Biofloc Technology
A Practical Guide Book

Third Edition

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With

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PREFACE

Aquaculture, the production of fish, crustaceous, mollusks, algae and others, is a dynamic industry. Aquaculture production has increased at an average annual rate of 8.9% since 1970, as compared with an annual growth rate of 1.2% and 2.8% for capture fisheries and terrestrial farmed meat production, respectively. Future demand for aquatic products and the inability to increase fishery catch call for a steeper increase of aquaculture production in the coming years. More and more people will join the industry. In addition, owing to water, land and feed constraints, more sophisticated technologies will be required.

Practicing aquaculture demands a good deal of knowledge and understanding. Unlike terrestrial culture of plants or animals, where you see symptoms of diseases or need for extra fertilizer, the aquaculturist does not see the fish. Management of pond production is facilitated through the determination of chemical, physical and biological processes in the water. These processes affect the well-being of the target animals and reflect conditions in the pond.

The control of fish growth (the term fish is used in this book to describe all cultured species) is in most cases obtained by controlling the pond environment. Even treating fish diseases is usually done by controlling the pond environment, and not by directly applying medicine to the fish. More research is required to better develop this goal.

In addition to the complex nature of the biological aspects of aquaculture production, the aquaculturist has to comply with additional constraints. In managing aquaculture, we deal with large quantities of water that are constantly enriched by nutrients and feed materials, and contain a biologic array that is different than that common in natural aquatic systems. Release of such water to the external environment may lead to the contamination of rivers, lakes and estuaries. Aquaculturists have to manage their farms in a way that will not pollute or damage the environment, and to follow numerous environmental regulations. On top of that, aquaculture is an industry that has to support the farmer and enable a decent income, while facing relatively expensive farm inputs and tough competition in the market.

Biofloc Technology: Past, Present and Future

Biofloc technology (BFT) is based upon a comprehensive philosophy of knowing and controlling the pond system. We do not look upon fish production as a separate entity in the pond, but rather as a part of a whole interactive eco-system, made out of the pond physical features, chemical interactions, a complex biota and the fish, as its components.

The operation of the pond involves understanding this complex eco-system and managing, or controlling it. Thus, we try to control the pond microbial community in a way that will improve water quality and maximize feed recycling (thus lowering expenses). We should plan and operate the pond, not barely as a means to supply oxygen to the fish, but to make sure that oxygen will be well distributed to prevent or minimize anaerobic processes, all these among the other additional processes discussed later- on.

Biofloc technology was developed as a means to better control the intrinsic microbial activity in aquaculture ponds. The initiative to develop this technology was based upon the spread of closed intensive ponds, where aeration is an integral part of the system and the buildup of organic substrates is the direct outcome of it.

Steven Serfling was the pioneer of commercial usage of biofloc technology practiced in a tilapia production farm, "Solar Aquafarms", during the years 1986-1994 (Serfling, 2000). The technology was nicknamed as the ODAS (organic detrital algae soup) system. This farm preceded, practiced and pioneered much of the research that was published later. Work on culturing shrimp in closed systems, similar to biofloc technology, was conducted at about the same period in Tahiti.

A series of publications on the development of biofloc technology originated in the early 1990s by two research groups (Chamberlain, 1994). The Technion Israeli group concentrated their efforts mostly on the intensive culturing of tilapia (Avnimelech and co-workers, 1992, 1994), while, at about the same time, work on intensive shrimp production was conducted in the Waddell Mariculture Center in South Carolina (Hopkins and co-workers, 1993).

Very often, it takes years for the application of research results in the field, especially so in agriculture, a relatively conservative industry. In the case of BFT, implementation of the research results was almost instantaneous, since the principles of BFT, limited water exchange, feed recycling and intensification were timely, and actually forced upon the aquaculture industry. As the technology developed, field experience preceded or concurrently developed with scientific work. Thus, in addition to results obtained through research efforts, information has been obtained and accumulated through field-proven experience. This so-called practical research needs to be taken into account as BFT matures as an applied science. The major problem in this context is that information and experience generated by private companies tends to be of limited public circulation, both due to justified or false interest in keeping information proprietary and/or due to lack of proper channels to distribute information.

One way of disseminating information on experience acquired in the field and in research institutes worldwide was achieved through the establishment, by the Aquaculture Engineering Society, of the Biofloc Technology Working Group. This working group holds special sessions, mostly during the regular WAS (World Aquaculture Society) meetings, and maintains a web site: **www.**

First, Second and The Present, Third Editions of The Book.

This book was originally written in response to great interest expressed by aquaculturists. Farmers from all over the world, as well as interested students and scientists, requested a source of general information on this technology. This book is a modest contribution and respect to the interested public.

In writing this book I have tried to have a conversation with the reader, to communicate in a way that will help the reader understand the story of biofloc technology, simplify explanations and minimize usage of complex technical terms. As I 'talk' with the reader, I introduce a smile from time to time, hoping this will ease the contacts between you, the reader, and me.

The first edition, published in August 2009, was completely sold out, and the need for a second edition was expressed. Editing the book included addition of new chapters, this time with co-authors, and updating of information. Updating the book was an exciting process. The amount of information added in just about 2 years is amazing.

Two years later, 2014, the 2nd edition was sold out. In consultation with Dr. Joseph Tomasso, WAS book editor, it was decided not to continue with another printout of the book, but to edit it again, considering the vast addition of information. It is exiting and inspiring to see how much information was added. (Personally, I am excited to see that a large percentage of the new science and practice originate from South Asian countries, an indication that - besides the increase of aquaculture production in this region - science and research is taking a large pace).

Road map: How to read this book:

This book was written so as to provide the needed information to farmers starting to manage biofloc systems, to field experts, students and to the academic community. It is not an easy job to provide understandable text, yet not to skip the basic science explanations. Moreover, in order to give both a practical and a scientific text, there was need to repeat some of the information. I believe that repetition is essential, in some cases, in order to facilitate retention of information and to enhance assimilation of the most important professional components, much as a farm manager makes decisions.

The reader familiar with the basics of scientific and aquaculture information may skip some parts. On the other hand, the book is written in a way that a reader not experienced in the highly scientific aspects may skip the equations or the more sophisticated information provided in "boxes", chapter brings us into new potential developments and hints on the special value of the natural feed to the growth and well-being of fish and shrimp.

The amount of biofloc produced, in most biofloc system, is higher than the amount harvested by the fish. Thus, in order to maintain sustainable conditions in the pond, we have to drain or siphon out excessive sludge. This may be an environmental point (see Chapter 17). Novel works published and summarized here by David Kuhn and coworkers, as well as more recent technology developed in Australia, demonstrate that the bioflocs can be used as an efficient feed component. Both groups demonstrated that feeding with bioflocs significantly raises shrimp production above and beyond feeding with conventional feeds. These works seem to be an exciting start of a break-through development.

Most of the aquaculture activity in the world takes place in small and medium scale family-owned farms. How can these take a giant leap from conventional farming to the intensive biofloc system? This is a critical and very important question. Some beginnings, described in chapter 9, may lead the way to achieve this goal. This chapter is dedicated to the memory of Professor N.C. Nandeesha, a prominent Indian scientist that devoted his life to a continuous struggle for advancing the development of family-owned sustainable aquaculture farms.

Aeration is an essential component in controlling ponds and enabling intensification in biofloc systems, as well as other aquaculture systems. The details of aeration are described in chapter 10. A discussion on the lack of suitable aerators and some guidelines as to the needed development of better aerators were added in the present edition, hoping this discussion will provoke the industry towards the production of new aeration devices.

Diseases of fish, and even more so shrimp diseases, are critical problems that caused failures, extensive losses, and, in cases, collapse of aquaculture production in many countries. Control of the pond environment is an important means to minimize the spread and impact of diseases. Chapter 11 gives data and prospects regarding the use of bioflocs and other biological controls on curbing diseases. The given information is based, to a large extent, on lectures and discussions in a special workshop on bioflocs and shrimp diseases, held in Vietnam on December 2013.

Different modes of pond construction are given in chapter 12 and field experience in leading farms are discussed in chapter 13 (written with Dr. Nyan Taw). The experience, prospects, as well as problems associated with the newly mode of super-intensive biofloc shrimp production are brought by Dr. Andrew J. Ray (Chapter 14).

Peter de Schryver and co-workers (Chapter 15) look at the biofloc technology from a basic scientific viewpoint. This chapter will be very interesting to readers looking forward to better understand the biofloc system and toward future development toward a better environment is discussed in Chapter 16.

Chapter 17 is a new addition in the present edition. It was written in response to readers' comments saying that, following reading the book, they are still confused as to how to do it. Giving a concise list of what to do in order to manage a BFT pond is not easy. There are different ways to operate a BFT pond, the choice of which depends on the local conditions. Yet, an effort was made to give as clear as possible guidelines for the beginner. In compiling this guide, I could not prevent making several repetitions. I certainly hope this chapter, with all of its limitations, will help those doing their best at jumping into the water.

I am confident in my belief that more information and insight will be pouring in the next few years, unveiling the details as well as the holistic aspects of biofloc technology.

Biofloc technology is a new field. There are numerous unknown variables and a need for more research and broader experience. I hope that we can add new information as it becomes available. A discussion group and electronic updating of this book can help in this. Readers are invited to comment, raise questions and share their knowledge via E-mail: biofloc@technion.ac.il.

Finally, I would like to acknowledge the help of many friends who assisted me in the writing process: Dr. Nyan Taw accompanied me in various technical meetings, shared his practical experience with me, and allowed me to use his data and pictures; Dr. Albert Tacon, whose long term cooperation was instrumental and educative, generously permitted the use of his data.

Similarly, Dr. Michele Burford, generously provided beautiful electron microscope pictures of bioflocs.

I am grateful to my friends, Drs. Ronald Malone and John Colt, for sharing with me their thoughts on aerators, ideas that are embedded in chapter 10.

I gained enormously by correspondence, meeting and consulting with farmers all over the world, posing questions to them and responding to their questions, data and observations. I have provided in this book one such letter, of Ms. Ninuk Sri Maharti, (Thanks, Ninuk, for letting me include it in this book). Mr. Boria Suryakumar, the owner, planner and manager of Hitide farm in Tamil Nadu, India, thought of his very efficient modifications of biofloc technology (see chapter 9) as examples of the useful cooperation with people having hands-on experience. As a matter of fact, correspondence and meetings with farmers all over the world led me to write this book.

As a professor who is too busy to manage orderly operations, I could not have brought this book to a neat shape without the help of Ms. Efrat Elimelech, Ms. Yifaat Baron and Ms. Tal Goldrath, all contributing in ways well beyond my own capabilities. Ms. Noa Hollender helped in shaping up the 2nd edition and Ms. Mor Mittelman helped in preparing the 3rd edition. I owe many

Chapter 1

Why Do We Need New Technologies For Aquaculture?

In Brief

Aquaculture production has increased at an average annual rate of 8.9% since 1970, as compared to an annual growth of 1.2% and 2.8% for capture fisheries and terrestrial farmed meat production respectively. Yet, to supply demands, aquaculture production must grow by 5 fold in the next 5 decades. This development has to overcome 3 major constraints:

a. Produce more fish without significantly increasing the usage of basic natural resources of water and land.
b. Develop sustainable systems that will not damage the environment.
c. Develop systems providing a reasonable cost/benefit ratio, to support the economic and social sustainability of aquaculture.

Aquaculture production comprises all forms of culturing aquatic animals and plants in fresh, brackish and marine environments. Aquaculture is one of the fastest growing food producing industries. Worldwide, aquaculture production has increased at an average annual rate of 8.9% since 1970, as compared to an annual growth of 1.2% and 2.8%, respectively, for capture fisheries and terrestrial farmed meat production. In 2006, total aquaculture production of fish (including crustaceans and mollusks) was estimated to be 51.7 million tons by fresh weight and 79 billion US$ by value. Fisheries fish production in 2006 was 91.994 million tons (FAO, 2008). Major groups of species produced in aquaculture systems are given in Table 1.1

Table1.1: Global aquaculture systems production in 2006

Products	Million Tons	Value, million US$
Fish, crustaceans, mollusks etc.	51.653	78,758.387
Inland water	31.593	41,433.732
Marine water	20.060	37,324.655
Aquatic plants	15.076	7,187.125
[illegible]	[illegible]	[illegible]

Global capture fisheries have reached their limit with 75% of the major sites being over-fished or fished at their carrying capacity (limit). The stagnating capture fisheries production is confronted with a growing demand for fish owing both to population growth and an increase in per capita fish consumption. In 2003, the global average per capita fish consumption was 16.3 kg (13.3 kg excluding China). The FAO (The UN Food and Agriculture Organization) recommends a 53-200% increase in per capita consumption, to 25 kg in 2025 and to 30-40 kg in 2050. **The growing gap between global fish demand and supply through capture fisheries is expected to be closed by aquaculture** (New, 1991; Wijkstrom, 2003).

The aquaculture industry carries the responsibility of increasing fish supply. A 5 fold increase in global aquaculture production is needed within the next 5 decades to maintain current aquatic food consumption levels. Past and predicted aquaculture productions are given in Table 1.2 and Table 1.3. A more detailed representation of the change in annual aquaculture production, 1953 – 2011 is given in Figure 1.1. It can be clearly seen that aquaculture production increased following an exponential growth. Will such growth continue in the future? Probably not. Further growth will be limited by external constraints.

Figure 1.1: Global aquaculture production of finfishes, crustaceans and mollusks 1953-2011

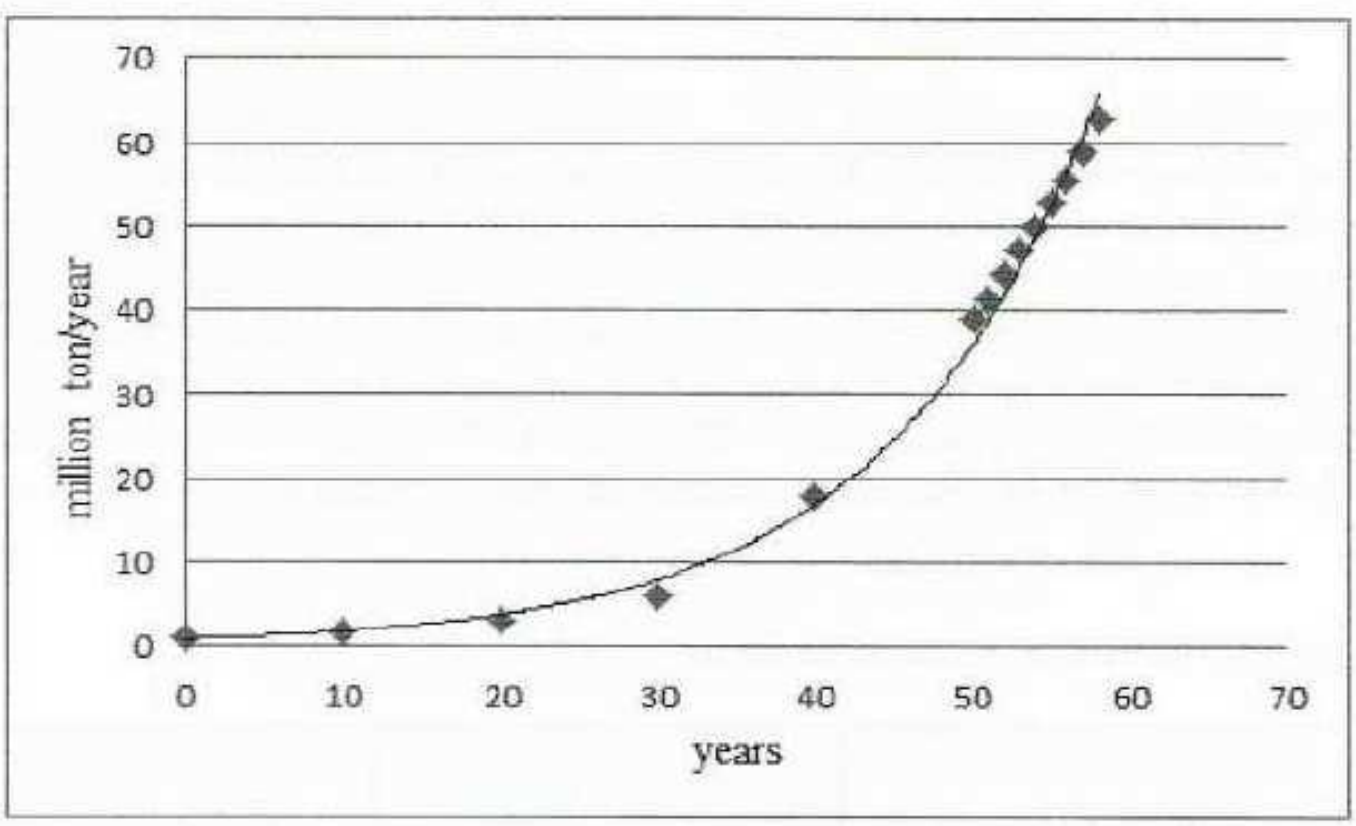


Table 1.2: Global aquaculture production of finfishes, crustaceans and mollusks

Year	1953	1963	1973	1983	1993	2003	2006	2010
Million MT	0.96	1.8	3.1	6.2	17.8	41.9	51.7	59

*FAOSTAT statistic database: www.fao.org; New, 1991; Wijkstrom, 2003; FAO, 2011

Table 1.3: Expected (or required) production

Year	2025[1]	2025[2]	2050
Million MT	120	150	210
Source	New, 1991	New, 1991	Wijkstrom, 2003

[1] Assuming per capita consumption identical to present one (i.e. no increase)

[2] Assuming recommended FAO's 25 kg per capita

Aquaculture production increased by more than 40 folds during the last 50 years and is expected to rise by another 5 folds in the upcoming 50 years. Such an increase in aquaculture production has to be planned, keeping in mind the need to minimize environmental impacts while optimizing resource utilization.

Sustainable Aquaculture: Rational Use of Natural Resources

Land based aquaculture operations use land and water (mostly fresh, but also brackish or marine water). On a global scale, fresh water resources are becoming scarce and expensive. About 41% of the world's population today lives in water-stressed river basins. In 2050, 70% of the world i.e. 20-100 m^3/ha, depending on seasonal temperature and humidity. Seepage from ponds built on highly permeable soil will be higher than 5 mm per day. Combined evaporation and seepage losses can be reasonably considered to be around 10 mm per day, or about 3,500 mm per year. In consequence, a 1-ha pond will use 35,000 m^3 of water per year to replace evaporation and seepage losses.

Aquaculture land sites must be more or less flat, easily drained and close to reliable water sources like rivers, lakes or artesian wells. Such lands are also in high demand for other purposes: Urbanization, agriculture, wetland and mangrove conservation, recreation and tourism. Marine aquaculture farms do not use large quantities of fresh water, but are developed in the coastal zone where land is extremely scarce: 60% of the world population lives within 60 km of the sea coast and more than two thirds of cities with over 2.5 million inhabitants, are situated in the coastal zone.

Coastal zones are ecologically sensitive, containing mangroves, coral reefs and other unique ecosystems. The need to protect these environments leads to public and legal objections to any further enlargement of pond areas in coastal regions. For instance, in India, population density in the coastal zone is very high, thus, the Indian Supreme Court decided in 1996 not to allow construction of ponds within 500 m of the high tide line. Other countries also limit construction of coastal ponds, demanding compliance with stringent environmental conditions. Getting permission to develop new aquaculture sites is becoming a difficult, time-demanding process all over the world.

- ***The prime goal of aquaculture development is to produce more fish without significantly increasing the usage of the basic natural resources of water and land (Avnimelech et al., 2008).***

The intensive development of the aquaculture industry has been accompanied by an increase in environmental impacts. The production process generates substantial amounts of polluted effluent, containing uneaten feed and feces. Discharges from aquaculture into the aquatic environment contain nutrients, various organic and inorganic compounds such as ammonium, phosphorus, dissolved organic carbon and organic matter. The high levels of nutrients cause environmental deterioration of the receiving water bodies. In addition, the drained water may increase the occurrence of pathogenic microorganisms and introduce invading pathogen species.

To produce 1 kg of live weight fish one needs 1-3 kg dry weight feed (assuming a food conversion ratio of about 1-3). About 36% of the feed is excreted as a form of organic waste. Around 75% of the feed N and P are unutilized and remain as waste in the water. An intensive aquaculture system, which contains 3 tons of tilapia, is equivalent to the weight of 50 people. This intensive aquaculture system can also be compared, in respect to waste generation, to a community of around 240 inhabitants. It can thus be concluded that live fish biomass generates approximately 5 times more waste than live human biomass. The reason is that the scope of digestion in fish is contains 65 to 75% protein. In addition, fish use proteins for energy production to a large extent, unlike terrestrial animals that use mostly carbohydrates and lipids. Fish protein requirements, therefore, are about two to three times higher than that of mammals. Ammonium is one of the end products of protein metabolism. All of these factors contribute to the high nitrogen residues in aquaculture water. In water, NH_3 (ammonia) and NH_4^+ (ammonium) are in equilibrium depending on the pH and the temperature. The sum of the two forms is called total ammonium nitrogen (TAN). Although both NH_3 and NH_4^+ may be toxic to fish, unionized ammonia is the more toxic form, attributable to the fact that it is uncharged and lipid soluble and consequently traverses biological membranes more readily than the charged and hydrated NH_4^+ ions. In most cases ammonia-N is toxic to commercially cultured fish at concentrations above 1.5 mg N/l, but, in most cases, the acceptable level of unionized ammonia in aquaculture systems is only 0.025 mg N/l. However, the toxicity threshold depends strongly on the species, size, fine solids, refractory organics, surface-active compounds, metals, nitrate, salinity and pH.

In addition to the generation of large amounts of waste, the use of fishmeal and fish oil as prime constituents of feed is another non-sustainable practice in aquaculture. Approximately one-third of the global fishmeal production is converted to aquaculture feeds. The proportion of fishmeal supplies used for fish production increased from 10% in 1988 to 17% in 1994 and 33% in 1997. Hence, aquaculture is a possible panacea, but also a promoter of the collapse of fisheries stocks worldwide. The ratio of wild fish-fed farmed fish (both live weight base) is about 1.41:1 for tilapia and 5.16:1 for marine finfish. Purchase of commercially prepared feed for fish culture comprises 50% or more of the production costs; this is primarily due to the cost of the protein component. On average some 25% of the nutrient input of these feed sources is converted into harvestable products. To make further sustainable increases in aquaculture production possible, inexpensive protein sources and ways to improve feed nutrient conversion efficiencies are needed (Tacon and Forster, 2003).

- ***The second goal, of present and future aquaculture development is to develop sustainable systems that will not damage the environment (Naylor et al., 2000).***

The already complex role of raising aquaculture production from 1 million tons in 1953 to more than 200 million tons in 2050 in tandem with environmental protection, is further complicated in light of the need to provide the fish farmer with ample income. Feed costs are rising steeply and are expected to rise further. Demand for agricultural products is rising, in large part, due to increased demands from the new emerging economies. This competition obviously pushes feed price to higher levels. In addition, the environmentally and economically driven rise of bio-fuel production takes away another significant portion of feed producing farming away, lowering supply of feed and driving feed prices up. Increasing fuel cost and other factors also contribute to rising production costs. Over the last decade, prices paid to farmers for most aquaculture products have dwindled. Many farms are being closed due to the tough competition.

In addition to the three constraints mentioned above, aquaculturists should be tuned in to the market demands for high quality, safe, attractive and socially acceptable products.

One of the major answers to the goals stated above is the use of the biofloc technology (BFT), a technology to be described in this manual.

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Verdegem, M.C.J., Kurup, M., Keshavanath, P. 2008. Sustainable land-based aquaculture: rational utilization of water, land and feed resources. Mediterranean Aquaculture Journal 1:45–55.*

2. *FAO. 2004. The state of the world fisheries and aquaculture. FAO Fisheries Department, Food and Agriculture Organization of the United Nations, Publishing Management Service, Rome, Italy.*

3. *FAO. 2011. The state of the world fisheries and aquaculture. FAO Fisheries Department, Food and Agriculture Organization of the United Nations, Publishing Management Service, Rome, Italy.*

4. *Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. 2000. Effects of aquaculture on world fish supplies. Nature 405: 1017-1024.*

5. *Tacon, A.G.J., Forster, I.P. 2003. Aquafeed and the environment: policy implications. Aquaculture 226: 181-189*

Chapter 2

Overview of Aquaculture Systems

In Brief

Aquaculture systems differ in their intensity, from the most extensive, growing fish or shrimp in impoundments with little or no feeding, up to the super-intensive systems supported by feed, aeration, and recycled water treatment. Each intensity level has its constraints and limitations.

Land and water scarcity calls for intensive systems where a large fish biomass can be produced with limited land and water. Feed materials are also limited and costly, thus food recycling is important.

Classical RAS (recirculating aquaculture systems) are efficient and operationally proven. These systems are expensive in both investment and operation, thus they are adapted mostly to specialty crops. Biofloc technology (BFT) systems are based upon the use of the water microbial community to both maintain proper water quality and to recycle un-utilized feed materials.

An overview of aquaculture systems, ranked by culture intensity from essentially enhanced fishing impoundments to super intensive recirculating systems, is given in Table 2.1.

The most extensive aquaculture systems are based on the stocking of fish in water impoundments. Ponds are not fed or are occasionally fed with grains, house and farm-yard residues. The annual yield is usually below 1,000 kg/ha. The factors limiting production in such systems are the production of organic matter by algae (primary production). The organic matter produced by the algae is the basis of the food web, supplying fish with energy, minerals and organic substrates needed for growth. **The limiting factor for higher productivity of these ponds is feed supply to the fish, limited by the rate of carbon assimilation by the algae.** Carbon assimilation in such ponds is in the order of 2-6 g carbon/m2 per day. Algae density in extensive ponds is usually limited by the supply of nutrients, mostly nitrogen and phosphorus. Adding fertilizers to such ponds may increase algal growth, carbon assimilation and fish productivity. It is important to note that fertilizer addition is needed only if nitrogen or phosphorus concentrations in the water are low (below about 10 and 1 mg/l, respectively). Excessive addition of fertilizers is a waste of money and can worsen the environmental impact of drained water.

Table 2.1: Schematic presentation of pond intensity levels, approximate annual fish yields and limiting factors

Pond type	Intervention	Approximate fish yields (Kg/Ha*Yr)	Approximate shrimp yields (Kg/ha* season)	Limiting factors
Minimal feed	minimal feeding with grains, farm and home residues	< 2,000	100 - 500	limits of primary production, food chain efficiency
Fed ponds	feeding by complete diet pellets	2,000-4,000	500 – 2,500	early morning oxygen
Night time aeration	Night time or emergency aerators, ~1-5 hp/ha	4,000-10,000	1,500 – 8,000	sludge accumulation, anaerobic pond bottom
Intensive mixed aerated ponds	24 hr. aeration >20 hp/ha –150 hp/ha, completely mixed	20-100 kg/m^3	8,000 – 20,000	water quality control. Selection of adapted fish
Super intensive tanks and raceways	Highly mixed and aerated (often added pure oxygen). Relatively small raceways or tanks (~100m^2), often in-house		Up to 100 kg/m^3	Energy utilization, economy

One of the features of natural aquatic environments is the ability to recycle nutrients. The feed supply of natural aquatic systems (and extensive aquaculture systems) is based upon the primary producers, algae that produce organic materials through the photo-synthetic process of binding solar energy, CO_2 and water to produce sugars and subsequently proteins and other cell components. Algae are grazed by different herbivores, plant eating animals, of different sizes, from microscopic zoo-plankton to algae-eating fish. Carnivores (meat eating), mostly fish, feed on smaller herbivores. Fish are then harvested by larger ones, by birds and by human beings. This seemingly simple sequence is more complex, and is described in much more detail in text books on aquatic biology (e.g. Wetzel, 1983).

A microbial community is always present in parallel to the food web described above. Micro-organisms (bacteria, fungi, protozoa) feed on organic substrates. Algae excrete organic matter and they are always accompanied by heterotrophic microorganisms, feeding on organic matter. Moreover, there are always dead cells, dead organisms, feces and other organic residues that serve as substrates for the activity of the micro-organisms. These organisms "eat", degrade the organic matter and use it for energy requirements and for growth and development of new cells.

All organisms, including micro-organisms that consume feed sources of a lower trophic level (algae by herbivores etc.), use the feed for essentially two goals: First, the feed is used to produce additional cells, tissues and increase the weight of the organism (As fish producers we are interested in this). The other use of the feed is to supply the energy required for different biochemical processes such as respiration, digestion, bio-synthesis etc., as well as energy required for movement, eating or feeding. The vast majority of aquatic animals do not maintain a constant body temperature and thus there is no energy consumed for temperature control. This last feature, as well as the fact that fish float and do not spend energy for standing against gravity, bring about a better feed utilization for growth as compared with other cultured animals (some say that fish do not waste energy for thinking, a topic not to be discussed here). In addition to energy production, feed is used to add new cells and increase the weight of the animal. Most aquatic organisms spend about 85-90% of the feed to produce energy and only 10-15% for growth.

Micro-organisms are much more efficient in converting feed to new cell material. Microbial efficiency (ME) is defined as the change in cell mass divided by the weight of feed metabolized. Micro-organism ME is in the range of 40-60%.

