# INTENSIVE TANK CULTURE OF TILAPIA WITH A SUSPENDED, BACTERIAL-BASED, TREATMENT PROCESS

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## Abstract

A 200-$m^3$ circular tank was evaluated in production trials stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) at 20 and 25 fish/$m^3$ in Trial 1 and 2, respectively. Water treatment methods consisted of aeration, water circulation (mixing), solids removal and nitrification in the water column. The fish were fed *ad libitum* twice a day with a complete (32% protein), floating pellet. After 175 and 201 days of growth, total production was 14.4 and 13.7 kg/$m^3$ in Trial 1 and 2, respectively. Ammonia and nitrite concentrations were generally acceptable for tilapia growth. The nitrate-nitrogen concentration increased throughout the trials and reached 654 and 707 mg/L in Trials 1 and 2, respectively, which indicated a high rate of nitrification and the need for a denitrification treatment process to be added to this closed system. Total suspended solids (TSS) increased throughout the trials and reached peaks of 1,300 and 1,960 mg/L in Trials 1 and 2, respectively. The horizontal water velocity was too high for effective sedimentation of suspended solids for removal by a cone situated in the center of the tank. The addition of an external clarifier to the system for the last 3 weeks of Trial 2 removed 360 kg of dry weight solids, resulting in the reduction of TSS levels from to 1,700 to 600 mg/L. The reduction of TSS improved other water quality parameters and fish feeding response.

## Introduction

Pond culture is the standard method of producing tilapia in the tropics. Pond culture depends on phytoplankton to generate oxygen and absorb dissolved nitrogenous waste. The feeding rate limit for fed ponds is determined by the ability of the pond's microbial community to assimilate fish waste products such as ammonia and solid waste, which undergoes microbial decomposition. The feeding rate limit determines a pond's production capacity. A standard production level for a fed pond is 5,000 kg/ha. The production level can be increased with aeration and/or water exchange.

Intensive tank culture system was developed at the University of the Virgin Islands, which reduces the limitations of pond culture (Rakocy *et al*. 2000; Rakocy *et al*. 2002). The tank is continuously aerated and does not depend on phytoplankton for oxygen production. The primary component of the microbial community is shifted from phytoplankton to autotrophic bacteria, which remove ammonia and nitrite. Settleable solid waste is removed

daily through a sedimentation process. The culture water is mixed to suspend the microbial community and maximize contact between bacteria and waste products. The culture water contains high concentrations of phytoplankton, and the system is referred to as greenwater tank culture. However, the phytoplankton community does not play as dominant a role in maintaining water quality as in pond culture.

**Materials and methods**

A 200-$m^3$ circular tank (surface area = 200 $m^2$) was constructed outdoors in St. Croix, U.S. Virgin Islands (Figures 1 and 2). The tank was 16 m wide by 1.22 m deep. The walls of the tank were constructed from six tiers of lintel blocks (knock out bond beam blocks), which were reinforced horizontally and vertically with steel reinforcement bar and core filled with concrete. A prefabricated plastic liner (30 mil HDPE) was installed inside the tank wall. The sides of the liner were pulled over the wall to the outside and secured by fastening lumber (5 cm by 20 cm) to the top of the wall. Soil was backfilled around the outside of the tank so that only 0.4 m of the tank wall was above grade.

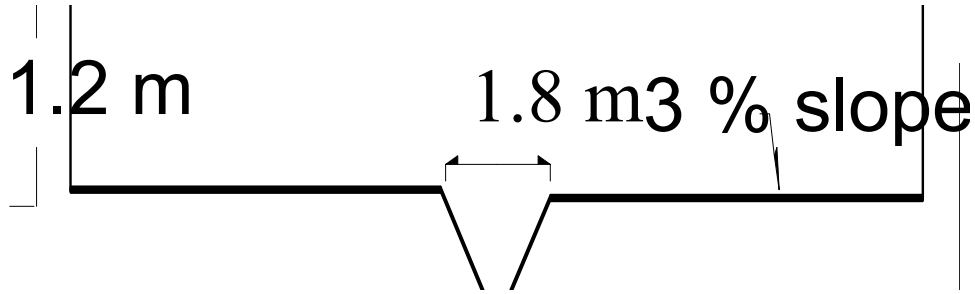


Figure 1. A 200-$m^3$ rearing tank with center cone and external drain line.

Figure 2. View of rearing tank with three vertical lift aerators.

The bottom of the tank sloped 3% to a central, 1-$m^3$, fiberglass cone with a 45° slope. The liner was attached to a wide flange around the top of the cone with double-sided tape. A 10-cm, PVC drainpipe extended from the apex of the cone to a 1-$m^3$ fiberglass tank located outside the rearing tank. By opening a gate valve in the drainpipe once a day, solid waste from the cone flowed into the small tank through an internal standpipe, and its volume was measured.

