Intensive Zero-Exchange Shrimp Production Systems – Incorporation of Filtration Technologies to Improve Survival and Growth

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ABSTRACT

Cost effective application of superintensive, biosecure marine production systems in the U.S. will depend upon proactive management of culture-water quality. More efficient production practices and effective management of waste materials from the shrimp aquaculture industry can allow for higher productivity, improved growth and survival, and pave the way for eventual application away from coastal areas. These improved production strategies are key factors contributing to profitability and environmental sustainability. Development of cost-effective management strategies includes application of mechanical and biological filtration devices to remove solids and nitrogenous products from culture systems. Accumulation of these waste products can limit system productivity and negatively impact cultured animals, increasing the potential for stress,

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disease, and mortality. Technologies developed to remove solids and maintain concentrations of nitrogenous waste products within acceptable limits include different types of filters used alone or in combination with a variety of media types. All of these technologies have achieved varying degrees of success. While use of expandable granular biofilters is not new, improvements have been made in the design and composition of the filtration media. This, in conjunction with an appropriate backwash regimen, encourages attachment and growth of nitrifying bacteria to accomplish clarification and nitrification in a single unit. The purpose of this study was to evaluate the effects of biological and mechanical filtration on production and selected water-quality criteria in zero-exchange, biosecure, superintensive shrimp production systems.

MATERIALS AND METHODS

The efficacy of two different filtration medias, alone and mixed 1:1 was evaluated using airlift-driven marine recirculating bubble-washed bead filters (MRBF). A foam fractionator (FF) using bubbled air was used to evaluate mechanical filtration. Both treatments were fitted to green-water tank systems stocked at high density (287 animals/m^2) with Pacific white shrimp (*Litopenaeus vannamei*). The two types of media used were Enhanced Nitrification (EN), a floating modified polyethylene bead (Beecher *et al.* 1997); Kaldnes Miljøteknologi moving bed filter media (KMT, Tonsberg, Norway), a neutrally buoyant polyethylene wheel (Lekang and Kleppe 2000); and a 1:1 mix of EN and KMT. Both EN and KMT media have a density <1 and a specific surface area of 500-1050 m^2/m^3 so that biofilm formation can occur while allowing the media to remain positively buoyant. Media used in this experiment were either new or bleached, reused beads which had no organic material associated with them.

Twenty 3.35 m diameter (8.8 m^2) polyethylene tanks were used to evaluate five treatments: no filtration (control); mechanical filtration (FF); biological filtration (EN Media), biological filtration (KMT Media); and biological filtration (mixed media EN/KMT). There were four replicate tanks (Figure 1) for each treatment. Tanks (each holding 6,279 L) were filled with filtered (25 μm) sea water from South Carolina's Colleton River (~28 g/L) and were maintained without water exchange. A commercial liquid fertilizer (Tri-Chek liquid polyphosphate pond fertilizer 10-34-0, Tri-Chek Seeds, Inc., Augusta, GA, USA) was applied on Days 1 and 3 (post fill) at 100 ml/tank and on Day 7 at 50 ml/tank to promote algal bloom development. Continuous aeration and circulation were supplied to the tanks and filters by two 5-hp regenerative blowers (Metek Model DR3D89, Rotron Industrial Products, Saugerties, NY, USA) delivering air to six fine pore airstones and one central 12-inch porous pipe diffuser per tank. Bead filters (Aquatic Systems Technologies LLC, New Orleans, LA, USA) were filled with 0.84 m^3 of media and automatically backwashed using air from a 1.6-hp compressor regulated at 40 psi (Ironforce 6.25 hp, Campbell Hausfeld, Harrison, OH, USA). Filter air flow was adjusted over a period of three weeks to establish a backwash periodicity of 2.5-3.0 h with a duration of roughly 60 s. The FF units were airlift-driven, prototype units (model # PS8 8.5", Aquaneering, Inc., San Diego, CA, USA) 20.3 cm x 169 cm with a rated flow of 45-94 L/m. Tanks were covered with white netting to prevent escape and juvenile (mean weight = 2.0 g) Pacific white shrimp were stocked at a density of 287/m^2 on Day 11 post-fill (June 13, 2003 - study Day 0). To better control tank temperatures the entire tank complex was covered with a roof of 63% shade cloth. Shrimp were fed a 35% protein, 8% lipid, 2.5% squid meal diet (Rangen, Inc., Buhl, ID, USA) applied twice daily (at 0800 and 1600 h) to single feed trays in each tank. Feed rate was adjusted based upon shrimp growth and feed consumption. Feed quantity applied and consumed was recorded at each feeding. Shrimp growth was measured weekly (treatments divided in half and each half measured every other week) by obtaining individual weights of 50 randomly collected shrimp from each tank.