(2.1) $$ME = C\ \textit{(added to cells)} / C\ \textit{(metabolized)} \sim 40\text{-}60\%$$

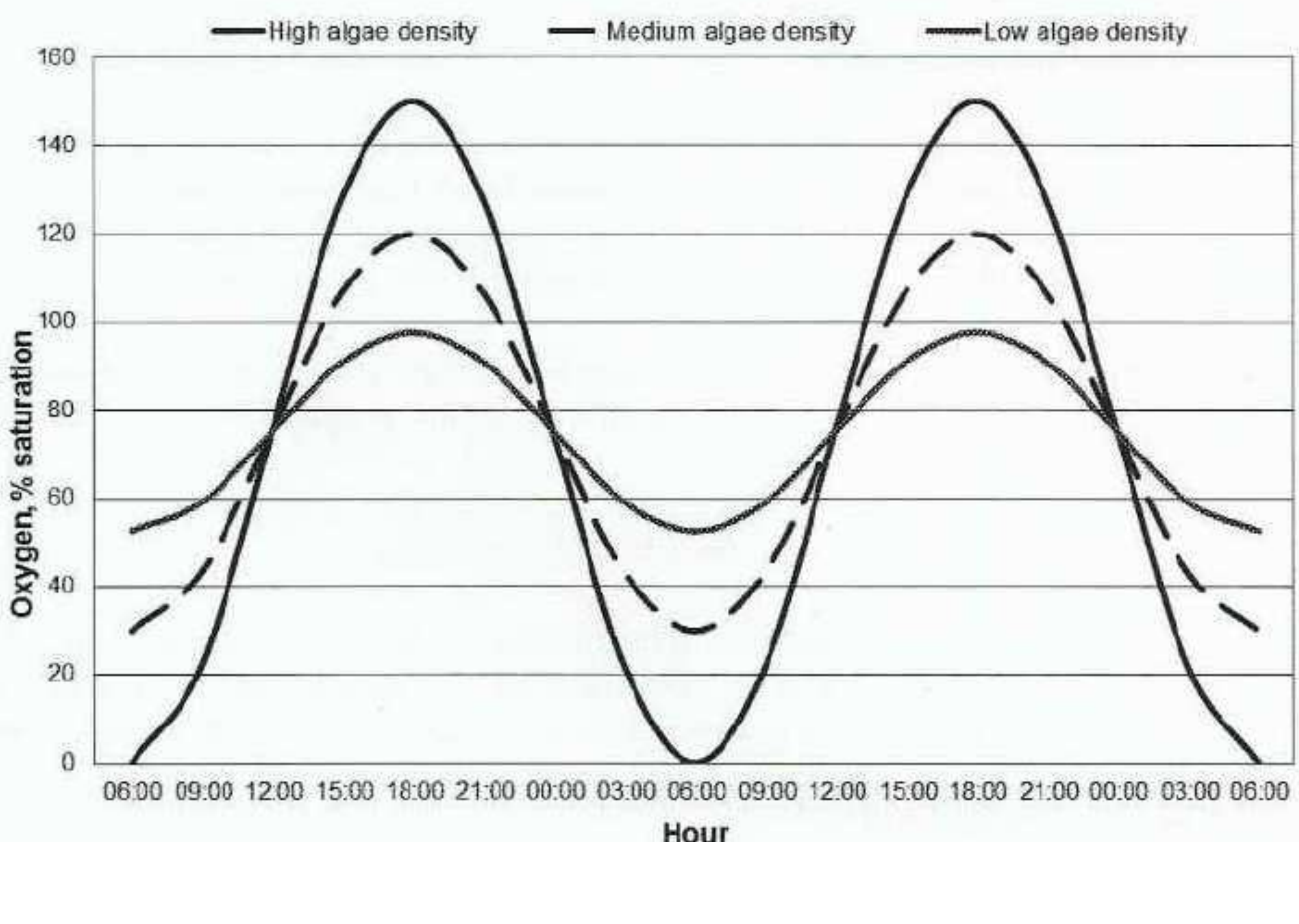
Feed conversion of bacteria is about 4 - 6 times higher than that of higher organisms. It has to be noted that utilization of feed by bacteria in the presence of oxygen (aerobic conditions) is higher than that of bacteria under conditions of low or zero oxygen (Anoxic or anaerobic conditions). Microbial feed utilization, and energy yield of feed metabolism is lower under anaerobic conditions than it is under aerobic conditions.

With the increase of feed input there is an ample supply of nutrients for algae development. Algae growth in these ponds is normally not limited by nutrient supply, especially in older ponds that have accumulated nitrogen, phosphorus and organic matter in the pond bottom. Algae development is now limited by the mutual shading and resultant light limitation. The maximal daily carbon assimilation in algae saturated ponds is in the range of 10 g carbon/m^2, however, values in the range of 2-5 g carbon/m^2 are the ones common in ponds. An interesting point is that when algae compete for light, algae that can float and reside near the water surface, such as blue green algae (cyano-bacteria), have an advantage in competing with other algae.

High oxygen concentration, often super saturation, may be found during daytime in algae rich ponds. Yet, at night, with no light and photosynthetic activity, with high oxygen consumption of the rich algae community, feed residues and fish respiration, (see Chapter 10) oxygen is depleted and anoxic conditions may develop, reaching lowest values in the early morning hours, as described in Figure 2.1.

Figure 2.1: Oxygen concentration in ponds as affected by time and algae density

***Oxygen increases by photosynthesis, starting on sunrise (6AM here) till sunset. Later, photosynthesis stops and respiration consumes the oxygen stored in the water.**



conditions is a function of fish and feed load, algae density, accumulation of organic substrates in the pond bottom and climatic conditions, such as temperature and wind. **The limiting factor for raising productivity in such ponds is the permanent or occasional development of low oxygen levels.**

The next level of pond intensification is the introduction of night time aeration systems. Night time aeration can be provided using permanent night time aeration or emergency aeration provided only when low oxygen is reached or expected. Most night time aeration systems are designed to supply oxygen to a certain part of the pond, a part that serves as a shelter for fish that are attracted to the relatively oxygen rich part of the pond. Fish (or shrimp) can survive in these safe-havens, yet, most of the pond area-- especially portions of the pond bottom -- undergoes anoxic conditions nightly.

The addition of aeration and prevention of catastrophic oxygen depletion enables the grower to raise the fish biomass in the pond, up to roughly 10,000 kg/ha. Yet, at about this level, the pond cannot carry higher biomass because of slow growth, stress, disease and mortality. As demonstrated in a number of studies (Avnimelech and Ritvo, 2003), **the limiting factor at this point is the presence of anaerobic conditions and production of anaerobic metabolites in the pond bottom.**

This limitation can be overcome by several means. One possibility is to remove pond water and settled materials from the pond and replace them with fresh water. This approach was once quite common in race-ways, shrimp farms close to the sea and other production units where water was plentiful. However, this approach has almost been phased out at present, due to restricted supply of water and, moreover, due to environmental concerns and regulations prohibiting release of nutrient and organic rich water to the environment. Growing dense fish biomass in cages is similar in a way, since water is exchanged with the sea or lake water surrounding the cage. The sustainability of this method is limited and it is viable only if the cage biomass does not exceed the dilution capacity of the surrounding water body.

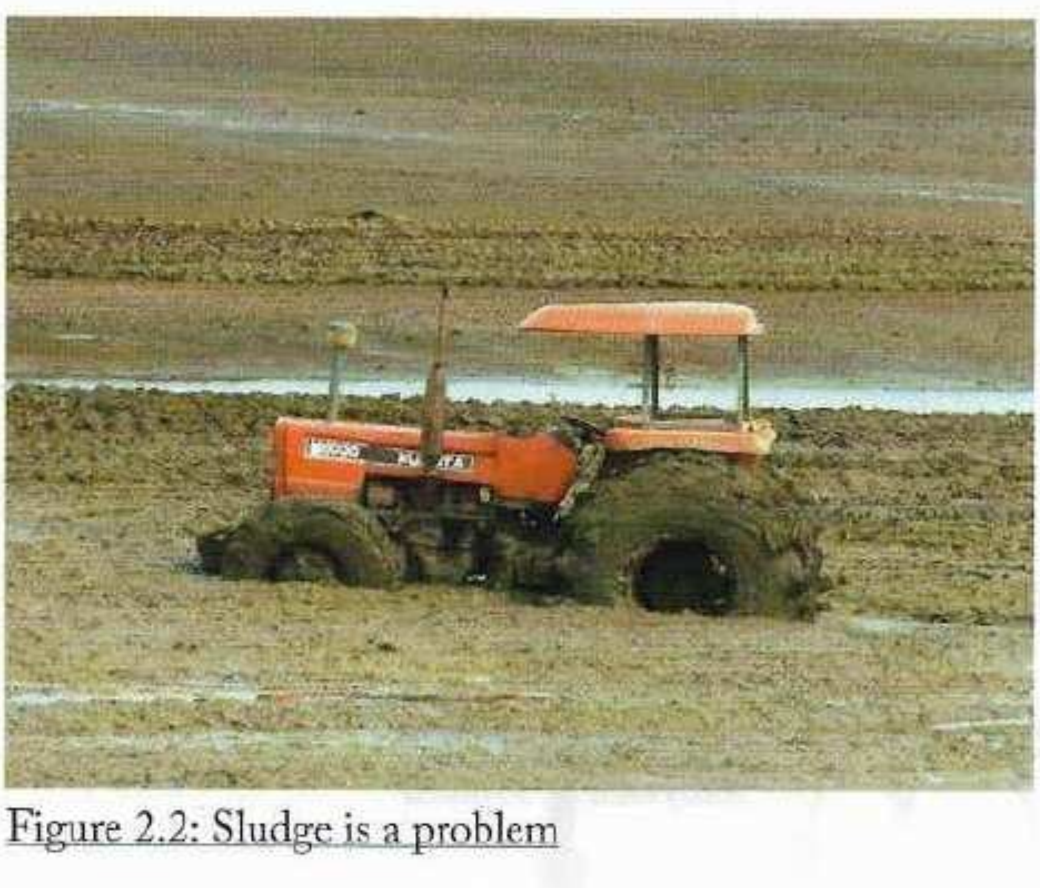


Figure 2.2: Sludge is a problem

The collected sludge is disposed (possibly treated and utilized as a feed material (Schneider et al., 2007) while the clear solution is treated in a bio-filter (e.g. Timmons & Ebeling, 2007). The bio-filter unit consists of different modes of suspended or stationary solid substrates of high surface area. Microbes covering these surfaces convert the TAN to nitrate and to some extent oxidize the water soluble organic matter. It has to be noted that the main role of the substrates is to hold the nitrifying bacteria in the bio-filter and prevent their flushing to the production unit. In the case of BFT systems, this problem (as we will see later) does not exist.

Figure 2.3: Scheme of recirculating aquaculture system (RAS)

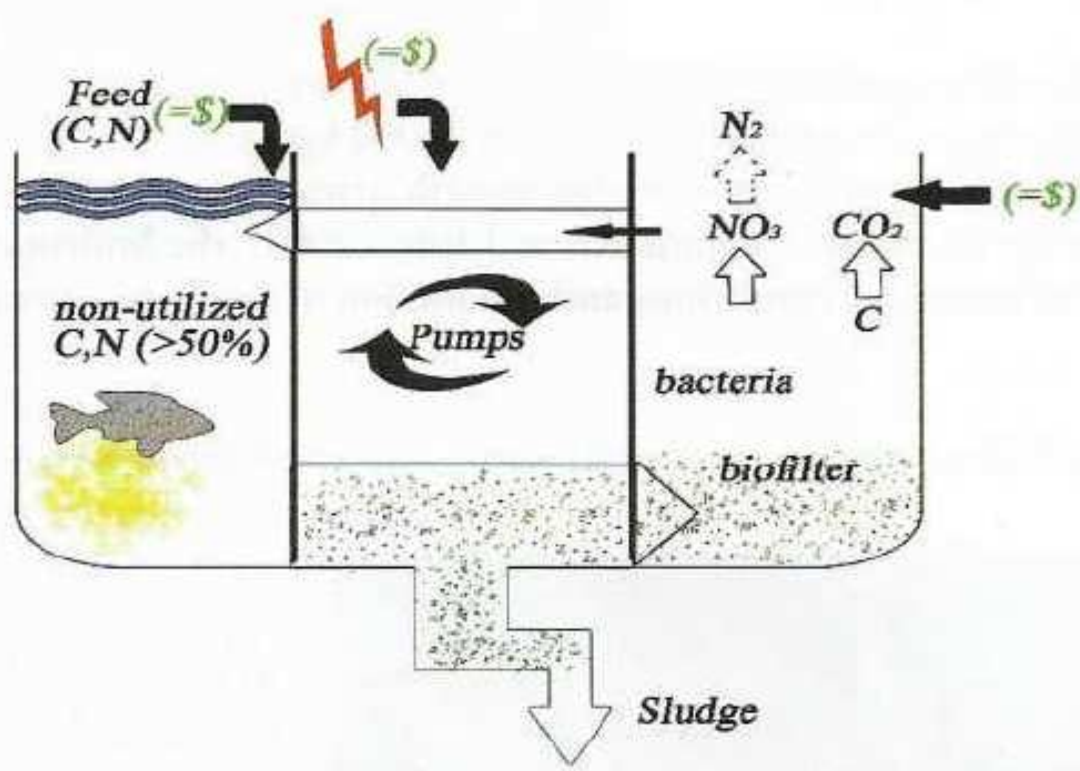
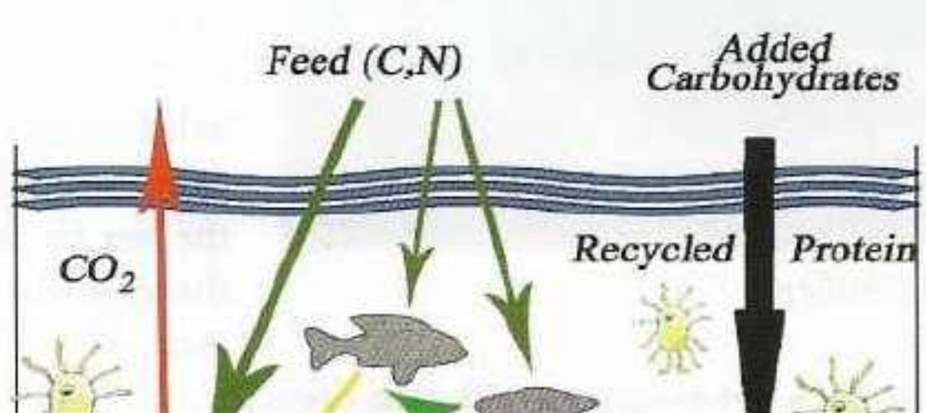


Figure 2.4: Scheme of Biofloc technology (BFT) system



Several RAS configurations contain denitrification modules to reduce the nitrates (van Rijn et al., 2006). Occasionally, water goes through a UV, ozone or other sterilizing device in order to reduce pathogen transfer. Pure oxygen may be added to maintain a very high fish biomass. Overall system oxygen consumption is minimized due to the very fast removal of oxygen consuming feed and other residues.

RAS technology has been reviewed extensively (e.g. Timmons & Ebeling, 2007). A clear advantage of this technology is the fact that it is an "off the shelf technology". Different systems and components of RAS have been used for many years and one can purchase any given system and can pretty much be assured as to the qualifications of the system. The RAS technology is to a large extent an all-purpose system enabling the culturing of a large variety of fish. The system needs very little area and water, enabling the growing of fish biomass in the order of 100 kg/m3. However, this system has a number of intrinsic drawbacks. Overall, RAS is expensive, both in investment and maintenance. Water is recycled several times an hour, requiring intensive pumping. Water filtration and bio-filtration require high investment and maintenance costs. Aeration devices have significant power requirements and are costly. Since these systems hold very dense fish biomass, they are not forgiving. Any failure in aeration or pumping may lead to mass mortality in a short time, in a matter of minutes in many cases. Bio-filters may be poisoned or damaged, requiring a few weeks recovery period. Thus, all systems require reliable backups, alarms and controls.

RAS ponds do not recycle feed. Any feed that is not consumed by the fish immediately and all un-utilized feed excretions are removed and disposed, at a high cost. This is contrary to natural and conventional water systems where feed and feed residues are recycled through the food web. This feature becomes more critical with the rising global competition for feed materials and the rising cost of feed. Ideally, a sustainable aquaculture system should be operating in a way as to maximize feed utilization.

Much effort has been devoted to developing more efficient and less costly RAS (Mozes et al., 2003). However, though these systems have already been in the market for a few decades, their use is limited mostly for high value aquaculture products, such as exotic fish, nurseries, and fish with high market value rather than for the large scale production of commodity fish.

A new approach, biofloc technology (BFT), was developed in order to facilitate intensive culture, while keeping initial investment and ongoing maintenance costs low and incorporating the potential to recycle feed. This technology will be described in detail in the following chapters. The development of BFT is based on a sequence of motivations, principles, and suitable operative technologies. Zero or minimal water exchange is imposed to save water, achieve maximal biosecurity and to minimize the external environmental effects of shrimp and fish cultures. With the closure of the pond, appreciable accumulation of residues takes place. To overcome the effects of

The management of the pond depends upon both the management of fish (or shrimp) culture as well as the management and control of the heterotrophic microbial community. One important aspect of microbial control is the control of toxic inorganic nitrogen in the water and the accompanying production of microbial protein, achieved by adjusting C/N ratios in feed. Uneaten nitrogen is being utilized to produce microbial protein, rather than generating toxic components. Microbial protein, suspended in the pond as microbial flocs, is utilized as fish feed. Protein utilization is twice as high in biofloc technology systems than in conventional ponds. There are indications that the dense diverse heterotrophic microbial biomass decreases the outbreaks of microbial diseases.

The novel concept of biofloc technology ponds is presently being successfully applied in fishponds (mostly tilapia culture) and in shrimp ponds. Present experimental and commercial results indicate that biofloc technology ponds enable growers to achieve high yields in environmentally and economically sustainable systems.

Practical Implications and Tips

1. *There are no intrinsically good or bad systems. The selection of intensity depends on conditions. Thus, if large water impoundments are available, it is possible that extensive systems are the optimal way to earn income, while in dense or water poor areas, such systems will make little sense.*

2. *The decision on the optimal intensity of a production system depends on existing conditions and future expectations.*

3. *Feed components are costly (and costs will probably rise). Thus, feed efficient utilization is essential.*

4. *Feed recycling through the pond food-web is a natural process and is suitable for almost any system. However, the net gain is limited due to losses in transfers along the food-web. The most efficient recycling route is through microbial heterotrophic processes.*

5. *Micro-organisms generate new cells and protein from about 50% of metabolized organics under aerobic conditions. The microbial efficiency under anaerobic conditions is less than half of this. Thus, organic matter accumulating on the oxygen limited pond bottom is wasteful. It is advisable to re-suspend the organic matter in the aerobic water column.*

6. *Algae add oxygen to the water during daytime (if there are no clouds). However, excessive algal density leads to an oxygen deficit at night. So, enough is enough also in regards to algae.*

Further Research Needs

1. *Though the choice of optimal aquaculture depends on site conditions (time, market, energy etc.), as mentioned above, it would be very useful to establish the outline of suitable economic model, listing all input items and components in a way enabling the farmer to fill in the relevant data and to get an estimation of the costs of building and operating different types of systems. Such compilation would be useful and respected.*

2. *Sludge is produced in all intensive aquaculture systems. Sludge cannot just be dumped as it used to be in the past. Safe disposal is not simple and may be quite costly. There are alternatives for reuse as an energy source, feed material or soil conditioner. Due to the fact that proper sludge disposal is a rather new requirement, not enough research has been conducted to provide the needed guidelines.*

3. *All aquaculture systems depend in one way or the other on the activities of algae, bacteria and other organisms. Existing information is rather empirical. More fundamental research is needed to better define the microbial population and to develop the means to control it.*

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220: 549-567.*

2. *Avnimelech, Y., Verdegem, M.C.J., Kurup, M., Keshavanath, P. 2008. Sustainable land-based aquaculture: rational utilization of water, land and feed resources. Mediterranean Aquaculture Journal, 1:45-55.*

3. *Mozes, N., Eshchar, M., Conijeski, D., Fediuk, M., Ashkenazy, A. and Milanez, F. 2003. Field report: marine recirculating systems in Israel- performance, production cost analysis and rational for desert conditions. The Israeli Journal of Aquaculture- Bamidgeh 55(4):243-257.*

4. *Schneider, O., Sereti, V., Eding, Ep. H., Verreth, J.A.J., Klapwijk, B., Kinetics. 2007. Design and biomass production of a bacteria reactor treating RAS effluent streams. Aquacultural Engineering 36: 24-35.*

5. *Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinvi, B.J. 2002. Recirculating aquaculture, NRAC publication 01-002. 2d Edition. 768 pp.*

6. *Van Rijn, J., Tal, Y., Schreier, H.J. 2006. Denitrification in recirculating systems: theory and applications. Aquacultural Engineering. 34: 364-376.*

7. *Books on Limnology, e.g.: Wetzel R. G. 1983. Limnology, 2d Edition, Saunders.*

8. *Books on Aquaculture, e.g.: Pillay, T.V.R., and Kutty, M.N. 2005. Aquaculture: principles and practice.*

Chapter 3

Microbial Processes and Communities Relevant to Aquaculture

In Brief

Photosynthesis and respiration are important processes in ponds.

Photosynthesis:

$$(3.1) \qquad 6CO_2 + 6H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6O_2$$

And (the opposite process) Respiration:

$$(3.2) \qquad C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Energy}$$

Algae based ponds, where carbon added by algae is an important component, have limited capacity and stability.

Heterotrophic microbial activity takes place in all aquaculture systems. The number of heterotrophic bacteria varies from 103/ml in extensive ponds up to 109/ml in highly intensive ones. The heterotrophic bacteria have a very short generation time and population depends on feed concentration. The microbial population rapidly adapts to varying conditions. Autotrophic nitrification is responsible for ammonia and nitrite oxidation. They have a long generation time and thus develop slowly (weeks) and respond slowly to changes in conditions.

When fish (including shrimp) biomass is higher than 5000 kg/ha, carbon added with feed exceeds that contributed by algae and the system is greatly affected by heterotrophic activity.

1. Autotrophic Organisms, Algae

Though we can grow fish in systems with practically no microbial activity (filtered sterilized water), fed only by formulated feeds, such conditions are rare. Usually, fish are grown in the presence of an array of micro-organisms, affected by the microbial communities present in the pond.

A commonly known microbial community coexisting with fish culture is the photosynthetic autotrophic, producing their own food by conversion of the solar energy into chemical energy, i.e. by the production of a series of organic compounds. The different organic compounds are used as feed for organisms that cannot produce their own food, the heterotrophic organisms. The autotrophs are the primary producers, serving as the base of life in ponds and all other natural systems.

The autotrophic community is composed of chemo-autotrophs, getting their energy by oxidizing chemical compounds (ammonium, divalent iron and manganese, reduced sulfur, methane) and photo-autotrophs, gaining their energy through the harvesting of solar energy, CO_2 and water. The light energy is captured by chlorophyll and other light adsorbing pigments and is used to reduce CO_2 to organic carbon in simple sugars:

$$6\,CO_2 + 6H_2O + \text{Light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (3.3)$$

The light energy is converted into chemical energy in carbohydrates. The chemical energy can be used for the different metabolic processes in the primary producers or subsequently in secondary producers by respiration that can be formulated as:

$$C_6H_{12}O_6 + 6O_2\,6 \rightarrow CO_2 + 6H_2O + \text{Energy} \quad (3.4)$$

The energy is released as bio-chemical energy (ATP) that subsequently can be converted to other types of chemical energy (Bio-synthesis), mechanical energy (movement) or heat. It is important to realize that respiration is the opposite process of photosynthesis. During active photosynthesis there is an uptake of CO_2 and release of oxygen. However, in the long run, practically all organic carbon is respired, CO_2 is released and practically all oxygen supplied by photosynthesis to the pond is consumed through the respiration and oxidation of virtually all organic matter that has accumulated in the system. Thus, one has to remember that in the long run, oxygen addition by algae is a loan and not a gift. The higher the primary production, the more respiration takes place at night. Schroeder (1975) found out that **night-time oxygen deficiencies are more severe in ponds with heavy algae population.** The same holds for the effect of photosynthesis on global warming. In steady state situations, all CO_2 sequestered by photosynthesis is eventually respired. Photosynthesis can counteract anthropogenic CO_2 release only if a new steady state is achieved, e.g. raising the steady algae density in the ocean by adding and maintaining higher trace elements level. In the past, some of the sequestered CO_2 was removed from the biosphere and stored as coal or as oil storage in deep strata. The recent anthropogenic CO_2 release is mostly due to the mining of this stored organic carbon and returning the carbon back to the atmosphere as CO_2. In a similar manner as regards to pond management, long term oxygen gain can be achieved in cases where the organic matter accumulated at the pond bottom is either mechanically removed or oxidized by air when pond bottom soil is dried in between cropping cycles.

in some cases CO_2 availability is limiting). Solar radiation may be a limiting factor. Light can be used by algae only during daytime, thus photosynthesis is active, on average, only on 50% of the time, while respiration of algae, other pond organisms and fish occurs 24 hours a day. This is an essential limitation, to be further discussed later. Light is also a limiting factor during cloudy days.

In addition, light often determines the potential for photosynthesis. Light harvesting by dense algae populations is limited due to the mutual shading of the plankton. In dense algae populations, light is adsorbed and its penetration is limited, as can be easily evaluated by the determination of Secchi depth (See Chapter 18). The mutual shading determines maximal saturation value for photosynthetic activity in ponds.

Algae need nutrients for normal development. Nitrogen is needed to produce proteins, nucleic acids and other cell components. Phosphorus is an essential element in cell membranes, energy transfer etc. A variety of other elements such as calcium, magnesium, potassium and iron are needed in rather high amounts and a number of trace elements are needed in minute amounts, yet are essential for proper activity and growth. The availability of nutrients in natural water bodies is often the limiting factor for algae development. On the other hand, an excessive availability of phosphorus or nitrogen often leads to excess density of algae and to eutrophication of rivers, lakes and regions in the ocean. Thus, enormous efforts are taken to cut down nutrient release into water bodies. This trend is an important constraint, determining the degree to which drained water can be released from ponds into the aquatic environment.

Nutrients are supplied with the feed, contributing usually to a supply higher than that needed by the algae, especially so in intensively fed fish ponds. Thus, in most cases nutrients do not limit algae development. This is not the case in new ponds that are just stocked, especially plastic-lined ponds that do not store nutrients from season to season. In such cases, ponds have to be fertilized initially, to support proper algae development start.

Gross photosynthesis in fertilized ponds is in the range of 1-8 gC/m^{2*}day. However, when pond water is continually mixed (e.g. in partitioned aquaculture systems), photosynthetic capacity is raised up to 10-12 gC/m^{2*}day. In following discussions, an estimated average algae production of 4 gC/m^{2*}day will be used.

The algae community is made up of a number of species and often a sequence of species. The succession of algae, characteristics of different algae and relevance to aquaculture is beyond the scope of the present discussion. It should be noted that algae populations may be un-stable, may experience crashes, changes of dominant algae, rises and decline of population and activity. All ing solar energy, CO_2 and water to produce sugars, subsequently bio-modified to proteins and other cell components. Algae are grazed by different herbivores, plant eating animals of different sizes, from microscopic zoo-plankton to algae eating fish. Carnivores (meat eating), mostly fish, feed on smaller herbivores. Small fish are then harvested by larger ones, by birds and by human beings. A microbial community is always present in parallel to the food web described above. Micro-organisms (bacteria, fungi, protozoa) feed on organic substrates. Algae excrete organic matter and they are always accompanied by heterotrophic microorganisms, organisms that feed on organic matter. Moreover, there are always dead cells, dead organisms, feces and other organic residues that serve as substrates for the activity of the microorganisms. These organisms degrade the organic matter and use it for energy requirements on the one hand and for growth and development of new cells on the other hand. Bacterial production is positively related to phytoplankton primary production. Even with no external feed addition, as is common in fish ponds, Bacterial Production (BP, μ C /litter x day) is related to Net Primary Production (NPP, same units) through:

(3.5) $$BP = CF \times (0.347 \times NPP0.8)$$

Where CF is a correction factor, equal to 1.56, required to convert the original log-log regression to arithmetic units (Cole et al., 1988).

Using this equation, we can estimate the bacterial production of a pond with a primary production of 4 gC/m^2 x day to be 1.64 gC/m^2 x day, 41% of the phytoplankton activity. When external feed is added, bacterial production is much higher. One important conclusion we can draw is that **in contrast with commonly used terminology, there are no "autotrophic" or "heterotrophic" ponds.** There are always both autotrophic and heterotrophic activities taking place at the same time. The ratios or the dominance of the given processes vary in different pond systems.

On average, microbial cells contain 25% dry matter. On a dry matter base, bacteria contain 48.9% carbon, 5.2% hydrogen, 24.8% oxygen, 9.46% nitrogen (=61% crude protein), and 9.2% ash (Rittmann & McCarty, 2001).

All organisms, including micro-organisms, consuming feed source from a lower level (algae by herbivores etc.), use the feed for two goals: converting the energy in the feed to energy spent for activity, and to gain weight and produce new cells.

The feed is used to supply the energy required for biochemical processes such as respiration, digestion, bio-synthesis etc., as well as the energy required for movement, eating or feeding. The vast majority of aquatic animals do not maintain a constant body temperature and thus there is no energy consumed for temperature control. This last feature, as well as the fact that fish float and do not use energy for standing and fighting against gravity results in better feed utilization by a large one, delivers to the final consumer just 1/1000 of the algae feed carbon (10% x 10% x 10%). Please note: The Feed Conversion Ratio, FCR, a term commonly used in aquaculture, usually in the range of 1-3, seems not to be in agreement with the above statement. However, for FCR computation we compare weight gain of the whole fish (the product sold in the market, containing about 75% water) with the input of dry feed bought by the farmer.

Though one should not relate two different entities (wet fish compared with dry feed), this relation makes sense, since the farmer pays for dry feed-pellets and is paid for the whole fish including the water in the fish. In previous computations we looked after the carbon in the feed and in the animal, both on the basis of dry matter organic carbon, a more scientific comparison. The fish farmer profit is not based upon scientific terms but upon market values.

Micro-organisms are much more efficient in converting feed to new cell material. Microbial efficiency, ME, is defined as the change in cell mass divided by weight of feed metabolized (Ritmann & McCarty, 2001):

$$ME = C\ (\text{added to cells})/C\ (\text{metabolized}) \sim 40\text{-}60\% \quad (3.6)$$

Microbial conversion efficiency for aerobic micro-organisms is in the range of 40-60%. Feed conversion of bacteria is about 4 times higher than that of higher organisms. It has to be noted, that utilization of feed by bacteria acting in the presence of oxygen (aerobic conditions) is higher than that of bacteria acting under conditions of low or no presence of oxygen (anoxic or anaerobic conditions). Microbial feed utilization, and energy yield of feed metabolism is lower under anaerobic conditions than it is under aerobic conditions (Reddy et al., 1986).