Figure 3. External clarifier.

This system of solids removal was modified in a second production trial. A 1.9-$m^3$ cylindro-conical clarifier was installed outside the rearing tank (Figure 3). The clarifier was constructed with fiberglass-reinforced rigid plastic sheeting (1 mm thick). The cylindrical portion of the clarifier was situated above ground and contained a central baffle that was perpendicular to the incoming water flow. The lower conical portion, with a 60$^o$ slope, was buried under ground. A 3-cm, PVC drainpipe extended from the apex of the cone to the top of the 1-$m^3$ sludge tank. Rearing tank effluent was drawn from a depth of 0.8 m along the side of the rearing tank through a 3.8-cm pipe and pumped, with a 0.25-hp centrifugal pump, into the clarifier just below the water surface at a rate of 38 liters/minute to create a 50-minute retention time. The incoming water was deflected upward by a 45$^o$ PVC elbow to dissipate the current. As water flowed under the baffle, turbulence diminished and solids settled to the bottom of the cone. A ball valve was opened to drain solids from the cone into the sludge tank for measurement. The clarifier was operated during the last 21 days of the trial. Solids were removed from the cone an average of eight times daily for the first 6 days.

During days 7-21, solids were removed once in the morning. During this 21-day period, solids were also removed from the cone in the center of the rearing tank once per day during late afternoon. The sludge was sampled several times to measure total suspended solids and determine the dry weight of solids removed.

The rearing tank was aerated with three ¾-hp vertical lift pumps (Figure 2). A single aerator was used for the first two months. Two aerators were employed during months 3-4, and three aerators were used during months 5-6. Another lift pump was positioned horizontally to provide horizontal water circulation (mixing). The amount of electricity used was calculated.

Two production trials were conducted. The tank was stocked with sex-reversed Nile tilapia (*Oreochromis niloticus*) fingerlings at a rate of 20 fish/$m^3$ in Trial 1 and 25 fish/$m^3$ in Trial 2. A nutritionally complete, floating pellet (32% protein) was offered twice daily *ad libitum* to satiation for 175 days in Trial 1 and 201 days in Trial 2. An initial 30-minute feeding period was eventually extended to 1 hour in Trial 1 and 30-40 minutes in Trial 2. Feed was restricted slightly during the first 4-6 weeks of the trials until populations of nitrifying bacteria in the water column were adequate to maintain low levels of ammonia and nitrite.

Water quality parameters were measured weekly (DO, water temperature, $NH_3$-N, $NO_2$-N, $NO_3$-N, pH, total alkalinity), biweekly (chlorophyll *a*, COD, settleable solids, TSS, TP, $PO_4$-P) or periodically (Cl). Base [$Ca(OH)_2$] was added frequently to maintain pH near 7.5. The base was added to a 0.2-$m^3$ tank through which a small stream of water flowed so that high-pH water was gradually added to the rearing tank. Water loss due to evaporation and sludge removal was volumetrically replaced. At the end of the trials all fish were harvested, weighed and counted.

**Results and discussion**

Total production was 14.4 kg/$m^3$ in Trial 1 and 13.7 kg/$m^3$ in Trial 2 (Table 1). The fish grew at a higher rate (4.0 g/day) and reached a larger size (912 g) in Trial 1 because larger fingerlings were stocked. Therefore initial growth rates were higher. In addition, the stocking rate was higher (25 fish/$m^3$) in Trial 2, which can reduce the growth rate of individual fish. The feed conversion ratios (2.2 in Trial 1 and 1.9 in Trial 2) were higher than expected. This may have been due in part to low survival rates (about 80%) in both trials caused by bird predation. Herons perched on the side of the tank and preyed on the fish during the beginning of the production cycle. Fish that were too large to swallow were found on the ground. An electric wire was strung along the top of the tank wall to repel birds midway through the first trial. This device failed in the second trial, and bird predation was heavy again.

Table 1. Production of tilapia - Trials 1 and 2.

| Trial | Stocking Rate (#/$m^3$) | Initial Size (g) | Final Size (g) | Culture Period (d) | Growth Rate (g/d) | Final Biomass (kg/$m^3$) | FCR | Survival (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 214 | 912 | 175 | 4.0 | 14.4 | 2.2 | 78.9 |
| 2 | 25 | 73.6 | 678 | 201 | 3.0 | 13.7 | 1.9 | 81.0 |

Another factor leading to a high feed conversion ratio appeared to result from the interaction of water quality and feed consumption. In both trials the daily feed consumption varied considerably, but there was an upward trend in consumption at the beginning of each trial (Figures 4 and 5). In Trial 1, feed consumption leveled off during the middle of the trial and declined slightly by the end. In Trial 2, feed consumption increased until day 113, leveled off until day 141, declined until day 180, and increased in the last 3 weeks. As the fish grow, a continuous increase in the daily feed ration is expected. If the daily ration reaches a limit due to water quality deterioration, gradually a smaller proportion of the daily ration goes to fish growth, which causes a decline in the growth rate and an increase in the feed conversion ratio.

