WAS America Meeting

Las Vegas

MICROBIAL CONTROLLED SYSTEMS

Special symposium, February 15, 2006

Program and Abstracts

Morning session, Basic principles

Chair-person: Greg Boardman

Co-Chair – Yoram Avnimelech

Yoram Avnimelech

MICROBIAL CONTROLLED PONDS - PRINCIPLES, IMPLEMENTATIONAND NEW DEVELOPMENTS (30min)

Lytha Conquest* and Albert Tacon
UTILIZATION OF MICROBIAL FLOC IN AQUACULTURE SYSTEMS: A REVIEW (30min)

D.E. Brune*, K. Kirk[1], and A. G. Eversole
ALGAL BACTERIAL INTERACTIONS IN A PARTITIONED AQUACULTURE SYSTEM FOR ZERO-DISCHARGE SHRIMP PRODUCION (30min)

Craig Browdy

INSIGHTS INTO THE FUNCTIONAL ROLES OF MAJOR COMPONENTS OF MICROBIAL COMMUNITIES IN ZERO EXCHANGE SUPERINTENSIVE SHRIMP SYSTEMS. (30 min)

Steve Serfling.

USE OF SUSPENDED MICROBIAL TREATMENT METHODS IN RECIRCULATING TANK SYSTEMS, FOR CULTURE OF FRESHWATER AND MARINE FISH, AND MARINE SHRIMP. (20 min)

James M. Ebeling, Michael B. Timmons, James J. Bisogni

EXPERIMENTAL RESULTS OF AUTOTROPHIC, HETEROTROPHIC BACTERIAL CONTROL OF AMMONIA-NITROGEN IN ZERO-EXCHANGE PRODUCTION SYSTEMS (30 min)

Discussion (45)

Total 3.5 hours

Afternoon Session, Lessons learned and future developments

Chair-person Yoram Avnimelech

Co-chair – Greg Boardman

Nyan Taw

SHRIMP PRODUCTION IN ASP SYSTEMS, C.P. INDONESIA: DEVELOPMENT OF TECHNOLOGY FROM R&D TO COMMERCIAL PRODUCTION (30)

Shaun Moss:

MICROBIAL CONTROL RESEARCH IN THE OCEANIC INSTITUTE (30)

Michael Mogollon.

OCEAN BOY, COMMERCIAL SCALE INLAND ASP SHRIMP PRODUCTION. (20)

Tzachi M. Samocha*, Susmita Patnaik, Josh M. Burger, Rodrigo V. Almeida, Abdul-Mehdi Ali, Zarrein Ayub, Margasanto Harisanto, Ami Horowitz, and David L. Brock

USE OF MOLASSES AS CARBON SOURCE IN LIMITED DISCHARGE GROW-OUT SYSTEMS FOR Litopenaeus vannamei (20)

Dean Farrel.

COMMERCIAL TILAPIA MICROBIAL CONTROLLED SYSTEMS IN CALIFORNIA (20)

James E. Rakocy, Donald S. Bailey, Eric S. Thoman
R. Charlie Shultz and Jason J. Danaher
INTENSIVE TANK CULTURE OF TILAPIA USING A SUSPENDED, BACTERIAL-BASED, TREATMENT PROCESS (20)

M.C.J. Verdegem[1], J. Schrama[1], B. Hari[2] and M. Kurup[2]

MICROBIAL CONTROLLED PRODUCTION OF P. MONODON IN EXTENSIVE PONDS (20)

Discussion

INTRODUCTION - George Chamberlain (40)

Total 3.5 hours

ABSTRACTS

MICROBIAL CONTROLLED PONDS - PRINCIPLES, IMPLEMENTATION AND NEW DEVELOPMENTS

Yoram Avnimelech
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Microbial controlled ponds (active suspension ponds, ASP), were designed to provide a relatively low cost solution to the control of intensive aquaculture based on low (or zero) water exchange rate. Such ponds, mixed and aerated, are typified by the build up of high organic matter (soluble and suspended) and the resulting high microbial biomass (ca 10^7 CFU/ml).
Accumulation of toxic inorganic nitrogen (Ammonium, nitrite) is minimized and controlled by adding carbonaceous substrates, raising the C/N ratio and the subsequent synthesis of microbial protein. Carbon addition is quantitatively related to the inorganic nitrogen flux through a set of microbial mass balance equations. Replacing nitrification by nitrogen immobilization as a nitrogen control mechanism saves significant quantities of oxygen.