Bacteria and other micro-organisms act as very efficient bio-chemical systems to degrade and metabolize organic residues. As mentioned, the conversion efficiencies of micro-organisms are several times higher than those found for higher organisms. In addition, their rate of multiplication and rate of action are remarkably high. Micro-organisms metabolizing organic substrates produce new cell materials and new cells. When substrates are available and conditions are suitable, it takes about 30 minutes for the multiplication of bacteria. i.e., one bacterium will theoretically produce 4, 16, and 64 bacterial cells after 1, 2 and 3 hours respectively. In 24 hours the bacterial population will potentially be 2 to the 48th power, i.e. 3*1014!! (Of course the actual population will be smaller, due to feed and other limitations as well as the mortality of cells).

The size of the microbial population, when environmental conditions are suitable, depends on feed substrate availability.

The equation most frequently used to represent bacterial growth kinetics is the

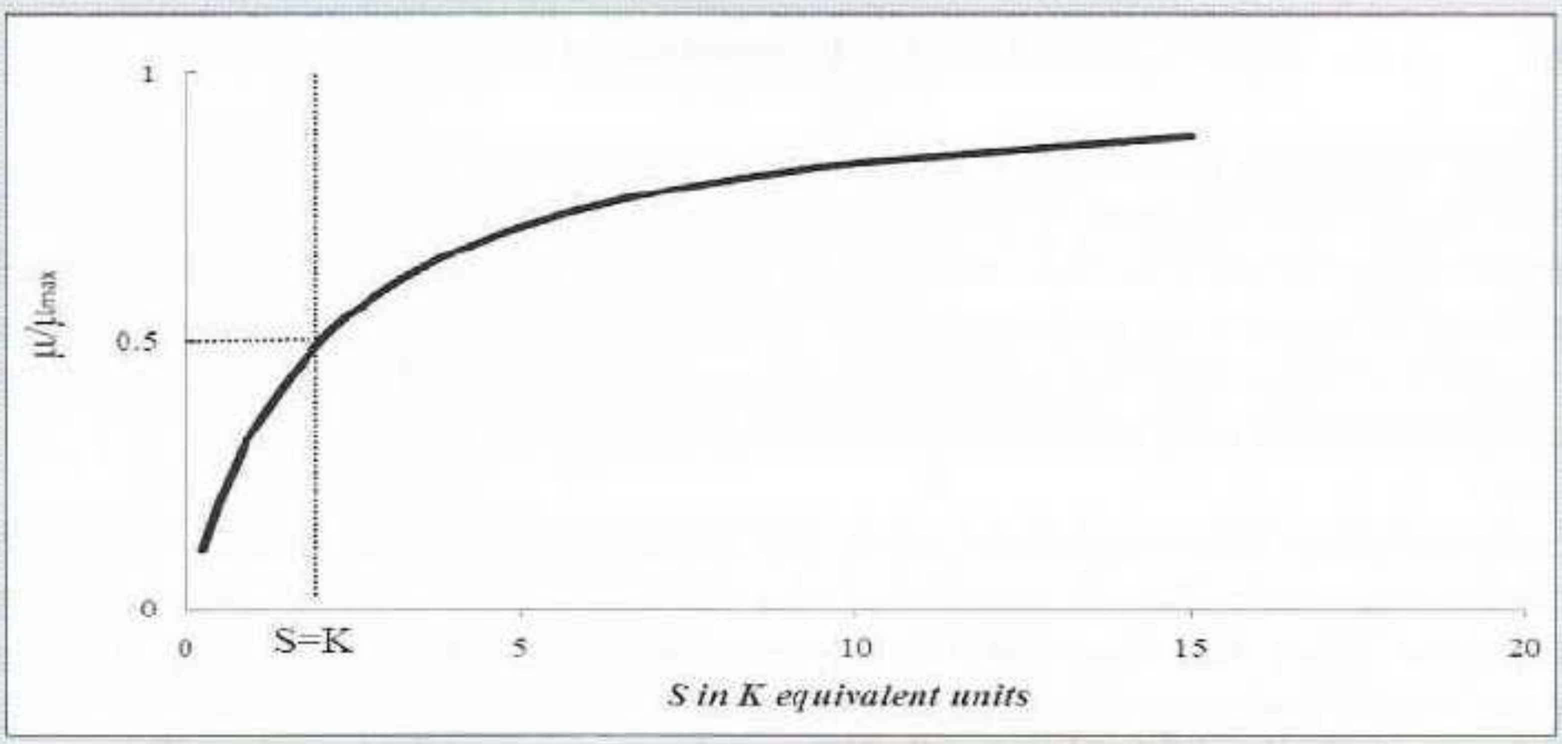
$\mathbf{m}_{max}$ is the maximum possible specific growth rate (T^{-1})

S is the rate limiting substrate concentration

K is a constant, representing the substrate concentration at which growth rate is 50% of maximal rate.

This equation demonstrates the features of the process: At low substrate concentrations growth is roughly a linear function of S: $\mu = \mu_{max}*[S/(S + Km)]$, yet, at high concentrations growth is not affected by S: $\mu = \mu_{max}$. This relationship is shown schematically in Figure 3.1.

Figure 3.1: The dependence of microbial growth rate on substrate concentration



* See equation 3.7

A practical conclusion stemming from microbial population growth dynamics is the exceptional adaptation of microbial population to the concentrations of organic matter in pond water. Microbial density in extensive ponds may be in the order of 10^3 (1 thousand) cells per ml water, and on the other-hand may reach a density of 10^9 (1 billion) cells per ml in limited exchange intensive ponds, where organic matter supply is very high. The adaptability of the microbial population to substrate concentrations implies that microbial potential to modify and control water quality in ponds is practically almost unlimited. **As we add more and more feed, the number of bacteria will rise and all added feed and feed products will be metabolized as long as we have enough oxygen and mixing in our ponds.**

Where S is the substrate concentration, Vmax the maximal rate of degradation and Km a constant equal to the concentration at which the rate is 50% of maximal. However, in most cases S is a limiting factor, whereupon this equation is reduced to a linear dependence on S concentration, a first order kinetic reaction:

(3.9) $dS/dT = K * S,$

Where K, a constant is equal to Vmax / Km and is given in units of 1/time (1/hour, day etc.). An important parameter is the half-life of the process, the time it takes to reach 50% of the complete degradation of a given substrate concentration. The rate of the degradation process and the half-life in ponds as a function of different substrates, is described below in Table 3.1.

Table 3.1: Rates of degradation of selected organic components

Organic component	K, first order rate constant (Day^{-1})	Half-life (Days)
Sugars	1.1500	0.6
Hemicellulose	0.1035	6.7
Cellulose	0.0495	14.0
Lignin	0.0019	364.7

* Adapted from Reddy et al., 1986.

Readily biodegradable substrates, in contrast with stable compounds, are effective in promoting bacterial growth. Most feed inputs into the pond are readily degradable. Feed pellets contain starch and proteins, having a first order decomposition rate (K) of about 0.8 day^{-1}. The mineralization rates of feed pellets tested in laboratory microcosms was found to be 0.27 day-1 under aerobic conditions, as compared to about 0.07 day^{-1} under anaerobic conditions (Torres-Beristain 2006). Avnimelech and co-workers (1995) found that degradation of organic matter in mixed – aerated tanks and in commercial BFT ponds followed a first order kinetics with a rate of 0.14-0.16 day^{-1}, which is in good agreement with the above mentioned laboratory data. A first order decomposition constant of dead algae cells and exudates has a rate of about 0.1 day^{-1}. On the other hand, refractive organic matter in sediments degrade at a rate of 0.4 yr^{-1}, and humic com-

3. Autotrophic Bacteria: Nitrification

Autotrophic nitrification is a two-step process in which ammonia is biologically oxidized into nitrite (nitritation) and then to nitrate (nitratation) with oxygen as terminal electron acceptor (Ritmann & McCarty, 2001):

$NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H^+ + H_2O$	ammonia-oxidising bacteria
$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$	nitrite-oxidising bacteria
$NH_3 + 2\ O_2 \rightarrow NO_3^- + H^+ + H_2O$	Overall process

The first step of the process is catalysed by ammonia-oxidising bacteria (such as: *Nitrosomonas* spp. and *Nitrosococcus* spp.). The second step is catalysed by nitrite-oxidising bacteria (such as: *Nitrobacter* spp. and *Nitrospira* spp.).

The nitrifying bacteria are obligate aerobic autotrophs. Being autotrophs they generate energy by the oxidation of NH_4 and NO_2 and produce their cell material by reducing CO_2 and producing reduced organic carbon components. The energy yield of NH_4 or NO_2 oxidation is rather low. This energy extensive process is primarily responsible for a very low conversion coefficient. Only about 10-14% of the yield of the chemo-oxidation process is converted toward the production of cell material, as compared with about 50% in heterotrophs.

The stoichiometry of the nitrification process (Ebeling et al., 2006) is (reactants and products of an oxidation of 1mole NH_4-N, quantities given in molar units):

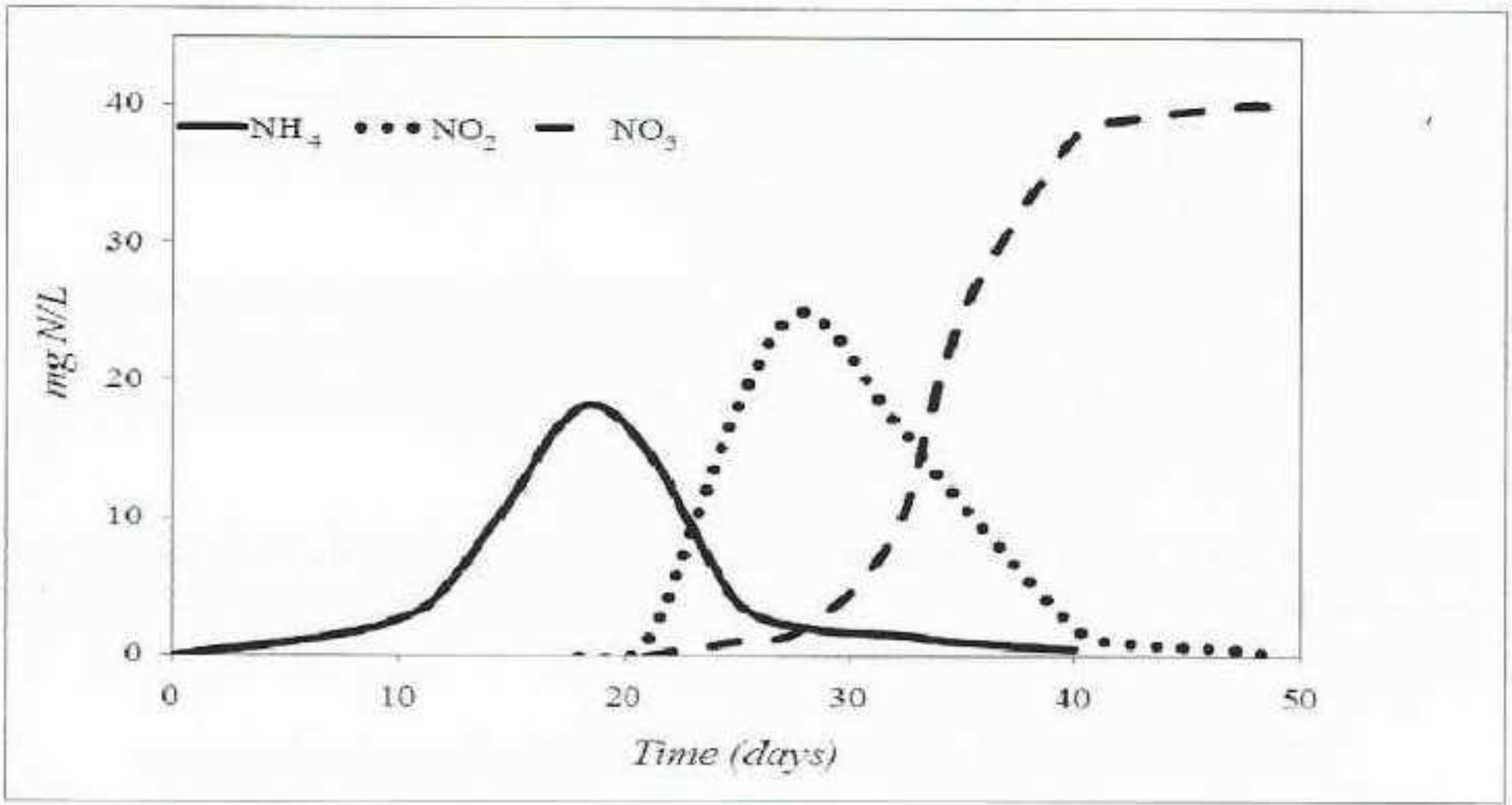
(3.10) $1\ NH_4^+ + 1.73\ O_2 + 0.154\ CO_2 + 0.038\ HCO_3^- \rightarrow 0.038\ C_5H_7O_2N + 0.962\ NO_3^- + 1.924\ H^+ + 0.923\ H_2O$ (where $C_5H_7O_2N$ represents the added cell mass.)

The same stoichiometry given in grams is:

(3.11) $1\ NH_4^+ + 3.95\ O_2 + 0.484\ CO_2 + 0.16\ HCO_3^- \rightarrow 0.31\ C_5H_7O_2N + 4.26\ NO_3^- + 0.137\ H^+ + 1.184\ H_2O$

The low conversion coefficient of nitrification leads to a small maximal specific growth rate and thus nitrifiers are slow growers, with a generation period in the order of 12 hours versus about 30 minutes for heterotrophs. The slow growth rate of nitrifying bacteria is of utmost practical importance. When a limited exchange pond is stocked and fed, processes leading to ammonium build-up in the water start very quickly and ammonium oxidizing bacteria population starts to

Figure 3.2: Sequence of nitrogen species in BFT pond



* Data from experimental pond, Dor, Israel.

At that point nitrite oxidizing bacteria start to develop (KNO_2 is 1.3 mg NO_2-N/l at 200C and 2.7 mg NO_2-N/l at 250C). During the transition time, before a stable population of nitrite oxidizers is achieved, nitrite concentration in the water rises over roughly 4 weeks, to a point when are enough bacteria and both ammonium and nitrite concentrations are controlled. The possibility of shortening these transition periods will be discussed in Chapter 11. Similarly, this slow build-up of nitrifying populations is also an issue in the acclimation of bio-filters mentioned before.

Nitrifiers are obligate aerobes and they need O_2 for respiration. The concentration of oxygen required for 50% of maximal activity is 0.5 mg O_2/l for ammonium oxidation and 0.7 mg O_2/l for nitrite oxidation. Note that **NO_2 oxidation is more sensitive to low oxygen than NH_4 oxidation.** Thus, when oxygen concentration is limiting, NH_4 may still be oxidized but NO_2 may accumulate in the water. This is often the case when aeration of ponds is not efficient or when anaerobic pockets exist in the pond.

Nitrification is affected by a variety of parameters such as substrate and dissolved oxygen concentrations, organic matter, temperature, pH, alkalinity, salinity and turbulence level. Nitrifying

4. Heterotrophic Vs Autotrophic Dominance

Fish biomass in conventional ponds is in the range of 1 to 10 ton ha^{-1} (0.1 to 1 kg m^{-2}), and reaches 100 kg m^{-2} in super intensive systems. Daily feed addition is roughly 2% of fish biomass, i.e. 2-10 g m^{-2} in conventional ponds and 2000 g m^{-2} in super intensive systems. The daily organic carbon input is half of the above-mentioned feed inputs since feed contains on average 50% organic carbon. A primary production of about

4g C m^{-2} d^{-1}, as the average value for ponds discussed here is equivalent to the daily carbon addition through the feed to a pond with a fish biomass of about 0.2 kg m^{-2}, or 2,000 kg ha^{-1}. Feed carbon additions to ponds with a higher fish biomass will supersede the carbon assimilatory capacity of the algae.

As mentioned above, **there are no totally autotrophic, algae controlled ponds, and there are no totally heterotrophic** ones. There is always a mix between the two. A useful concept can be the Heterotrophic Ratio (HR) that is defined as:

(3.12) HR = Daily external C addition / Autotrophic carbon assimilation rate

Feed application in ponds of different biomass is given in Table 3.2, in comparison with expected algae primary production. It can be seen that when fish biomass is higher than 5,000 kg/ha (0.5 kg/m^2), carbon added with feed is higher than that added by algae and HR is higher than 1. We can conclude that ponds with a fish biomass below 5000 kg/ha are dominated by algae activity, while ponds with higher biomass are dominated by heterotrophic organisms activity. Yet, both contain algae and bacteria.

Table 3.2: Carbon addition to fish (shrimp) ponds with feed and algal production

Biomass (kg/ha)	Daily feeding (Kg/ha)	Daily carbon added (Kg/ha)	HR
200	4	2	0.05
500	10	5	0.125
1000	20	10	0.25
2000	40	20	0.5
5000	100	50	1.25
10,000	200	100	2.5

Microbial growth rate (dx/dt) is related to the concentration of organic substrate (S) through the Monod equation as mentioned above:

(3.13) $\mu = \mu_{max} * (S/ (K+S)$

When K is higher than S (i.e. substrate concentration is the limiting factor), as is usually the case, equation (3.11) is reduced to a first order kinetic:

(3.14) $\mu = (\mu_{max}/K) \times S$

i.e., microbial growth rate becomes a direct linear function of substrate availability.

The degradation of the organic substrate (dS/dt) is considered to follow first order kinetics (-K x S), where K is a degradation constant.

Organic substrates are added daily to the pond as feed or as organic fertilizers and through primary production. Assuming that the daily addition of organic matter (B) is constant over a given time, then the equation becomes (Avnimelech, 1989):

(3.15) $dS/dt = B - K * S$

This after integration becomes:

(3.16) $S = (B - e^{-Kt} * (B - KS_0))/K$

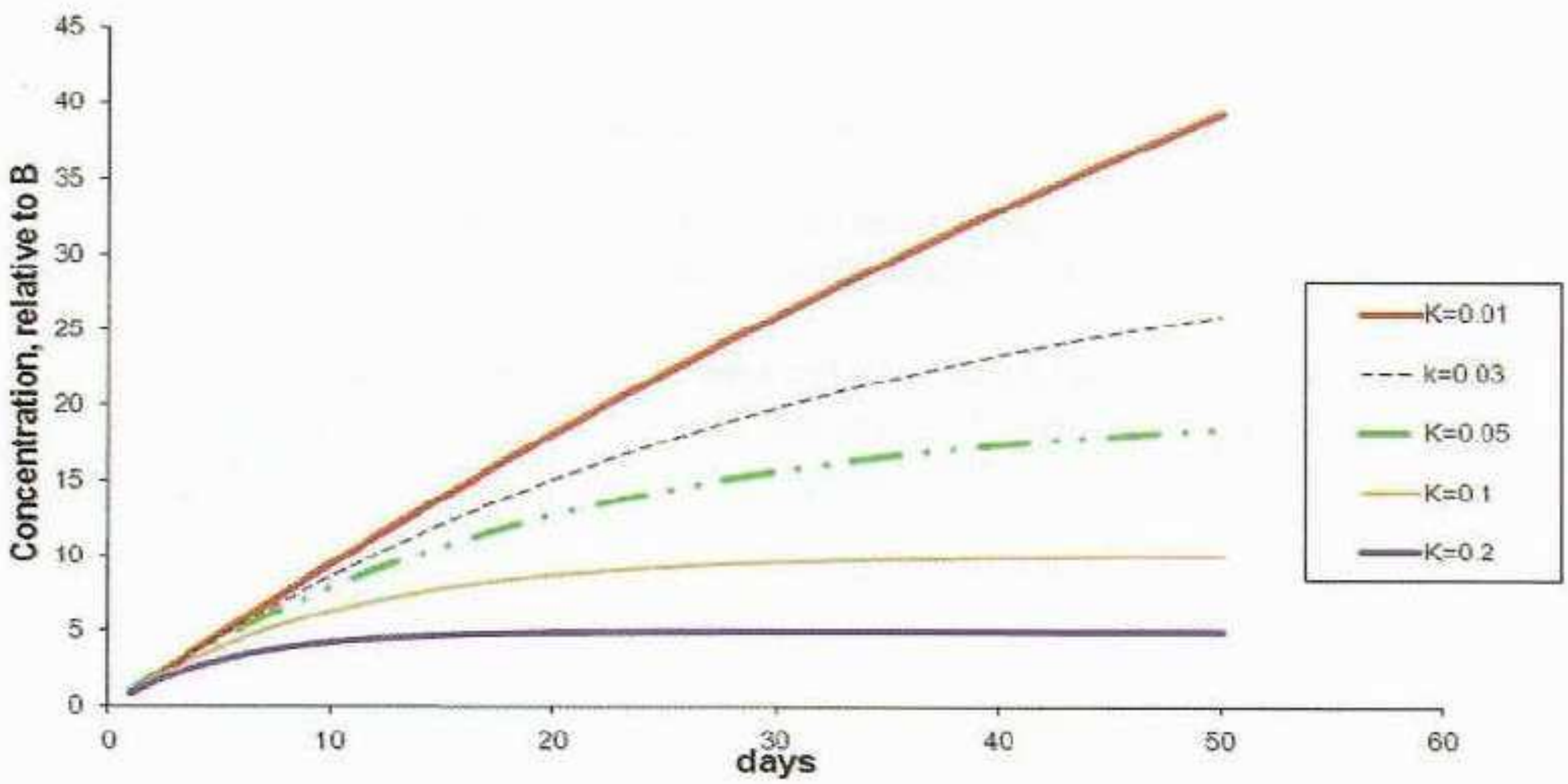
Equation (3.16) describes the evolution in time of the substrate concentration S, with S_0 as the initial concentration and a simplifying assumption of constant feeding. However, when time is long, the exponent e^{-Kt} approaches zero, and equation (3.16) becomes:

(3.17) $S = B/K$

i.e., the substrate concentration approaches a steady state, where daily addition is equal to daily degradation and the concentration remains constant over time.

Computed substrate concentrations as a function of time, for several rate constants are presented in Figure 3.3.

Figure 3.3: Ponds approaching biological steady state: Organic substrates concentrations as a function of time and rate constants



* According to eq. 3.16.

It can be seen, that the time required to achieve a steady state decreases with the increase of the degradation rate constant. For a rate constant of about 0.15 day-1, typical to fish ponds, a steady state is approached within a period of a few weeks.

The model described above is an approximated model, not taking in to account cloudy days, algae die-offs, fish diseases or changes in feeding rate over time. Moreover, the uptake by fish of the bioflocs, or other organic residues, an important process in the system is not considered. Nevertheless, the model leads to interesting conclusions: (1) the organic substrate level and the

Beatrize 2006). These findings suggest that the overall features of microbial communities in ponds are similar. Thus, we can expect, as a first approximation, to find similar organic matter concentrations and similar bacterial biomasses in aerated-mixed ponds with similar feed addition, worldwide. The addition of large amounts of labile organic matter to the pond raises the bacterial biomass and bacterial activity. The percentage of the assimilated carbon with respect to the metabolized organic carbon is defined as the microbial conversion efficiency (EM) and is in the range of 40-60%. Carbohydrate digestibility by fish is 40-60% so it can be assumed that at least 50% of the organic carbon input through the feed ends up in the pond as un-utilized feed or feces. In consequence, one kg of feed generates about 125 g bacterial biomass on a dry weight basis (1,000g feed x 50% carbon x 50% released x 50% microbial efficiency). This is equivalent to a floc volume of about 10 liters (see Chapter18).

The rate of feed conversion into microbial biomass depends on the conditions in the pond. Under aerobic conditions, the degradation rate of feed materials is about 10-80% per day (K=0.1-0.8 day^{-1}), thus the conversion takes place within hours or up to a few days. However, if the added organic substrate settles down to the anoxic pond bottom, the degradation rate is slower and the bacterial conversion efficiency will be much lower. Therefore, maintaining the organic matter under aerobic conditions is important in order to get better recycling of feed residues through the pond's food web. (This point will be discussed further on, e.g. Chapter 9)

Practical Implications and Tips

1. *In the long run, algae do not contribute oxygen to the pond, since they respire and add to the accumulated oxygen consumption of the pond.*

2. *Removal of accumulated sludge or drying of bottom sludge is equivalent to adding oxygen. These are the cheapest means to adding oxygen.*

3. *You do not have to worry as to the origin and development of the heterotrophic microbial community in the pond. Just feed the pond and they will be there.*

4. *Adding 1 kg feed supports the generation of 0.125 kg microbial cells or 10 ml/l floc volume, FV, (not considering losses in the microbial community). Assuming we want to get a FV of 5ml/l, a feed addition of 0.5 kg/m3 or about 5 ton/ha is necessary. This addition should be made by adding starter substrates and lateron by feeding the fish.*

5. *Any organic substrate will do, yet very slow-reacting substrates (e.g. cellulose) are less effective.*

6. *If oxygen is low, one may get a buildup of nitrite in the pond, due to the higher demand for oxygen of the second nitrification stage.*

Further Research Needs

1. *The role of algae was discussed in this chapter. However, different algae have different roles and functions in extensive, intensive and BFT ponds. Diatoms may drastically differ from blue green algae (cyano-bacteria) as well as others. We do not know enough of the role of different algae in aquaculture.*

2. *How can we control algae species distribution in ponds?*

3. *Specifically, there is a need to further study the role of algae in BFT systems. Some claim that we need to encourage algae (e.g. by providing enough light) while others try to eliminate algae (see Chapter 13).*

4. *The interaction of algae and bacteria in ponds should be further studied.*

5. *We need more quantitative data in order to develop reliable simulation models.*

Recommended Reading and Cited Literature

1. *Avnimelech, Y. 1989. Modeling the accumulation of organic matter in the sediments of a newly constructed reservoir. Water Research 23: 1327– 1329.*

2. *Avnimelech, Y., Mozes, N., Diab, S., Kochba, M. 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture 134 pp, 211-216.*

3. *Avnimelech Y., and Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220: 549–567.*

4. *Cole, J.J., Findlay, S., Pace, M. L. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Marine Ecology- progress series 43:1-10.*

5. *Ebeling, J.M., Timmons, M.B., Bisogni, J.J. 2006. Engineering analysis of the stoichiometry of photoautotrophic, autotrophic and heterotrophic removal of ammonia-nitrogen in aquaculture systems. Aquaculture 257:346-358.*

6. *Ford, T.E. (Editor) 1993. Aquatic microbiology, an ecological approach, Blackwell Scientific Publ. Boston USA 518 pp.*

7. *Hargreaves, J.A. 2006. Photosynthetic suspended growth systems in aquaculture. Aquacultural Engineering 34: 344–363.*

8. *Ram, N., Zur, O., Avnimelech, Y. 1982. Microbial changes occurring at the sediment–water interface in an intensively stocked and fed fish pond. Aquaculture 27: 63–72.*

9. *Reddy, K.R., Feijtel, T.C., Patrick, W.H. 1986. Effect of soil redox conditions on microbial oxidation of organic matter pp 117–156, Chen, Y., and Avnimelech, Y., (Eds.), The role of organic matter in modern agriculture, Martinus Nijhoff Pub., Dordrecht, The Netherlands.*

10. *Rittmann, B.E., McCarty, P.L. 2001. Environmental biotechnology- principles and applications, McGraw Hill International Edition, Singapore 754 pp.*

11. *Schroeder, G.L. 1975. Night time material balance for oxygen in ponds receiving organic waste. The Israeli Journal of Aquaculture-Bamidgeh 27:65–74.*

12. *Torres–Beristain, B., Verdegem, M., Kerepeczki, E., Verreth, J, 2006. Decomposition of high protein aquaculture feed under variable oxic conditions. Water Res. 40:1341–1350.*

Chapter 4

Biofloc Systems

In Brief

Biofloc technology evolved as a consequence of the development of permanently mixed and aerated ponds, systems resembling bio-technological plants and maximizing the potential of microbial processes. BFT systems introduction is in line with the restriction of water exchange due to costs and environmental regulation and as a means to provide biosecure systems to minimize disease, especially viral disease of shrimp.

The basis of BFT systems are the bioflocs, conglomerates of microbes, algae, protozoa and others, together with detritus, dead organic particles. The biofloc is a unique ecosystem of rich and potent particles suspended in the water. Bioflocs found in ponds are porous, light and have a diameter of 0.1 to a few mm.

The scientific and practical concepts of biofloc technology (BFT) evolved concurrently and independently at about the same time (Mid 90's), by Steve Hopkins and co-workers at the Waddell Mariculture Center, South Carolina and by Avnimelech and co-workers in Israel (Avnimelech, 1993; Avnimelech et al., 1994; Chamberlain & Hopkins, 1994; Hopkins et al., 1993).

In both cases zero or minimal water exchange was practiced. Organic residues accumulating in the pond under such conditions degrade, and ammonium is nitrified or assimilated, by an intensive microbial community. This series of processes replaces the conventional high water exchange systems or the use of external bio-filter. In essence, the microbial processes within these ponds serve as the pond water quality treatment system (built in bio-filter) and microbial protein serves as a feed additive.

The pioneers of this technology include Steve Surfling, who established Solar Aquafarms in California, a shrimp and fish farm based upon developing active microbial suspension ("Microbial soup"), French scientists in Tahiti (Cuzon and co-workers) , and Hepher, Schroeder, Moav & Wohlfarth in Israel, who developed the concept of the "Heterotrophic feed web" (Hepher, 1985).