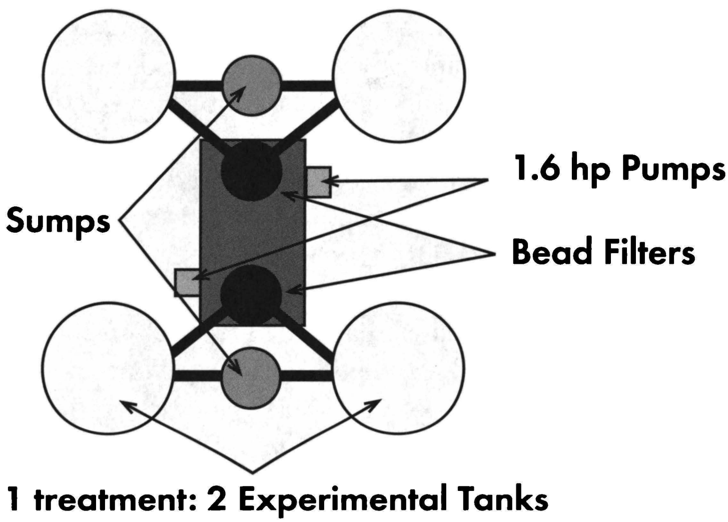


Figure 1. Design of experimental system.

Dissolved oxygen (mg/L), temperature (C), salinity (g/L), and pH (YSI Model 556 Multiprobe System, Yellow Springs Instrument, Yellow Springs, OH, USA) were recorded every morning (~0800 h). Temperature and pH were again recorded every afternoon (~1600 h). To maintain pH levels >7.0 and alkalinity >120 mg/L (as $CaCO_3$), sodium bicarbonate was added to each system. Alkalinity, total ammonia-N, nitrite-N, nitrate-N, and chlorophyll *a* were measured weekly according to standard water quality methods (APHA 1989). Total suspended solids (TSS) was measured weekly and turbidity (ntu) was measured daily using a turbidometer (Micro 100 Turbidometer, HF Scientific, Fort Myers, FL, USA) and by Secchi disc depth. Light and dark BOD bottles were used to measure water column gross oxygen production (change in light bottle minus change in dark bottle) and demand (change in dark bottle) and calculate net primary productivity (gross oxygen production divided by oxygen demand) once a week (Bratvold and Browdy 1998). Bead-filter maintenance included monitoring flow rates, inlet/outlet dissolved oxygen levels, sludge volume, percent solids, and filter backwash regularity. Flow rates and dissolved oxygen levels associated with bead-filter intake/outflow and foam fractionator return flow rates were measured twice during the week. Flow rate through the filter was adjusted for a turn over rate of roughly 10 water exchanges daily. Sludge was removed and total volume measured twice a week. To ensure maximum removal, sludge was purged from the filters until the discharge was clear (tank volume lost to sludge removal was <1%). The sludge was then mixed to remove bead-filter media and create a homogenous sample, and total volume was recorded before aliquots were removed and allowed to settle to determine percent solids. Discharge from the foam fractionators was also collected (collection buckets were removed and replaced every morning and afternoon), quantified and allowed to settle for percent solids determination. Sludge samples were collected weekly for total and volatile suspended solids (TSS, VSS), Kjeldahl nitrogen (TKN), and total organic carbon (TOC), and either frozen or the pH reduced to <2 by addition of H_2SO_4 for later analysis. Filter backwash periodicity and duration were monitored, recorded, and adjusted once a week. Filter air flow was checked daily. For all measured parameters, the treatments were compared with ANOVA tests when data exhibited normal distribution. Student's t-test was used to compare treatment means ($\rho = 0.05$). An ANOVA on ranks (Wilcoxon test) was performed for data that were not normally distributed.