The microbial proteins produced may serve as a feed source to fish and shrimp, especially when bio-flocs are developing. These are harvested by the cultured fish, probably through a filtration process. Floc formation, characteristics stability and potential control will be discussed.

Harvesting and utilization of flocs was studied by a number of authors, using ^{15}N tracing, ^{13}C ratios and growth analysis. Protein utilization was doubled through the control of C/N ratio in commercial production systems.

Future developments, including the probiotics effects of ASP systems, the use of the same principles in extensive ponds and expected future user friendly design will be discussed.

Steve Serfling.

USE OF SUSPENDED MICROBIAL TREATMENT METHODS IN RECIRCULATING TANK SYSTEMS, FOR CULTURE OF FRESHWATER AND MARINE FISH, AND MARINE SHRIMP.

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This paper describes the use of a suspended microbial type treatment system for culture of fish and shrimp in recirculating tank systems. The process has been referred to as "ODAS", short for "Organic Detrital Algae Soup". The ODAS consisted of a diverse community of photosynthetic micro-algae (brown and green), bacteria, fungi, ciliates, rotifers and other zooplankton, and microinvertebrates, plus detritus. The suspended detritus functioned as high-surface area habitat for beneficial bacteria, attached ciliates, and surface grazing microinvertebrates. This process was originally developed and refined for large commercial scale production of tilapia during 1982-1992 (Solar Aquafarms in S. California). Tilapia were cultured in large greenhouse covered tanks at yield rates of 65-70 kg/m3/yr. (600,000 lbs/ac/yr), and the ODAS was the sole water treatment component (no fixed-film media or other biofilters).

The ODAS was consumed by the filter-feeding tilapia as a natural food, reducing feed costs, and eliminated the need for expensive biofilm substrates and particulate filters. The ODAS contained many strains of probiotic bacteria, which dominated and excluded pathogens. No disease problems occurred during 10 years of tilapia production. Final waste solids were pumped to a settling pond/marsh. No effluent was discharged off-site. Net water exchange averaged less than 1.0% of total water volume per day, supplied by a small on-site well. A similar tilapia farm has been operating successfully in Jordan since 1995, using technology provided by Solar Aquafarms.

During that period (1982-86), the ODAS process was also tested and used successfully as the sole water treatment method for culture of hybrid striped bass, pacu, and marine shrimp (*P. vannamei*) in recirculating tank systems. The striped bass and pacu, not being filter-feeders, could not utilize the ODAS as food, but the marine shrimp did. The water quality remained very stable, the cultured species were healthy, and no diseases occurred.

In more recent years (1995 to present), the ODAS process has been used successfully to culture marine fish (snook, several species of snapper, pompano and permit), and sturgeon (primarily Caspian Sea/Russian species - bester hybrids, Russian, Siberian, and sterlet) in pilot-scale recirculating tank systems in Florida, and the shortnose sturgeon in commercial scale tank systems in Canada. With some of the more sensitive marine and sturgeon species, the treatment process needs to be modified to reduce inhibitory compounds accumulating in the recirculated water.

UTILIZATION OF MICROBIAL FLOC IN AQUACULTURE SYSTEMS: A REVIEW

Lytha Conquest* and Albert Tacon

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In aquaculture production, the practice of minimal or zero-water exchange systems has become a standard. While this method provides for increased biosecurity of the system, it also allows for increased buildup of particulate material (floc) and metabolic wastes. This paper presents an overview of the composition of the floc systems, potential nutritional value, and a review of the utilization of the floc within marine shrimp and freshwater fish aquaculture.