Most water quality parameters were in acceptable ranges for tilapia culture (Table 2). Total ammonia-nitrogen (TAN) averaged 1.15 mg/L in Trail 1 and 1.85 mg/L in Trial 2. The TAN concentration reached a peak of 8.55 mg/L in Trial 2 for a short period (Figure 6). There was no observed mortality during this period, and the feeding ration did not decline (Figure 5). Nitrite-nitrogen concentrations averaged 0.58 mg/L in Trial 1 and 2.68 mg/L in Trial 2. There was a peak concentration of $NO_2$-N in Trial 1 of 13.62 mg/L, but this value was not included in the average because it was caused by mistakenly adding chlorinated water to replace evaporative losses. The chlorine appeared to affect *Nitrobacter* bacteria but not *Nitrosomonas*, as TAN levels did not increase during this period. In Trial 2 there was a peak $NO_2$-N concentration of 18.27 mg/L, which followed the peak in the TAN concentration by a week (Figures 6 and 7).

To avoid ammonia toxicity, pH was maintained near 7.5 so that most ammonia was in the ionized, nontoxic form. However, during system startup, the pH of the well water was close to 9.0 and nitrifying bacteria were not established. Therefore, pH, TAN and $NO_2$-N were monitored frequently for 4-6 weeks, and CaCl was added as a prophylactic to prevent nitrite toxicity. The Cl concentration averaged 301 mg/L in Trial 1 and 319 mg/L in Trial 2.

The $NO_3$-N concentration increased steadily throughout the production trials and reached peak concentrations of 654 mg/L in Trial 1 and 707 mg/L in Trial 2, indicating that nitrification was occurring in the water column (Figure 8). The high $NO_3$-N concentrations near the end of the trials could have affected the feeding response of tilapia.

The tank system required very little water exchange. Average daily makeup water was 880 liters (0.44% of the tank volume) in Trial 1 and 401 liters (0.20% of the tank volume) in Trial 2 (Table 3). The average volume recovered as sludge was 470 liters/day in

Trial 1 and 366 liters/day in Trial 2. Therefore, average net water loss was just 0.1% of the system volume over the two production trials.

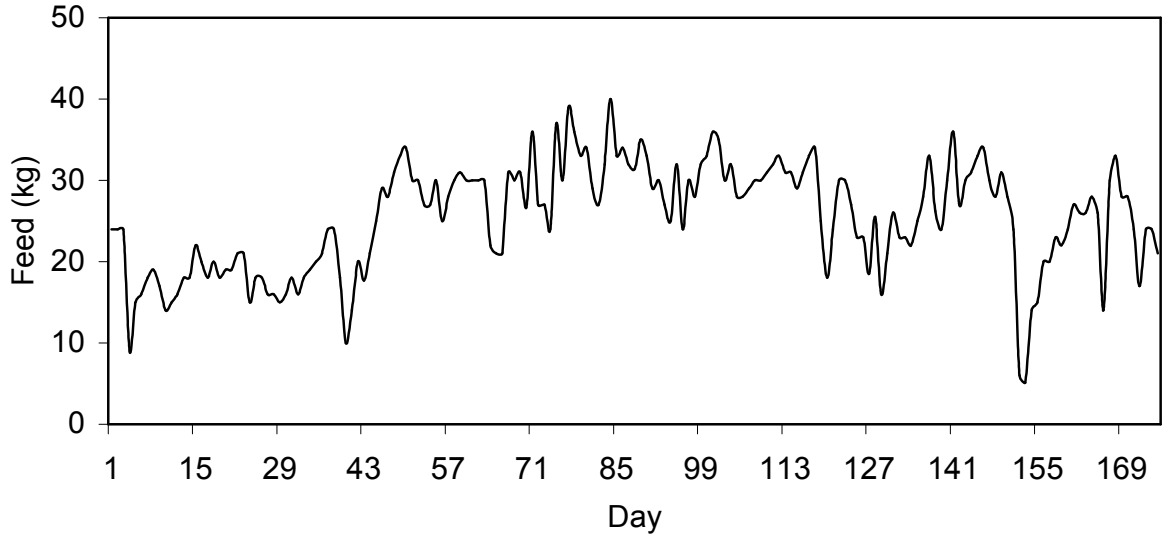


Figure 4. Feed input - Trial 1.

