9 m pond in experiment #3 to 81% in the 18 m pond of the same experiment (Table 5). A 2-way ANOVA showed that pond position significantly influenced the N uptake efficiency calculated by TAN analyses, while experiment # had no effect. The TAN uptake efficiency in the first pond was higher than in the other two ponds in a Post Hoc Tukey test.

The overall N-uptake efficiency values assessed by N in harvested seaweed (bottom of Table 4) ranged from 44% in experiment #4 (High TAN load) to 64% in experiment #1 (low TAN load). They were lower by 20–40 percent and were much more variable, compared to the overall efficiency values assessed by TAN analyses of the inflow to the 18 m pond and the outflow from the 4.5 m pond (bottom of Table 5), which were about 90% in experiments #1, 3 and 4, and dropped, but only to 86%, in experiment #2 (high TAN load).

Discussion

Seasonality

Three of the four experiments took place in winter, with three different TAN loads. The 3-way ANOVA analyses found that the only significant effects between the experiments were related to differences in TAN load. This was true for the pH and DO concentrations and the main biofilter performance parameters. It therefore seems that, as in Israel et al. (1995), there were no clear seasonal effects on the biofilter performance in these data. Of course, the seasonal question is irrelevant when examining variance within each separate experiment.

General water quality

In the sea, the autotrophic processes (plant uptake and bacterial nitrification) dynamically balance the environmental consequences of heterotrophic metabolism with respect to nutrients, pH and DO (e.g., Clarke, 1954). With the integration of seaweed biofilters in the right proportion to fish culture, a similar dynamic balance is created within the intensive fishfarm. Using the nomenclature from Chopin et al. (2001), the *extractive* seaweed biofilters counteract the deleterious effects of the *fed* fish culture, not only with respect to ammonia, but also with respect to dissolved carbon dioxide, pH and dissolved oxygen.

The novel biofilter system performed this role and maintained good water quality criteria of DO, pH and ammonia under all conditions. The particular combination of these three parameters is critical, since the toxicity of ammonia to fish can be exacerbated by extremes of high pH and low DO (see in Wajsbrot et al., 1991). The values of these measures in the effluent of the 3-stage biofilter system were dependably maintained at safe levels, that is to say, outside the respective ranges of TAN, pH and DO whose composite combination could become toxic to seabream (see in Wajsbrot et al., 1991). This situation made the biofiltered effluent suitable for both recirculation and discharge (Neori et al., 1996; Neori et al., 2000).

BOD_5 levels in the fishpond effluents presented here require some kind of treatment, as they usually exceeded the 20 mg L^{-1} levels allowable in aquaculture (USA standards), and exceeded those reported as normal for fish culture effluents by Boyd & Gross (1999). The degradation of the organic matter by the seaweed biofilters is therefore an important environmental as well as practical function provided by this seaweed biofilter system, even with rather short water residence times. The seaweed biofilter system was highly effective in the breakdown of the water's organic matter content, as expressed by the BOD data, probably due to the water agitation, the large surface area of the tank walls and the seaweed fronds, and the high concentrations of DO. With a residence time

of 9.25 h, all BOD was eliminated by the seaweed system. With shorter water residence times, some BOD remained in the seaweed effluent, as described in Schuenhoff (2001). Yet even under the shortest water residence time of 2.35 h for the entire seaweed pond system, most fishpond effluent BOD was removed by the biofilter.

The TAN uptake rate by a single-stage seaweed biofilter is predictable, as are the TAN uptake efficiency, seaweed yield and seaweed protein content (Neori et al., 1996). To justify the added complexity of the three-stage seaweed biofilter system, it should perform quantifiably better in most of the main parameters defined above. Namely, for a given fishpond effluent, an improved seaweed biofilter should take up TAN at a higher areal rate and efficiency, and produce higher seaweed yield with higher protein content. To do that, the second and the third successive seaweed ponds should perform as well as the first pond in all the parameters, i.e., react to TAN load and not to the reduced concentration of TAN in its water or the increased water exchange rate. Our data confirm that this occurred. The ANOVAs show that TAN load and not pond position was the main variable that determined seaweed pond performance – TAN uptake rate, seaweed yield and seaweed protein content. Only nitrogen uptake efficiency measured by the in/out differences in TAN concentrations, was affected by pond position, while TAN uptake efficiency measured by protein N harvest was neither affected by TAN load nor pond position. Even the meaning of this significant difference in nitrogen uptake efficiency between the first pond and the two other ponds is questionable, related in our opinion to the large errors involved in such calculation and also possibly to poor water circulation and some short-circuiting of the water flow in the two smaller ponds. The important lesson from the uptake efficiency data is that a reasonably good treatment performance was obtained even from the last pond, in spite of the large drop in TAN concentrations in the preceding ponds. This seemingly counter-intuitive result most likely arises from the existence around the fronds of a water boundary layer, which modifies the exchange of substances between the algae and the bulk water (Gonen et al., 1993). Sufficient water turbulence thins this layer so that apparently nutrient uptake is not a problem for the algae (Msuya & Neori, unpublished).