The suspended floc material is typically comprised of aggregations of autotrophs and heterotrophs: phytoplankton, bacteria, protozoans, and metazoans and detritus, which can beneficially provide additional nutrition and mediate water quality. Through tracer studies, shrimp and tilapia are known to utilize the floc as supplemental nutrition, potentially reducing feed costs. A current study at the Oceanic Institute is evaluating the biochemical content (fatty acids, amino acids, and vitamins) of a diatom-dominated shrimp culture floc with regard to the nutritional needs of *Litopenaeus vannamei* for growth.

There is a large interest in managing heterotrophic floc systems to reduce the harmful levels of ammonia and nitrate in the zero exchange systems. In *L. vannamei* culture systems, additions of carbonaceous compounds are being used balance to C:N ratios, encouraging bacterial protein synthesis and improving water quality.

D.E. Brune*, K. Kirk[2], and A. G. Eversole

ALGAL BACTERIAL INTERACTIONS IN A PARTITIONED AQUACULTURE SYSTEM FOR ZERO-DISCHARGE SHRIMP PRODUCION

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Four 250 m^2 marine Partitioned Aquaculture System (PAS) units were used to culture the Pacific white shrimp, *Litpenaeus vannamei*, in growing seasons 2003, 2004 and 2005 at densities of 125, 180 and 500 animals /m^2, respectively. In 2003, water from three of the PAS units was exchanged on an "as needed basis" (to maintain water quality) with a single 250 m^2 unit containing tilapia. In 2004, the shrimp and tilapia units were exchanged on a routine basis with average detention times of 2 days. In 2005, a solids settling basin, suspended culture, aerated, reactor and tilapia polishing basin was added (in sequence) with average detention times of 2 to 5 days. In 2003, feed application rates reached 281 kg/ha-day, with an average shrimp yield of 16,800 kg/ha. In 2004 feed application rates reached 787 kg/ha-day, with an average shrimp yield of 25,600 kg/ha. By mid-season of 2005, feed application rates have already reached 1067 kg/ha-day, at an average shrimp standing crop of 24,700 kg/ha of 5 gm individuals. If successful, this unit should approach shrimp yields of 56-67,000 kg/ha. In 2003, the PAS units averaged 53% overall nitrogen removal via photosynthesis, with 40% N-removal through water-column nitrification. The system reached organic-C loadings of 12.5 gm C/m^2-day, at algal productivity of 7.5 gm C/m^2-day with average seasonal nitrogen application rates of 0.9 gm N/m^2-day. In 2004, overall algal N-removal averaged 23%, with 69% of nitrogen treatment arising from water-column nitrification, at an average N-application rate of 2 gm N/m^2-day. At maximum feed application rates in 2004 (35 gm organic C/m^2-day), system net photosynthesis approached zero. PAS system modifications in 2005 (a solids settling basin, and aerated basin prior to a tilapia polishing basin) provided much improved solids removal and oxidation. As a result of the improved water clarity, photosynthesis rates are currently exceeding 5 gm C/m^2-day at feed application rates of 47 gm organic C/m^2-day. By midseason 2005, algal N-uptake is ranging from 25-50% of N-loading (at 4 gm N/m^2-day) with water-column nitrification removing 40-50% of system N-loading. The data from 3 years of PAS culture of marine shrimp at feed application rates ranging from 281 to 1067 kg/ha-day suggest that decreases in algal productivity as a result of increased organic loading is likely due to algal light limitation arising from heterotrophic and chemoautotrophic bacterial biomass within the water column. Aggressive water-column solids management, using a combination of settling and aeration basins, coupled with tilapia polishing basins, may be used to reduce this light limitation. The advantages of maintaining enhanced algal production at high organic load within the PAS, include increased feed application and shrimp

carrying capacity, reduced system oxygen demand, and reduced alkalinity consumption, with improved pH management.

Craig Browdy

INSIGHTS INTO THE FUNCTIONAL ROLES OF MAJOR COMPONENTS OF MICROBIAL COMMUNITIES IN ZERO EXCHANGE SUPER-INTENSIVE SHRIMP SYSTEMS.