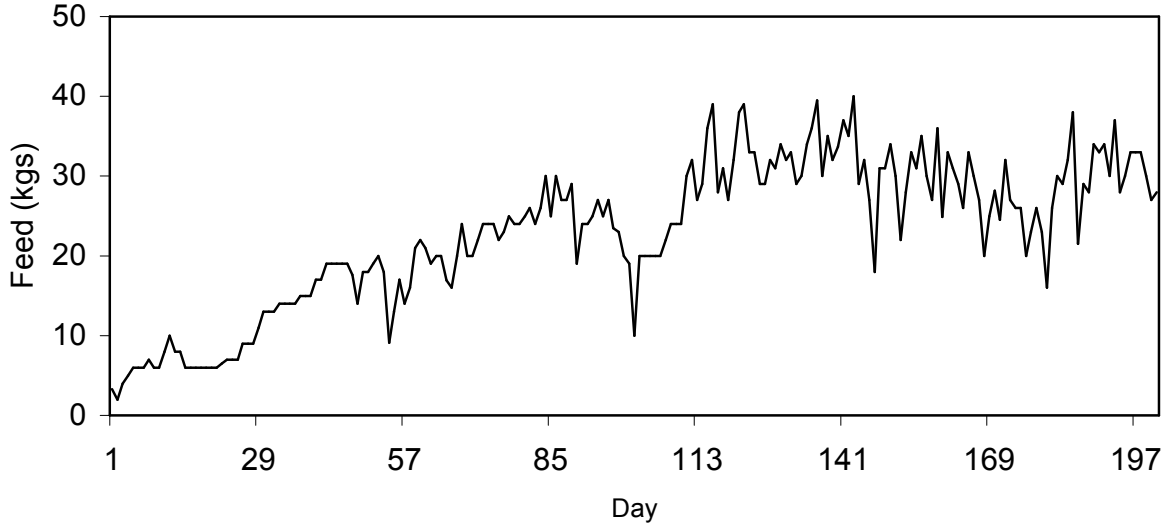


Figure 5. Feed input - Trial 2.

Table 2. Water quality values - Trial 1 and 2.

| | Trial 1 | Trial 2 |
|---|---|---|
| Dissolved Oxygen (mg/L) | 5.5 (4.2-7.5) | 7.9 (4.2-11.1) |
| pH | 7.8 (6.9-8.9) | 7.8 (6.8-8.8) |
| Alkalinity (mg/L, as $CaCO_3$) | 224 (76-416) | 204 (126-306) |
| Temperature ($^oC$) | 28.6 (25.8-31.3) | 28.5 (26.6-31.4) |
| Total Ammonia-N (mg/L) | 1.15 (0.41-3.85) | 1.85 (0.21-8.55) |
| Nitrite-Nitrogen (mg/L) | 0.58 (0.03-2.69)* | 2.68 (.04-18.27) |
| Nitrate-Nitrogen (mg/L) | 289 (62-654) | 397 (182-707) |
| Total Phosphorous (mg/L) | 41.9 (17.5-71.7) | 64.5 (4.1-143.0) |
| Orthophosphate (mg/L) | 16.9 (6.7-32.2) | 19.2 (0.08-40.8) |
| COD (mg/L) | 353.3 (219-606) | 363 (125.4-639.8) |
| Total Suspended Solids (mg/L) | 476 (220-1300) | 898 (100-1960) |
| Total Settleable Solids (ml/L) | 29 (11-100) | 48 (2-136) |
| Turbidity (FTU) | 328 (140-1030) | 506 (76-1160) |
| Chlorophyll-a (ug/L) | 1895 (670-6488) | 924 (219-1690) |

* Indicates removal of two data points for $NO_2$-N, 10.65 and 13.62 mg/L, resulting from addition of chlorinated water

Table 3. Inputs and outputs.

| Trial | Initial Water ($m^3$) | Makeup Water (L/day) | Sludge (L/d) | Feed (kg/day) | Base Addition (kg/day) | Electricity (kWh/day) |
|---|---|---|---|---|---|---|
| 1 | 200 | 880 | 470 | 25.4 | 1.5 | 52.8 |
| 2 | 200 | 401 | 366 | 23.0 | 1.7 | 52.8 |

Another variable that may have affected tilapia feeding response and growth was total suspended solids (TSS), which steadily increased during the production trials and reached 1,300 mg/liter in Trial 1 and 1,960 mg/liter in Trial 2 (Table 2). The central settling cone removed an average of 470 liters of sludge in Trial 1 and 366 liters in Trial 2 (Table 3). Daily sludge removal was somewhat variable over Trial 1 (Figure 9). On some days large amounts of sludge were removed for an unknown reason. These were called “sludge events.” Near the end of Trial 1 daily sludge removal was consistently low (150-350 liters). In Trial 2, daily sludge removal in general was consistently low (150-300 liters) until the last 3 weeks of the trial when the external clarifier was activated (Figure 10).

