

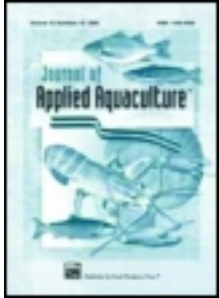
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Effects of Biofilter/Culture Tank Volume Ratios on Productivity of a Recirculating Fish/Vegetable Co-Culture System

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Effects of Biofilter/Culture Tank Volume Ratios on Productivity of a Recirculating Fish/Vegetable Co-Culture System

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ABSTRACT. The effects of four biofilter volume (BFV)/culture tank volume ratios (0.67/1, 1.00/1, 1.50/1, and 2.25/1) on biofilter function were examined in a recirculating fish/vegetable production system in a greenhouse. Sand beds served as biofilters, as substrate for vegetable growth, and as location for decomposition of waste solids. No fertilizer was used. Three experiments were conducted over the course of one year. In Experiment 1, as the BFV/tank volume ratio increased, total ammoniacal nitrogen (TAN) and nitrite concentrations decreased (9.0 to 3.6 mg/L and 0.39 to 0.20 mg/L, respectively), and biomass increase over the culture period and oxygen levels increased significantly (13.34 to 16.03 kg/m³ and 6.03 to 6.47 mg/L, respectively). pH was maintained at 5.8-6.2 without the addition of lime. Yield per plant of the tomato variety 'Laura' tended to decrease (3.4 to 2.3 kg/plant), and yield per plot increased (13.6 to 31.6 kg/plant) with increasing BFV/tank ratio. In Experiment 2, the system was operated for 42 days without plants. pH dropped rapidly to near 4.0. Cucumbers were then planted, and weekly additions of lime and CaO were made. Significantly less CaO was required to achieve target pH

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in systems with the largest BFV/tank ratios. pH levels conducive to good plant growth were only slowly stabilized, and cucumber yields were erratic. TAN and nitrite levels were not measured, but fish grew well (5.2 to 7.2 kg/m³ with increasing BFV/tank ratio). By Experiment 3, with the tomato variety 'Kewalo,' TAN and nitrite concentrations decreased from 0.96 to 0.48 mg/L and from 0.06 to 0.02 mg/L, respectively, with increasing BFV/tank ratio, and in the latter part of the experiment, pH was stabilized at 6.3-6.5 without lime. Yield/plant decreased from 5.0 to 2.4 kg/plant and yield per plot increased from 19.9 to 33.1 kg/plot with increasing BFV/tank ratio. Daily water exchanges averaged 2.8%. Nutrient concentrations of the irrigation water after a year's operation were low overall. Although plants showed no deficiency or toxicity symptoms, K⁺ was found to be low and Zn⁺⁺ high relative to other ions. No clogging was observed in the sand beds. Carbon measurements \pm SEM of the sand medium at the wastewater inlet of the smallest and largest BFV/tank ratio systems were $0.23 \pm 0.03\%$, and $0.15 \pm 0.01\%$, respectively. Nitrogen was below detectable levels ($<0.04\%$). The enhanced biofilter/culture tank ratios used here resulted in a functionally well balanced fish/vegetable co-culture system. While needing refinement, this design represents a step towards a highly productive, low-tech system with efficient use of water, chemical, and labor resources. [Article copies available for a fee from The Haworth Document Delivery Service: 1-800-342-9678. E-mail address: getinfo@haworth.com]

INTRODUCTION

The need for improved methods of producing high quality protein and vegetable foods on limited resources has led to innovative designs of recirculating aquacultural systems (Lewis et al. 1978, 1981; Nair et al. 1985; Rakocy 1989, 1990; Watten and Busch 1984; Rakocy and Hargreaves 1993). A major problem of these systems has been maintenance of sufficient O₂ in the biofilters for efficient microbial conversion of total ammoniacal nitrogen (TAN) to NO₃⁻, which is less toxic to fish. Other problems have included clogging of the biofilter with particulates and bacterial growth, as well as channeling within the filter (Lewis et al. 1978; Paller and Lewis 1982). The introduction of the reciprocating biofilter (Lewis et al. 1978) addressed two of these problems: fresh air was drawn into the filter as it was alternately flooded and drained, greatly increasing oxygenation of the biofilter, and the nutrient-laden water was more uniformly distributed throughout the filter volume, eliminating the problem of channeling.

Historically, in systems based on biofiltration of recirculated water, nitrate-N and phosphate-P accumulation was controlled through partial flushing and anaerobic denitrification (Meade 1974). The use of hydroponic plant culture to reduce NO_3^- concentrations in the recirculating water through uptake has reduced the need for relatively expensive microbial denitrification and has provided an additional economic crop (Naegel 1977; Lewis et al. 1978, 1981; Paller and Lewis 1982; Rakocy 1989a, 1990). However, sedimentation of aquacultural water sequestered the nutrients in the particulate fraction, making these unavailable to the plants and resulting in the need for fertilizer amendments to achieve good vegetable crop yields.