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The elimination of water exchange during all or part of the growing season has become a standard procedure for many shrimp producers. Although implemented primarily as a response to biosecurity concerns, other advantages of reduced exchange have been shown. Without water exchange, as long as supplemental aeration is maintained at appropriate levels, nutrient discharge is reduced, water quality fluctuations associated with algal blooms and crashes are minimized, and nitrogen assimilation efficiency is improved while production costs are potentially reduced, improving profitability. Therefore, interest in the application of 'heterotrophic' production strategies has received increasing attention.

The microbial community plays a key role in waste cycling, directly and indirectly affecting both water quality and growth. At the Waddell Mariculture Center in South Carolina a series of studies have been carried out over the past ten years to better understand the activity of and potential for manipulation of these communities in shrimp production systems operated without water exchange. Relatively simple techniques for characterizing microbial activity and abundance have been applied including bacterial counts, monitoring of chlorophyll A, tracking of inorganic nitrogen species, determination of nitrification rates, and measurement of short term oxygen production and consumption in light and dark bottles. Similarly, studies have evaluated methods of managing the microbial community by fertilizing and manipulating carbon nitrogen ratios; by altering microhabitats; by adding substrates; by using bacterial amendments with and without system disinfection; and by cropping microbial flora from the water column.

The term 'heterotrophic' oversimplifies the nature of the microbial community in these systems ignoring several critical components which in fact should be carefully managed to maximize production benefits. Based on functional activity, the microbial community can be divided into three important groups: heterotrophic bacteria, photoautotrophic phytoplankton and chemoautotrophic nitrifiers. Although the importance of anaerobic chemoautotrophs in pond sediments can not be overlooked, current trends towards use of lined production units and resuspension of organic matter can reduce or eliminate the harmful metabolites they produce resulting in much improved target crop production dynamics. In the water column, heterotrophic bacteria can play a dual role: 1) contributing to the formation of microbial flocs which can function as microhabitats for nitrification and denitrifcation and 2) assimilating excess nitrogen into bacterial biomass. The latter can be particularly important in low salinity systems where nitrite toxicity is particularly acute. The role of phytoplankton in contributing to growth of *L. vannamei* is well recognized and should not be underestimated. In fact, cropping of the microbes in the water column to increase light transmission and stimulate photosynthesis can have positive effect on growth of this species. Nitrifiers can provide very effective cycling of excess nitrogen to nitrate so long as adequate substrates are available and alkalinity is maintained. Effectiveness is highly dependant on the population density of these often slow growing bacteria and water reuse between crops can minimize potential for ammonia and nitrite spikes. An understanding of these functional roles can allow for effective management at surprisingly high levels of intensification without sacrificing water quality. System carrying capacity, shrimp growth and nitrogen transformation efficiency can be maximized while reducing waste production by carefully controlling nutrient inputs, by maintaining alkalinity and high dissolved oxygen levels and by cropping as necessary to allow photosynthesis while not over-cropping nitrifying communities.

Experimental results of autotrophic, heterotrophic bacterial control of ammonia-nitrogen in zero-exchange production systems

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In intensive aquaculture systems, ammonia-nitrogen buildup from the metabolism of feed is usually the limiting factor after dissolved oxygen to increasing production levels. Currently, large fixed-cell bioreactors are the primary strategy for controlling inorganic nitrogen in intensive recirculating systems. This option utilizes chemosynthetic autotrophic bacteria, Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB), for the nitrification of ammonia-nitrogen to nitrite-nitrogen and finally to nitrate-nitrogen. In the past several years, zero-exchange management systems have been developed based on heterotrophic bacteria and promoted for the intensive production of marine shrimp and tilapia. In this pathway, heterotrophic bacterial growth was stimulated through the addition of carbonaceous substrate. At high carbon to nitrogen (C/N) feed ratios, heterotrophic bacteria assimilate ammonia-nitrogen directly from the water yielding cellular protein. This presentation reviews the two nitrogen conversion pathways used for the removal of ammonia-nitrogen in aquaculture systems, autotrophic bacterial conversion of ammonia-nitrogen to nitrate nitrogen, and heterotrophic bacterial conversion of ammonia-nitrogen directly to microbial biomass. The first part reviews in detail the three ammonia removal pathways, presents a set of stoichiometric balanced relationships, and discusses their impact on water quality. In addition, microbial growth fundamentals are used to characterize production of volatile and total suspended solids for autotrophic and heterotrophic systems. In the second part, the results of a study are presented on the impact C/N ratio on water quality. In this experimental trial, sufficient carbon in the form of sucrose (sugar) was added daily at 0%, 50%, and 100% of the feed rate to three proto-type zero-exchange systems. The system was stocked with marine shrimp (*L. vannamei*) at a modest density (150 /m^2) and water quality measured daily. Significant differences were seen between the three systems in the key water quality parameters of ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen, pH, and alkalinity. The control tank exhibited water quality characteristics of a mixed autotrophic/heterotrophic system and the two receiving supplemental carbon, water quality characteristics of pure heterotrophic systems.