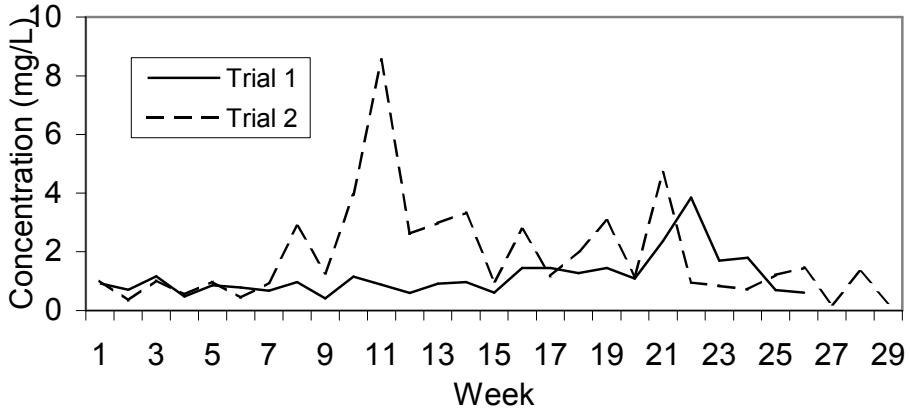


Figure 6. Total ammonia nitrogen - Trials 1 and 2.

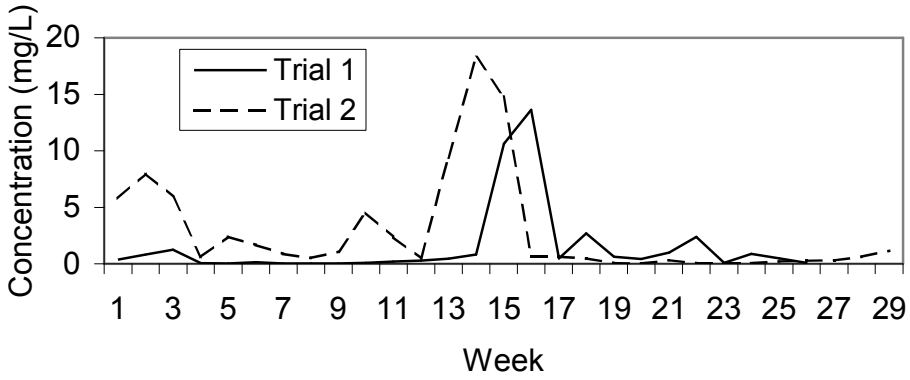


Figure 7. Nitrite nitrogen - Trials 1 and 2.

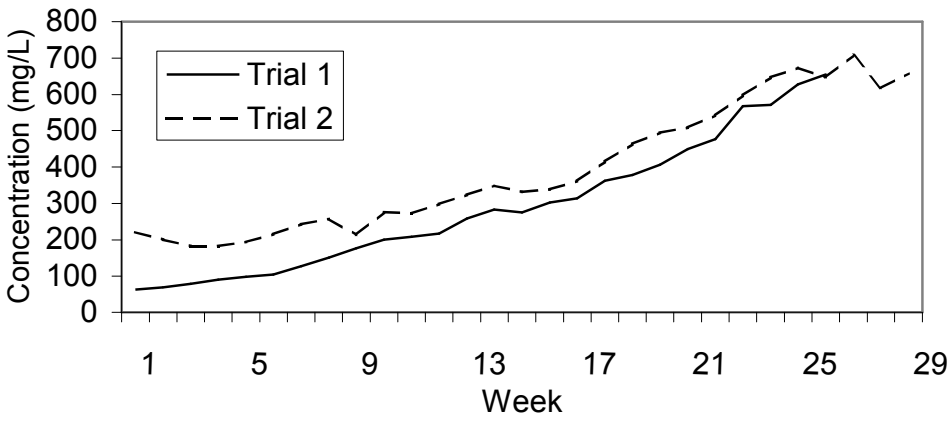


Figure 8. Nitrate nitrogen - Trials 1 and 2.