Though based upon rather simple principles, the BFT concept ran against conventional common wisdom. Aquaculturists were trained and accustomed to believe that water in the pond had to be clear, the clearer the better. Farmers hesitated to jump into the turbid water. It took about 10 years to get this approach into full swing.

the major means to control water quality and production in semi intensive and intensive fish and shrimp farms. Water was exchanged, often at a very high rate, on a routine base. Whenever water quality tended to deteriorate, pond water was disposed of and replaced by "clean" water. This approach came to a deadlock for several reasons. First of all, fresh non-saline water became scarce or expensive to the extent of limiting aquaculture development. Secondly, the release of untreated pond water into the environment, be it a river or marine system, became prohibited by the environmental authorities in most countries. In addition, a very important factor encouraging aquaculturists to stop high water exchange was the severe outbreaks of viral diseases of shrimp. Water from one farm drained into an estuary or other outlet, where it was pumped by other farmers as "fresh" water. Once a given farm, or even a single pond, was inflicted by a disease, the different vectors distributed that disease effectively in all the neighboring farms and within days the disease was spread all over the region. A major element of bio-security needed to inhibit the breakout and spread of disease, was minimizing or totally avoiding water exchange.

When water exchange is limited (zero exchange or minimal exchange regime), organic matter in the water is built up. Organic matter is the substrate needed for the development of a heterotrophic microbial community, microbes that get their energy by metabolizing organic molecules. Intensification, aeration, mixing and limited water exchange, all necessarily led to the development of microbial dominance in the pond.

Intensive fish culture systems using heterotrophic microbial control were studied and implemented over the past two decades. Typical features of microbial dominant systems as compared to algae dominant systems are given in Table 4.1.

Table 4.1: Comparison of algae and bacteria controlled systems

Property	Algae control	Bacteria control
Energy source	Solar radiation	Mostly organic matter
Occurrence	Ponds with low organic matter concentration. Algae density increases with the availability of nutrients up to limitation of light.	Dominance in ponds with high supply and concentration of organic substrate, normally limited to intensive ponds with zero or low water exchange, though common in nutrient rich sites (sediments, surfaces)
Sensitivity toward environmental variables	Light is essential (activity lowered in cloudy days). Crashes are common.	Does not need light. Adapts to a variety of conditions. Crashes are exceptional.
Effect on oxygen	Oxygen is produced during the day, consumed at night	Oxygen is consumed
Relevant activities	Primary production: Produces organic matter and oxygen. Ammonium uptake.	Degradation of organic matter. Nitrification. Production of microbial protein.
Inorganic nitrogen control	Uptake driven by primary production. Maximal capacity ca 0.7 g NH_4/m^2 day.	Uptake of nitrogen affected by the C/N ratio of organic matter. Practically unlimited capacity.
Potential capacity	Normally, daily primary production does not exceed $4gC/m^2$.	Limited by substrate concentration and rate constant of degradation.

* Avnimelech et al., 1994; Chamberlain et al., 2001.

Algae and heterotrophic micro-organisms differ regarding their energy source. Algae use solar energy, while microbes depend on the availability of organic substrates. Thus, algae can grow and are dominant in ponds having a low concentration of organic matter. They do not need organic matter but need and respond to nutrients and solar radiation. Typically, extensive ponds will be dominated by algae. Microbes, on the other-hand, prosper when organic matter is high, as typical selection of those species that out-compete others in light harvesting, concentrating at the water surface (cyano-bacteria). Algae are less active on cloudy days, sometimes down to almost no activity. Algal populations often go through crashes or massive die-out due to high light intensity or other factors. These events introduce a dramatic instability to fish ponds, depleting oxygen, raising TAN concentration etc. Microbes do not need light so their activity is about the same, 24 hours per day. Populations are stable and if conditions change, microbes adapt to new sets of conditions rather quickly. Algae contribute oxygen to the pond, as a product of the photosynthetic process. At night, oxygen is consumed by the algae and microbes that thrive on the algae production. Algae systems (ponds or natural eutrophic water bodies) tend to have night time oxygen depletion events associated with a very high algae population. Bacteria consume oxygen and their activity may contribute much to the oxygen demand of a fish pond.

The size of the microbial population depends on the supply of organic matter. The stability of the aerobic community depends on an ample supply of oxygen, though their sensitivity toward low oxygen is far less than that of fish.

The driving force for the proliferation of microbes is the addition of organic matter, the major source of which is the feed. The number of bacteria in zero exchange intensive ponds was found to be in the order of 10^7- 10^8 cells/ml. In some studies a higher order of magnitude, up to 109 cells/ ml was found (Burford et al., 2003; Chamberlain et al., 2001; De Schryver et al., 2008).

The level of organic matter in ponds tends to reach a stable steady state, due to a balance between the addition of organic matter and its microbial degradation, as discussed in the previous chapter. Here we evaluate the steady state concentration in BFT ponds. The assumptions used to develop this theory are relevant in the case of BFT ponds; ponds are mixed and uniform, have negligible or controlled sedimentation and a rather simple material balance. An estimate of the concentration of organic matter in the pond can be obtained using the following approximate equations:

1. We consider that the pond gets a constant amount of feed, (or concentration, as amount per m^3) = B. This assumption is an approximated one. In reality, feed levels increase with the rise of biomass in the pond.

2. Fish retain in their cells about 50% of the feed, about 50% of the feed is excreted to the water eventually either directly or following metabolism.

(4.1) Excretion $0.5*B$

The overall process as described in eq. 3.14 in the previous chapter is:

$$C = [0.5B - e^{-KT} * (0.5B - KC_0)]/K \qquad (4.3)$$

Equation 4.3 was schematically plotted in Figure 3.3. It can be seen that the concentration of the organic matter rises linearly in the beginning and then it starts to slow down until it reaches a steady state, at which it remains stable with time. The flattening of the curve is obtained when the concentration of the organic matter increases to a level where the daily degradation rate is equal to the daily feed excreted to the water. Achievement of a steady state is a very important feature of many natural systems.

Equation 4.3 is simplified if long time periods are considered and a steady state is approached. At this point, the term e^{-KT} becomes very small (T becomes a large number), approaching zero. Thus, eq. 4.3 is reduced to:

$$C = 0.5\ B/\ K \qquad (4.4)$$

Though the above computations do not take all relevant processes into account, they give a very good concept of the range of processes taking place in the pond.

The degradation rate constant, K, was found, in a large number of ponds and laboratory trials (Avnimelech et al., 1992, 1995) to be around 0.15 day^{-1} (i.e. 15% of the organic matter in the water degrades every day). Using this constant and assuming a 20 ton/ha shrimp pond (2 kg shrimp/m^3, fed daily by 40 g feed (2% body weight)), the steady state concentration of organic matter in the water is expected to be about 130 g/m^3 (= 130 mg/l). With intensive fish ponds holding fish biomass of 20 and more kg/m^3, the expected steady state organic matter concentration is 10 times higher, more than 1,000 mg/l. This is a non-realistic figure, not taking in account any sedimentation of suspended organic matter and drainage of excessive sludge out of the pond.

The stability achieved with properly run biofloc systems is an important property of such systems. One of the conclusions of the bioflocs and shrimp diseases workshop, Vietnam, December 2013 (See chapter 11 and http://www.aesweb.org/bioflocs) was that a basic features leading to good health and growth of shrimp is the achievement of stable conditions in the pond. Algae dominated ponds having extreme fluctuations of oxygen along the diurnal cycle (see figure 2.1) yet, aerated BFT ponds have a relatively constant time dependent oxygen centration profile.

Figure 4.1: Dissolved oxygen comparison between autotrophic and heterotrophic system

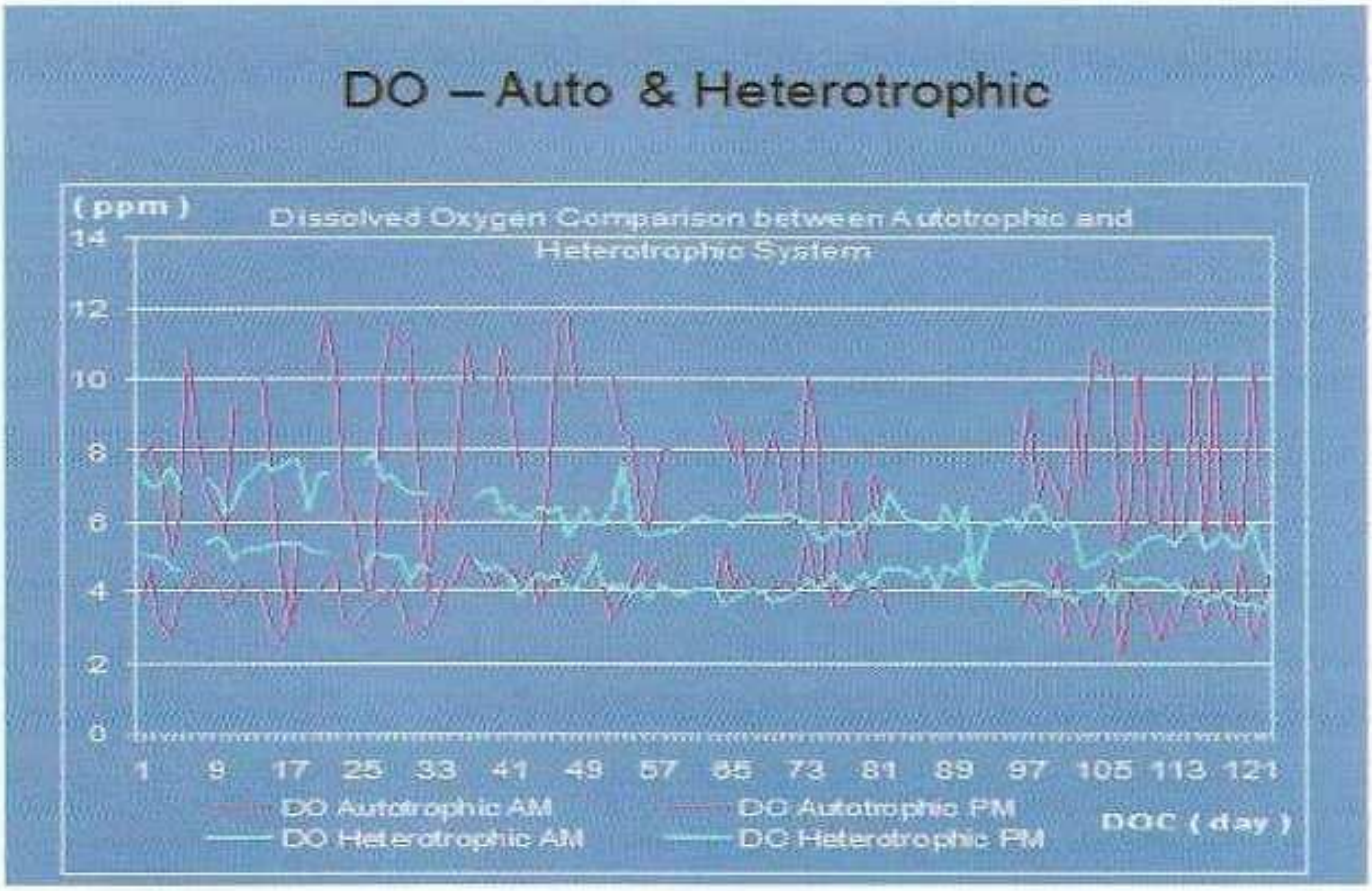
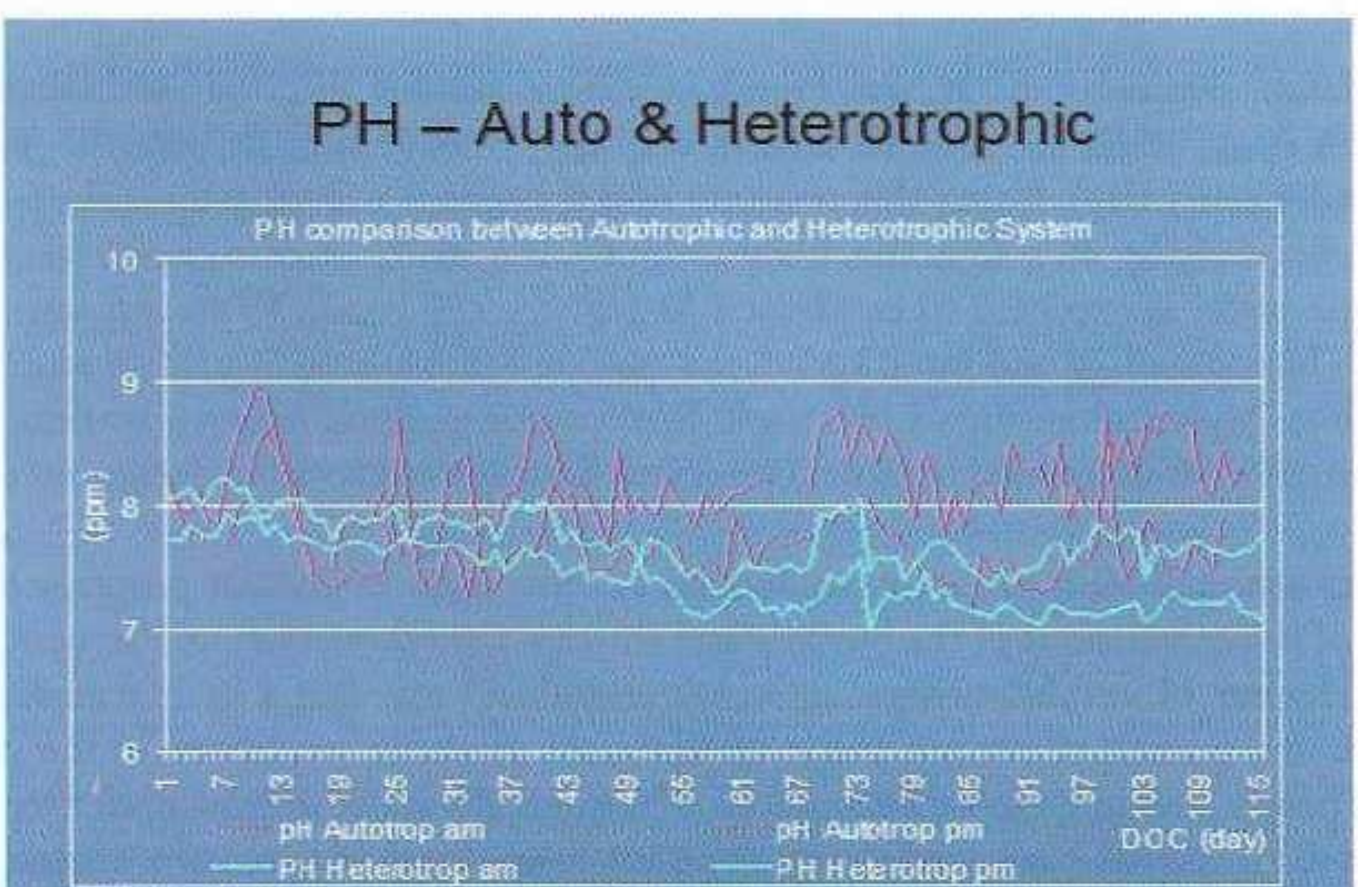
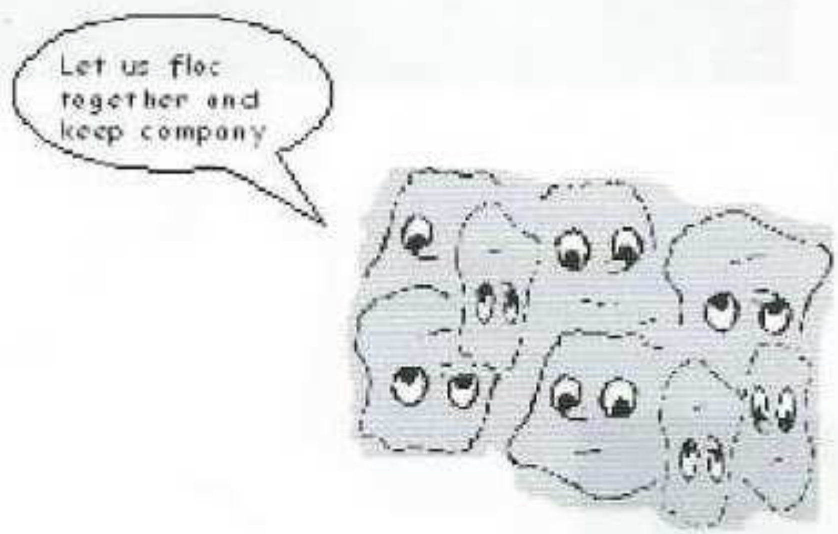


Figure 4.2: pH comparison between autotrophic and heterotrophic system



environment at an acceptable quality. However, if suspended organic matter (or the more often used factor TSS- Total Suspended Solids) is too high and the pond mixing system cannot keep it in suspension, organic matter accumulates at the pond bottom, anaerobic conditions develop and the pond environment deteriorates. In such cases the in-situ biological balance cannot control the system without intervention. The most common and practical intervention in this case is drainage of the excessive suspended water, either through water exchange (or recycling through a solid removal component) or by draining the sludge that accumulates at the pond bottom.

The determination of the point at which intervention is needed is quite complicated and will be discussed in Chapter 18



Bacteria are very small, having a typical diameter of about 1 μm. However, in most cases where we have dense microbial biomass, microbes tend to congregate and create flocs, conglomerates of microbes having a diameter in the range of 0.1 mm to several mm. There are several binding mechanisms that affect floc formation, shape and stability. Many organisms are coated by and excrete extra-cellular polymers, made of polysaccharides, proteins, humic compounds etc. These slimy polymers act as glue, embedding cells and other particles together. Another mechanism is related to the balance between short range attractive forces (molecular interactions, dipoles, hydrogen bonding) and electrostatic repulsive forces. Most organisms are negatively charged and induce a mutual electrostatic repulsion. If this repulsion is lowered, then the strong attractive forces can take place. Such is the case when salt concentration is high (high ionic strength) or polyvalent ions prevail. Thus addition of calcium or aluminum ions induces stable flocculation. In addition, filamentous organisms (algae, fungi or bacteria) help in bridging between the different floc forming components (De Schryver et al., 2008). The flocs are made of a mixture of live and dead cells, detritus (organic matter particles).

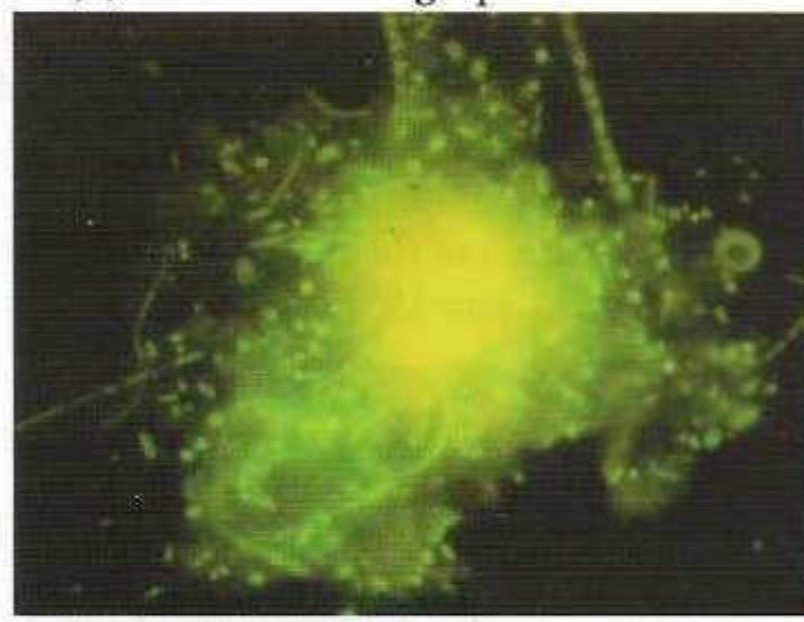
Examples of flocs are shown in photographs taken in the field (Figure 4.1 a-d).

Figure 4.3: Bioflocs

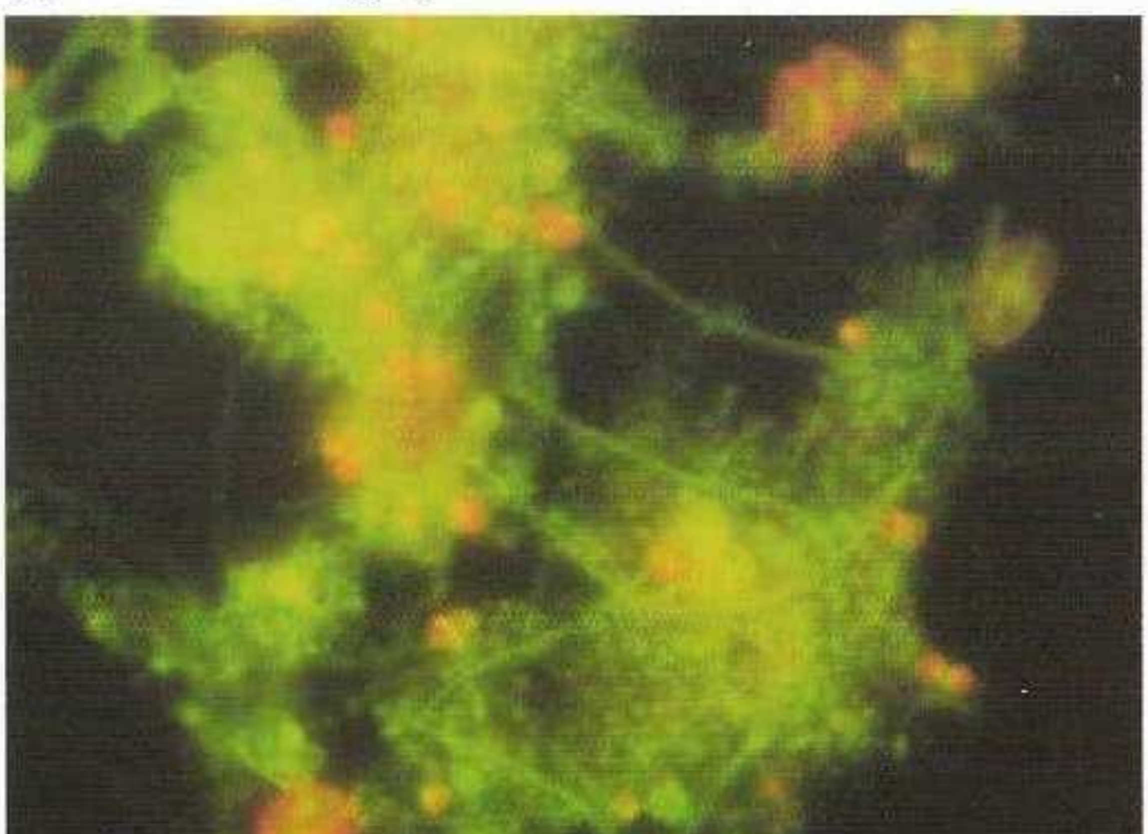
(a) Imhoff settling cone

Bioflocs, a few mm in size can be noticed

(b) Electron micrograph

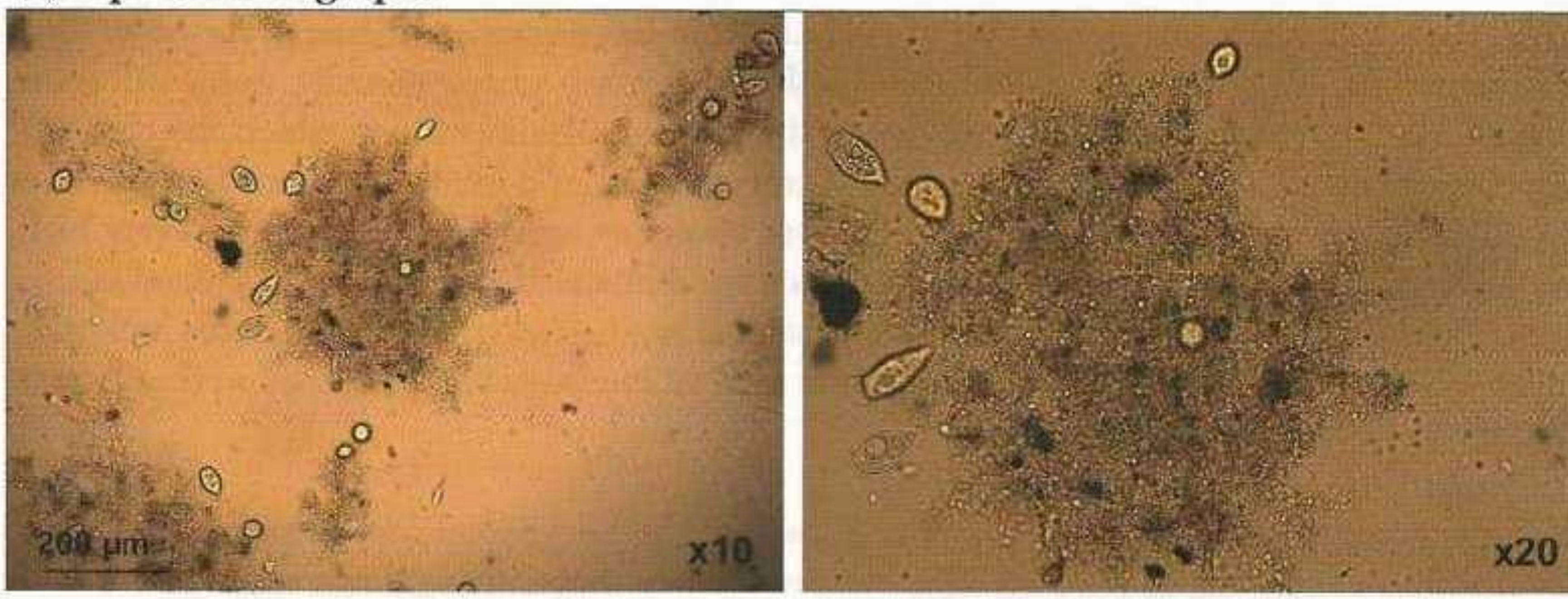


(c) Electron micrograph



Note the open structure of the biofloc

(d) Optical micrographs



Note the presence of grazers, protozoa and other.

In the first illustration (4.1.a), taken in a tilapia BFT pond in California with no magnification at all bioflocs are seen in the Imhoff settling cone. Flocs that are a few mm in diameters are seen by the naked eye. Other images (Figures 4.1 (b-d)) show higher magnifications of flocs, using optical and electron microscopy. It can be seen that the flocs are made up of a conglomeration of different organisms: Bacteria, filamentous algae, protozoa and zooplankton, a conglomeration of a large spectrum of organisms, and probably also a large spectrum of activities.

The floc system represents an interesting ecological system. The flocs are located in the water phase, a phase that is relatively poor with respect to nutrients and organic substrates. Within the poor water phase, we find micro-zones, flocs that are made of a mass of detritus, microbes, algae and higher organisms. These micro-zones may be equivalent to oases in the middle of the aquatic desert. These sites certainly attract bacteria of many kinds, algae and higher animals, all responsible for a dynamic ecological system. In a study of a biofloc system in tanks stocked with tilapia, where the bioflocs were tagged by $15NH_4$, it was estimated, based upon the measured nitrogen fluxes, that the average residence time of organisms (actually the protein fraction of organisms) in the biofloc was about 10 hours (Avnimelech & Kochba, 2009). This means that due, on one hand to destruction of organisms through harvesting by fish and probably other organisms and

The possible flow of water through the floc may have an important ecological implication, as demonstrated in studies performed by oceanographers. An individual microbial cell floating in the water gets supplies of nutrients (and oxygen) and removes metabolites by diffusion across an immobile water layer surrounding the cell. This is a relatively slow and inefficient process. A biofloc that has a higher mass than that of the individual cell, moves in relation to the water mass (sedimentation and other changes of location). If the floc has a high porosity, water will move through the pores and will effectively decrease the diffusion distance along which the chemical interchange with the organisms takes place. A microbe within a biofloc has an advantage over an individual one in terms of having more efficient transport, unless water agitation is very high, way above that used in aquaculture. Another advantage of the bioflocs, from the microbial species point of view, is that the majority of cells are protected against harvesting by protozoa and other predators since harvesting is limited to the outer shell of the flocs. Further detail on these topics are given in this book in Chapter 15.

The high porosity of the biofloc makes the density of the flocs rather low, just a bit above that of water. This effectively keeps the bioflocs suspended in water, slowing down sedimentation. In activated sludge waste water treatment plants, a fast sedimentation of flocs is essential, in order to obtain a fast separation between the settling sludge and clear water. In BFT systems, we need light bioflocs that stay in the aerated water column, facilitating aerobic processes and providing extended contact among bioflocs and fish, to facilitate harvesting.

As mentioned here, bioflocs are an important component in water treatment systems and their use there is extensively studied. Interest in a system similar to bioflocs is raised presently in ocean sciences. Microbial aggregates called "Marine snow" are found in the ocean (e.g. Azam and Long, 2001). These particles, formed through the agglomeration of organisms and detritus around organic particles such as feces, are found in the ocean water, though their occurrence is way below what we find in our ponds. These sedimenting particles are considered to be an important vector of organic carbon removal from the ocean water. An interesting study, most probably relevant to our bioflocs, deals with microbial physiology in relation to presence in the floc (Alldrege and Silver 2003). The working hypothesis is that when bacteria lives in the clear water phase, where feed and organic substrates are very low, then bacteria has to preserve its reserves and minimize its energy consumption (otherwise, with no feed it will be distinct). However, when the same bacteria is a part of the marine snow aggregate (biofloc in our case), it needs, in order to be competitive, to mobilize all available biochemical systems to grow fast, multiply and compete in digesting the available feed. It was found that the activity of microbial species when present in the marine snow particle is several folds higher than that found for the same species in the free water. This finding corroborates the finding, as reported in this chapter, on the fast microbial turn-over rates in bioflocs.