USE OF MOLASSES AS CARBON SOURCE IN LIMITED DISCHARGE GROW-OUT SYSTEMS FOR Litopenaeus vannamei

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Increased environmental regulations and loss of crops due to viral disease outbreaks have created a demand for productive, pathogen viral free, efficient and sustainable shrimp production practices. These methods, for the most part, call for raising the shrimp under limited water exchange where careful monitoring of the water quality is used to avoid adverse effect on the cultured organisms. In these systems, feed, feed management and water treatment can affect water quality and shrimp performance. Thus, optimization of protein utilization and manipulation of microbial communities can improve water quality and shrimp yields while reducing feed costs. The autotrophic and heterotrophic microbial communities play a major role in limited discharge shrimp culture systems, particularly in maintaining optimum water quality, enhancing natural productivity and nutrient cycling. Different studies suggest that detritus-rich culture water can enhance shrimp growth in intensive culture systems operated with limited water exchange. Beside the nutritional benefit from bacterial flocs, the use of carbon supplementation was suggested as a management tool to control ammonium buildup in the culture system.

Two studies were conducted with the Pacific white shrimp *Litopenaeus vannamei* to evaluate the effect of carbon supplementation on selected water quality indicators and shrimp performance in two outdoor membrane-lined ponds (working water volume of 2,000 m^3) stocked at a density of about 22 juveniles/m^3 and in 24 small tank-system (working water volume of 7.8 m^3) stocked at a density of 75 juveniles/m^3 and operated with limited discharge. Each pond was provided with four aspirator-type aerators and the shrimp were fed a commercial diet containing 30% crude protein (30% Eco, Rangen Inc.). Carbon supplementation in the ponds was based on the ammonium levels found in the water. Six grams of carbon from molasses was provided for each 1 g of ammonium found. Shrimp in the tank-system were fed two commercial shrimp diets (the same 30% CP diet used in the ponds and a 45% CP diet –"45/10" Rangen Inc.). Shrimp in twenty tanks were fed the low-protein diet while shrimp in four tanks were fed the high-protein diet. The high-protein diet was fed on iso-nitrogenous basis to the low-protein diet. Rations were determined based on assumed growth of 1 g/wk, FCR of 1.5 and predicted survival rate. Feed was offered daily at four equal portions. Four levels of carbon supplementation in a four-replicate treatment were tested in this system. The amount of carbon to be added was

calculated to provide 50%, 100%, 150% of the theoretical level of carbon needed to convert ammonium into bacteria biomass assuming 50% of the nitrogen contributed by the feed is converted into ammonium. Carbon supplementation in the fourth treatment was based on the actual level of ammonium in the water as explained for the ponds. The paper discusses the effect of the diets and the addition of carbon on selected water quality indicators and on shrimp performance in these systems.

Key Words: *Litopenaeus vannamei* limited discharge grow-out, water quality, molasses, carbon