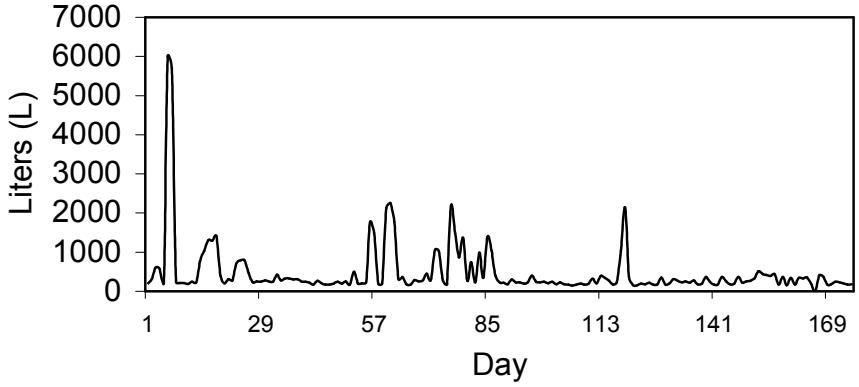


Figure 9. Daily sludge removal - Trial 1.

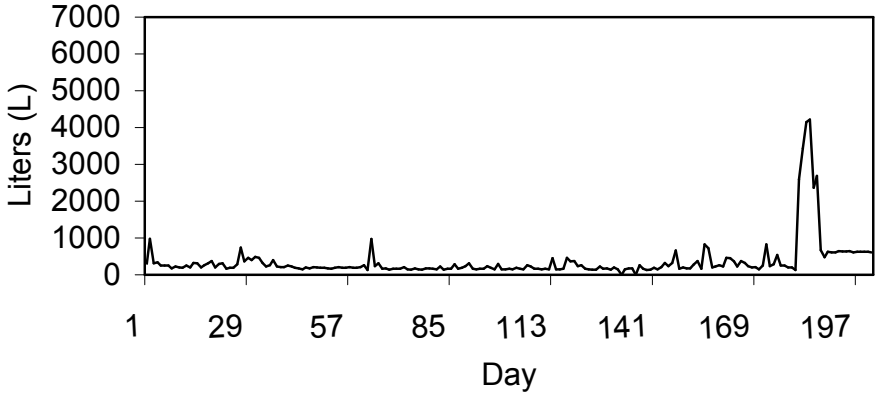


Figure 10. Daily sludge removal - Trial 2.

As each trial progressed, the buildup of TSS could be observed by the appearance of the water. There were clear streaks in the culture water that looked like solids were settling as the water circulated around the perimeter. However, the current was fast and re-suspended the solids on each pass through the horizontal mixing device. Water circulated completely around the perimeter of the tank every 2.5 minutes, a horizontal velocity of 20 m/minute. This phenomenon was most apparent in the last few weeks of the production trial when TSS levels were near their peak. At the end of each trial a sample of culture water was collected and sampled for TSS every 5 minutes over a 30-minute period. The settling curves show that 89% of the solids settled out in 30 minutes (Figure 11). In Trial 2, 84% of the solids settled out in 5 minutes. These results showed that the mixing was too rapid for suspended solids to settle out in the central cone for effective removal on a daily basis. As TSS increased, it likely affected the fish directly through physical irritation of the gills, and also exerted a high biochemical oxygen demand (BOD) and led to secondary ammonification. Paradoxically, rapid mixing and high TSS levels created an effective biofilter. An external clarifier was installed to reduce TSS levels in Trial 2.

The solids removal efficiency of the external clarifier was 88.5% (Table 4). The effectiveness of the external clarifier is clearly indicated in Figure 12. Sludge TSS was 26,230 mg/L, which is 2.6% dry weight solids. During the first 6 days of operation, the external clarifier removed 175.5 kg of dry weight solids from the rearing tank compared to 5.9 kg of dry weight solids removal by the central cone (Table 5). During this 6-day period, the external clarifier removed 96.7% of the total amount of solids that were collected. During days 7-21, the external clarifier removed 184.4 kg of dry weight solids compared to 4.8 kg of dry weight solids removal by the central cone. During this 15-day period, the clarifier removed 97.5% of the total amount of solids that were collected. During the 3-week period, TSS concentrations in the rearing tank declined from to 1700 mg/L to 600 mg/L, a 65% reduction (Figure 13). There were also decreases in total phosphorus from 172 to 64 mg/L (Figure 14) and chlorophyll *a* (Figure 15). Concentrations of ammonia and nitrite remained low, which indicates that sufficient levels of nitrifying bacteria remained in the water column (Figures 6 and 7). With substantially lower TSS levels, there would be less secondary ammonia production caused by the decomposition of suspended organic matter. Dissolved oxygen concentrations (data not shown) and the feeding response of the fish increased.