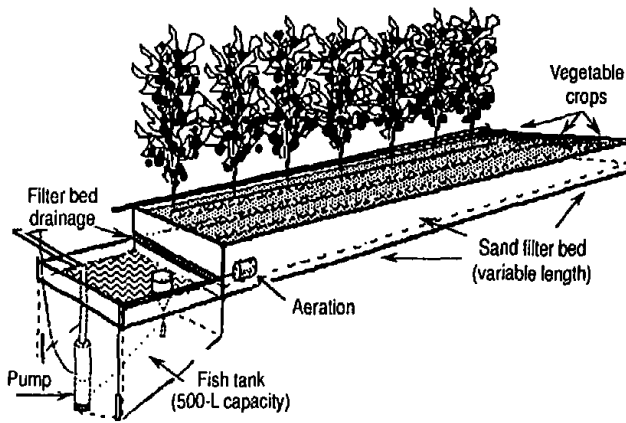
Objectives of this study were to design a recirculatory fish culture and vegetable crop production system which would be functionally simple, easy to maintain and operate, with improved water- and nutrient-utilization efficiencies. In this system, therefore, water was pumped in the reciprocating fashion directly from the bottom of the fish tank to the surface of sand beds. The sand beds served as biofilters, as substrate for vegetable crops, and as location for decomposition of waste solids. The ratios of biofilter volume (BFV)/culture tank volume were higher than in existing systems, so that nitrate-N and phosphate-P concentrations would be more completely controlled through plant uptake, and water flushing would be reduced or unnecessary. Four biofilter/tank volume ratios were studied in three successive experiments over the course of a year to evaluate the effects of these ratios on the performance of the system. Fish and crop growth, water quality measures, organic content of the sand beds, and signs of clogging were evaluated.

MATERIALS AND METHODS

All male (sex-reversed) hybrid tilapia, *Tilapia mossambicus* × *O. niloticus*, were cultivated in 500-L in-ground tanks with aeration provided by regenerative blowers at 0.7 L/second and through two (3.8 × 3.8 × 15 cm) airstones per tank. Water (City of Raleigh, North Carolina) temperatures were kept above 25°C by two Visitherm 250 W thermostatic aquarium heaters (Visitherm, Mentor, Ohio¹) per tank. The rectangular tanks were formed with plywood, the bottom sloped to 45° and lined with 0.50 mm (2 layers each 10 mil.) black polyethylene (Figure 1). Each tank was coupled to a biofilter employing a builder's grade sand as substrate. Tank water level at capacity was 10 cm below the bottom of the biofilter.

1. Use of trade or manufacturer's name does not imply endorsement.

FIGURE 1. Schematic diagram of the recirculatory fish/vegetable co-culture system.



Biofilters were 1.2 m wide, 0.33 m deep and 0.86, 1.25, 1.90 or 2.90 m long to achieve 4 ratios (0.67/1, 1.00/1, 1.50/1, 2.25/1) by volume to the fish tanks. Biofilters were lined with a 0.45 mm (three layers each 6 mil.) polyethylene plastic and the bottom sloped 1/200 along the length for drainage to the associated tank. Composition of the medium, which was optimized to avoid clogging, was 99.25% quartz sand and 0.75% clay. The sand fractionation was: very fine sand (0.10-0.05 mm), 2.2%; fine sand (0.25-0.10 mm), 5.2%; medium sand (0.50-0.25 mm), 21.0%; coarse sand (1.00-0.50 mm), 38.3%; and very coarse sand (2.00-1.00 mm), 33.3% (USDA particle size system; Brady 1990). Each BFV/tank volume ratio treatment was replicated with four independent systems per treatment.

Experiments were conducted in a polyethylene greenhouse in Raleigh, North Carolina. Preplant fumigation of the biofilters with methyl-bromide-chloropicrin (trichloronitromethane) (98-2 v/v) was made at 250 kg/ha. Each biofilter was inoculated with 1.0 L of Fritz-zyme #7 (a suspension of *Nitrosomonas* Winogradsky spp. and *Nitrobacter* Winogradsky spp.; Fritz Pet Products, Dallas, Texas) and was irrigated with aquaculture effluents for nine days prior to planting the first vegetable crop. Tomato, *Lycopersicon esculentum*, or cucumber, *Cucumis sativus* seedlings were transplanted into each biofilter at four plants/m². Plant populations of 4, 6, 9, or 14 plants per biofilter were directly proportional to the respective BFV. Blue-green algae grew on the surface of the beds soon after planting. This

was broken up manually and disappeared spontaneously as the plant canopy developed.

Insect pests were controlled principally through the use of beneficial insects including encarsia, *Encarsia formosa*, and lacewings, *Chrysopa carnea*, for greenhouse whitefly, *Trialeurodes vaporariorum*; and ladybugs, *Hippodamia convergens*, for potato aphid, *Macrosiphum euphorbiae*. They were applied according to directions. Insecticidal Soap (Safer Inc., Newton, Massachusetts) was applied as necessary to control sweet potato whitefly, *Bemisia tabaci*, populations below threshold levels. An in-ground subsonic alarm was effective against shrews, *Balarina* spp.