RESULTS

Average shrimp weight at harvest, mean growth/week, survival, production, and food conversion ratio (FCR) are listed in Table 1. KMT treatments showed significant increases in growth rate relative to mixed media, foam fractionation, and control treatments. A similar trend was observed for the EN treatment. Survival and production, on the other hand, were not significantly different than control and FF treatments. Mixed-media filtered tanks performed least effectively, with production and FCR significantly different from other treatments.

Table 1. Mean values for harvest weight, growth/week, survival, food conversion ratio (FCR) and production. Values in a column with different superscripts are significantly different.

	Weight (g)	Growth/week (g)	Survival (%)	FCR	Production (kg/m³)
Control	7.9 ± 0.7^{b}	0.8 ± 0.1^{b}	77.0 ± 3.1^{a}	1.9 ± 0.1^{ab}	2.4 ± 0.1^{b}
FF	8.5 ± 1.0^{ab}	0.8 ± 0.1^{ab}	63.9 ± 5.4^{ab}	2.2 ± 0.1^{ab}	2.2 ± 0.2^{b}
EN	9.4 ± 0.5^{ab}	0.9 ± 0.1^{ab}	65.8 ± 24.3^{ab}	2.3 ± 1.2^{ab}	2.5 ± 1.0^{b}
KMT	9.9 ± 1.0^{a}	0.9 ± 0.1^{a}	69.3 ± 3.9^{ab}	1.8 ± 0.2^{a}	2.8 ± 0.4^{b}
EN/KMT	8.4 ± 1.6^{b}	0.8 ± 0.2^{b}	43.8 ± 35.0^{b}	4.7 ± 2.9^{b}	1.6 ± 1.4^{a}

There were significant water-quality differences between treatments (Table 2). Dissolved oxygen levels and daily pH values were significantly higher in bead-filter treatments than in unfiltered treatments. The DO range reflects two power outages that interrupted tank aeration. Salinity in FF tanks was significantly different from other treatments, including controls.

Total ammonia-nitrogen (TA-N) and nitrite-nitrogen (NO_2-N) were significantly different in filtered and unfiltered treatments (Table 3). Mixed media tanks had significantly higher TA-N and NO_2-N concentrations than all other treatments and all filtered treatments had higher NO_2-N concentration than unfiltered treatments. Unfiltered treatments had significantly higher NO_3-N concentrations. Unfiltered tank TA-N dropped to <1.0 mg/L by Day 14 while filtered tanks, especially those with KMT media, never appeared to stabilize and decrease. By day 45 the NO_2-N in unfiltered tanks dropped to <0.5 mg/L while filtered tanks continued to have higher, fluctuating nitrite levels. In all tanks NO_3-N concentration

Table 2. Mean values for salinity, temperature, pH, and dissolved oxygen. Values in a column with different superscripts are significantly different.