Table 4. External clarifier efficiency - Trial 2.

| Parameter | Concentration |
|---|---|
| Influent TSS (mg/L) | 1178 |
| Effluent TSS (mg/L) | 136 |
| Sludge TSS (mg/L) | 26,230 |
| Removal (%) | 88.5 |

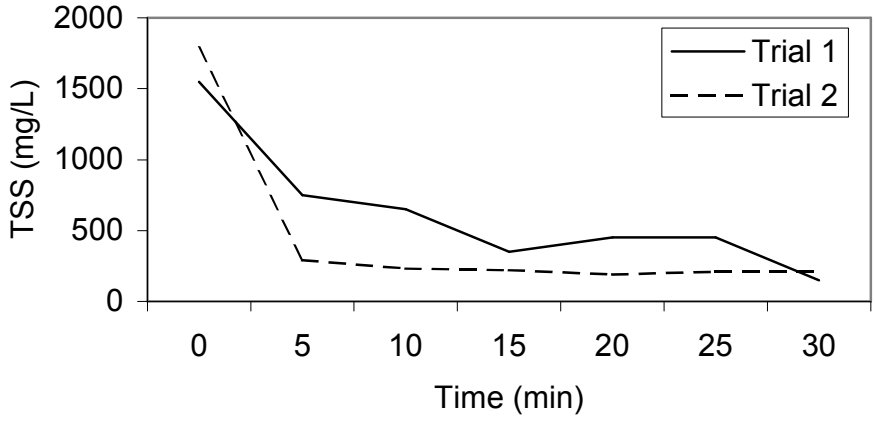


Figure 11. TSS settling curve - Trials 1 and 2.

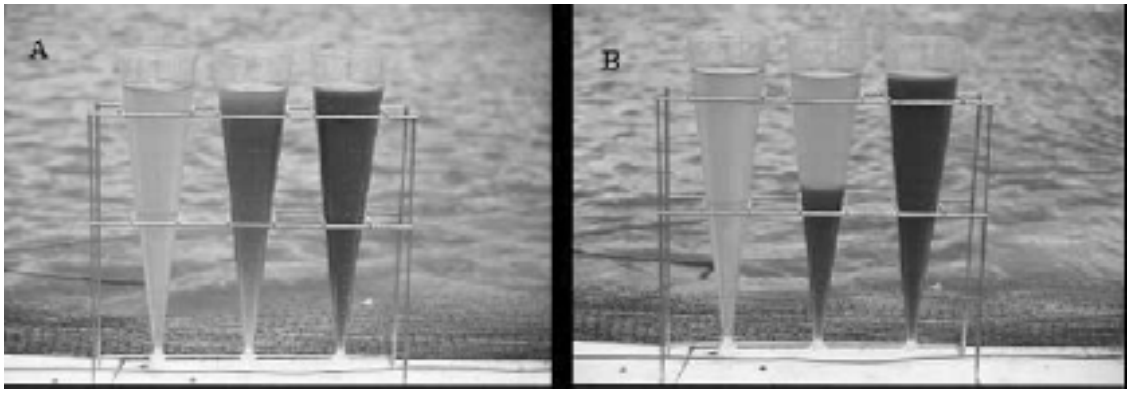


Figure 12. (A) Clarifier effluent from culture tank water, (B) Sludge from clarifier after 10 minutes settling.

Table 5. Sludge removal after external clarifier installed - Trial 2.

| | Sludge Removed, Day 1-6 | Sludge Removed, Day 7-21 |
|---|---|---|
| Clarifier | | |
| Total (kg) | 175.5 | 184.4 |
| Mean (kg/d) | 29.2 | 12.3 |
| Cone | | |
| Total (kg) | 5.9 | 4.8 |
| Mean (kg/d) | 1.0 | 0.3 |
| Percentage | | |
| Clarifier (%) | 96.7 | 97.5 |
| Cone (%) | 3.3 | 2.5 |

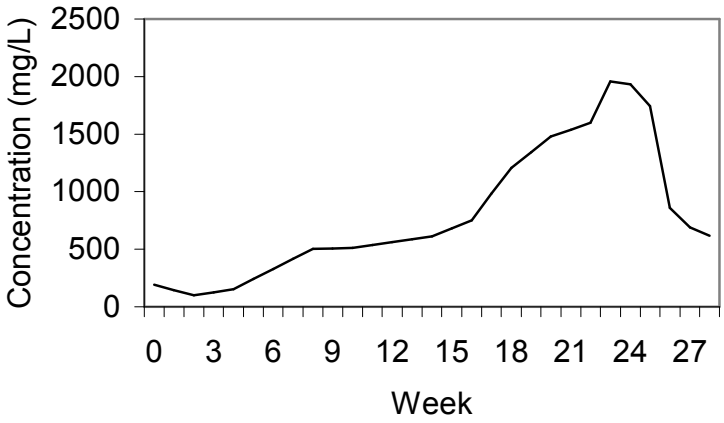


Figure 13. TSS - Trial 2.