The fish were fed a diet of modified Purina Fish Chow 5140 with a minimum analysis of 32% protein, 3.5% crude fat, and not more than 7.0% crude fiber. Since large amounts of water were never exchanged from the system, the feed was not fortified with vitamins or trace elements in order to avoid trace element toxicity for the plant crops. The initial daily feed input rate varied from experiment to experiment and was based on a percentage of standing fish biomass. The amount fed was adjusted to what the fish would consume in 15 minutes. The daily ration was divided equally into two feedings administered at 0800 and 1300. The fish also were observed grazing on algae, *Oscillatoria* spp. and *Ulothrix* spp., which grew in the water and on the tank sides.

Irrigation water was pumped from the bottom of the fish tanks eight times daily and delivered to the biofilter surfaces at a rate of 500 L/m² of biofilter surface each day. The water flooded the biofilter surfaces, percolated through the medium, and drained back to the fish tank. The tank water level dropped approximately 25 cm during each irrigation event. Therefore, the returning water provided additional aeration resulting from the cascade effect. Biofilters drained rapidly for approximately 15 minutes following cessation of irrigation and at a diminished rate for one hour. Evapotranspiration losses were replaced weekly with city water (McMurtry et al. in press).

Water temperature and pH measurements were made *in situ* at random times daily. Diurnal modulation of pH, temperature, total ammoniacal-N (TAN), NO₂⁻-N and NO₃⁻-N levels were assayed weekly. In the diurnal assay, the culture water of each tank was sampled prior to each filtration event, the irrigate sampled during each filtration event, and drainage from each biofilter was sampled prior to tank return. Values obtained from the random assays were compared with those taken at the same hour in the diurnal sampling of the same week. Water samples of 190 mL were drawn at the time of each pH assay from the top of each tank, titrated to pH 2.0, sealed, and stored at 5°C for up to 2 weeks prior to assays for nitroge-

nous compounds. Aqueous TAN and NO_2^- concentrations were assayed on an Orion SA270 Ion Specific Electrode (ISE) meter using Fisher ($\text{NH}_{(3+4)}$ and NO_2^-) ISE electrodes. Aqueous NO_3^- concentrations were assayed on an Orion Research Ionalyzer model 407 A meter with a Fisher NO_3^- ISE electrode and were verified using a modified salicylic acid and NaOH colorimetric procedure (Cataldo et al. 1975) with a Beckman DB-G grating spectrophotometer. Culture tank dissolved oxygen measurements were made at 0730 and 1300 *in situ* with an Otterbine Barebo 111 oxygen meter at least weekly. Methyl orange alkalinity was determined by titration. Samples of input water and irrigation water at the conclusion of the study were analyzed by the North Carolina Department of Agriculture Agronomic Division. Feed composition was analyzed by standard techniques (McMurtry 1990).

Samples of the sand bed medium were collected at the end of the study. The upper 11 cm at the inlet for the irrigation water were ground in a jar mill (Paul O. Abbe, Inc., Little Falls, New Jersey) until all the sample passed through a US Standard sieve No. 40 (420 microns). Subsamples of the mixed material were analyzed for carbon and nitrogen on a Perkin-Elmer 2400 CHN Elemental Analyzer at the Soils Analytical Laboratory at the North Carolina State University Soil Science Department.

For biomass determination, fish were removed from the tank and sedated with 20 ppm Quinaldine (Aquacenter, Leland, Mississippi), blotted dry, and weighed individually. Fish biomass increase per time interval was calculated by subtraction. Fish were returned to the tanks with adjustments made (fish added or removed) to maintain a uniform ($\pm 2.5\%$) biomass among all tanks. Feed conversion ratio (FCR), monthly production rate (MP), monthly specific growth rate (MSG), increase over the culture period (I), and the daily rate of increase in biomass (DRIB) were calculated, with adjustments in fish populations taken into account. All adjustments among tanks were made with fish from this study (no new individuals were introduced).

The experiments were conducted as a randomized complete block with four replicates. This design was selected to account for a temperature gradient in the greenhouse. Analyses of variance were made for factorial experiments with Statview TM512+ (Abacus Concepts, Berkeley, California). When F-tests warranted ($P \leq 0.05$), Least Significant Differences (LSDs) were calculated.

For Experiment 1, fish were stocked on 5 May 1988 at a uniform stocking density, mean individual weight (P_{m_i}) and initial biomass (B_i) as seen in Table 1. The initial daily feeding rate was 4.3% of B_i . Feed was adjusted upwards based on feeding response. At the final harvest, daily

TABLE 1. Fish stocking, growth, and harvest variables as influenced by BFV/tank volume ratio and experiment. Abbreviations: P_{m_i} , mean individual fish weight at stocking; B_i , mean initial fish biomass/tank; G , mean individual growth rate over the culture period; MSG , average monthly specific growth rate; $DRIB$, daily rate of increase of the biomass calculated from $B_f = B_i (1 + i)^n$ where n = interval in days and $i = (DRIB/100)$; I = increase over the culture period; FCR , feed conversion ratio (g feed consumed/g increase in biomass); P_{m_f} , mean individual weight at harvest; B_f , mean fish biomass/tank at final harvest; MP , average monthly production— $(B_f - B_i)$ observed and recalculated on a 30.4-d basis. NS = not significant at $P \leq 0.05$.