Practical Implications and Tips

1. *The biofloc system is a dynamic and versatile system. Biological turn-over rate of the biofloc is less than a day. This short biological residence time indicates that new flocs are created, while the old ones have been harvested and degraded. A period of a few days with lowered feed input to the pond may change the system.*

2. *Due to the transient nature of the flocs, it seems that changes of feeding with time may affect the concentration and properties of the flocs. It is advisable to keep a rather constant feeding regime with minimal perturbations.*

3. *A steady state is achieved within a few weeks if feed input is relatively steady.*

4. *The bioflocs contain microbes, algae, protozoa and zooplankton, all together supplying the fish with nutritious sources of feed.*

5. *Whenever TSS concentration is too high to allow for perfect mixing and maintenance of particles in suspension (ca 200-500 mg/l), an intervention is needed: Either water exchange or drainage of sludge.*

Further Research Needs

1. *The study of the biofloc ecosystem seems to be an exciting field of research. How do the different organisms in the biofloc interact? What are the effects of the biofloc density, porosity or size on processes within the floc?*

2. *How do environmental factors affect the biofloc? Some observations suggest that different feeding regimes (feed composition, rate and frequency) greatly affect the biofloc shape, density or stability.*

3. *How can pond management details affect biofloc properties?*

4. *Determine species composition of bioflocs.*

5. *What is the effect of TSS concentration on the performance of the pond? What are the low and high preferred limits of TSS concentrations?*

6. *And, a last and very important research topic: Development and maintenance of optimal biofloc consortia.*

Recommended Reading and Cited Literature

1. *Allgredge, A.L., Silver M.W. 2003, Characteristics, dynamics and significance of marine snow. Progress in Oceanography, 20:41–82*

2. *Avnimelech, Y., Mozes, N., Weber B. 1992. Effects of aeration and mixing on nitrogen and organic matter transformations in simulated fish ponds. Aquaculture Engineering 11:157–169.*

3. *Avnimelech, Y. 1993. Control of microbial activity in aquaculture systems: active suspension ponds. World Aquaculture 34:19–21.*

4. *Avnimelech, Y., Kochva, M., Diab, S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israel Journal of Aquaculture- Bamidgeh, 46:119–131.*

5. *Avnimelech, Y., Kochva, M., Diab, S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio, Israel Journal of Aquaculture- Bamidgeh, 46:119–131.*

6. *Avnimelech Y., Mozes N., Diab S., Kochba M. 1995. Rates of organic carbon and nitrogen degradation in intensive fish ponds. Aquaculture, 134: 211–216.*

7. *Avnimelech, Y., Kochba, M. 2009. Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using ^{15}N tracing. Aquaculture 287:163–168.*

8. *Azam, F., Long, R.W. 2001. Oceanography: Sea snow microcosms, Nature, 414: 495–498*

9. *Browdy, C.L., Bratvold D., Stokes A., McIntosh R.P. 2001. Perspectives on the application of a closed shrimp culture system. In: Browdy C.K. and Jory E (Eds.). The new wave. Proc. special session sustainable shrimp farming. WAS 2001:20–34.*

10. *Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219: 393–411.*

11. *Chamberlain, G.W., Hopkins, S.J. 1994. Reducing water use and feed cost in intensive ponds. World Aquaculture 25: 29–32.*

12. *Chamberlain, G., Avnimelech, Y., McIntosh R. P., Velasco, M. 2001. Advantages of aerated microbial reuse systems with balanced C/N. In: Nutrient Transformation and Water Quality Benefits. Global Aquaculture Alliance Advocate 4: 53–56.*

14. *Hepher, B. 1985. Aquaculture intensification under land and water limitations. GeoJournal 10 (3):253-259.*

15. *Hopkins, J. S., Hamilton, R.D., Sandifer, P.A., Browdy, C.L. 1993. Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budget of intensive shrimp ponds. Journal of the World Aquaculture Society 24:304–320.*

16. *Ray. A.J., Shuler, A.J., Leffler, J.W., Browdy C.L. 2009. Microbial ecology and management of biofloc systems. In: C.L. Browdy and D.E. Jory (Eds.). The rising tide, proceedings of the special session on sustainable shrimp farming. World Aquaculture 2009. World Aquaculture Society, Baton Rouge Louisiana, USA.*

17. *Rittmann, B.E., McCarty, P.L. 2001. Environmental biotechnology– principles and applications. McGraw-Hill International Edition. Singapore 754 pp.*

18. *Wasielesky, W. (Jr), Atwood, H., Stokes, A., Browdy, L.C. 2006. Effect of natural production in a zero water exchange suspended microbial floc based super-intensive culture system for white shrimp Litpenaeus vennamei. Aquaculture 258: 396–403.*

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By
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Chapter 5

The Nitrogen Syndrome: Problem and Solutions

In Brief

Nitrogen enrichment of the water is a common process in all aquaculture systems. The extent of this process rises with the increase of biomass and feeding. Both ammonia (NH_3) and nitrite (NO_2) are toxic and may dramatically affect fish growth, health and existence. Means to remove these species or transform them to non-toxic nitrogenous species are essential in high density systems. Major processes discussed here are algal uptake of inorganic nitrogen and nitrification. Nitrogen control in biofloc systems is described in the next chapter.

Aquaculture ponds are always enriched in respect to nitrogen. The higher the intensity the higher is this enrichment. Fish are fed by natural and formulated feeds containing protein, usually in the range of 30-40% protein. In terrestrial animals, the majority of ingested feed proteins are assimilated into the body proteins. However, fish use much of the protein as a source of energy, by oxidizing it and using the energy stored in the proteins (Hepher, 1988). A major end product of this metabolic route is the formation of ammonium and ammonia and its excretion through the gills to the surrounding water. An additional source of total ammonium nitrogen, TAN, in the pond, is the decomposition of organic matter, especially decomposition under low oxygen conditions, such as those existing in the pond bottom.

Ammonium excretion by fish makes up an appreciable fraction of nitrogen in the feed. Averaging data, obtained by many scientists under a variable set of aquaculture systems (Table 5.1), show that just about 25% of the nitrogen in the fish feed is recovered in the fish body on harvest, while about 75% of the nitrogen is released to the pond, mostly as TAN (Avnimelech & Ritvo, 2003). As an example, a fishpond holding 500 g fish/m^2, is fed (2% fish weight per day) with 10 g feed of 30% protein/day*m^2, i.e. 3 g protein or 0.465 gN per day. Excretion of 75% of this amounts to about 350 mgN/m^2. For a 1 m deep pond this amounts to a daily buildup of 0.350 mgN/l. If this nitrogen accumulates in the pond will be built up (as TAN), toxic levels will be built up within about 5 days. The nitrogen buildup is 10 times higher for a pond holding 5 kg fish/m^2. It is obvious that removing TAN and NO_2 is essential.

Table 5.1: Estimated carbon, nitrogen, and phosphorus recovery in fish or shrimp expressed as a percentage of total pond budget (nutrients added as feed and fertilizers) [1]

Organism	Carbon	Nitrogen	Phosphorous	Reference
Shrimp	30		10.5	Muthuwani and Lin, 1996
Shrimp[2]	11.5	45.3	21.3	Boyd and Teichert Condding-ton, 1995
Shrimp	12-14		11-14	Ritvo et al., 1998
Shrimp			17-21	Lemonnier and Brizard, 1998
Shrimp		25	9	Lin and Nash, 1996
Shrimp	6-11	18-27	10-15	Funge-Smith and Briggs, 1998
Shrimp		35.5	6.1	Paez-Osuna et al., 1997
Shrimp		22.7	10.6	Paez-Osuna, 1999
Shrimp		17-34.6 [3]		Martin et al., 1998
Carp	16	26.7	31.8	Avnimelech and Lacher, 1979
Catfish	9.1	24.8	29.7	Boyd, 1985
Average	13	29	16	
SD	8	8	9	

(1) Organic carbon input via primary production was not considered in most surveys.

(2) Calculation based upon the determination of harvested yield C, N and P and assumed FCR=2.

(3) 17% in low density ponds (1 PL/m^2) and 34.6 for high density (30 PL/m^2)

Where PTAN is the production rate of total ammonia nitrogen (kg/day), F is the feeding rate (kg/day) and PC is the protein concentration in the feed (decimal value). Using this equation to estimate TAN excretion for the example given above, we get a somewhat lower result a daily enrichment of 0.28 gN/m^3 as compared to 0.35 gN/m^3 (as calculated by Avnimelech & Ritvo compilations above). However, Timmons and co-workers (2002) suggest a higher constant - 0.144 for marine shrimp. Inserting this constant to Eq. 5.1, a higher value is obtained and the different estimates get closer to one another.

Rather good estimates can be made for nitrogen enrichment in ponds, whichever way we choose to compute it. It is obvious that unless TAN is removed or converted to other forms of nitrogen, a significant and dangerous rise of TAN will take place within a few days.

Ammonium is an end product of protein bio-degradation. Ammonium, or mostly its un-ionized species- ammonia, is toxic. Mammals detoxify ammonium by converting it to urea in the kidneys and excreting it by urination. Fishes excrete TAN by the diffusion of ammonia through the gills, fulfilling a similar role as the kidneys. When ammonia concentration in the water is high, outward diffusion is slow and ammonia is built up within the fish, affecting the central nervous system and causing other damages to the organism. Chronic exposure to ammonia leads to reduced growth and greater susceptibility to disease. The lethal level of un-ionized ammonia is in the range of 0.2-2 mg/l, a bit different for various fishes. It has to be noted that ammonia toxicity increases when oxygen concentration is low.

TAN is made of two species, the ammonium ion, NH_4^+, that dissolves in the water and is not volatile, and the un-ionized species NH_3 that can be volatilized out of the water. The two species are in a state of equilibrium, as determined by the pH:

(5.2) $$NH_4^+ \leftrightarrow NH_3 + H^+$$

The ratio of the two species is determined by the pH according to eq. 5.2:

(5.3) $$(NH_3) / (NH_4^+) = Kd / (H^+) \quad \text{or:}$$

(5.4) $$\text{Log}\,[(NH_3) / (NH_4^+)] = pKd - pH$$

Where the parenthesis denote the activities (corrected concentration term) of the given species and Kd is the dissociation constant, 10-9.3 (at 250C), or pK = 9.3 (Boyd & Tucker, 1998).

At pH = 9.3, 50% of TAN is in the form of ammonia (at pH = 9.3, (NH_3) = (NH_4+)). When pH is low, ((H^+) is high), most of the TAN is stored in the water as NH_4+, the ammonia effect is lower and it does not volatilize. When pH rises, the fraction of NH_3 rises. Ammonia toxicity the onset of the nitrification process or as a result of improper pond aeration conditions (Note: A rise of NO_2 concentration in the pond may be an indication of insufficient aeration or an indication for the accumulation of sludge and the creation of anaerobic pockets).

Nitrite entering the blood stream oxidizes the iron in the hemoglobin molecule from Fe^{++} to Fe^{+++}, changing the hemoglobin to methemoglobin and poisoning the respiration process. Nitrite crosses the gill membrane by the same mechanism normally used to transfer chloride. Thus, when chloride concentration is high (10-20 times higher than NO_2 concentration), chloride competes with nitrite uptake and nitrite toxicity is reduced. Thus, NO_2 toxicity to shrimp growing in marine water is lower than that to shrimp growing in brackish water .The different aspects of inorganic nitrogen control were reviewed by Crab and co-workers, (2007). The critical factor in relation to the potential damage of nitrogen buildup in the pond is the rate and capacity of mechanisms removing nitrogen out of the pond or transforming the toxic ammonia (and nitrite) into non-toxic components. Removal of the inorganic species is essential to maintain pond productivity, especially when intensity is increased.

Both carbonaceous and protein rich components of the feed are metabolized, releasing TAN and carbon as CO_2. The emitted CO_2 reaches equilibrium with the air and excess CO_2 is volatized and does not accumulate over time in the water. There are no similar rapid mechanisms to remove the equivalent TAN, from the pond system.

Means To Control Inorganic Nitrogen Accumulation

A. Water exchange

In the past, many aquaculturists exchanged nitrogen rich pond water with external clean water. This solution is practically phased out presently due to both water scarcity and environmental regulations.

B. Algae control

A conventional mechanism controlling inorganic nitrogen buildup in ponds, especially in extensive ones, is algal control of nitrogen.

As discussed above (Chapter 3), algae assimilate CO_2 and water to produce simple sugars, serving as a source of chemical energy and a prime building block for production of new cells. However, an essential component of algal cells is protein (on average, 12% of algae dry matter,). To produce protein from the assimilated sugars, the algae need an available nitrogen source. New algal cell production is tied to an uptake of soluble inorganic species (primarily TAN) from the daily capacity to immobilize nitrogen is 0.4-1 g N/m^2. This capacity is high enough to control nitrogen buildup in ponds holding between 0.5 to 1.2 kg $fish/m^2$. In more intensive ponds this mechanism is insufficient. Another limitation of the algal control is the instability of algae activity. Algal carbon assimilation depends on light and algae do not remove TAN during night. Thus, TAN in ponds rises during the night. Moreover, the algal nitrogen control may crash during a series of cloudy days, leading to fish stress or the need to stop feeding when solar radiation is limited. On the other hand, it is possible to raise algal capacity by improving growing conditions for the algae, through the mixing of the water, as practiced in partition aquaculture systems (Brune et al., 2003).

C. Nitrification

TAN undergoes a series of biological oxidation (nitrification) that takes place in two stages, oxidation to nitrite and subsequently to nitrate (see Chapter 3). Of the 3 inorganic nitrogen species, NH_4^+, NO_2^-, and NO_3^-, the first two inflict a degree (often severe degree) of toxicity to fish. Nitrate is not toxic, unless it is present in very high levels- more than 100's mg/l (Colt and Armstrong, 1979).

Nitrification is the major means of controlling nitrogen concentrations in recirculating aquaculture systems- RAS (Timmons et al., 2002). Water from the production component of the pond is recycled through a treatment unit that has a high surface area, the biofilter. This unit can be made of sand, plastic beads or sheets of different structures. Bacteria adhere to the surfaces and if properly used are not washed-out. The bio-filter has to be acclimated, i.e. to be in contact with solution containing TAN and nitrite, so that nitrifying population can develop. The acclimation takes a few weeks. The nitrification capacity of bio-filters is high, due to the high surface area and large number of bacteria adhering to the surface. In addition, conditions for nitrification are optimized: Oxygen is purged to maintain high enough levels, water flow or mixing minimize diffusion distances, organic matter in water entering the bio-filter is reduced by sedimentation or sieving and if needed water chemistry (pH, alkalinity) is controlled. In some cases the system has also an anaerobic cell to discard NO_3 by denitrification. These devices are known to support efficient nitrification, yet the construction and operation of those systems can be costly. Relying on nitrification as the only means to control TAN and nitrite levels is rather complicated (Rittmann and McCarty, 2001; Crab et al., 2008). Nitrification is a slow process and as discussed, it takes a few weeks in order to fully develop the nitrifying community. Moreover, nitrification responds very slowly to fast changes in TAN or nitrite concentration. Nitrification in stagnant ponds is usually not fully developed, possibly due to the fact that the bottom layers of the pond are not well aerated. In intensive aerated and mixed ponds nitrification develops and proper conditions exist, yet it is sensitive to improper aeration. Moreover, if water is exchanged between the mixed aerated intensive pond and another water body, such as a reservoir, the nitrifying bacteria are washed out and if water exchange rates are high (above about 30% per day), it does not have enough time to recuperate (Diab et al., 1992). It was found that a hydraulic residence time of about 24 hours is the minimal residence enabling a steady nitrification process to take place.

of carbon to nitrogen ratio, and the assimilation of inorganic nitrogen into the microbial protein pool, as discussed in the following chapter. In addition, as discussed later, bioflocs contain nitrifying bacteria and seem to provide proper conditions for nitrification, even in systems containing high organic matter concentrations. Thus, it should be noticed that there is no contradiction between using nitrification and nitrogen immobilization of nitrogen. By varying the level of carbohydrates addition, one can control the ratio between these two mechanisms.

Practical Implications and Tips

It is important to know the fraction of the toxic NH_3 species within total ammonium nitrogen (TAN). Detailed values of this are given in general chemistry and water quality text books (e.g. Boyd & Tucker, 1998). The following table is useful to get an approximate idea.

Table 5.2: Percentage of NH3 species within total TAN

pH \ Temp (°C)	24°C	32°C
7	0.5	0.9
8	4.9	8.7
9	34.4	49
10	84	90.5

As seen, the NH_3 fraction rises with temperature. However, this fraction is slightly lowered with an increase of salinity. For example, at 24°C and pH = 8.0, this fraction is 4.9, 4.5, 4.3 and 4.2% for fresh water, 20, 25 and 30 ppt, respectively.

Adding acid in case of an acute rise oNH_3 to slightly lower the pH is sometimes a good emergency measure to save the fish. Yet, be careful when adding concentrated acid not to over-shoot, acidify the pond water and eventually kill your fish

Frequent recycling of pond water (from the production unit to an earthen pond or to a biofilter) flushes out nitrifying bacteria and hinders nitrification in the production unit. You can't have it all: water can be treated either inside the production component water or outside.

Further Research Needs

1. *Unlike most biofilters, BFT systems support nitrification even when organic matter and BOD are high. Thus, both nitrification and inorganic nitrogen immobilization into microbial protein take place at the same time. The addition of carbonaceous substrates is needed in order to support immobilization. The optimal biological and economical balance between the two processes is still un-clear and should be further studied.*

2. *Ammonium species are readily immobilized. In cases when nitrite accumulate, its control is slow. What prevents efficient NO_2 removal?*

3. *Recently, the annamox process was revealed and studied (The process is an anaerobic oxidation of ammonia:*

   $$NH_4 + NO_2 \rightarrow N_2 + 2H_2O$$

   A recent study (Lahav and co-workers, 2009) revealed the significance of this process in a denitrification reactor for a recirculation aquaculture system, affected apparently by the presence of annamox bacteria in the digestive tract and feces of fish. How important is this process in BFT systems? Can it be controlled?

Recommended Reading and Cited Literature

4. *Avnimelech, Y., Lacher, M. 1979. A Tentative nutrient budget for intensive fish ponds. Bamidgeh – Israel Journal of Aquaculture 31:3-8.*

5. *Boyd, C. E. 1985. Chemical budget for channel catfish ponds. Trans. Amer. Fisheries Society 114: 291-298.*

6. *Boyd, C.E., Teichert-Coddington, D. 1995. Dry matter, ash, and elemental composition of pond-cultured Penaeus vannamei and Penaeus stylirostris. Journal of the World Aquaculture Society 26: 88-92.*

7. *Boyd, C.E., Tucker, C.S. 1998. Pond aquaculture water quality management. Kluwer Academic Publication 700 pp.*

8. *Brune, D.E., Schwartz, G., Eversole, A.G., Collier, J.A., Schwedler, T.E. 2003. Intensification of pond aquaculture and high rate photosynthetic systems. Aquacultural Engineering 28: 65-86.*

9. *Colt, J., Armstrong, G. 1979. Nitrogen toxicity to fish, crustaceans and mollusks. Department of Civil Engineering, University of California Davis.*

10. *Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., Verstraete, W. 2007. Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270: 1-14.*

11. *Diab, S., Kochva, M., Avnimelech, Y. 1999. Nitrification pattern in a fluctuating anaerobic-aerobic pond environment. Water research 27 (9): 1469-1475.*

12. *Diab, S., Kochba, M., Mires, D., Avnimelech, Y. 1992. Combined intensive – extensive (cie) pond system, a: inorganic nitrogen transformations. Aquaculture101: 33-39.*

13. *Funge-Smith, S.J., Briggs, M.R.P. 1998. Nutrient budgets in intensive shrimp ponds: implications for sustainability. Aquaculture 164:117-133.*

14. *Hepher, B. 1988. Nutrition of pond fish, Cambridge University Press, UK 388 pp.*

15. *Lahav, O., Bar Massada, I., Yackoubov, D., Zelikson, R., Mozes, N., Tal, Y., Tarre, S. 2009. Quantification of annamox activity in a denitrification reactor for a recirculation aquaculture system. Aquaculture 288: 76-82.*

16. *Lemonnier, H., Brizard, R. 1998. Effect of water exchange on pond ecosystem in shrimp semi-intensive rearing in New Caledonia during cold season, Ifremer, Noumea, New Caledonia*

18. *Martin, J.L.M., Veran, Y., Guelorget, O., Pham, D. 1998. Shrimp rearing: stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds. Aquaculture 164:135-149.*

19. *Muthuwani, V., Lin, C. K. 1995. Water quality and nutrient budget in intensive shrimp culture ponds. World Aquaculture 96, Book of Abstracts, World Aquaculture Society, Baton Rouge, LA. USA 270 pp.*

20. *Paez-Osuna, F., Guerrero-Galvan, S.R., Ruiz-Fernandez, A.C., Espioza-Angulo, R.E. 1997. Fluxes and mass balances of nutrients in semi-intensive shrimp farm in north-western Mexico. Marine Pollution Bulletin 34:290-297.*

21. *Paez-Osuna, F., Guerrero-Galvan, S.R., Ruiz-Fernandez, A.C. 1999. Discharge of nutrients from shrimp farming to coastal waters of the Gulf of California. Marine Pollution Bulletin. 38:585-592.*

22. *Rittmann, B.E., McCarty, P.L. 2001. Environmental biotechnology- principles and applications. McGraw-Hill International Edition, Singapore 754 pp.*

23. *Ritvo, G., Dixon, J.B., Lawrence, A.L., Neill, W.H., Speed, M.F. 1998. Accumulation of chemical elements in Texas shrimp pond soils. Journal of the World Aquaculture Society 29:422-431.*

24. *Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T., Vinvi, B.J. 2002. Recirculating Aquaculture. NRAC Publication 01-002, 2d Edition 768 pp.*

Chapter 6

Using BFT to Control Inorganic Nitrogen Buildup

In Brief

Bacteria fed with carbonaceous substrates take up nitrogen from the water, because it is required for producing protein. By doing so, they reduce the concentration of inorganic nitrogen (especially TAN) in the water. Adding carbonaceous substrates to ponds in prescribed amounts enables precise control of inorganic N in the water and eliminates the danger associated with the buildup of toxic ammonia and nitrite.

Emergency post factum and preventive strategies are presented, as well as the means to calculate the amounts of carbonaceous substrates required as a routine part of feeding to prevent the buildup of inorganic nitrogen in the water.

The major components making bacterial cells are proteins. The Carbon: Nitrogen ratio of most microbial cells is about 4-5. When bacteria are fed with organic substrates that contain mostly carbon and little or no nitrogen (sugar, starch, molasses, cassava meal etc.), they have to take up nitrogen from the water in order to produce the protein needed for cell growth and multiplication.

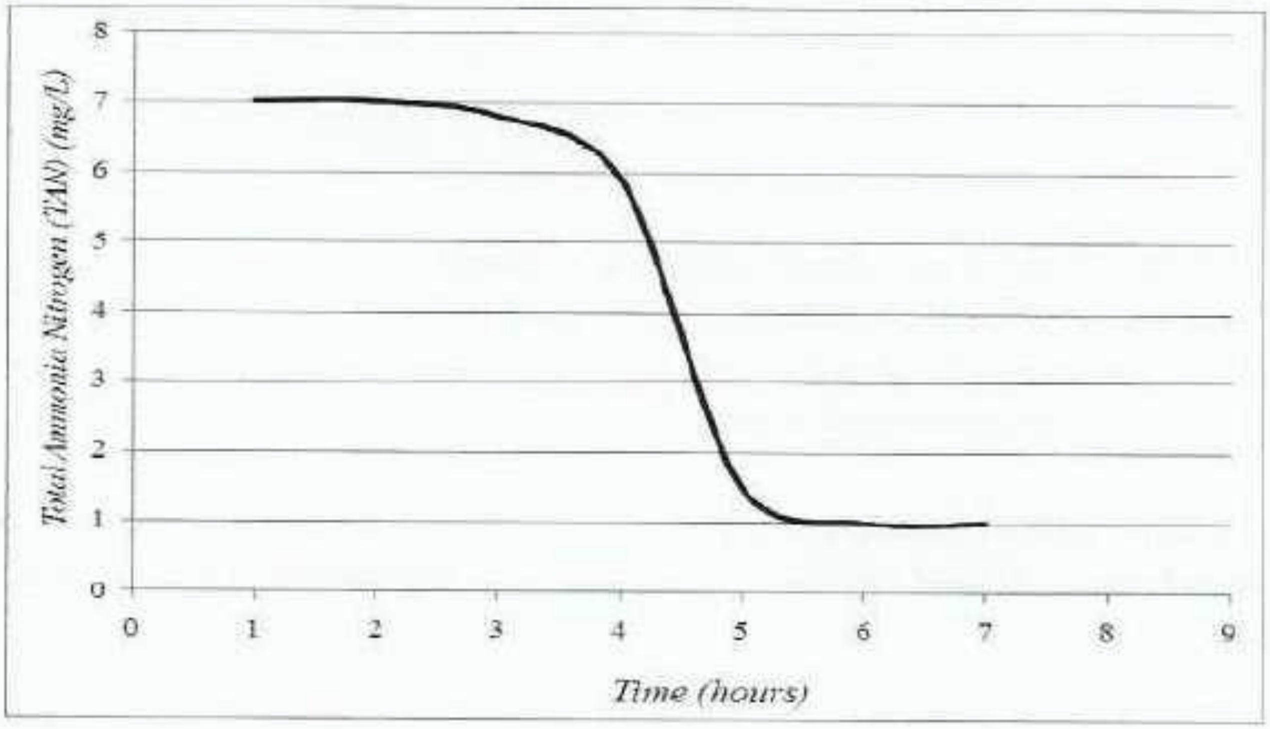
The control of inorganic nitrogen accumulation in the pond is based upon carbon metabolism and nitrogen immobilization into microbial cells (Avnimelech, 1999; Crab et al., 2008). Bacteria and other microorganisms use carbohydrates (sugars, starch and cellulose) as a food, to generate energy and to grow:

(6.1) Organic C $\leftarrow$ CO_2 + Energy + C assimilated in microbial cells.

The percentage of the assimilated carbon with respect to the metabolized feed carbon is defined as the microbial conversion efficiency (E) and is in the range of 40-60%. Nitrogen is required as an important building block of the microbial cell, since the major component of the new cell material is protein. Thus, microbial utilization of carbohydrate (or any other low nitrogen feed) is accompanied by the immobilization of inorganic nitrogen. This is a basic microbial process and practically every microbial assemblage performs it.

TAN. This process is relatively fast, if the availability of the carbonaceous substrate added is high. A demonstration of adding sugar to pond water containing about 7 mg/l NH_4-N is shown in Figure 6.1. As shown, and easily demonstrated in class or otherwise, within a few hours after sugar addition, ammonium concentration was lowered to about 1 of mg/l.

Figure 6.1: Changes in TAN concentration in a suspension of pond bottom soil (2% dry soil) following the addition of glucose (TAN/glucose ratio of 1/20)



The amount of carbohydrate supplement (ΔCH) required to reduce the ammonium can be evaluated (Avnimelech, 1999). According to eq. 6.1 and the definition of the microbial conversion coefficient, E (eq.6.2) the potential amount of microbial carbon assimilation when a given amount of carbohydrate is metabolized (ΔCH), is:

(6.2) $$\Delta Cmic = \Delta CH \times \%C \times E$$

Where DCmic is the amount of carbon assimilated by microorganisms and %C is the carbon contents of the added carbohydrate (roughly 50% for most substrates).

The amount of nitrogen needed for the production of new cell material (ΔN) depends on the C/N ratio in the microbial biomass (about 4).

(6.3) $$\Delta N = \Delta Cmic \;/\; [C/N]mic = \Delta CH \times \%C \times E \;/\; [C/N]mic$$

According to eq. 6.4 (assuming the approximation as above), the CH addition needed to reduce TAN concentration by 1 mg/l N (i.e. 1g N/m^3) is 20 mg (20 g/m^3).

This relationship may enable a pond operator finding a high TAN concentration in the pond (following cloudy days, algae crash, high fish biomass etc.), to calculate how much carbonaceous substrate he has to add to correct the dangerous situation. This mode of action may be considered as an emergency, post factum mode. The pond operator reacts following the excessive rise of TAN or NO_2. This mode of action is practiced by many farm operators all over the world. Pond operators in Thailand used to keep bags of cassava meal to be used when cloudy days lead to the dangerous rise of TAN in the pond. Others use molasses addition when TAN concentration rise. A farmer in Israel, having easy access to a bakery, used spoiled flour addition when ammonium levels in the pond rose.

WARNING: **Adding large rations of carbohydrates at once may lead to high oxygen consumption and oxygen deficiency. It is better to apply it using partial additions and to monitor oxygen along this process.**

A different, pro-active approach is to add the right amounts of carbohydrate with the feed in order to prevent un-wanted TAN increase and to optimize the process. One has to estimate the amount of carbohydrate that has to be added in order to immobilize the ammonium excreted by the fish or the shrimp in real time.

As mentioned, fish or shrimp in the pond assimilate only about 25% of the nitrogen added in the feed. The rest is excreted mostly as NH_4 (some as organic N in feces or feed residue). It can be assumed that the ammonium flux into the water, ΔNH_4, by fish excretion or indirectly by microbial degradation of the organic N residues, is at least 50% of the feed nitrogen flux:

(6.5) $$\Delta NH_4 = \text{Feed} \times \%\text{N feed} \times \%\ NH_4\ \text{excretion}$$

A partial water exchange, sedimentation or removal of sludge reduces the ammonium flux in a manner that can be calculated or estimated. In zero exchange ponds all the ammonium remains in the pond. The amount of carbohydrate addition needed to assimilate the ammonium flux into microbial proteins is calculated using eq. 6.6 and 6.7:

(6.6) $$\Delta CH = \Delta\text{Feed} \times \%\text{N feed} \times \%\ NH_4\ \text{excretion} / 0.05$$

Example: Assuming 30% protein feed pellets (4.65% N) and assuming that 50% of the feed nitrogen is excreted (% N excretion), we get:

(6.7) $$\Delta CH = \text{Feed} \times 0.0465 \times 0.5 / 0.05 = 0.465 \times \text{Feed}$$

An approximate calculation of the C/N ratio of different feed or feed mixtures is rather simple:

a. The amount of carbon in the feed is very close to 50% of the total feed weight (almost all feed materials have about 50% carbon).

b. The amount of protein is the protein percentage times the feed component(s) that contains protein and the amount of nitrogen is protein x 0.155 (on the average, 15.5% nitrogen in protein)

c. C/N is obtained by dividing C (from a) by N (from b).