	Salinity (g/L)	AM Temp. (C)	PM Temp. (C)	AM pH	PM pH	D.O. (mg/L)
Control	21.9 ± 2.1^{b}	27.0 ± 0.9^{a}	28.2 ± 1.0^{a}	7.3 ± 0.3^{d}	7.4 ± 0.2^{e}	5.7 ± 0.8^{c}
FF	22.8 ± 2.0^{a}	27.0 ± 0.9^{a}	28.1 ± 1.0^{a}	7.4 ± 0.3^{c}	7.5 ± 0.2^{d}	6.0 ± 0.7^{b}
EN	21.9 ± 2.0^{b}	26.8 ± 0.9^{a}	28.2 ± 1.1^{a}	7.5 ± 0.2^{b}	7.7 ± 0.2^{c}	6.3 ± 0.6^{a}
KMT	22.0 ± 2.5^{b}	26.9 ± 0.9a	28.2 ± 1.1^{a}	7.6 ± 0.2^{a}	7.8 ± 0.2^{a}	6.2 ± 0.7^{a}
EN/KMT	21.9 ± 2.2^{b}	26.9 ± 0.9^{a}	28.2 ± 1.1^{a}	7.6 ± 0.2^{a}	7.7 ± 0.2^{b}	6.2 ± 0.7^{a}
Mean	22.1 ± 2.1	26.9 ± 0.9	28.2 ± 1.0	7.5 ± 0.3	7.6 ± 0.3	6.1 ± 0.8
Range	18.0 - 22.1	24.5 - 30.9		7.5 - 8.5		2.1 - 8.2

showed a slight decrease around Day 30 but then increased again and continued to increase for the duration of the production trial. Survival was similar to that of previous production trials with the exception of three filtered tanks which experienced elevated NO_2-N levels.

Table 3. Mean values for dissolved inorganic nitrogen: A. TA-N; B. NO_2-N; C. NO_3-N. Values in a column with different superscripts are significantly different.

	TA-N (mg/L)	NO_2- N (mg/L)	NO_3-N (mg/L)
Control	1.1 ± 2.0^{b}	3.1 ± 5.0^{d}	25.5 ± 13.9^{a}
FF	1.3 ± 1.9^{b}	3.2 ± 4.4^{d}	24.3 ± 14.2^{ab}
EN	1.2 ± 1.8^{b}	9.1 ± 9.5^{abc}	17.5 ± 11.1^{cd}
KMT	1.6 ± 1.5^{b}	7.4 ± 5.3^{c}	14.5 ± 11.6^{cd}
EN/KMT	3.9 ± 9.6^{a}	11.9 ± 8.9^{a}	19.4 ± 13.1^{bc}
Mean	2.1 ± 5.2	7.8 ± 7.9	18.6 ± 13.0
Range	0 -57.0	0 - 34.1	0 - 64.0

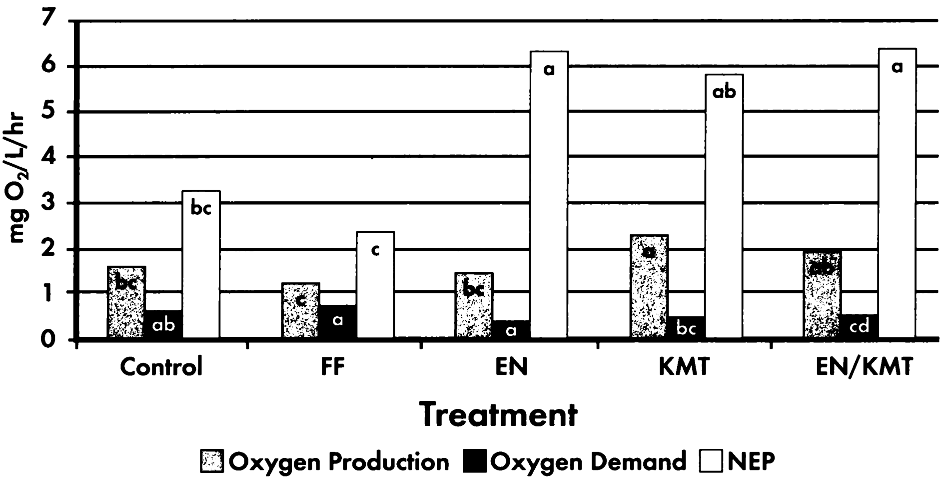


Figure 2. Overall mean treatment values for oxygen production, oxygen demand, and net ecosystem production (NEP). Values across columns not sharing the same letter are significantly different.

Figure 2 illustrates the relationship between oxygen production, demand, and net ecosystem productivity (NEP). An NEP >1 indicates that there is more oxygen production than oxygen consumption while an NEP <1 indicates that consumption exceeds production. Filtered tanks containing KMT media (alone or with EN media) had the highest oxygen production while tanks containing EN media (alone or with KMT media) had the lowest oxygen consumption compared to unfiltered treatments. Filtered tanks also had the highest NEP.