BFV/tank vol. ratio	Stocking data		Growth data					Harvesting data		
	P_{m_i} (g)	B_i (kg/m ³)	G (g/d)	MSG (%)	$DRIB$ (%)	I (kg/m ³)	FCR	P_{m_f} (g)	B_f (kg/m ³)	MP (kg/m ³)
<u>Experiment 1, 'Laura' tomato (103 days)</u>										
0.67/1	15.4	1.11	1.72	385.8	2.52	13.34	1.51	204.5	14.76	3.94
1.00/1	14.3	1.25	1.98	405.9	2.57	15.74	1.29	227.6	16.82	4.65
1.50/1	14.5	1.11	1.86	420.4	2.61	14.58	1.40	216.4	15.66	4.32
2.25/1	15.1	1.09	1.99	469.3	2.71	16.03	1.27	217.3	16.73	4.74
LSD _(0.05)	NS	NS	0.21	NS	NS	2.35	NS	NS	1.95	0.70
<u>Experiment 2, No crop interval (42 days) and 'Fidello' cucumber (65 days)</u>										
0.67/1	277.2	3.93	1.90	55.7	0.67	5.21	2.60	453.7	9.14	1.25
1.00/1	244.5	3.90	1.89	59.6	0.71	5.79	2.09	406.7	9.69	1.39
1.50/1	266.3	3.87	2.33	69.8	0.84	7.41	1.95	454.5	11.29	1.77
2.25/1	250.5	3.87	2.41	68.7	0.83	7.22	2.03	452.3	11.08	1.73
LSD _(0.05)	NS	NS	0.49	10.5	0.13	1.65	0.41	NS	1.62	0.39
<u>Experiment 3, 'Kewalo' tomato (132 days)</u>										
0.67/1	440.0	8.80	1.80	35.8	0.33	5.16	2.83	652.0	13.67	1.19
1.00/1	421.4	8.83	1.93	36.4	0.35	5.57	2.59	715.7	13.93	1.28
1.50/1	438.0	8.72	1.99	36.6	0.35	5.35	2.74	711.2	13.85	1.23
2.25/1	438.3	8.55	2.06	38.1	0.38	5.16	2.87	689.2	14.10	1.19
LSD _(0.05)	NS	NS	NS	NS	NS	NS	NS	55.4	NS	NS

feeding rate was 2.2% of B_f . Tomato 'Laura' was transplanted into the biofilters on 13 May 1988. This indeterminate greenhouse variety was grown as a single stem. Because of excessive heat ($>40^\circ\text{C}$) fruit set occurred only on the first 4 trusses. These fruit were harvested at the incipient color stage, weighed, and graded according to U.S. grade standards (McMurtry et al. 1993b). The experiment was terminated at 95 days after planting the tomatoes, 103 days after stocking the fish.

For Experiment 2, fish were restocked on 16 August 1988. Stocking densities, Pm_i , and B_i are given in Table 1. The system was operated for 42 days without plants grown in the biofilters, in order to assess the contribution of olericulture to pH buffering of the water. The initial daily feed rate, based on fish biomass, was 5.0% of B_i . This amount was constant for 42 days and was equivalent to 3.1% of biomass at 42 days. When pH fell to nearly 4.0, incremental additions of $\text{CaMg}(\text{CO}_3)_2$ were made to each biofilter in order to raise pH and reestablish nitrification. At 42 days (27 September), fish biomass per tank was equalized across treatments, and cucumber 'Fidello' was transplanted into the biofilters. A new daily feeding rate for the fish, 1.0% of B_f , was established. Following $\text{CaMg}(\text{CO}_3)_2$ inputs, water pH in most tanks remained below pH 6.0 which is too low for balanced nutrient assimilation by cucumber. Therefore, CaO was added to the tank water approximately twice weekly in quantities sufficient to raise pH in each tank to above 6.5 following each application. Experiment 2 was terminated 85 days after planting the cucumbers (127 days total), on 21 December.

Experiment 3 was initiated with fish stocking and crop planting on 22 December. Fish were stocked at a uniform Pm_i , and B_i (Table 1). Initial daily feed rate was 1.8% of B_i . The final feeding rate was 0.6% of B_f . The semi-determinate, bacterial wilt-resistant tomato 'Kewalo' was planted 23 December, and grown as a single stem. Fruit were harvested, weighed, and graded as for Experiment 1. Fish were harvested, and the experiment was terminated 132 days from stocking, on 2 May 1989.

RESULTS

Experiment 1

At the beginning of the study, individual fish weight and total fish biomass after stocking were statistically uniform (Table 1). However, a slightly high mean biomass at stocking in the 1.0/1 BFV/tank ratio treatment resulted in discernibly higher (or lower) values for this treatment than might be expected on the basis of the trends for several of the vari-

ables in the table. Individual growth rate (G), increase in total biomass during the culture period (I), B_f , and MP all increased significantly as the BFV/tank ratio increased from 0.67/1 to 1.00/1. MSG and $DRIB$ were not significantly different but tended to increase with increasing BFV (Table 1). The decreasing trend in FCR with increasing BFV/tank ratio was not statistically significant ($P \leq 0.05$).