Examples:

1. Pellets containing 40% protein:

C = 500 g/kg feed

N: Protein = 400g/kg feed,

N = 62 gN/kg feed (Protein x 0.155)

C/N = 500/62 = 8.06

2. Tilapia pond fed daily with 5 kg 30% protein pellets + 4.5 kg corn starch:

$C = (5 + 4.5)\times 50\% = 4.75$ kg

N: Protein = $5 \times 30\% = 1.5$ kg;

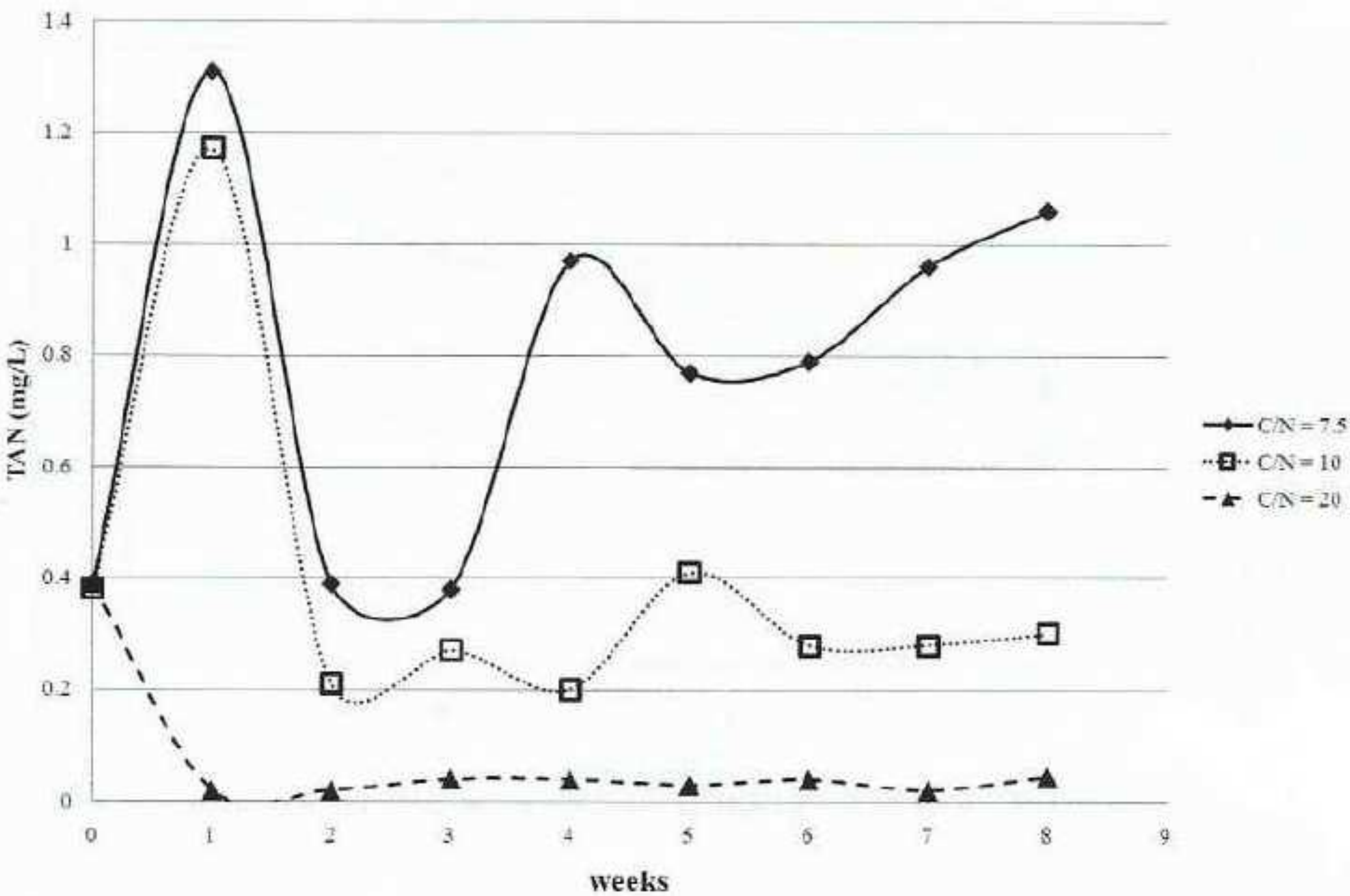
$N = 1.5 \times 0.155 = 0.233$

C/N = 4.75/0.233 = 20.4

The theoretical considerations for controlling inorganic nitrogen buildup by adjusting the C/N ratio of feed are verified by many laboratory and field results. Two examples are given here:

3. Tank trial with shrimp (Avnimelech and Panjaitan, 2006.)

Figure 6.2: TAN concentrations vs. time in zero water exchange tanks experiment, growing shrimp (Monodon, at a density equivalent to $30/m^2$)



It can be seen that TAN level increased to about 1 mg/l for C/N ratios of 6.5 and 15, was 0.4 when C/N was 17.5 and was practically zero when C/N ratios were 20 and 22.5.

4. Pond experiment with tilapia over-wintering using BFT (Crab et al., 2009)

Ponds ($50m^3$) were stocked with tilapia fingerlings at a fish biomass of close to 20 kg/m^3. Feeding with 30% and 23% protein pellets with, or without addition of corn starch to adjust C/N to about 20. All ponds received the same amount of protein. No water exchange during the reported period. Results are given in Figure 6.3; a-c (Crab et al., 2009).

Figure 6.3 (a): Changes over time of total nitrogen concentration in ponds fed with 23 or 30% protein pellets, with or without starch addition

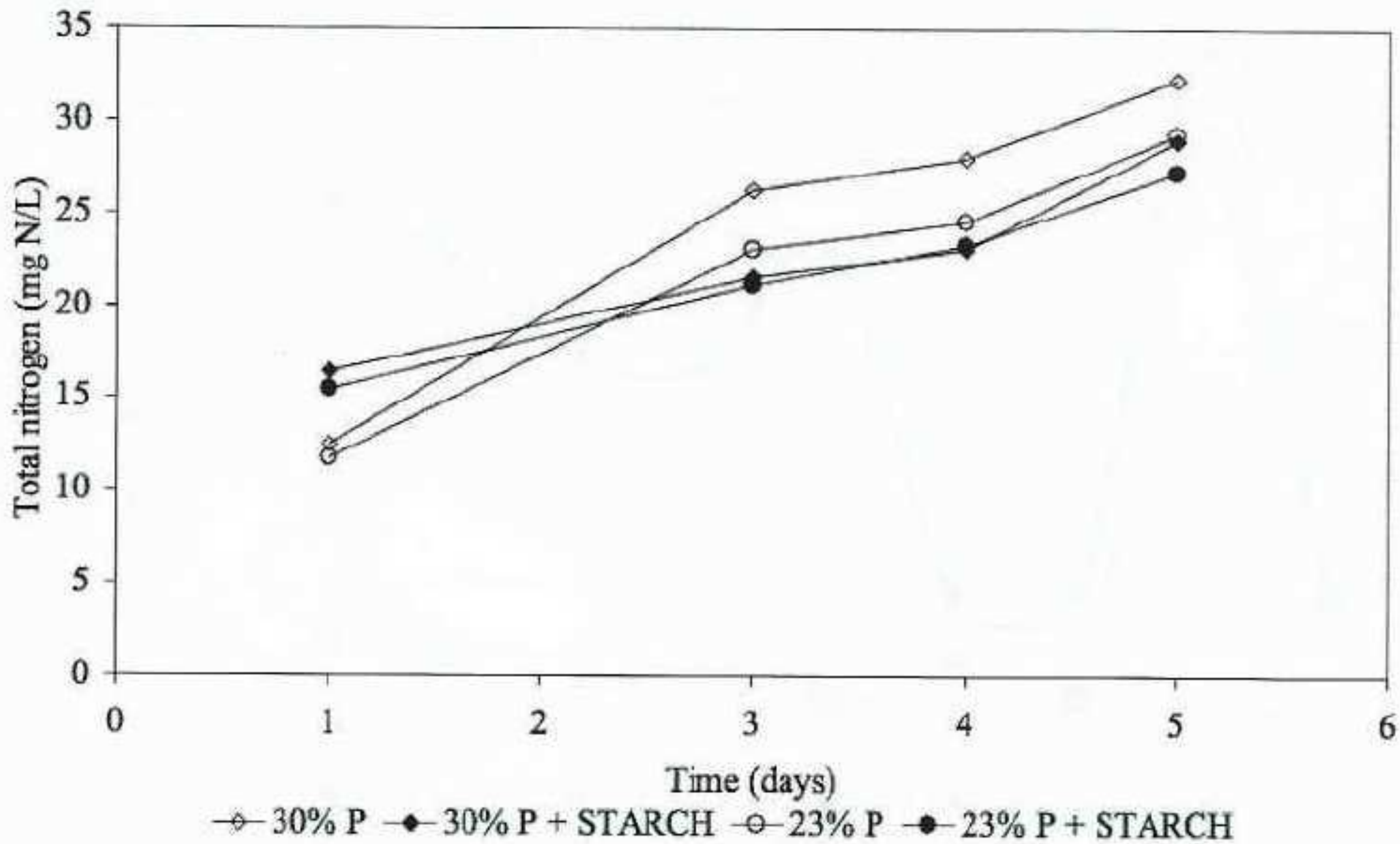


Figure 6.3 (b): Evolution over time of inorganic nitrogen concentration in pondswith 4 different treatments

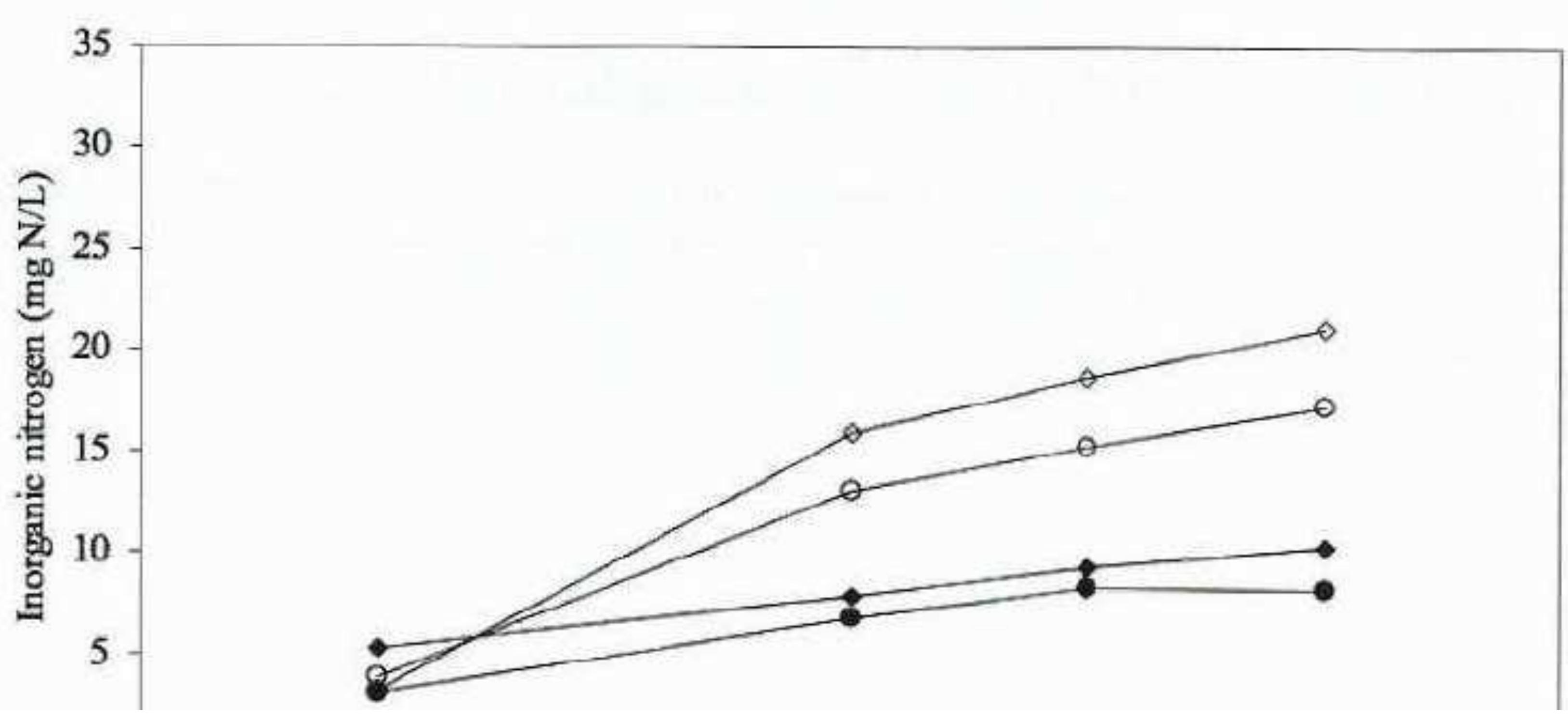
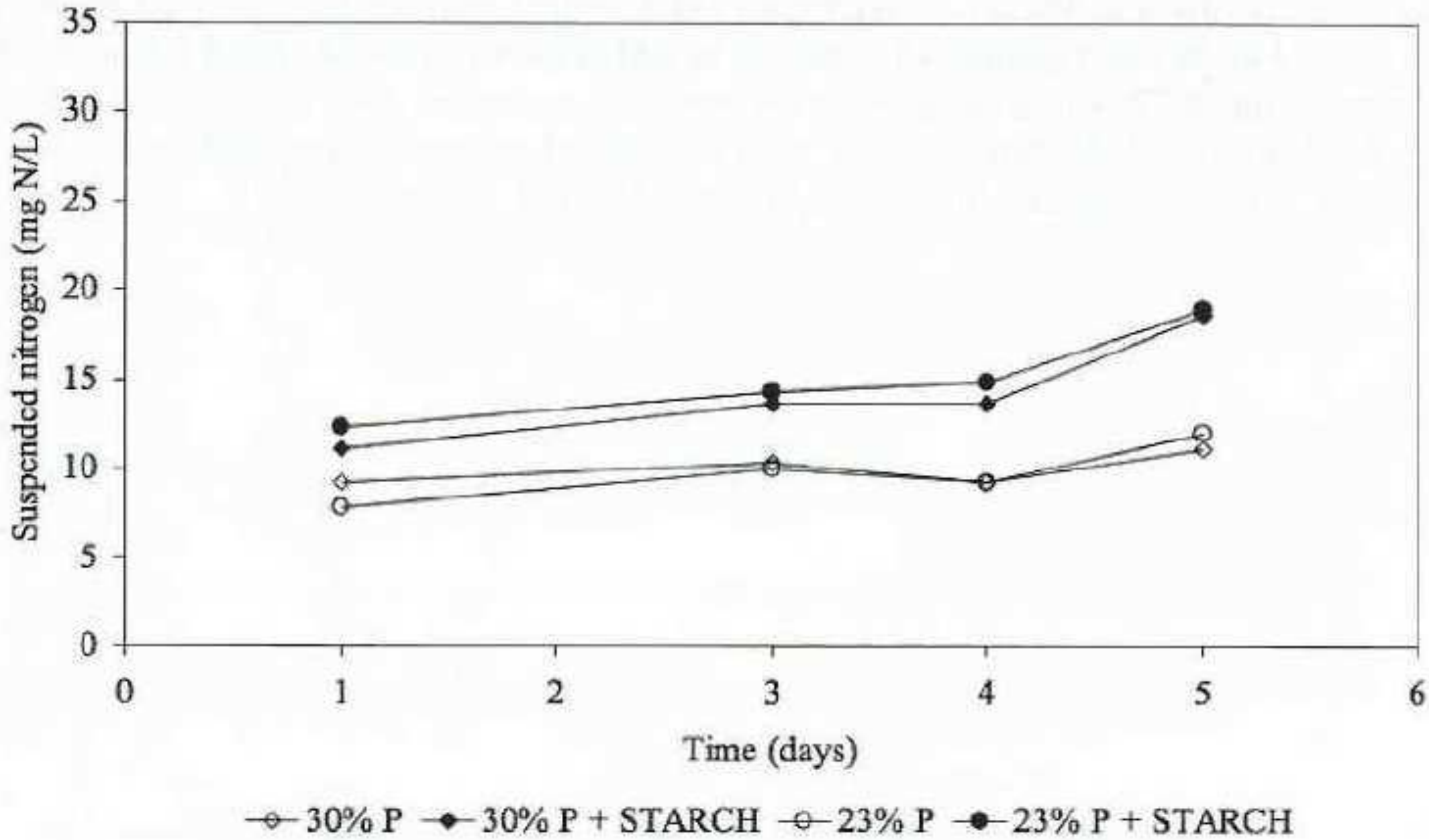


Figure 6.3 (c): Changes in suspended nitrogen concentration over time in ponds with different treatments



In Figure 6.3 (a), it is shown that total nitrogen (inorganic + suspended organic nitrogen) rose at the same rate in all treatments, equal to the amount of nitrogen applied with the feed. However, the change over time of inorganic nitrogen (NH_4 + NO_2 + NO_3), shows that there was a significant increase of inorganic nitrogen in the two treatments that got feed with C/N ratios of 10 and 14, while just a slight increase of inorganic nitrogen was found in the treatments fed with pellets + starch, having a C/N ratio of about 20 (Figure 6.3 (b)). Figure 6.3 (c) gives the changes of suspended organic nitrogen (nitrogen in bioflocs) over time. Here, the opposite trend is seen. While there was no change in organic N concentrations in the treatments without starch, practically all added nitrogen was converted to suspended organic nitrogen when starch was added and C/N ratio rose to about 20.

It can be concluded that controlling inorganic nitrogen buildup by adjusting the C/N ratio of feed material is feasible and reliable. Moreover, since we can describe the relevant processes by rather simple equations, it is possible to simulate the process and predict the outcomes. A modeling program was developed and verified. Details of this are given in the suggested reading list.

However, reality in ponds is not so simple.

carbohydrates we have to add to prevent TAN build-up if N microbial assimilation is the only mechanism. In reality we have to add less, in cases appreciably less.

In practice (as discussed in details in Chapter 18) the calculations as above are used as a starting point, but the exact amount of carbon to be added has to be re-calculated following the determination of TAN concentration in the water. In cases when there is a low level of TAN, you should lower carbohydrates addition and if TAN is high or increasing, application rate has to be raised. This is a rather simple and straight forward control process.

Practical Implications and Tips

4. *Removal and control of excessive inorganic nitrogen concentrations is provided by a combination of algae activity, nitrification and nitrogen assimilation. It is difficult to control the capacities of the first two mechanisms. The control of nitrogen assimilation by adding carbohydrate allows to take advantage of algae uptake and nitrification, yet to obtain a steady balanced system.*

5. *Ammonium (TAN) monitoring is essential to adjust carbohydrates addition to the pond.*

6. *3 Basic parameters one should know are the protein percentage and the C/N ratio of the feed. Protein percentage is given by the feed producer. The overall C/N ratio of the organic substrates in the pond has to be adjusted taking into account the C/N ratio of the feed. The C/N ratios of different feeds are given in Table 6.1 below.*

Table 6.1: C/N ratios of feed materials

Protein content %	C/N
15	21.5
20	16.1
25	12.9
30	10.8
35	9.2
40	8.1

Further Research Needs

1. *The assimilation of inorganic nitrogen into microbial proteins is a very common microbial process. However, efficiency of the process may vary with different microorganisms or microbial assembly. As of yet, we know very little about the population structure of bioflocs and the ability to select favorable microorganisms.*

2. *The effect of different carbonaceous substrate addition on nitrogen assimilation was discussed in this chapter. We know that particle size is important (fine particles are essential for effective processing) and cost is an important criterion. However, it is possible that different substrates will induce different nitrogen assimilation rates. Thus, Crab and co-workers (2009) found in a laboratory study that glycerol leads to a protein assimilation twice as high as compared to glucose. Such effects should be further studied.*

3. *As mentioned in the previous chapter, the combination of algae and bacteria and its effects should be clarified.*

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Diab S., Kochva, M., Mokady S. 1992. Control and utilization of inorganic nitrogen in intensive fish culture ponds. Aquaculture Research 23: 421-430.*

2. *Avnimelech, Y. 1999. Carbon/Nitrogen ratio as a control element in aquaculture systems, Aquaculture, 176: 227-235.*

3. *Avnimelech, Y., Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220: 549-567.*

4. *Avnimelech, Y., Panjaitan, P. 2006. Effects of carbon: nitrogen ratio control on water quality and shrimp growth in zero water exchange microcosms. Abstracts. World Aquaculture, Firenze Italy.*

5. *Crab, R., Kochva, M., Verstraete, W., Avnimelech, Y. 2009. Bioflocs technology application in over-wintering of Tilapia. Aquacultural Engineering 40(3): 105-112.*

6. *Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W. 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for macrobrachium Rosenbergii Postlarvae. Aquaculture Research41: 559-567*

7. *Kochba, M., Diab, S., Avnimelech, Y. 1994. Modeling of Nitrogen Transformation in Intensively Aerated Fish Ponds. Aquaculture 120: 95-104.*

Chapter 7
Feeding with Bioflocs

In Brief

Two related, but different issues are dealt with in relation to feeding with bioflocs. The first, the quantitative aspect, is related to the recycling of feed and the resultant increase in the added feed efficiency. Field experience, and limited controlled research hint the feed rationed could be reduced in about 20%.

The second issue is related to the quality of the bioflocs as a feed source having added values that are absent in industrially processed feed. . Evidences are rapidly accumulating showing that the BFT as feed improves growth of shrimp and fish, improves enzymatic and possibly hormonal activity and possibly reduces risk of disease. An even more exciting development is the potential ability to control the quality and quantity of these added values. It is anticipated that this direction of work will develop exponentially in the coming years. Biofloc meal produced in bioreactors added to compounded feeds is currently focus of intensive research related to the feed industry (see chapter 8). One successful example developed by CSIRO – Australia is Novacq®, a bacterial biomass additive added to aquaculture diets that enhanced about 30% of P. monodon growth in pond trials performed in Australia farms. Biofloc might possess a "growth factor". However, nutritional characteristics could be affected by temperature during drying process and the "native" properties could be altered. Certainly more research is needed on this field.

Microbes are manipulated in BFT systems in order to control and reduce toxic inorganic nitrogen concentrations. Extensive development of microbial biomass is an integral part of this process. Suspended bioflocs in intensive limited water exchange ponds amount to high feed equivalent. For floc volume of, say, 5 ml/l, the equivalent feed potential stored in the pond is about 700 kg/ha. Can fish or shrimp utilize this potential feed storage? This is not obvious and depends on the properties of the flocs, on the ability of any given fish species to harvest the bioflocs and on the ability to digest the suspended organic matter.

Research and commercial scale results show that for certains pecies, mostly shrimp (of different varieties) tilapia and carp, we have solid evidence that the suspended bioflocs are utilized and enhance formulated feeds. New information on additional fish that can utilize the bioflocs as a feed supplement is accumulating and should be validated. Such studies can be aided by different experimental methods enabling quantitative evaluation of harvesting and utilization of the bioflocs by fish.

Controlling inorganic nitrogen in biofloc technology ponds generates large amounts of microbial proteins. We can estimate this protein pool using different approximations. Assuming that fish excrete about 75% of the nitrogen applied with the feed, and taking a low estimate, assuming that only one half of this will be incorporated into available microbial protein (the rest settled as feed at a daily rate of 100 g/m^3 (feed protein application = 30 g/m^3) the amount of microbial protein generated daily is about 11 g/m^3.

A different way to estimate the potential of microbial protein production is by evaluating the amount of suspended protein per m^3 in a pond containing 200 mg/l suspended bioflocs (200 g/m^3). As discussed in Chapter 3, the bacterial biomass contains 61% crude protein. Thus, the water in that pond contains about 120 g crude protein per m^3, equivalent to 4 daily feeding rations for the above mentioned pond.

The above are just rough approximations and examples. However, every farm manager can easily determine suspended matter, or even better, suspended nitrogen, to find out how much protein is stored in his pond.

The potential use of the gross microbial protein accumulating in the water is not trivial. The bacteria have to be harvested by the fish, digested and then assimilated, in a way so as to support proper functioning and growth of the fish. All of these steps are essential for the beneficial use of the microbial protein.

The use of microbial protein has been practiced by fish farmers for centuries, as they fed pond fish with available organic wastes. The contribution of microbial metabolism to fish nutrition was defined by a group of investigators in Israel (Moav et al., 1977; Schroeder, 1978) as the heterotrophic food web. According to this concept, fish can feed, directly or indirectly, on primary producers, yet they can also feed on bacteria degrading residues such as manure, applied to the pond.

Recent practical experience is accumulating following the increase in use of BFT ponds around the world. Shrimp, or fish, growing in BFT ponds have a relatively low FCR, grow well and are found to have a full digestive tract all day long, due to constant feeding on the biofloc suspension. A more direct indication of efficient feeding is the high protein utilization in BFT ponds. Fish, or shrimp in conventional ponds, accumulate in their body only about 25% of the protein applied with the feed. The rest is excreted, mostly as TAN. Thus, we have to supply 4 kg protein with the feed in order to obtain 1 kg protein in the fish. The term Protein Conversion Ratio (PCR) as equivalent to FCR, the feed conversion ratio was proposed. The average PCR in fish or shrimp production is 4, i.e. we need to feed with 4 kg protein to obtain 1 kg protein in our marketed fish. It has to be noted that protein is the most expensive feed component, thus, raising protein uptake efficiency is of prime importance. In addition, since much of the protein used to produce aquaculture feeds is made of fish meal, adding protein to feeds has a grave environmental cost. Protein utilization is significantly higher in BFT systems: Adding carbonaceous substrates, causes microbes to harvest the excreted nitrogen and to produce microbial proteins that are then consumed by the fish. It may be said that the fish eat the protein twice: once with the feed and then again as microbial proteins. The PCR obtained in BFT commercial systems is about enough formulated feed.

However, there were and there still are questions related to the use of the microbial protein generated in biofloc systems. Is this protein pool available to any fish species? Can it be utilized? Can we save in applying expensive feed protein by replacing some of it with microbial proteins?

The microbial proteins have to be harvested by the fish in order to be utilized. The dimensions of an individual bacterium are in the order of 1 micron, too small to be filtered out of the water by the gill net of fish. Silver carp can filter out particles larger than 20-50 microns, *Mugil cephalus* take up particles larger than 10 microns, all larger than the size of an individual bacterium. It was shown, though, that even very small particles can adhere to the mucus layer covering the gills, yet, this may be an inefficient means to harvest the microbial proteins. Yet, as discussed in Chapter 4, a large fraction of the microbes in the pond are associated with biofloc, the size of which is in the range of 0.1 – 3 mm, large enough to be filtered out by many fish.

Studying these issues is not trivial. Moreover, the demonstrations of biofloc uptake as discussed above are obtained following a long exposure of fish BFT systems. The observations and measurements made over a long growing season cannot demonstrate short term processes, such as the differences in biofloc harvesting as affected by the age of the fish, the density of the biofloc suspension or the size of the flocs. For all of these questions we need more sensitive experimental techniques. We need to quantitatively evaluate the harvesting and utilization of microbial protein by different fish under different conditions. A very sensitive technique to get such information is to tag the biofloc with ^{15}N isotope determine the fate of these isotopes (Burford 2003; Avnimelech 2007).

Most of the nitrogen in nature is made of atoms having an atomic mass of 14. Yet, a small fraction (about 0.3%) is made of heavier atoms, with an atomic mass of 15. ^{15}Nitrogen is a stable, non-radioactive isotope that chemically behaves practically the same as the lighter ^{14}N atom. If we enrich the bioflocs with ^{15}N and then determine the enrichment of ^{15}N in the fish, we can quantitatively determine harvesting and utilization of the microbial protein by the fish. The percentage of ^{15}N as related to total nitrogen is determined by mass spectrographs and presently, by special instruments determining $\%^{15}N$ in a rapid, automatic and reliable way. Tagging the bioflocs with ^{15}N is also quite easy, by adding ^{15}N enriched salt (usually NH_4Cl or $(NH_4)_2SO_4$, enriched up to 99% with ^{15}N). We can enhance the uptake of the tagged NH_4 by the bacteria by adding starch. It has been found that within a few hours, practically all added ammonium is contained in the biofloc. Harvesting of the biofloc by fish can then be evaluated by the determination of the ^{15}N enrichment in the fish.

The determination of ^{15}N enrichment in the suspended microbial biomass and in the fish is rather straight forward, especially by using the modern automatic ^{15}N analyzers. Due to the high sensitivity of ^{15}N enrichment determinations, it is possible to run short term experiments and to evaluate microbial protein uptake along short periods, and under variable sets of conditions.

However, the interpretation of the results is rather difficult. The system to be monitored is very complex and a series of processes affect the distribution of the tracer in the system. The added ^{15}N ammonium is readily introduced into the microbial biomass, reaching an isotopic equilibrium within a relatively short time, due to the relatively short residence time of the microbial cells in the system and the fast regeneration of new cells. At the same time, the microbial biomass undergoes a constant process of degradation and ammonium is released to the solution over time. The changes with time of the ^{15}N pool are rather complex, as described by the following equations:

(7.1) $$\Delta^{15}N_{fish} = {}^{15}N\ \text{Harvest} - {}^{15}N\ \text{Excretion}$$

Where $\Delta^{15}N_{fish}$ is the change over time of ^{15}N in the fish tissues.

The tagged protein harvested by the fish is partially digested, and the rest is excreted as fecal material. In addition, metabolic processes in the fish lead to an excretion of ammonium, including some of the digested tagged nitrogen. The measured enrichment of ^{15}N in the fish tissues is the difference between the gross uptake and the excretion processes, and thus represents the "net uptake", an important entity, but only part of the picture. The ratio of gross uptake to net uptake may differ under different conditions such as fish age, fish variety, environmental conditions, properties of substrate etc. Various kinds of metabolic chambers are used in studies related to the nutrition of terrestrial animals. Using such chambers, it is possible to directly determine uptake of feed, determine different routes of excretion and the accumulation of feed elements in the animal. This is not possible in nutritional studies with aquatic animals. Thus, the required computation and interpretation of results are more complicated.

The amount of ^{15}N in the bioflocs is also a dynamic entity:

(7.2) $$\Delta^{15}N_{BF} = (-)\ \text{Fish Harvest} - \text{Microbial degradation} + \text{BFproduction}$$

Where $\Delta^{15}N_{BF}$ is the change over time of ^{15}N associated with the bioflocs.