The next question is, therefore, whether the three-stage pond system outperforms a similarly sized one-stage pond. To better understand this issue, the results in this paper have been compared to our published results, rescaled to equal bases of overall size and total TAN input (Table 6). The new design has increased the areal TAN load – the parameter that chiefly determines the biofilter performance – by 45% in the low TAN-load experiments and by up to 80% in the high TAN load experiment. The biofilter system apparently did not even reach the upper limit of its TAN uptake potential of about 7 g N m^{-2} d^{-1} (Cohen & Neori, 1991; Neori, 1991). It is anticipated that the new design will best perform under TAN loads that approach and even surpass this limit. Possibly with higher TAN loads, a seasonal effect would also be apparent, since eventually the biofilter performance would become light and temperature limited.

The performance parameters of the biofilter in three of the four experiments were improved by the new design, except for the yield. In the low TAN load experiment (#1), only protein content of the harvest

Table 6. Performance of the three-stage seaweed pond biofilter design compared to the performance of a hypothetical similarly-sized one-stage design, and the ratio between the values in the 3-stage and 1-stage designs, respectively

Experiment:	1	2	3	4
Seaweed system design	TAN load, g $m^{-2}d^{-1}$			
One-stage pond*	0.73	3.1	2.0	1.4
3-stage data	1.06	5.6	2.7	2.0
Improvement ratio	1.45	1.8	1.35	1.43
	Seaweed fresh yield, g $m^{-2}d^{-1}$			
One-stage pond*	125	242	208	179
Present data	70	176	146	117
Improvement ratio	0.56	0.72	0.70	0.65
	Average protein content, % in dw			
One-stage pond *	19	36	31	27
3-stage data	24	41	35	31
Improvement ratio	1.26	1.14	1.13	1.15
	Overall areal TAN uptake (g $m^{-2}d^{-1}$)			
One-stage pond*	0.67	2.15	1.56	1.17
3-stage data	0.62	2.5	1.9	1.3
Improvement ratio	0.92	1.16	1.22	1.11
	Average areal TAN uptake efficiency, % of TAN load			
One-stage pond*	91	69	78	83
3-stage data	90	86	91.5	91
Improvement ratio	0.99	1.25	1.17	1.1

* Based on the equations from Cohen & Neori, 1991, and from Neori et al., 1991, modified by more recent results (Neori, 1991 and unpublished).

increased by a large margin of 26%. However, yield in the four experiments was lower by as much as 44% relative to the predicted values for a 1-stage pond. No single explanation for this difference can be given, but it is probably a result of the difference in pond design and the loss of fragments through the outflow screens, a possibility that can also explain the differences in TAN uptake values when estimated by TAN concentration analyses and by seaweed N content analyses. The lower nitrogen removal rates calculated from protein yield relative to the results from TAN analyses are also probably due to seaweed loss through the outflow screens, coupled with minor nitrification-denitrification processes that developed in the system over time (Dvir et al., 1999; Schuenhoff, 2001); both issues deserve a continuing study.

In conclusion, qualitatively, the new design improves three of four main performance parameters of the seaweed biofilter. The best benefit from the three-stage seaweed biofiltration approach is expected under high nitrogen load situations. From the practical angle, only the highest N load experiment (#2) is relevant, since it makes no economic sense to operate seaweed biofilters under low TAN loads. Clearly, as areal TAN load to the seaweed biofilter increases, the three-stage design obtains high TAN uptake rates and a good quality high-protein harvest together with an acceptably high TAN uptake efficiency, and this is what it was designed to do. Therefore, to fully realize the value of the three-stage seaweed system, it is necessary to push the TAN load upward toward 10 g TAN m^{-2} d^{-1}, and to improve seaweed pond design.

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