Chlorophyll *a* levels were significantly lower in filtered treatments (Table 4) even though oxygen production was significantly higher than unfiltered tanks. In addition to reduced chlorophyll *a*, filtered treatments had less VSS and TSS. An exception to this trend was observed in two EN tanks where TSS and VSS increased at about Day 30. Despite demonstrated solids removal, filtered treatments turbidity fluctuated during the production trial and the turbidity actually increased in EN media tanks (Table 4) even though sludge output remained high. This increase in solids load was accompanied by an increase in dissolved oxygen consumption within the two affected EN filters (Figure 3). Unfiltered tank turbidity increased and Secchi depth decreased throughout the production trial.

Table 4. Overall mean values for suspended solids and water clarity. All levels were significantly different for filtered tanks.

	Chlorophyll *a* (mg/m^3)	VSS (mg/L)	TSS (mg/L)	Turbidity (ntu)	Secchi (cm)
Control	226.7 ± 95.9[a]	263.3 ± 96.9[a]	383.6 ± 134.2[a]	119.4 ± 37.4[a]	17.6 ±5.4[b]
FF	195.7 ± 97.9[ab]	225.6 ± 100.6[b]	318.6 ± 125.3[b]	100.8 ± 24.5[b]	18.2 ± 5.5[b]
EN	150.8 ± 100.4[b]	74.7 ± 89.9[c]	113.4 ± 132.8[c]	30.1 ± 13.8[c]	42.4 ± 8.2[a]
KMT	179.5 ± 132.9[b]	40.0 ± 43.4[de]	58.4 ± 61.8[d]	20.8 ± 7.3[c]	44.4 ± 9.9[a]
ENKMT	153.3 ± 109.8[b]	34.0 ± 20.6[e]	50.5 ± 29.0[d]	20.2 ± 7.2[c]	41.7 ± 8.0[a]
Mean	176.5 ± 114.8	97.0 ± 112.3	141.1 ± 159.0	58.3 ± 47.9	33.1 ± 14.3
Range	2.0 - 533.2	0 - 486.0	5.0 - 636.0	9.4 - 210.0	10.3 - 63.0

Feed rates were adjusted as shrimp growth and water quality parameters changed. Throughout the production trial feed loading never exceeded 600 g/day or 0.7 kg/m^3 of bead media. Cumulative sludge removal and % settleable solids were highest for EN media filters and lowest for FF tanks with no significant difference in either sludge removed or settleable solids between KMT and EN/KMT filters. In sampled filters there was no appreciable change in TKN across the EN filter. TKN increased in water returning from the KMT filter and was only reduced after passing through the EN/KMT filter. TKN increased in sampled sludge from all three filters with the greatest increase in organic nitrogen loading occurring in the EN filter (six times higher than the initial sample) which also had the highest initial concentration.