SHRIMP PRODUCTION IN ASP SYSTEMS, C.P. INDONESIA: DEVELOPMENT OF TECHNOLOGY FROM R&D TO COMMERCIAL PRODUCTION

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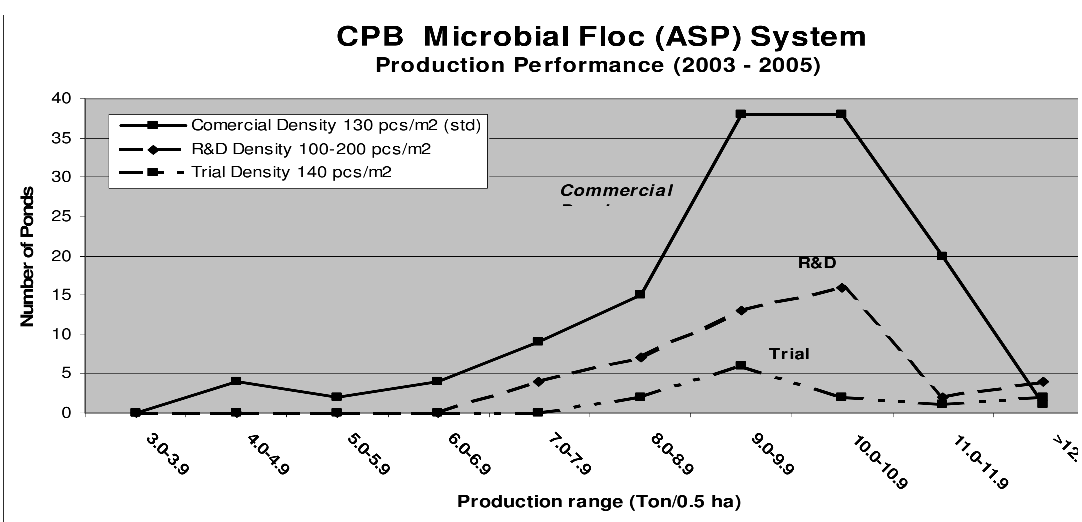
PT. Central Pertiwi Bahari (Charoen Pokphand, Indonesia), integrated shrimp farm has over 3,200 commercial ponds, technically supported by the Technology Development Division, which consists of R&D, trial and commercial ponds. The R&D and trial sections have nearly 100 intensive culture ponds of various sizes (2,000 – 6,000 m^2), shapes (round, square, rectangular) and construction types (earth & HDPE lined). The Division also has 301 production commercial ponds for commercial feasibility study using technologies proven through R&D and trials. The studies on microbial controlled aquaculture systems (ASP) were carried out in ponds of diverse sizes, shapes and construction types since mid 2002 (Taw & Chandaeng, 2005; Kopot & Taw, 2003). Chandaeng, et. al., (2005) gave an account on the production potential of the system. Based on the R&D and trial findings, the ASP system was successfully transferred to commercial production in 2003 for the first time. A standard for commercial implementation has been established and a sustainable production was achieved. The standard system was successfully applied in three modules consisting of 131 commercial ponds. The sustainable production of between 18.0-22 tons/hectare with MBW (mean body weight) and FCR of 16-18.0 gram and 1.1-1.3 was realized respectively. On bio-security, the ponds were WSSV free although a few incidents of viral disease in the vicinity of the modules were experienced.



Shaun Moss:

(30)

INTENSIVE MICROBIAL REUSE SYSTEMS FOR SUPER-INTENSIVE SHRIMP PRODUCTION: EXPERIENCES FROM THE OCEANIC INSTITUTE

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To reduce the risk of disease introduction from waterborne vectors, many shrimp farmers worldwide have decreased water exchange rates or eliminated water exchange as a management strategy. Reducing or eliminating water exchange during the growout period provides shrimp farmers with an opportunity to take advantage of the *in situ* microbial community in a unique way. Microbes can be managed to prevent the accumulation of toxic nitrogenous wastes in the culture environment, while simultaneously serving as a supplemental food source for the shrimp. Such systems are referred to as intensive microbial reuse (IMR) systems and have been used to culture both shrimp and fish.

As part of an on-going effort to develop a biosecure system for super-intensive shrimp production, researchers at the Oceanic Institute (OI, Hawaii, USA) have conducted growout trials in enclosed raceways using a modified IMR approach. Trials were designed to sustain high shrimp biomass while minimizing capital and operating costs. To date, we have produced 8.9 kg shrimp/m^3 at an estimated cost of $4.38/kg. To better understand the role of microbes in this system, we have constructed preliminary carbon and nitrogen budgets using stable isotope analysis. In addition, researchers from OI and Scripps Institution of Oceanography (California, USA) have conducted small-scale studies to examine carbon- and nitrogen-flux partitioning between shrimp, feed, and *in situ* microbes. Results from these experiments will be used to refine our IMR approach to intensive shrimp production.