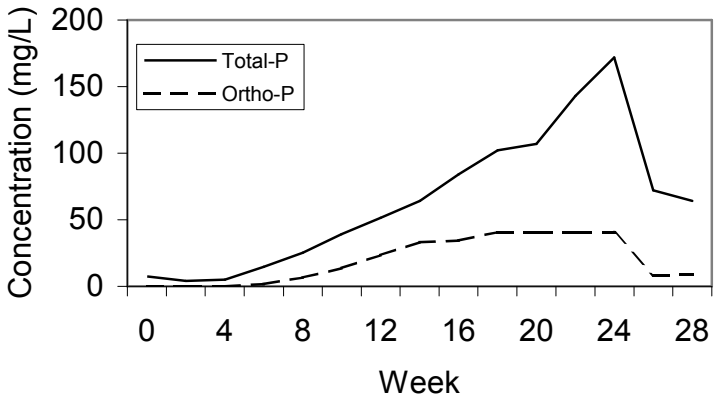


Figure 14. Phosphates - Trial 2.

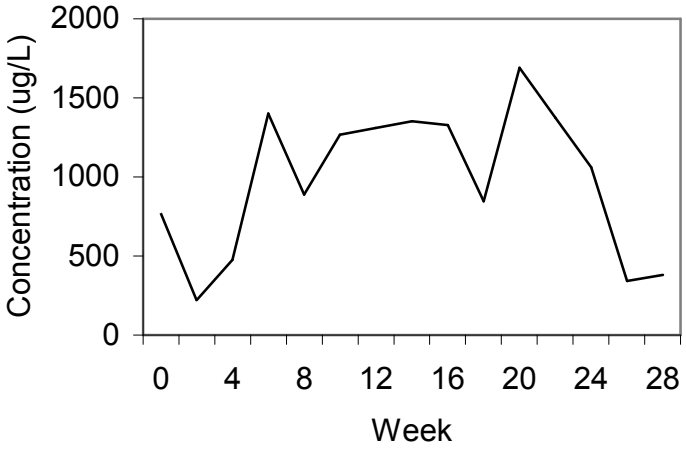


Figure 15. Chlorophyll *a* - Trial 2.

Base and electricity were the other major inputs to the system (Table 3). Addition of $Ca(OH)_2$ averaged 1.6 kg/day, which was 6.6% of the average daily feed input (24.4 kg) over the two production trials. The maximum sustainable daily feed input was approximately 32 kg/day. The average electrical usage was calculated to be 52.8 kWh/day.

**Conclusion**

This tank system was easy to manage and produced high densities of fish. Tank culture production of tilapia was 28.8 times higher in Trial 1 and 27.4 times higher in Trial 2 than levels typically obtained by un-aerated pond culture.

High mortality resulting from bird predation needs to be addressed in a future trial. Plans have been formulated to install small support rods (0.6 m high) inside the top ledge of the tank and secure plastic netting to the rods. This will eliminate the space on which the herons perch.

Limiting the accumulation of nitrates is important to fish health and growth. Denitrification is an anaerobic process that can be used to reduce $NO_3$-N to $N_2$. Two denitrification channels (30.5 m x 1.2 m x 0.6 m deep) have been constructed next to the rearing tank and will be tested in the next production trial. These tanks will be filled with dilute sludge to create anaerobic conditions, and culture water will be circulated through these tanks at a low flow rate to remove nitrates. Flowering macrophytes will be cultured in an attempt to produce a plant crop with commercial value.

An alternative option to reduce $NO_3$-N concentrations is increased water exchange. This approach is not feasible in the Virgin Islands and in many other areas that have limited water resources. The reuse of system water after the fish harvest is encouraged to conserve water and eliminate the acclimation period for nitrifying bacteria.

Effective management of suspended solids with an external clarifier was a major finding of this project. The central cone and the 3% bottom slope are unnecessary and can be eliminated. This will make future construction faster, easier and less expensive. The external clarifier provides the ability to control TSS levels. Work is now needed to determine the optimum TSS concentration for nitrification and fish growth.

The tank described in this paper is compared to ponds because it may be possible to scale it up to the size of a commercial pond and greatly increase production or substantially reduce resource requirements. For example, 100 ha of un-aerated ponds could be replaced with 3.6 ha of tanks, based on the average production levels of these two trials. Research is needed to determine the effect of scale-up on inputs, management, production and cost. An economic comparison of large tanks and ponds will be required. Evaluation of risk factors and environmental benefits will be an important element of an economic comparison. Intensive tank culture of tilapia, utilizing a suspended, bacterial-based treatment process, has great potential in the development of the tilapia industry.

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