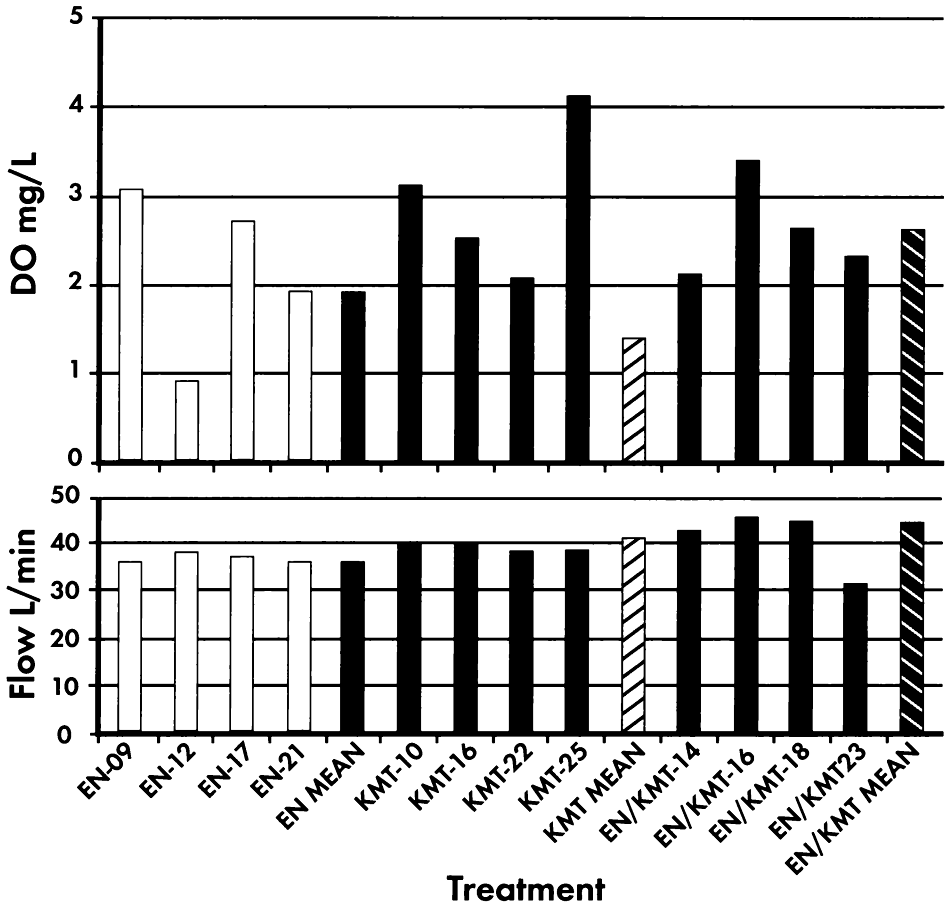
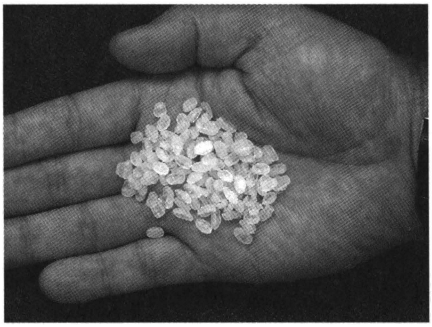


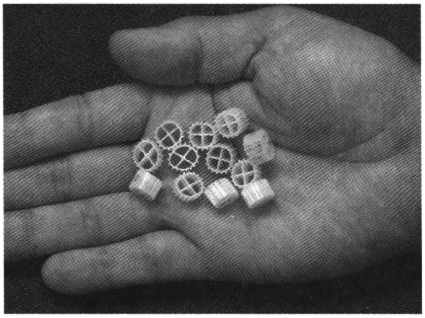
Figure 3. Filter respiration and tank return flow for all filtered tanks. Although mean filter flow appears consistent, there was a significant decrease in flow in both FF and filtered treatments over the course of the production trial.

DISCUSSION

The purpose of this study was to evaluate air lift-driven bead filters and foam fractionation units for their potential as management strategies for suspended solids and nitrogenous waste removal in superintensive, zero exchange shrimp production systems. The type of bead filter used was particularly attractive because it had the capacity to function as both a biological and mechanical filtration unit while requiring no electric pump for operation or removal of accumulated solids. Filtration was to be accomplished through the use of two dissimilar polyethylene media (Figure 4). EN media as a small modified bead has a much greater composite surface area and smaller packed volume pore space than the larger wheel shaped KMT media. The larger surface area for adhesion coupled with the smaller pore space was expected to remove more small particulate material. The modified shape provided a concave surface to protect nitrifying bacteria under backwash conditions. The KMT media, with its protected interior surface for colonization, was expected to remove and accumulate larger solids while retaining a greater population of nitrifying bacteria under backwash conditions. Because solids capture efficiency varies when media size is fixed, it was expected that the combination of these two dissimilar media would achieve the solids capture efficiency of the individual media types. Especially under the green-water conditions of this study, determining the appropriate backwash frequency to maximize solids removal while enhancing biofiltration is critical if the detrimental effects of retained solids decay and subsequent ammonia loading is to be avoided. Under normal organic loads the small, tightly packed EN media should be backwashed more frequently than the larger, less densely packed KMT media for optimal function (Moore *et al.* 2001). Foam fractionation as a mechanical filtration unit was expected to efficiently remove fine suspended solids and dissolved solids using bubbled air moving upwards against the downward flow of water from the tank (Cripps and Bergheim 2000). As with the bead filter used, the design of the foam fractionator was attractive because it required no electric pump for operation.