Diurnal mean dissolved oxygen concentrations increased and water temperatures decreased with increasing BFV/tank ratio (Table 2). Dissolved oxygen concentrations ranged from 4.8 and 7.8 mg/L with minimum day-to-day variation. The TAN and NO_2^- -N concentrations decreased with increasing BFV/tank ratio (Table 2). Initial TAN concentrations increased from 0.0 mg/L over the first 7 weeks to mean high levels ranging from 30.2 to 10.8 mg/L with increasing BFV/tank ratio, and initial NO_2^- -N concentrations increased from 0.0 mg/L over the first 4 weeks to mean high levels ranging from 2.6 to 1.0 mg/L with increasing BFV/tank ratio (data not shown). At the end of the tomato crop, TAN and NO_2^- -N concentrations ranged from 1.1 to 0.7 mg/L and from 0.022 to 0.006 mg/L, respectively, with increasing BFV/tank ratio. Total alkalinity increased from 40 mg/L week 1 to 180 mg/L by week 5 but remained stable through week 8 and was not assayed thereafter (data not shown).

pH was slightly higher in the lowest BFV/tank ratio treatment (Table 2). pH increased from about 6.5 to 7.4 in all treatments over the first 2 weeks, as bacterial and plant populations became established. By week 5, pH declined to approximately 6.0 in all treatments and remained stable thereafter.

In Experiment 1, plants grew well in the biofilters (Table 3). Fruit yields calculated per plant decreased with increasing BFV/tank ratio. Average yield per plot increased 132% in the 2.25/1 BFV/tank ratio treatment compared to the 0.67/1 BFV/tank ratio treatment, an increase of 235% in area.

Experiment 2

The Pm_i and B_i for Experiment 2 are listed in Table 1. During the 42-day interval in which there were no plants growing in the biofilters, pH dropped rapidly from approximately 6.0 in all treatments to 4.3 or less. Subsequently, 2 kg/tank $CaMg(CO_3)_2$ amendment raised the pH to 5.5 or greater. At the end of the first 42 days, mean fish biomass increase ranged from 1.88 to 3.04 kg/m³ and G ranged from 1.85 to 2.74 g/fish/d (data not shown). The FCR ranged from 1.43 to 3.50, but there was no consistent trend with BFV/tank ratio.

Overall responses of fish growth variables to BFV/tank ratios showed

TABLE 2. Water quality and pH adjustment as influenced by BFV/tank volume ratio and experiment. Abbreviations: DO, dissolved oxygen; TAN, total ammoniacal nitrogen. NS = not significant at $P \leq 0.05$.

BFV/tank vol. ratio	Water quality (average over growth interval)						pH adjustment	
	DO (mg/L)	Temp. (°C)	TAN (mg/L)	NO ₂ ⁻ -N (mg/L)	NO ₃ ⁻ -N (mg/L)	Avg. pH	Lime (g/tank)	CaO (g/tank)
<u>Experiment 1. 'Laura' tomato (103 days)</u>								
0.67 / 1	6.03	28.4	9.01	0.39	-	6.18	0.00	0.00
1.00 / 1	6.15	28.4	7.70	0.32	-	5.95	0.00	0.00
1.50 / 1	6.36	28.1	5.78	0.28	-	5.95	0.00	0.00
2.25 / 1	6.47	27.5	3.65	0.20	-	5.84	0.00	0.00
LSD _(0.05)	0.28	0.8	0.73	0.07	-	0.23	NS	NS
<u>Experiment 2. no crop interval (42 days) and 'Fidello' cucumber (85 days)</u>								
0.67 / 1	-	26.9	-	-	-	5.32	2000	12.5
1.00 / 1	-	26.9	-	-	-	5.06	2000	23.8
1.50 / 1	-	26.7	-	-	-	5.35	2000	18.8
2.25 / 1	-	25.6	-	-	-	6.00	2000	0.0
LSD _(0.05)	-	NS	-	-	-	0.45	NS	16.4
<u>Experiment 3. 'Kewalo' tomato (132 days)</u>								
0.67 / 1	5.43	29.0	0.96	0.06	52	6.33	0.00	265
1.00 / 1	5.79	28.9	0.79	0.05	54	6.29	0.00	324
1.50 / 1	5.91	28.2	0.61	0.04	47	6.35	0.00	221
2.25 / 1	6.10	27.8	0.48	0.02	21	6.52	0.00	51
LSD _(0.05)	0.42	NS	0.33	0.01	16	NS	NS	150

trends similar to Experiment 1 (Table 1). Composite 127-day G, MSG, DRIB I, B_f and MP all increased significantly as BFV/tank ratio increased to 1.50/1 treatment. The FCR decreased with the first increment (0.67/1 to 1.00/1) but was not further improved with larger biofilters. Final Pm_f did not differ among treatments.

TABLE 3. The influence of BFV/tank volume ratio on vegetable yields. Yields of 'Fidello' cucumber were compromised by pH fluctuations during Experiment 2. NS = not significant at $P \leq 0.05$.