The ^{15}N in the bioflocs is consumed through fish harvesting. In addition, it is constantly degraded through the different microbial degradation processes. The $^{15}NH_4$-N released by both the degradation processes and excretion by fish may be subse-

An additional means to evaluate fish utilization of natural feed webs is by evaluation of the ^{13}C enrichment of fish tissues. ^{13}Carbon is a stable isotope found naturally. The ^{13}C enrichment of different carbon sources may vary, e.g. it is possible to find differences of ^{13}C enrichment between formulated feeds and algae. Avnimelech and co-workers (1989) fed tilapia stocked in experimental tanks with cellulose powder and fertilizer nitrogen. Tilapia cannot digest cellulose, yet bacteria in the water can metabolize the cellulose and generate microbial protein from the cellulose derived carbon and the ammonia of the added fertilizer. It was found that the ^{13}C enrichment in the fish tissues approached that of the cellulose, rather than that of the formulated feed, proving that the fish utilized the cellulose by harvesting and digesting the bacterial flocs.

Biofloc contribution to protein nutrition of shrimp was studied by a number of scientists over the last two decades. Michele Burford and co-workers (2003, 2004) performed a number of studies in both research tank systems and in the Belize Aquaculture (BAL) farm ponds. BAL was the first commercial scale farm using biofloc technology for shrimp production. It was found that about 20-30% of the protein assimilation by shrimp originated from biofloc harvesting. Similar results on shrimp uptake of biofloc were obtained by other scientists (e.g. Abreu et al., 2007; Epp et al., 2002; Nunes et al., 1997; Velasco et al, 1998). Avnimelech studied protein assimilation by tilapia (Avnimelech, 2007; Avnimelech and Kochba, 2008). It was found that net protein uptake from bioflocs was equivalent to 25-50% of conventional protein feeding. Excretion of ^{15}N was found to be about twice as high as the net ^{15}N uptake. It was found that the turnover rate of bioflocs (i.e. the length of time needed for an equivalent replacement of bioflocs with freshly synthesized material) was about 8 hours. This very fast turnover is an indication of the very dynamic nature of the bioflocs and to the fact that most cells are young and active.

The data on the contribution of bioflocs to fish nutrition give the approximate values of bioflocs feeding. It seems probable that different results will be obtained as a function of fish variety and age, density of biofloc suspension and size of flocs (it seems that feeding efficiency is higher with larger flocs (Ekasari 2014). Moreover, the ratio of net to gross uptake may also vary as a function of fish physiology. It can be concluded, though, that bioflocs contribute a significant fraction for most fish protein nutrition. Presently, we have good data on the utilization of microbial proteins by tilapia, shrimp (Mostly *for Litopenaeus vannamei* and *Penaeus monodon*) and possibly for carp. There are preliminary results indicating that Mugul can readily take up bioflocs. Yet it was found, in a preliminary study (Avnimelech, un-published), that African Catfish hardly take up bioflocs, probably due to the fact that gills of this fish are not developed. Similarily, In-Kwon Jang and Su-Kyoung Kim from Korea, found a difference in uptake of bioflocs by 3 species of shrimp. *L. Vannamei* had the highest potential, higher than those of *F. Chinesis* and *M. Japonicus*. These differences could be correlated with the development of the 3d maxilliped and the development of the network of seta on the 3d mxp, forming a mesh structure which potentially trap suspended matter (>10 μ) More data, relating uptake to specific fish and bioflocs parameters are needed and will probably be added in the future, possibly using the above mentioned techniques.

Although bacterial floc can be an important nutritional supplement, it probably should not be used as a sole source of nutrition. Microbial cells contain higher levels of nucleic acid nitrogen ment to feed proteins. BFT systems are based on using microbial proteins as a partial source of protein and not as the only one. However, we know that bioflocs may contain algae and fungi as well as significant amounts of grazers, such as protozoa and zooplankton, possibly decreasing the percentage of nucleic acids in the overall crude protein pool.

There is a need to develop a set of quantitative instructions and economical evaluation related to feeding with bioflocs. As mentioned, shrimp seem to take about 20% of their protein diet by harvesting bioflocs. With tilapia, BFT protein uptake is higher, up to 50%. We can assume that other feed component, such as energy yielding components, are taken up as bioflocs in a similar proportion. These findings can lead to the reduction of applied feed rations (and saving money). In some tilapia farms, added feed in BFT ponds is reduced by 20%. Pohan Panjaitan (2006) found that he could reduce feed rations to shrimp in BFT microcosms, by 30%, without any reduction of production. We need more data on this aspect, data that could reliably help farmers in making important decisions, potentially enabling a significant cut in production costs.

The discussion above assesses the harvesting of microbial protein sources by different cultivated fish and shrimp. Yet, a number of studies specify the added, or extra, nutritive value of bioflocs. There are indications concerning special effects of the bioflocs feeding on fish or shrimp, above the expected conventional effects of protein supply. Leber and Pruder (1988) of the Oceanic Institute demonstrated that shrimp reared in tanks receiving flow-through pond water and fed a medium to high quality diet grew 50% faster than shrimp fed identical diets but maintained in clear well water from a seawater aquifer. Moss reported that suspended solids, taken from an intensive shrimp pond, stimulated the growth of fed shrimp reared in clear well water by 89%. Suspended solids in the water were characterized as microalgae and microbial-detrital aggregates. The growth-enhancing factor in the suspended solids was not identified, but bacteria were mentioned as a strong candidate. Kuhn and co-workers (2008), found that inclusion of processed bioflocs from tilapia ponds into shrimp feed, at levels of about 8-16%, raised shrimp yields by 160% over those obtained by commercial diets (see Chapter 8 for details). It has to be noticed that all known feed components were kept equal in the different commercial and bioflocs containing diets. Similar results were obtained in a study performed in the Oceanic Institute (Ju et al., 2008) where bioflocs were collected from marine shrimp culture tanks and subsequently used, either intact (as whole floc), or following several extractions , as diets or diet components for shrimp. Feed including whole floc improved the growth of shrimp over that of shrimp fed with a control conventional feed, assumed to contain all needed components. Some of the added values in the whole flocs were lost during extraction, yet, even the extracted floc fractions raised shrimp performance. There are no final conclusions as to the specific compounds attributing to improved performance. Yet, it was clearly demonstrated that bioflocs, either present in the pond water or supplied with feed, improve shrimp performance. Biofloc meal produced in bioreactors added to compounded feeds is currently focus of intensive research in nutrition fields (See chapter 8). An interesting case study is a feed additive developed by CSIRO-Australia Novacq®, a bactefish oil.

McIntosh and co-workers (2001) analyzed suspended flocs from commercial BFT shrimp ponds in Belize. They found higher protein levels in suspended floc than in the feeds they were derived from (Table 7.1).

Table 7.1: Composition of suspended detritus filtered from the water column of intensive zero water exchange shrimp ponds fed with 31.5% or 22.5% protein feed

Composition of suspended detritus	Crude protein level of feed (%)		
	Low- 31.5	High- 22.5	Mean
Organic matter (%)	78	66	72
Ash (%)	21	32	26
Protein (%)	51	35	43
Fat (%)	10	15	12.5
Arginine (%)	2.3	1.61	1.95
Methionine (%)	0.61	0.35	0.48
Lysine (%)	2.5	1.7	2.1

* Adapted from Chamberlinand co-workers, 2001.

Tacon (2000) filtered suspended detrital floc from outdoor BFT tanks at the Oceanic Institute in Hawaii at the end of a 56-day shrimp feeding trial. Chemical analyses of this material revealed a protein content similar to the 35% protein in pelleted shrimp diet (Table 7.2).

Table 7.2: Proximate, mineral, and amino acid composition of the microbial floc collected from outdoor shrimp rearing tanks managed as intensive microbial reuse systems[1]

Nutrient	Lowest value	Highest value	Mean
Suspended microbial floc, mg/l	31.7	340.1	156.5
Crude protein (N x 6.25), %	24.64	40.6	33.45
Crude lipid, %	0.46	0.83	0.61
Ash, %	22.91	38.54	30.21
Gross energy, cal/g	2656	3207	3014
Carotenoid, mg/kg	60	163	122.7
Phosphorus (P), %	0.38	2.29	1.44
Potassium (K), %	0.14	0.95	0.68
Calcium (Ca), %	0.45	3.06	1.81
Magnesium (Mg), %	0.13	0.48	0.28
Sodium (Na), %	0.43	4.59	2.94
Manganese (Mn), mg/kg	9.58	49.64	30.47
Iron (Fe), mg/kg	182.42	394.04	342.82
Copper (Cu), mg/kg	4.12	95.53	24.5
Zinc (Zn), mg/kg	83.58	618.34	365.81
Boron (B), mg/kg	9.46	48.53	29.19
Amino acid (g/100g protein)			
Isoleucine	1.99	5.69	3.75

Nutrient	Lowest value	Highest value	Mean
Phenylalanine	1.24	9.05	6.09
Histidine	1.2	1.65	1.4
Threonine	3.98	6.21	4.94
Lysine	2.98	5.32	3.93
Valine	2.76	10.14	6.07
Arginine	5.62	7.5	6.45
Tryptophan	N.A.	N.A.	N.A.

Values are ranges and means (dry matter basis) of 21 samples, except for amino acids, which are based on 12 samples (Adapted courtesy of Tacon, 2000).

Lipid contents in floc published by McIntosh (in Chamberlein et al., 2001) revealed different results. McIntosh found high lipid levels (mean = 12.5%), while Tacon found low levels (mean = 0.61%). Data presented by Tacon in 2002 differ to some extent from his data of 2000. According to Litchfield (2000), the composition of microbial cells in suspended flocs varies widely depending on the specific organism and the conditions under which they are grown. Substrate C/N ratios of 10:1 or less favor bacteria with high protein contents, while higher C/N ratios favor accumulation of lipid in algae, yeasts, and molds and accumulation of poly-â-hydroxybutyrate (PHB) in bacteria. The composition of the organisms used as feed is commonly reflected in the composition of the consumer fish. High concentrations of certain fatty acids (HUFA, others) are important as to the nutritional value of the fish and PHB is reported to be an important disease protection agent (Chapters 11, 15). These findings emphasize the need for further study of floc composition under different conditions and raise the possibility of manipulating microbial bio-floc composition in order to achieve a desired nutritional outcome (see chapters 8, 15). Bioflocs may contain different levels of fatty acids of different composition and chain length depending on the type of carbohydrates in feed. Crab and co-workers (Crab et al., 2010) reported that feeding the system with different carbon sources (glucose, starch, glycerol, and acetate) leads to significant differences in the concentrations of the different fatty acid groups (See Chapter 15).

McIntosh reported that amino acid levels in bioflocs were adequate in respect to lysine and arexpected to contain higher proportions of grazers. Certainly we need more data to control the nutritive value of the bioflocs.

Both McIntosh and Tacon measured high levels of ash (mean of 26.0 and 30.2, respectively) in suspended flocs. (it has to be noted that both worked with bioflocs developing in sea water, where salt accumulation is expected). Analysis of minerals indicates that microbial flocs are rich in phosphorus as well as a wide range of other minerals (Table 7.2). Much of these minerals may be bound to bacteria in a bio-available organic form.

Tacon found in a shrimp BFT microcosm system that supplemental vitamins and trace metals could be completely omitted since they are supplied by the bioflocs.

Several findings lead to the conclusion that bioflocs contain different growth and probiotic factors. As mentioned above, Tacon found that feeding with bioflocs can replace the vitamins typically supplied in commercial shrimp feed. Moss and co-workers (2001) found that feeding shrimp with bioflocs raise specific activity of enzymes (serine protease, collagenase, amylase, cellulose, lipase and acid phosphates) by about two fold, possibly contributing to shrimp growth enhancement.

Bioflocs may contain components that have a probiotic effect. One of the probable probiotic factors is PHB, poly-β-hydroxybutyrate, a bio-polymer stored in microbial cells involved in bacterial carbon and energy storage. This polymer can depolymerize in the fish gut and release butyric acid, a known anti-microbial agent. The nutritional composition of the bioflocs can be important to economical production of healthy, high quality crops (Chapter 15).

The effects of bioflocs on the growth and health of fish, the role of different specific components and the ability to direct bioflocs composition to enhance fish production is still far from being resolved. The potential and importance of such information is exciting. Yet, it is a new field of study, and rather complicated. Most probably, new information will evolve in the future, information that may direct biofloc technology to new horizons (See Chapter 15).

Practical Implications and Tips

1. *Monitoring floc volume (FV) is an easy and efficient means to follow and evaluate bioflocs development and abundance. It should be done at least once a week and results kept and recorded.*

2. *The interpretation of FV in terms of equivalent feed weight or equivalent gross protein is not trivial and probably varies for different ponds. It is recommended to determine TSS and Kjeldahl nitrogen to get a calibration for your farm.*

3. *The content of bioflocs in the settled floc plug as found in a BFT tilapia farm were 1.4% or 14 mg dry matter per ml. This implies that the equivalent feed stored in the pond is 140 kg/ha per each one FV ml. The protein concentration in the bioflocs are about 25 –30%, i.e., the gross protein stored in the pond is 35– 42 kg/ha for each FV ml.*

4. *Experiences in both research and farm systems show that if the pond is not fed for a few days, FV drops due to its consumption by the fish. Biofloc concentration can be built up by additional feeding.*

5. *We got information on the ability to control and improve the nutritional value of bioflocs. This can be of a very high importance in raising fish growth nutritive value and fish health. This complex needs to be intensively further studied.*

Further Research Needs

In some cases basic research data are combined into a comprehensive framework enabling the application of the data in design and operation of a practical system. However, in many cases, such as the use of bioflocs as feed, the sequence is reversed: field results are retrieved, followed by proper design of the scientific work needed toward the understanding and optimization of these systems.

1. *Harvesting of bioflocs by the different aquatic animals is most probably a function of bioflocs properties (concentration, size, density, composition, surface properties such as adherence etc.) and those of the animal (e.g. gills structure, amount of water filtration, effect of environmental conditions on activity etc.). All of these factors may be formulated by an equation similar to:* ***Harvesting = Biofloc concentration x biofloc properties x fish harvesting efficiency.*** *Presently, we do not have the right parameters to predict harvesting of bioflocs. There are consistent data proving uptake of bioflocs by some fish (tilapia) and by a variety of shrimp. Biofloc harvesting efficiency is not expected to be equal for all fish. African Catfish harvesting efficiency is very low, probably due to poorly developed gills (Avnimelech, unpublished data). Similarly there is a difference in biofloc harvesting potential of different shrimp. There is a need to study harvesting efficiency of different fish and possibly relate it to anatomy and physiology of the different species studied in order to be able to predict harvesting efficiency. We do have some preliminary data defining harvesting efficiency by tilapia, much less information regarding shrimp. Preliminary observations hint that large bioflocs are better harvested by tilapia, yet these observations are far from being quantitative or conclusive.*

2. *As reported in this chapter, a number of studies that demonstrated the added nutritive value of bioflocs. Tacon and co-workers demonstrated the fact that bioflocs contain vitamins and essential trace elements, Moss and co-workers showed that feeding with bioflocs raises some enzymes activity, Khun et al. (2008) showed that shrimp fed with bioflocs grew better than those fed with artificial diets and the group in Gent demonstrated that bioflocs have some probiotic effects. These are a few findings demonstrating that biofloc feeding carries dramatic advantages that should be further studied. It is quite possible that bioflocs made with specific microbial communities might be nutritionally better than others.*

3. *Further research on the means of growing bioflocs to both enhance harvesting efficiency and improve the selection of more favorable microbial communities, will probably lead to a sophisticated biotechnological development of BFT.*

Recommended Reading and Cited Literature

1. *Abreu, P.C., Ballester, E.L.C., Odebrecht, C., Wasielesky, W. (Jr), Cavalli, R.O., Graneli, W., Anesio, A.M. 2007. Importance of biofilm as a food source for shrimp (Farafatepanaeus Paulensis) evaluated by stable isotopes (□ ^{13}C and d^{15}N). Journal of Experimental Marine Biology and Ecology 347: 88–96.*

2. *Avnimelech, Y., Mokady, S., Schroeder, G.L. 1989. Circulated ponds as efficient bioreactors for single-cell protein production. The Israeli Journal of Aquaculture– Bamidgeh 41(2): 58–66.*

3. *Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture 264:140–147.*

4. *Avnimelech, Y., Panjaitan P. Effects of carbon : nitrogen ratio control on water exchange microcosms , WAS conference, Firenze, May 9–13 2006*

5. *Avnimelech, Y., Kochba, M. 2008. Evaluation of nitrogen uptake and excretion by tilapia in biofloc tanks, using ^{15}N tracing. Aquaculture 287 (1–2): 163–168.*

6. *Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219: 393–411.*

7. *Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C. 2004. The contribution of flocculated material to shrimp (Litopenaeus Vannamei) nutrition in a high-intensity, zero-exchange system. Aquaculture 232: 525–537.*

8. *Chamberlain, G., Avnimelech, Y., McIntosh, R.P., Velasco, M. 2001. Advantages of aerated microbial reuse systems with balanced C/N. II. Composition and nutritional value of organic detritus. Global Aquaculture Advocate 4 (4): 22–24.*

9. *Cheng-Sheng, L., O'Brien. P., (Eds.). 2002. Microbial approaches to aquatic nutrition within environmentally sound aquaculture production systems. World Aquaculture Society, Baton Rouge, LA 188 pp.*

10. *Crab, R., Chielens, B., Bossier, P., and Verstraete, W. 2010. The effbect of different carbon sources on the nutritional value of bioflocs, a feed for Macrobrachium rosenbergii postlarvae. Aquaculture Research 41:559–567*

The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture, 426:105-111 shrimp grown in biofloc systems. www.aesweb.org.bioflocs. shrimphealth

14. *Izquierdo, M., Forster, I., Divakaran, S., Conquest, L., Decamp, O, Tacon, A. 2006. Effect of green and clear water and lipid source on survival, growth and biochemical composition of Pacific white shrimp Litopenaeus Vannamei. Aquaculture Nutrition 12: 192-202*

15. *Ju, Z.Y., Forster, I., Conquest, L., Dominy, W. 2008. Enhanced growth effects on shrimp (Litopenaeus Vannamei) from the inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquaculture Nutrition 14: 533-543.*

16. *Kuhn, D.D., Boardman, G.D., Craig, S.R., Flick, G.J., Mclean, E. 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, Litopenaeus Vannamei in recirculating aquaculture systems. Journal of the World Aquaculture Society 39: 72-82.*

17. *Leber, K.M., Pruder, G.D. 1988. Using experimental microcosms in shrimp research: the growth-enhancing effect of shrimp pond water. Journal of the World Aquaculture Society 19 (4):197-203.*

18. *Litchfield, J.H. 1990. Food supplements from microbial protein. P 65-109. In: Goldberg I. and Williams R. (Eds.) Biotechnology and food ingredients. Van Nostrand Reinhold, NY*

19. *Moav, R., Wohlfart, G., Schroeder G. L., Barash H. 1977. Intensive polyculture of fish in freshwater ponds, 1. Substitution of expensive feeds by liquid cow manure, Aquaculture 10: 25-43.*

20. *Moss, S.M., Divakaran, S., Kim, B.G. 2001. Stimulating effects of pond water on digestive enzyme activity in the pacific white shrimp, Litopenaeus Vannamei (Boone) Aquaculture Research 32(2): 125-131.*

21. *Nunes, A.J.P., Gestiera, T.C.V., Goddard, S. 1997. Food ingestion and assimilation by the southern brown shrimp Panaeus Subtilis under semi-intensive culture in NE Brazil. Aquaculture 149: 121-136.*

22. *Schroeder, G.L. 1978. Autotrophic and heterotrophic production of microorganisms in intensively manured fish ponds, and related fish yields. Aquaculture 14:303-325.*

23. *Tacon, A., G.J. 2000. Shrimp feeds and feeding regime in zero exchange outdoor tanks. Global Aquaculture Advocate 3 (2):15-16.*

24. *Tacon, A., G.J., Cody, J.J., Conquest, L.D., Divakaran, S., Forster, I.P. Decamp, O.E. 2002. Effect*

Chapter 8

Ex-Situ Biofloc Technology

Using Bioreactors To Treat Aquacultural Effluents While Producing Bioflocs for Shrimp Feed

David D. Kuhn, Addison L. Lawrence

Edited for 3d edition by ***Yoram Avnimelech***

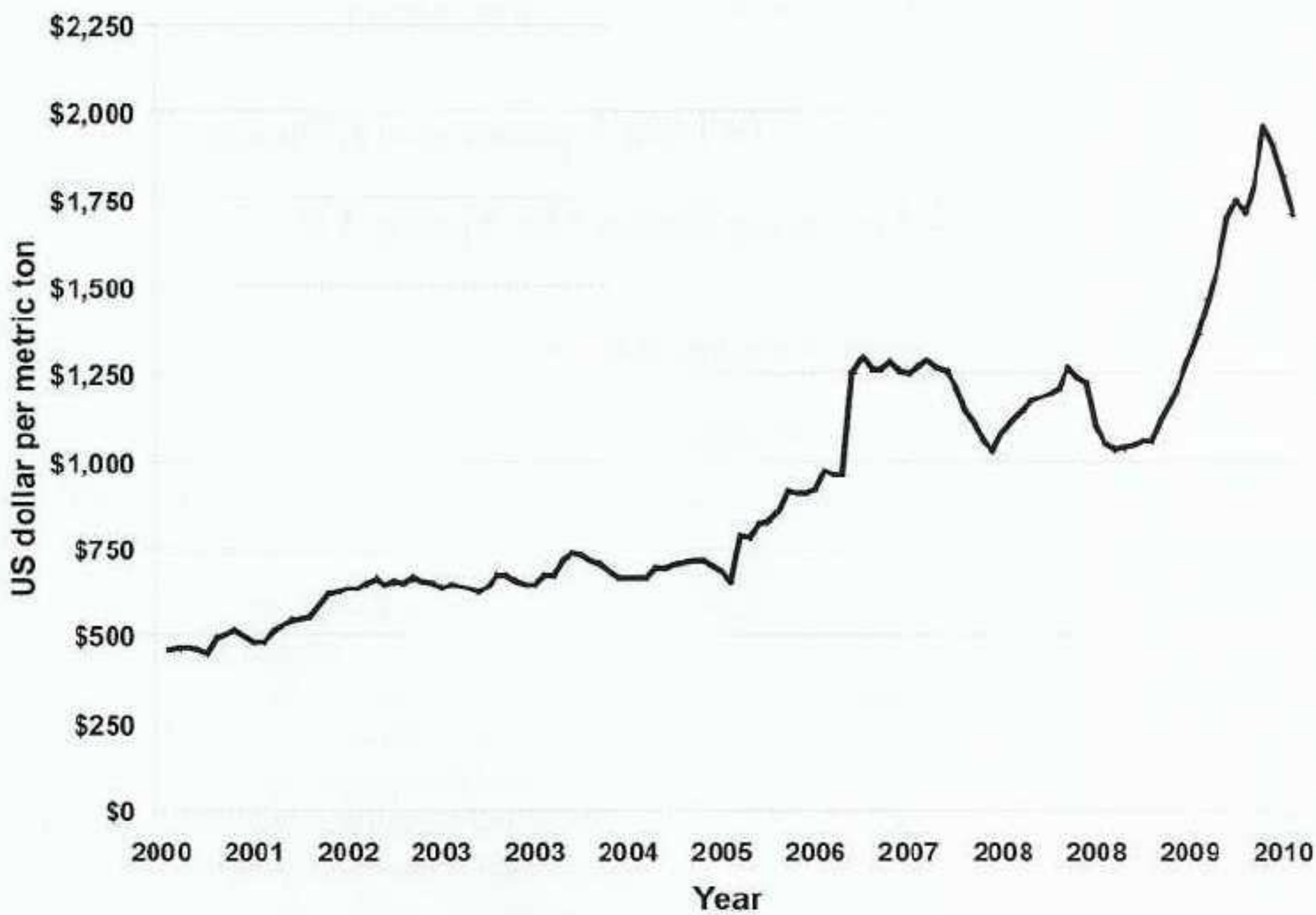
In Brief

Shrimp farming has traditionally relied on fish-meal for the formulation of a nutritionally complete diet. Fish-meal is becoming more expensive, and the oceans are being over exploited due to an increase in demand as the global human population continues to grow. This is prompting the aquaculture industry to investigate and implement alternative sources of protein to replace less sustainable protein ingredients in aquaculture feeds. Traditional alternative proteins are derived from plants; e.g. soybean meal. Recent developments in research are demonstrating that yeast-based and biofloc-based proteins are suitable replacements for fish-meal in aquaculture diets. This chapter covers biofloc-based proteins in shrimp diets. Since ex-situ bioflocs can be produced while treating aquaculture effluents in external biological reactors, bioflocs may have an additional advantage over plant-based proteins. This is because we are effectively converting a waste (dirty water high in pollutants such as solids and nitrogen) into a valuable resource for the industry. This ex-situ biofloc technology is in the experimental stage but has shown promise. Accordingly, this Chapter outlines research thus far and the potential for this technology to be helpful for the aquaculture industry.

Further verification and development of this approach was obtained since the publication of the 2d edition of this book. An extensive research efforts by Australian scientists led to the development of a product, made of a selection of marine bacteria(Novacq™) that can replace fish meal and increase yields of Panaeus monodon by about 30% over conventional feeds. More development in this direction is expected.

have increased dramatically over the last decade (Figure 8.1). Feed costs can account for 50% of operational expense (Van Wyk et al., 1999), so, reducing ingredient costs will have significant impacts on business economics.

Figure 8.1: Cost of fishmeal over the last decade



* Source: International Monetary Fund

Researchers and the aquaculture industry are advancing the use of alternative feed ingredients. One success story has been the use of soy protein in place of fish-meal in some aquaculture feeds. Other alternative ingredients are being investigated, such as single-celled protein meals. In recent years several researchers and industry groups have begun exploring novel ways to produce these single-celled ingredients, not only for use as a potential feed ingredient, but also to accomplish other goals. The research group at Virginia Tech, for example, has demonstrated that single-celled proteins produced in biological reactors can clean up effluent waters from aquaculture facilities

Fish farming can also generate pollutants that can be detrimental to animal health and to the environment. Accordingly, researchers and farmers have developed and implemented numerous technologies and strategies to combat these issues, especially when considering aquaculture systems that minimize water use. Technologies for cleaning culture water for reuse include biological, chemical, and physical processes. Biological processes have traditionally focused on fixed-film processes, more commonly known as nitrification. Recently, suspended-growth biological processes are getting more attention in the aquaculture industry.

Suspended-growth bioreactors

In suspended-growth bioreactors, microorganisms are maintained in suspension by well mixing the reactor using pneumatic (aeration) or mechanical agitation. Microorganisms in these bioreactors form biofloc particles (between 50 and 200 µm in diameter) and are conglomerates of microorganisms that are bridged together by polysaccharides and proteins. Pollutants (organic material and nutrients) in the wastewater are removed as these bioflocs move through the water. Figure 8.2 illustrates the effectiveness of biological reactors for cleaning aquaculture effluents.

Figure 8.2: Untreated fish wastewater (left) compared to settled bioflocs after suspended-growth biological treatment (right)

1. Fill stage: "Dirty" wastewater is pumped or gravity fed into the SBR. Bioflocs are already in the bioreactor from the previous batch (cycle). Estimated time for this stage is 10 to 120 minutes.

2. React stage: The "dirty" wastewater is well-mixed with bioflocs until treatment objectives are met; e.g. greater than 90% reduction of nutrients. The estimated time for this stage can vary from hours to days depending primarily on substrate rate of degradation. If effluent is available every day it is recommended that the react stage be 24 hours or less.

3. Settle stage: The mixing cycle is terminated allowing bioflocs to settle out of the quiescent water column (45 and 120 minutes). Longer times are required if solids have not adequately settled out. Typically, the longer the bioreactor is under operations (in terms of weeks), the more stable and settleable the bioflocs will become.

4. Decant stage: Treated "clean" wastewater is removed from the bioreactor while leaving the settled bioflocs behind. This stage can range from 10 to 120 minutes.

5. Note: An MBR is similar, but instead of using a settle and decant stage, the well mixed bioflocs and treated wastewater are separated from each other using a membrane or filter.

Every aquaculture system has a limiting factor that requires wastewater to be discharged (Figure 8.3(a)). Typical limiting factors in aquaculture operations are either elevated suspended solids or accumulated nitrate levels. Biological reactors remove nutrients and suspended solids thereby enhancing water reuse at aquaculture facilities (Figure 8.3(b)).

Figure 8.3 (a): Production flow diagram using systems without suspended growth bioreactors

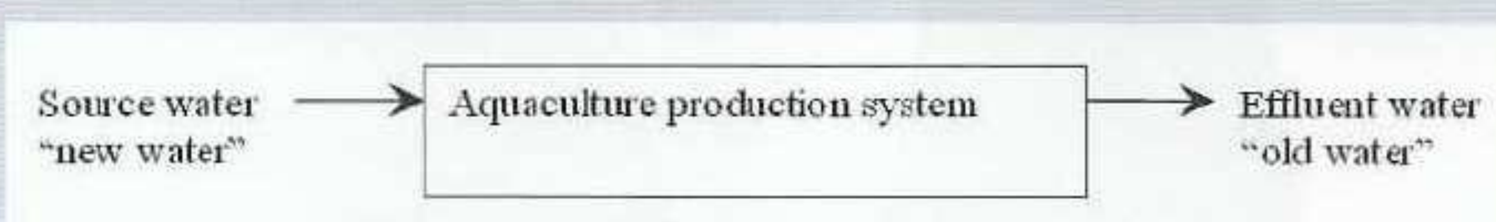
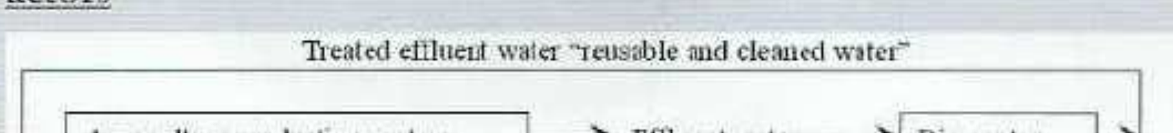


Figure 8.3 (b): Proposed flow diagram using systems with suspended growth bioreactors



Evaluation methods

To determine the feasibility of implementing suspended-growth bioreactors, researchers performed the following studies. Wastewater from an indoor recirculating aquaculture facility that cultures freshwater fish was diverted into bioreactors to determine removal rates of pollutants. Three 5,100 L pilot-scale SBRs (Figure 8.4) and a single 42,000 L pilot-scale MBR were used in the evaluation process. Bioreactors were operated using both aerobic and anaerobic stages. Furthermore, carbon supplementation, in the form of sucrose, was used in the SBR experimental trials. No external carbon source was used for the MBR process. When the C:N ratio is low, carbon supplementation is required (Avnimelech, 1999; Schneider et al., 2006), as was the case for effluents being received by the SBRs. (See Chapter 6 for more information regarding nitrogen management and carbon supplementation).