EN Media



KMT Media

Figure 4. Comparison of EN media to KMT media.

High-density production trials conducted in zero-exchange superintensive raceway systems at the Waddell Mariculture Center have achieved production rates approaching 3 kg/m^2 (McAbee *et al.* 2001, Weirich *et al.* 2002). At these biomass levels, problems with unexplained reductions in survival rates, cuticular lesions and chronic mortality were thought to be indicative of a system at or near its carrying capacity (Weirich *et al.* 2002). During this experiment, weight at harvest and mean growth rate (g/week) were less than that seen in previous production trials under similar conditions while survival, FCR, and production numbers, however, were consistent with those achieved during previous production trials. Although bead-filtered treatments were expected to improve survival and growth, results were quite variable.

Previous studies in these systems demonstrated that cropping of organic material increased system carrying capacity while enhancing growth of the target crop (Weirich *et al.* 2002, McAbee *et al.* 2001). The results of this study confirm that incorporating filtration into a eutrophic, zero-water-exchange intensive production system can offer clear benefits as a mechanical means of reducing solids, managing the algal community and improving general water quality. This improvement is reflected by higher pH and oxygen levels in the presence of reduced chlorophyll *a* and increased water clarity in filtered treatments. Significant reduction in TSS and VSS were also as expected for bead filtered systems which function well at removing particles larger than 50 μm (Chen *et al.* 1993). The mean percentage of TSS that was VSS ranged from 62-74%, consistent with observed values for aquacultural (Ning 1996) and domestic sludge (Metcalf and Eddy Inc. 1991). The percentage of nitrogen (as TKN) in sludge was much greater than in aquacultural (Ning 1986) and domestic sludge (Metcalf and Eddy Inc. 1991) with mean values ranging as high as 74%.

Although this may at first appear to be counterintuitive, cropping of senescent algal cells and suspended organic material by filter media may have been conducive to the production of a younger, more active phototrophic community. This type of natural productivity has been suggested to contribute to the improved growth of Pacific white shrimp associated with pond water based systems (Moss *et al.* 1992, Moss 1995). In the present study this may have been reflected in the significant improvement in growth rate in the KMT filter treatment.