IMR systems represent a viable alternative to traditional shrimp farming. They provide farmers with a strategy to minimize the introduction of shrimp pathogens into the culture environment by eliminating water exchange as a management option. In addition, they allow farmers to grow shrimp at high densities without having to invest in external biofiltration to control the accumulation of toxic nitrogenous compounds. Finally, IMR systems promote the presence of a rich microbial floc which can serve as a supplemental food source for the shrimp, thereby reducing feed costs and increasing profitability.

THE USE OF MIXED DETRITAL HETEROTROPHIC FLOC CULTURE SYSTEMS IN INTENSIVE CULTURE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*) IN AN INLAND SHRIMP FARM (OCEANBOY FARMS INC) IN FLORIDA, USA, UTILIZING VERY LOW-SALINITY GROUND WATER AND ZERO DISCHARGE.

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In an effort to find a potential solution to disease outbreaks and to avoid the environmental degradation associated with coastal shrimp farming, OceanBoy Farms Inc. (OBF) embarked during 2001 in the development of commercial protocols for the intensive and zero water discharge culture of specific-pathogen-free *Litopenaeus vannamei* in inland ponds in South Florida, USA. During the initial pond culture trials it became evident that phytoplankton populations predicted to decline over time in the culture cycle in fact lasted through out and coexisted with heterotrophic populations of bacteria that emerged at mid cycle, contrary to information published about pond culture at Belize Aquaculture. The flocs that developed in the ponds were in fact detrital/bacterial and also very fine at times, also contrary to Belize aquaculture. The nursery system for the ponds, 320 m3 raceways enclosed in greenhouse, also showed the same characteristic mixed floc as the ponds and similar techniques were developed to manage both culture areas. During 2003, OBF developed the first commercial scale inland hatchery for specific-pathogen-free *Litopenaeus vannamei*, again following the same philosophy of zero discharge. Techniques were developed to recycle all larviculture water and manage the mixed floc in larviculture tanks for the 18 day larviculture cycle. The growout in the hatchery of broodstock, selected from ponds just prior to pond harvests, is likewise carried out in 100 m3 raceways best described as mixed floc systems with zero discharge of water. These production processes are described in general terms.

Dean Farrel.

COMMERCIAL TILAPIA MICROBIAL CONTROLLED SYSTEMS IN CALIFORNIA (20)

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Two types of microbial controlled production systems presently used for commercial production of tilapia will be discussed.

Several farms recirculate the same water after feces removal without using biofilters. The uncontrolled microbial activity results in, as yet, undocumented floc uptake by the tilapia. Inorganic nitrogen is reduced. This, along with a pH control, allows for intensive production without the capital and operation costs of a biofilter.

The main focus, however, of this report will be on the use of the controlled active suspension pond (ASP) system in the semi – intensive production of tilapia using a 20% protein feed. The detailed results of this system include documented floc uptake by the tilapia, using ^{15}N tracing, protein conversion and comparative growth. This system enables to lower production costs. Potential methods to increase stocking density will be suggested.

James E. Rakocy, Donald S. Bailey, Eric S. Thoman
R. Charlie Shultz and Jason J. Danaher

INTENSIVE TANK CULTURE OF TILAPIA USING A SUSPENDED, BACTERIAL-BASED, TREATMENT PROCESS (20)