Experiment	Units	BFV/tank vol. ratio				LSD _(0.05)
		0.67 / 1	1.00 / 1	1.50 / 1	2.25 / 1	
1 ('Laura' tomato)	kg/plant	3.4	2.8	2.4	2.3	NS
	kg/plot	13.6	17.0	21.9	31.6	4.4
3 ('Kewalo' tomato)	kg/plant	5.0	3.7	3.0	2.4	1.0
	kg/plot	19.9	22.1	27.3	33.1	9.1

A trend towards declining temperature with increasing BFV/tank ratio similar to Experiment 1 was not statistically significant (Table 2). pH readings tended to be higher for the largest BFV/tank ratio, so that CaO adjustment was not necessary for that treatment (Table 2). pH measurements at the end of the experiment were, respectively, 6.0, 5.5, 5.8, and 6.4 with increasing BFV/tank ratio.

When the system was operated without growing plants, pH of the aquacultural water dropped rapidly to levels below optimum for cucumbers. Cucumber growth and yield was erratic, as target pH was only slowly regained. Cucumber yield per biofilter was 11.2, 10.0, 11.4, and 33.3 kg, respectively, and yield/plant was 2.8, 1.7, 1.3 and 2.4 kg, respectively, with increasing BFV. Correlation of diurnal mean pH and fruit yield within treatments, in the same order, were 0.99, 0.90, 0.97 and 0.93 ($r^2 = 0.98, 0.81, 0.94$ and 0.86 with $P = 0.01, 0.10, 0.03$ and 0.07 , respectively).

Experiment 3

There were no statistically significant ($P \leq 0.05$) differences among treatments for any of the fish stocking, growth, and harvesting variables except for Pm_6 , which increased for the 1.00/1 and 1.50/1 BFV/tank ratio treatments, compared with the 0.67/1 BFV/tank treatment (Table 1).

Dissolved oxygen increased with increasing BFV (Table 2). Individual readings ranged from 5.6 to 6.1 mg/L, with minimal day-to-day variation ($SD = 0.31$). Water temperature tended to decrease with increasing BFV/tank ratio.

The mean TAN, NO_2^- -N, and NO_3^- -N concentrations over the 132-day experimental period decreased with increasing BFV/tank ratio

(Table 2). The TAN and NO_2^- -N concentrations initially ranged from 0.20 to 0.03 mg/L and 0.03 to 0.02 mg/L, respectively, and increased over 10 weeks to mean high levels ranging from 1.49 to 1.18 mg/L and 0.11 to 0.02 mg/L, respectively, with increasing BFV/tank ratio. At peak tomato harvest the TAN and NO_2^- -N concentrations ranged from 0.32 to 0.29 mg/L, respectively, and from 0.03 to 0.02 mg/L, respectively, with increasing BFV/tank ratio. Mean NO_3^- -N concentration differed only in the 2.25/1 BFV/tank ratio treatment, where it was significantly ($P \leq 0.05$) smaller than for the other ratios (Table 2). The NO_3^- -N concentrations initially ranged from 52 to 20 mg/L with increasing BFV/tank ratio, then increased for 2 weeks to a range of 56 to 22 mg/L, and at peak tomato harvest had declined to 54 to 7 mg/L.

Mean pH tended to increase with BFV/tank ratio through the course of Experiment 3, but differences in overall means were not statistically significant ($P \leq 0.05$; Table 2). The pH initially remained low following Experiment 2, and weekly additions of CaO were made until pH stabilized above 6.0 in all the tanks (1-3 weeks). Significantly less CaO was required to bring the pH of the high BFV/tank ratio treatment up to acceptable levels (Table 2).

Crop yield for 'Kewalo' tomato in Experiment 3 was higher than in Experiment 1, but treatment effects were similar (Table 3). Yield per plant decreased with increasing BFV/tank ratio, and yield per plot (biofilter) increased with size of the biofilter.