The bead filters, however, did not effectively reduce nitrogenous wastes. Although all tanks were filled and fertilized prior to being stocked, it is likely that there was inadequate time for nitrifying bacteria to become well established and to maintain a density sufficient for effective nitrification while backwash frequency and periodicity were being adjusted. Additionally, while backwash frequency and periodicity were adequate for removing solids from the filtered systems, sludge removal needed to be more frequent to mitigate the effects of organic solids accumulation and decomposition. Conditions within the filters tended to favor proliferation of faster growing heterotrophic bacteria rather than the slower growing nitrifying bacteria (Sastry *et al.* 1999). It was apparent that even with a relatively low feed loading, media type affected filter performance under the gentle backwash conditions present in the airlift driven MRBF system. The configuration of the KMT media did not function effectively under the relatively low feed loading at the established backwash frequency. Solids trapped within the interior of the bead clogged the media, reduced the available surface area for nitrification and reduced bead buoyancy causing the media to sink and become mired in the bottom of the filter. The EN media was also problematic. Because it removed smaller particles more efficiently than the larger, less densely packed KMT or mixed media, it removed too much of the suspended material associated with attached nitrifying bacteria and beneficial flocculent material which can contribute to shrimp growth (Moss *et al.* 1992, Burford *et al.* 2004). Although EN tanks clearly removed more solids throughout the production trial, at about Day 30, two of the EN filters had significant increases in suspended solids and decreases in water clarity. The increase in TSS and VSS, indicative of reduced filter function possibly due to excessive solids retention and to clogging, was accompanied by a significant increase in filter oxygen consumption. This increase in consumption is to be expected as every 1 mg increase in VSS requires roughly 1.42 mg O_2 for oxidation (McCarty 1965). Mixed-media tanks operated with efficiency intermediate to the filters filled with a single media type. Despite constant cleaning both bead filters and foam fractionators were affected by fouling within the pipes by filamentous algae and other organisms which attached themselves to the air stones driving the system. Because the filters were gravity-fed, they had to be buried below ground to circulate water with airlifts. As a result, sludge removal above ground was incomplete in all filters. Effective removal was complicated by media, especially the larger KMT media, which was subject to fouling as discussed previously, becoming trapped in the sludge drain. Repetitive backwashes, forcing air back through the filter and "bumping" the filter did not always break up bead/biofloc clumps and when the systems were taken down at the end of the study a substantial amount of solids with embedded media were stuck in the bottoms of the filters.

It was expected that the FF units would function better in conjunction with a marine system than a freshwater system and would have been capable of removing both fine and suspended solids (Chen *et al.* 1992; Cripps and Bergheim 2000). Although turbidity and suspended solids were significantly less in FF tanks as compared to control tanks, solids removal was inconsistent and directly related to the intermittent generation of bubbles with a consistency capable of entrapping and removing solids from the water column. When foam was produced it was *en masse* and despite trying several strategies to cause the bubbles to break up and collect as sludge, much of the material remained stuck to the inside of the clear plastic sleeve at the top of the unit.

Aquaculture systems with or without filtration have some nitrification capacity. Nitrifying bacteria actively colonize available tank surfaces when optimal water conditions are met. Indeed, in the bacterial floc dominated, zero-exchange shrimp production systems, nitrification *in situ* has been shown to effectively control ammonia and nitrite levels within the system (Browdy *et al.* 2001, Bratvold and Browdy 2001, Weirich *et al.* 2002). With environmental parameters within tolerance in both filtered and unfiltered tanks, *in situ* nitrification should account for 30-60% of total nitrification (Malone *et al.* 1993). Control and FF treatments had successful populations of nitrifying bacteria and had no problems with ammonia or nitrite. Filtered treatments having the same available surface area in addition to filter media were not as successful in managing these compounds. It seems likely that while not optimized for nitrification, the efficiency of solids removal may have depleted this inherent nitrifying capacity. As a result, high nitrite levels may account for the negative results on survival and production found in the mixed media treatment.

CONCLUSION

Both the bead filters and foam fractionators were effective at removing solids from the high-density zero exchange shrimp production tanks. Although the cropping of organic material stimulated algal primary productivity and did result in improved growth in one treatment, overall results were variable. In many tanks the removal of too much of the bacterial floc from the system and the breakdown of organic material within the filter may have contributed to increased ammonia and nitrite levels and reductions in growth and survival. Operated as they were in this production trial, filtration did not make a positive contribution to the management of nitrogenous waste. To optimize filtration and nitrification and to negate these problems in subsequent production trials, refinements to the filter systems and operating protocols have been designed. The goal of these refinements is to improve the operation of the filters by reducing organic loading and improving control of the level of cropping of organic material from production tanks. Refinements include using EN media exclusively, reducing the backwash rate to once every eight hours, raising the filters above ground level using 1/3-hp pumps to circulate the water, and removing settled solids more frequently. To preclude over-cropping of algae and beneficial flocculent material in the tank water from cycling through the filter, filtration will be more precisely controlled through the use of a sump coupled with a recirculation loop. The ultimate goal of these studies is to maximize growth and carrying capacity while improving production efficiency, as measured in terms of nitrogen transfer from feeds to the target crop and waste recycling within the system.

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