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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, 25 and 25 fish/m^3 in Trials 1, 2 and 3, respectively. Water treatment methods consisted of aeration, water circulation (mixing), solids removal, nitrification in the water column and denitrification (Trial 3). The fish were fed *ad libitum* twice a day with a complete diet (32% protein) consisting of floating pellets. After 175, 201 and 182 days of growth, total production was 14.4, 13.7 and 15.3 kg/m^3 in Trials 1, 2 and 3, 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. In Trial 3, a side stream of water was pumped through two denitrification channels (15.2 m x 1.2 m by 0.6 m, water volume = 9.46 m^3) with retention times of 1 or 2 days. Solids from the culture water accumulated on the bottom of the channels, creating anaerobic conditions. Nitrate-nitrogen levels decreased by an average of 20.5 and 45.8 mg/L on passage through the denitrification tanks with 1 and 2-day retention times, respectively. The effect of both denitrification channels was calculated to be an overall reduction in the NO_3 -N concentration of 373.7 mg/L in the rearing tank over the course of the production trial. This calculation was verified by the rearing-tank concentration of NO_3 -N, which reached a peak value of 341.3 mg/L. Total suspended solids (TSS) increased throughout Trials 1 and 2 and reached peak values of 1,300 and 1,960 mg/L, 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 1,700 to 600 mg/L. The reduction of TSS improved other water quality parameters and fish feeding response. The external clarifier was used throughout Trial 3, and TSS reached a peak concentration of 560 mg/L. External clarification and denitrification proved to be effective treatment methods for the sustainable production of tilapia in a bacterial-based tank culture system.

MICROBIAL CONTROLLED PRODUCTION OF P. MONODON IN EXTENSIVE PONDS

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Relying on extensive production technologies, small-scale farmers contribute significantly to global shrimp production. With *P. monodon,* stocking densities of 2 - 10 PLs per m^2 are commonly used in ponds fed high protein diets. The costs linked to PL stocking, but more so to feeding, make shrimp farming a high risk business for small-scale farmers. Therefore, strategies to reduce feeding costs in extensive ponds should be developed.

The infrastructure needed to apply ASP technology, including tanks or lined ponds and constant water column mixing, is out of reach for small-scale rural farmers. Rural ponds are mostly shallow (< 1 m deep) water bodies with earthen bottoms in which intensive water column mixing to stimulated microbial floc development cannot be applied. Nevertheless, microbial communities are also present in non-mixed extensively managed ponds (with primary productivities of 3-5 g C m^{-2} d^{-1}) where they might contribute to production in a similar way as in active suspension ponds.

Experiments were conducted in Cochin, India, to evaluate the combined effects of reducing the protein content in supplemental feeds and carbohydrate addition on production in extensively managed ponds. To reduce costs, an indoor trial in small replicate tanks was conducted to develop the technique before applying it to farmers' ponds. In the experiments, 20 g of tapioca flour was added for each g of TAN released. The amount of TAN released was estimated assuming that 50% of the dietary protein input was converted to ammonia. The indoor experiment was carried out in triplicate 1,200-l tanks for each of four diet treatments. Twenty-five and 40% crude protein diets were applied, with or without carbohydrate addition, resulting in treatments referred to as P25, P40, P25+CH, and P40+CH. A stocking density of 6 PL_{20}/m^2 was applied. The addition of carbohydrate reduced the TAN and nitrite levels. The high protein diet resulted in higher levels of TAN, nitrite and total nitrogen concentrations. There was no effect on the organic carbon content of the sediment, but the addition of carbohydrate caused a significant reduction of sediment-TAN. The total heterotrophic bacteria count in the water column and sediment were higher in treatments with added carbohydrate. The specific growth rate and feed conversion ratio were similar in the P40 and P25+CH treatments. The protein efficiency ratio was highest in treatment P25+CH.

In the farm trial, eight 250-m^2 earthen ponds were stocked at 6 PL_{20}/m^2. Treatments P40 and P25+CH were applied to four replicated ponds. TAN concentrations in the water column and sediment were lower in treatment P25+CH than P40. The addition of carbohydrate had a profound effect on the heterotrophic bacteria count. Shrimp yield and individual shrimp weight at harvest were higher in treatment P25+CH than P40. Growth rates, feed conversion, and protein efficiency were also better for P25+CH. Survival was 36-42 % and not different between treatments.

The revenue/ha from the harvested shrimp was 54% higher in P25+CH than P40 due to the combined effect of better yield and higher prices for bigger shrimp. A 35% reduction in feed cost was recorded in the P25+CH treatment when compared to treatment P40. The benefit:cost ratio was 1.3 in treatment P25+CH compared to 0.2 in P40.