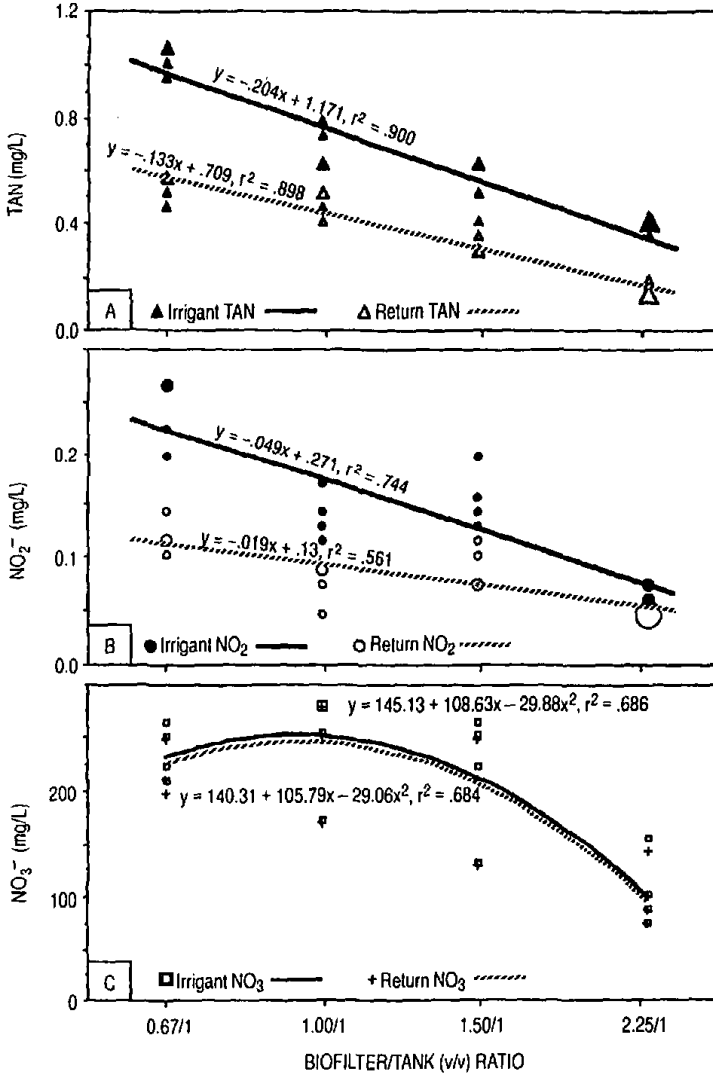
Elemental composition of the fish feed, input water, and tank/irrigation water at the termination of Experiment 3 are represented in Table 4. Levels of phosphorus, magnesium, chloride, iron, and zinc were significantly lower ($P \leq 0.05$) in the largest BFV/tank volume ratio treatment. Other nutrient data showed similar trends but were not significant statistically. No symptoms of nutrient deficiencies or toxicities were apparent. Water returning from the filters to the fish tanks had TAN and NO_2^- -N concentrations approximately half that of the irrigation water drawn from the bottom of the fish tanks (Figure 2, A and B). The larger BFV/tank ratios filtered the aquacultural water more effectively than the smaller ratios. The percentage reduction in TAN and NO_2^- -N concentrations with each filtration event decreased with increasing BFV/tank ratio (data not shown). The proportional reduction in NO_3^- -N concentration with each filtration event was much less than for TAN or NO_2^- -N (Figure 2), but over the long term was sufficient to keep NO_3^- -N in the 20-50 mg/L range (Table 2).

At no time was clogging or channeling observed in the biofilters. In fact, these biofilters were run for three years without clogging after this

TABLE 4. Elemental composition of the fish feed and input water, and of the irrigation water after Experiment 3. NS = not significant at $P \leq 0.05$.

		N	P	K	Ca	Mg	Cl	S	Fe	Mn	Zn	Cu	B	Mo
		(% dry wt)						($\mu\text{g/g dry wt}$)						
Fish Feed		4.65	0.88	1.20	1.31	0.28	0.6	1600	201	52	65	12	22	0.4
	BFV/tank vol ratio	(mg/L)												
Input H ₂ O	mean	2.0	0.04	1.60	6.62	2.8	4.0	5.8	2.91	0.07	0.25	0.00	0.01	0.00
Irrig. H ₂ O	0.67/1	173	1.31	11.5	184	58	31	60	0.01	0.02	0.46	0.01	0.17	0.02
	1.00/1	184	0.60	3.5	167	57	32	47	0.02	0.02	0.34	0.02	0.17	0.02
	1.50/1	160	0.09	1.3	167	65	39	50	0.01	0.01	0.38	0.01	0.27	0.02
	2.25/1	47	0.00	0.7	30	17	4	36	0.00	0.00	0.11	0.01	0.09	0.02
LSD _(0.05)		NS	0.54	NS	NS	45	34	NS	0.01	NS	0.34	NS	NS	NS

FIGURE 2. Diurnal mean total ammoniacal nitrogen (TAN), NO_2^- , and NO_3^- concentrations out of the fish tanks (irrigate) and out of the biofilters (return), as influenced by biofilter volume/tank volume ratio. In A and B, increasing symbol sizes represent increasing observation number from one to four.



work (Sanders, unpublished observation). Samples of the medium were collected at the termination of Experiment 3 from all the biofilters. Samples were collected near the inlet for the wastewater from the top 11 cm, which should contain the highest concentration of organic material. These samples contained 0.23 ± 0.03 (SEM)% and $0.15 \pm 0.01\%$ carbon, in the 0.67/1 and 2.25/1 ratio treatments, respectively. Nitrogen was below the level of detection (0.04%).

DISCUSSION

A functionally simplified fish/vegetable production facility was designed and operated over the course of a year, demonstrating good productivity with excellent economy of water, nutrient, and lime amendment. The biofilter/culture tank volume ratios were greatly expanded over most previous systems (Rakocy and Hargreaves 1993). This single factor permitted high water quality without high rates of water exchange, stable pH without liming, and good vegetable yields without fertilizer additions, as well as good fish growth.

The purpose of this work was not to maximize fish growth rates but to examine the effects of component ratios on biofilter function. Equalizing fish biomass among replicates and treatments on a monthly basis maintained uniform nutrient input, so that differences in biofilter function would not be confounded with grossly different fish populations over time. Thus, differences in fish growth among treatments may be understated. Direct comparisons of fish growth rates with other systems are difficult, due to differences in stocking density, feed quality, etc. However, the growth rates observed in this work, generally around 2 g/day, compared well with those in other studies (2.5 g/day, Watten and Busch 1984; 1.6 g/day, Nair et al. 1985; 0.6 g/day, Kane 1987).

The treatments demonstrated how biofilter/culture tank ratios affected biofilter function. To achieve equal irrigation rate per m^2 of sand bed, each liter of water in systems with higher BFV/tank ratios passed through the sand filters more frequently than a liter of water in the lower BFV/tank ratio systems (McMurtry et al. in press). Although concentrations of TAN and NO_2^- were low in all the treatments (Redner and Stickney 1979; Balarin and Haller 1982), they were significantly lower in systems with the larger BFV/tank ratios. There were also higher oxygen levels and lower temperatures in the hot summer season in these treatments. Greater fish growth rates reflected the improved water quality in the systems with relatively larger biofilters.

Over the course of the year, on average 2.8% of the system water was

consumed per day, to replace evapotranspiration and leakage losses. A more durable material for the culture tank would eliminate or reduce leakage losses and further improve water consumption. Details of water consumption are presented in a separate study (McMurtry et al. in press).

pH, traditionally maintained at levels >7.0 with carbonate inputs (Rakocy 1990), was allowed to be self-regulating in this system between 5.5 and 6.5. Larger biofilters tended to require less CaO to reestablish a stable pH after the run without plants in Experiment 2. While it is well established that optimum pH for nitrification is 7-8, substantial rates of these reactions also occur at pH 6-7 or even lower (Anthonisen et al. 1976; Fochte and Verstraete 1977). This was demonstrated here (Figure 2) and reflected in the low levels of TAN and NO_2 seen in the aquacultural water, especially in Experiment 3 (Table 2). Indeed, at pH values <7.0 , free ammonia is not present to inhibit *Nitrobacter* activity or to inhibit fish growth. TAN is almost entirely in the form of NH_4^+ , the non-toxic, plant-available form. In the absence of plant growth in the first part of Experiment 2, pH fell rapidly, indicating active nitrification. After plant growth was reestablished in Experiment 3, pH was stable for the rest of the study in the 6.3-6.5 range with no liming. The acidification characteristic of nitrification was probably being counteracted by the production of OH^- or HCO_3^- which is produced when NO_3^- , H_2PO_4^- , or other anions are absorbed by roots of actively growing plants (Marschner 1995).

Nutrients were sufficient for plant growth without fertilizer additions because the sand beds served as sedimentation tank, and organic matter was allowed to decompose there. Oxygen as well as nutrients were replenished eight times daily, so conditions were ideal for rapid oxidation of the organic matter: well aerated, moist, and warm. Clogging was never observed. Wastewater percolation rate through the beds did not change noticeably with time, and no evidence of channeling or localized anaerobic conditions was observed.

Nutrient concentrations in the irrigation water were low overall (Table 4), reflecting good nutrient sequestration in the plant material. Good plant growth can be achieved with low nutrient concentrations where roots are constantly replenished with fresh solution, as in the stirred hydroponic system (Marschner 1995) or the reciprocating biofilter (McMurtry et al. 1993a). Nutrient concentrations decreased as the ratio of BFV/tank volume ratio increased, suggesting greater efficiency in nutrient extraction from the effluent with increasing BFV/tank ratio. This is consistent with increased fruit yield per biofilter in the higher-ratio treatments. The relationship between fruit yield and nutrient inputs is examined in greater detail (McMurtry et al. 1993b).

Some nutrients were not in ideal ratios for plant growth. Potassium stands out as very low relative to nitrogen, calcium, and magnesium. To maximize plant yields, this imbalance could be addressed with the addition of sea kelp or a salt fertilizer. Also, zinc was high in relation to other micronutrients. Nonetheless, visible signs of nutrient imbalance in the plant material were not observed, and plant growth was good.

Yields of 'Laura' tomato in Experiment 1 were low because heat stress in the greenhouse during the summer caused abortion of flowers and fruits above the fourth truss. However, yield of 'Kewalo' tomatoes in the 0.67/1 BFV/tank volume ratio treatment in Experiment 3, expressed either as 5.0 kg/plant or as 19.9 kg/m², compares well with average regional commercial greenhouse growers (M. Peet, pers. comm.). This is in the lower range of values for US-wide greenhouse tomato yields given by Snyder (1996), a range shared by the smaller greenhouse operations.

While this work was not without flaws, its main contribution is that it deals directly with the question of water quality in the context of biofilter/culture tank ratios and demonstrates the value of an enhanced plant growth-filtration component in a balanced fish/vegetable co-culture system. Such balance is important if a system is to be low-tech in filtration device; low-input in labor, water, fertilizer, and lime; and high-yielding. Remaining work includes intensifying fish and vegetable production while maintaining balance. In this work the upper limit of fish stocking density per unit plant carrying capacity was not found. Clogging was not a problem, and water quality measures were good in all ratio treatments. Larger fish culture tanks and higher stocking density, therefore, might well increase fish production rate and in turn tomato yields. Further filtration at night might improve water quality as stocking density and volume are increased. Potassium amendment should also be tested for improvement of tomato yield as well as, possibly, the uptake rates for other nutrients. Finally, continuous culture of both fish and vegetables would eliminate swings in water quality that result from the batch approach used in these experiments.

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