Figure 8.4: Three independent sequencing batch reactors used for experimental trials

All bioreactors were monitored for water quality. Treatment efficiencies were assessed by measuring water quality parameters before and after treatment. Biofloc quantities can be estimated by measuring total suspended solids or more appropriately, volatile suspended solids. Biofloc concentrations in SBRs are operated to contain between 1,000 and 3,000 mg/L. MBRs are typically operated at higher biofloc concentrations, often, at levels that exceed 10,000 mg/L (Rittman and McCarty, 2001). Bioflocs from SBRs and the MBR were harvested, dried, and analyzed for nutritional composition (see part II: Bioflocs as Shrimp Feed Ingredient).

Results and implications

Both reactor types removed ammonia, nitrite, and suspended solids with great efficacy. However, the MBR removes higher rates of suspended solids compared to the SBR. This is to be expected, because the MBR has a filter membrane which is more efficient in removing solids compared to settling. It should also be noted that an anaerobic stage is required for effective denitrification. Implementing anaerobic stages complicates the process but should be considered if nitrate is the limiting factor at an aquaculture facility. The most efficient means of denitrification is to keep the bioflocs "gently" suspended with mechanical agitation without introducing ambient air (oxygen). More information on utilizing biological reactors for aquaculture effluents can be obtained in Kuhn et al. (2010a)

Table 8.1: Typical removal rates of various constituents using suspended-growth biological treatment.

	Removal rates			
	Ammonia	Nitrite	Nitrate	Suspended solids
SBR	>90%	>90%	>90%	>95%
MBR	>90%	>90%	>95%	>99.5%

If bioreactors are properly operated and maintained, no offensive odors will be produced (Rittman and McCarty, 2001). Bioflocs are typically withdrawn (wasted) from the bioreactor to control and stabilize the population of microorganisms. For example, wasting 10% of the solids each day will maintain a biofloc that is, on average, 10 days old. The age of the bioflocs influences the types of organisms that make up the bioflocs. Typically, a young biofloc age would yield predominately heterotrophic organisms and an old biofloc age could be dominated by fungi. Traditionally, wasting bioflocs to control biofloc age is an issue for a bioreactor operator. However, if the aquaculture industry could harvest bioflocs to be used as an alternative protein source they could potentially reduce the amount of fishmeal required in aquaculture diets.

Bioflocs as Shrimp Feed Ingredient

a. Research in the USA

than the other bioreactor . Research evaluating factors that can influence nutritional properties are currently underway to optimize the nutritional value of the biofloc produced from SBR and MBR type reactors (e.g. essential amino acids and fatty acids, protein levels, crude fat levels).

In studies with SBR and MBR type reactors, the following ranges of nutritional values (on a dry matter basis) have been observed as presented in Table 8.2.

Table 8.2: Range of nutritional composition of ex-situ produced bioflocs from two primary types of bioreactors

Parameter	Bioflocs from SRBs using sucrose as carbon supplement	Bioflocs from MBR without external carbon supplement
	[g/100g]	**[g/100g]**
Crude protein	41 to 49	38 to 42
Carbohydrate[1]	31 to 36	22 to 28
Total ash	12 to 13	21 to 29
Crude fat	0 to 1.0	0 to 1.0
Crude fiber	13 to 16	14 to 18
Calcium	1.3 to 1.5	2.5 to 2.8
Phosphorus	1.2 to 1.3	2.1 to 2.3
Sodium	1.2 to 1.8	2.2 to 2.6
Potassium	0.6 to 0.8	1.1 to 1.7
Magnesium	0.4 to 0.5	0.7 to 0.9
	[mg/kg]	**[mg/kg]**
Zinc	180 to 350	1,100 to 1,900

In reference to amino acids, non-essential amino acids can be synthesized by shrimp. However, the essential or indispensable amino acids (EAA) cannot be synthesized by shrimp. The EAA in a high quality shrimp feed and biofloc typical of our studies are compared in Figure 8.5. Overall, the levels of essential amino acids for shrimp compare well, but there is a notable difference (≥ 0.5% dry weight basis) in the percent compositions of leucine, lysine and isoleucine. It is anticipated that the biofloc protein level can be increased to about 65% and the quality of the EAA profile in biofloc can be further optimized by changing the effluent used, bioreactor methodology and/or carbon source and level.

Figure 8.5: Essential amino acid profiles of a representative biofloc ingredient sample versus a typical high quality shrimp feed

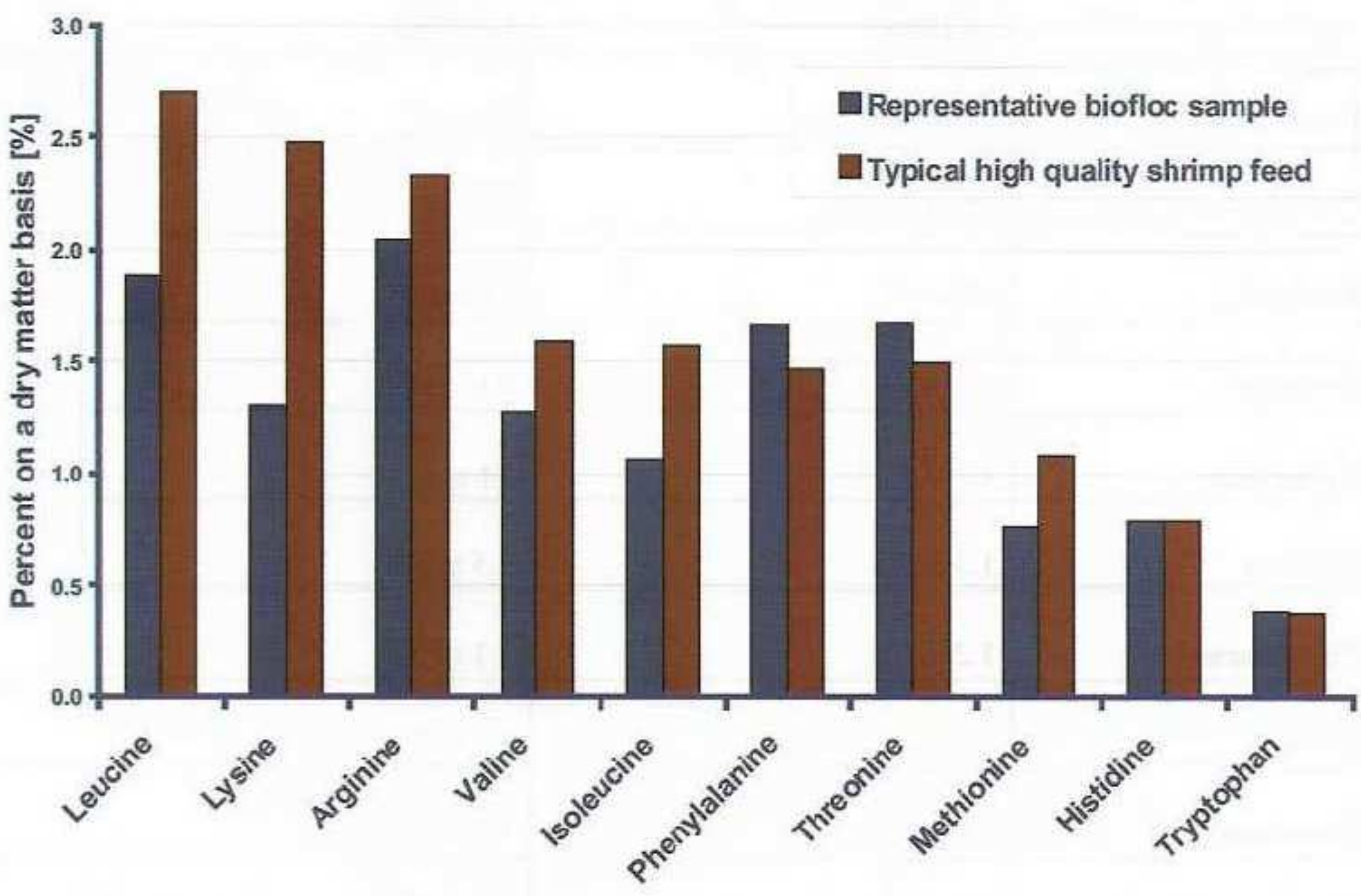
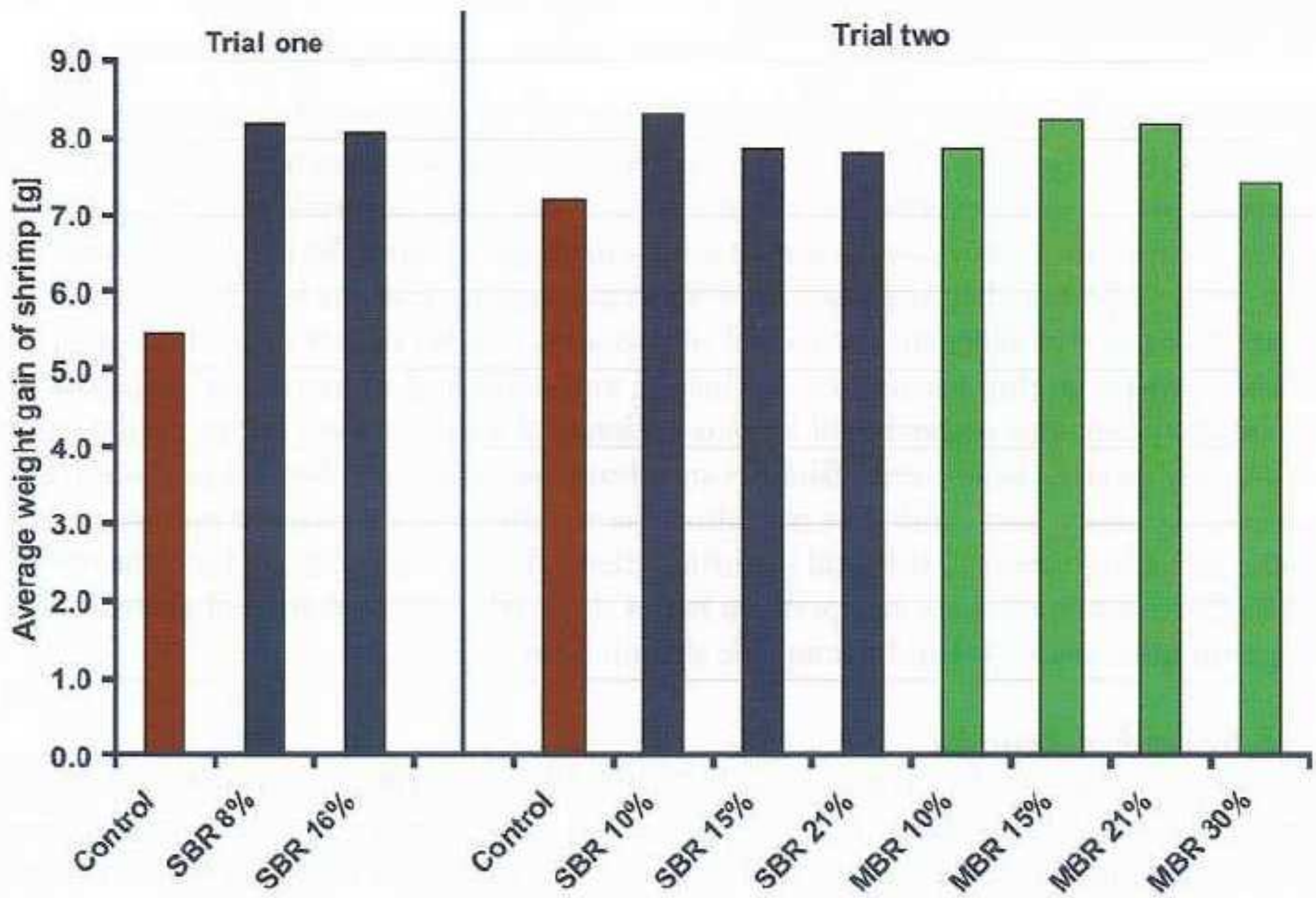


Figure 8.6: Weight gain of shrimp fed various diets over two 5 week trials with bioflocs from sequencing batch reactor (SBR) and membrane batch reactor (MBR). Biofloc inclusion level is given as percent of dry matter



Shrimp Feeding Trials

Two feeding trials were conducted to determine if biofloc can be used to replace fish-meal and/or soybean protein in shrimp diets. Every 5 weeks, a trial was conducted in recirculating aquaculture systems where water quality was maintained at an optimum for shrimp culture. Bioflocs were dried and incorporated into experimental diets that were formulated to be equivalent for crude protein (35%) and total fat (8%). The biofloc experimental diets were compared against high quality control diets by replacing fishmeal and/or soy protein. In the first trial, SBR bioflocs only were tested. Both SBR and MBR bioflocs were used in the second trial at twice the inclusion rate of the first trial. Weight gain of shrimp in the trials is presented in Figure 8.6.

In the aforementioned biofloc feeding trials, feeds were formulated to be equivalent for crude protein, carbohydrate, crude fat, crude fiber, and energy. Using standard laboratory methods and semi-purified diets in which all the ingredients are reagent grade or purified, we validated that all diets with or without bioflocs contained identical levels of many nutrients. Thus, none of these nutrients which were constant or in excess in diets would not contribute to the increased growth rates observed in the biofloc-fed shrimp.

Even though we have not yet identified what nutritional component of bioflocs is contributing to accelerated shrimp growth in some of the experimental feeds, we know a lot of things that do not contribute such as crude protein, a particular amino acid, crude fat, crude fiber, energy, sterols, vitamins, or minerals. In this case, demonstrating what is not causing the enhanced shrimp growth is extremely important, because it provides information as to what the beneficial nutrients might be and indicates that there are nutritional components that we do not fully understand. From a scientific viewpoint, this is extremely fascinating and could lead to innovative projects and findings in the future. This research will help us understand nutrition even better than we do today and possibly produce better feeds. Bioflocs are advantageous because they are produced in a manner that is not only sustainable, but may also offer valuable water treatment options, resulting in an alternative ingredient to fishmeal in shrimp diets. These findings, along with the observation that biofloc-containing diets may perform better than either soy or fish-meal proteins, may lead to an even more successful and sustainable shrimp farming industry.

- ***b. Research in Australia***

After 10 years of research, and investment of $10 millions, CSIRO scientists have perfected the Novacq™ prawn feed additive. Farmed prawns fed with *Novacq* grow on average 30 per cent faster, are healthier and can be produced with no fish products in their diet (Glencross and co-workers, 2014).

The Novacq formula is a closely guarded secret, thus information given here is limited in part to commercial publications period *Novacq* is an entirely natural food source based on the smallest organisms in the marine environment, the marine microbes which are the foundation of the marine food pyramid.

Production of *Novacq* relies on the controlled production of these marine microbes. CSIRO researchers have discovered how to feed and harvest them, and convert them into a product that can then be added to feeds as a bioactive ingredient, like a dietary supplement for prawns.

They are harvested when they are 40 days old. "We then de-water the product. We drain it down and filter it and then we harvest the product as sludge or ... a mud," said CSIRO's Dr Brett Glencross. "That product is dried before it gets milled and then included in a prawn feed."

Including *Novacq* in the diet of farmed prawns has shown for the first time that fish meal and

Ju and coworkers (2008) found that microbial biomass inclusion in the feed pellets raised growth rates of L. vannamei by 21% as compared to commercial feed. These authors fractionated the microbial biomass and suggested that the potential bioactive component was in the acetone soluble fraction, possibly a carotenoid of some sort. This hypothesis has still to be verified.

Conclusions

Although more work is needed to understand the fundamental aspects of using biofloc in shrimp or fish feeds, and ascertain the reproducibility of the responses, it was demonstrated that bioflocs can suitably replace fishmeal and/or soybean protein in shrimp feeds. The capacity to generate the bioflocs by treating effluents makes biofloc technology even more attractive. The potential for the production of bioflocs having desired nutrient levels for specific aquatic species by manipulating aquaculture effluent, bioreactor production method, and/or carbon source and level is very great.

The expansion of these findings by the Australian research seems to further open exiting developments. Developing sustainable protein sources, such as bioflocs, could offer the aquaculture industry a viable option for the future.

If this biofloc technology can be successfully implemented, the aquaculture industry could make significant progress by improving resource conservation and enhancing economic viability. This is becoming more critical as our human population continues to increase, per capita consumption of fish rises, while natural resources are being depleted.

Further Research Needs and Prospects

The example set by the CSIRO research efforts and results whould probably encourage more such research world-wide. It can be expected that different microbial consortia could be found to be beneficial as feed supplements or feed components. Different shrimp or fish, and different eco-systems, may require new developments. It is interesting the the Australian developers are already working on the development of bioactive ingredient for fish. Industrial production of such microbial preparations can be reproducible and consistent, possibly more than the inoculation of ponds by probiotic organisms. A fast increase of technology in this direction may be expected and can possibly lead to a dramatic development of aquaculture.

Characterization of the microbial communities in these ex-situ bioflocs needs to be performed to better understand the fundamentals of this technology.

The isolation of the different bioactive components is an extremely interesting and important field of required research.

The technology and economy of the bioactive ingredients production should also be advanced.

Recommended Reading and Cited Literature

1. *Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176: 227-235.*

2. *FAO (Food and Agricultural Organization). 2007. State of the World Fisheries and Aquaculture. 2006. Fisheries and Aquaculture Department, Food and Agricultural Organization of the United Nations, Rome, 2007. 162 pgs.*

3. *Glencross, B. Irvin S. Arnold, S. Blyth, D. Bourne, N. Preston, N. 2014 Effective use of microbial biomass products to facilitate the complete replacement of fisheru resources in diets for the black tiger shrimp. Panaeus monodon. Aquaculture, 431:12-19*

4. *Ju, Z.Y., Forster, L.,Conquest. L., Dominy, W., 2008 Enhanced growth effects on shrimp (Litopenaaeus vannamei) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aqua. Nutr. 14:533-543.*

5. *Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick, G.J. 2009. Microbial flocs generated in bioreactors is a superior replacement ingredient for fishmeal or soybean meal in shrimp feed. Aquaculture 296: 51-57.*

6. *Kuhn, D.D., Boardman, G.D., Flick, G.J. 2010a. Production of microbial flocs using laboratory-scale sequencing batch reactors and tilapia wastewaters. International Journal of Recirculating Aquaculture 11: 37-53.*

7. *Kuhn, D.D., Lawrence, A.L., Boardman, G.D., Patnaik, S., Marsh, L., Flick, G.J. 2010b. Evaluation of two types of biofloc meals derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, Litopenaeus vannamei. Aquaculture 303: 28-33.*

8. *Merrill, A.L., Watt, B.K., 1973. Energy value of foods: basis and derivation. United States Department of Agriculture (USDA), Handbook No. 74.*

9. *Metcalf, Eddy. 2003. Wastewater engineering: treatment and reuse. 4th ed. Edited by Tchobanoglous, Burton, and Stensel. McGraw Hill. New York, New York.*

10. *Rittmann, B.E., McCarty, P.L. 2001. Environmental biotechnology: principles and applications. 1st ed. Edited by Tchobanoglous, Burton, and Stensel. McGraw Hill. New York, New York.*

11. *Schneider, O., Sereti, V., Eding, E.H. 2006. Molasses as C source for heterotrophic bacteria production on solid fish waste. Aquaculture 261: 1239-1248.*

Chapter 9

Optimizing Microbial Activity in Extensive Ponds

In Brief

Aerated – mixed ponds are most suitable for BFT applications, due to the dominance of aerobic conditions throughout most of the pond. Without mixing, sedimentation of feed residues and other organics lead to the development of anaerobic conditions at or near the pond bottom. These conditions reduce the potential for efficient recycling of organic matter and effective nitrogen control in extensive ponds. It is possible though, to minimize the effect of the anaerobic conditions at the pond bottom by properly aerating and cleaning it between production cycles. In addition, it is possible to exchange at least part of organic sedimentation by the absorption of organic residues onto vertical substrates, natural or synthetic. Both of these strategies were shown to enable the usage of BFT and C/N control in extensive and semi extensive ponds. Moreover, these approaches are a way to introduce biofloc systems to presently extensive farm systems and to gradually get farmers into the more intensive technology.

Comment:

The ideas proposed by Mr. B. Suryakumar, *of Hitide Sea Farms, Tamil Nadu India, visiting his farm and having numerous discussions, helped in developing this chapter. This is deeply appreciated.*

Very high productivity can be achieved in super-intensive BFT ponds. These ponds are thoroughly mixed and aerated 24 hours a day. The whole volume of the pond is mixed and aerobic. The dominance of aerobic conditions is a pre-requisite to the operation of such ponds. However, the construction and maintenance of such ponds is expensive and complex, demanding elaborate infrastructure, beyond the reach in wide areas of developing countries, regions where aquaculture is needed for local food production and for export. Yet, there are indications that principles similar to the ones used for BFT, can be used to significantly improve production of extensive, or semi extensive ponds, especially ponds in tropical and warm regions.

The conditions and water characteristics in stagnant ponds is different from those in aerated-mixed ponds. Organic residues, un-eaten feed, fecal pellets and dead algae, all settle down to the pond bottom, creating a site of high biological oxygen demand. Oxygen supply to the pond bottom is meager, with the exception of periods when intensive wind mixes the water column. Usually the pond bottom has very low oxygen concentrations and it is very often anaerobic.

The situation at the pond bottom becomes more severe with increasing stocking, when high quantities of organic matter are added to the pond. The low or even zero concentrations of oxygen at or near the bottom affect fish (and especially shrimp) functioning and activity. In addition, organic matter recycling is driven towards anaerobic processes that are inefficient and generate toxic compounds. There is a reason to suspect that the addition of carbonaceous substrates which can enhance microbial dominance and protein production may worsen the oxygen deficit and not be effective in extensive ponds.

A work by Hari and co-workers (2004, 2006) in India, demonstrated that this is not necessarily true. Microbial development and protein recycling was studied in 25 m^2 ponds stocked at a rate of 6 *Penaeus monodon* Pl/m^2. Feed rations consisted of a relatively low protein feed (25%) and carbohydrates, as Tapioca flour, was added at a rate of 20 g for every 1 g expected NH_4 excretion (assuming 50% of eaten nitrogen is excreted). Results obtained in this study (Table 9.1) demonstrated high similarity to those obtained with BFT, intensive, fully mixed and aerated fish or shrimp ponds. Nitrogen retention in the control ponds, 20%, was similar to that obtained in most conventional ponds. Nitrogen retention was 45% in ponds receiving 25% protein + carbohydrates. Net yields was higher and FCR lower in the BFT treatment.

Table 9.1: Summary of shrimp production in extensive earthen ponds, as affected by feed C/N ratio[1]

	P25 + CH	P 40
FCR	1.6	2.2
N retention, %[2]	45	20
Net yield, g/m^2	64	45
Gross return (Rs/ha)	193,275	125,406
Production costs (Rs/ha)	83,202	103,420
Net profit (Rs/ha)	110,073	21,986

* Adapted from Hari et al., 2004.

(1) Control ponds, P 40 got feed pellets with 40% protein. Treated ponds, P25 + CH, got 25% protein pellets + tapioca flour, at levels adjusted to induce full recycling of N excreted by shrimp.

(2) Nitrogen accumulation in shrimp as a percentage of nitrogen added with feed

stantiate, explain and develop this approach.

Though this work has to be repeated and expanded, it can be considered as a break-through and an indication of the feasibility of enhancing microbial processes as a method of raising yields and at the same time lowering production costs, mainly by reducing protein in the feed. It has to be noted that shrimp yields were raised by 50%, protein utilization doubled and net profit increased by a factor of 5.

Another means of enhancing the beneficial aerobic microbial activity under aerobic conditions is to divert organic carbon accumulation from the pond bottom to vertical substrates, periphyton. Periphyton is quite similar to bioflocs, made of a complex mixture of algae, cyanobacteria, heterotrophic microbes, detritus and others.

Suspended organic matter (dead algae, feces, feed residues etc.) settles down and accumulates on or within the pond bottom, where oxygen supply is meager and anaerobic conditions prevail in most cases. Anaerobic metabolism of organic residues is appreciably slower than aerobic one. Thus, recycling of organic residues accumulated on the pond bottom is inefficient. In addition, anaerobic compounds generated through the anaerobic processes are often toxic. This may be reversed, or modified, by placing either natural or synthetic vertical substrates (such as bamboo stems, plastic substrates etc.), that lead to the absorption of suspended organic matter on the vertical surfaces, at least partially replacing the downward sedimentation and accumulation in the pond bottom. The addition of vertical substrates can be visualized as a change in the rules of the game: Replacing the accumulation of the excessive organic residues within the anoxic pond bottom by attachment to vertical surfaces in regions where oxygen is available. The organic matter attached to the vertical substrates is exposed to aerated water, degrades aerobically and thus contributes to a beneficial food web. The microbial community developing under these conditions can be manipulated using C/N control and other means. In a way, placement of vertical surfaces reduces the extent of artificial aeration needed to prevent anoxic conditions at the pond bottom and utilizes natural aeration processes to support aerobic microbial activity.

Extensive work was conducted and published in the last decades on periphyton role and ecology in aquaculture ponds (Azim et al., 2005). Microbial communities, similar to bioflocs, containing algae, blue green algae, bacteria, protists, zooplankton and fungi embedded in an extracellular polysaccharide matrix develop on submerged surfaces. Autotrophic or heterotrophic biomass dominates, depending on light and nutrient availability.

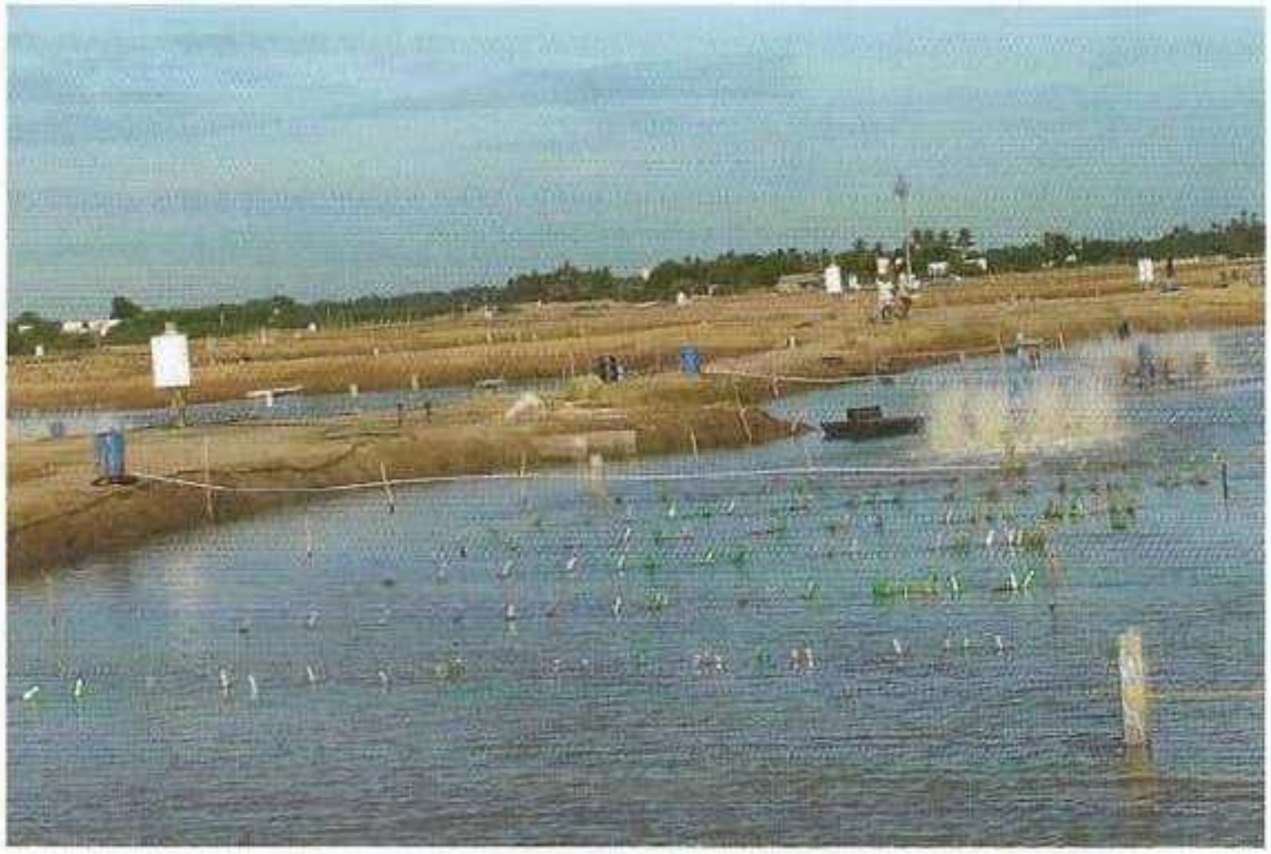
Providing substrate at a density of 1 m^2 per m^2 of pond area increase the production of Rohu (Labeo rohita) by 80% in fertilized ponds. A similar production increase can be seen with Kalbaush (L. calbasu) using a substrate density of 0.5 m^2 per m^2 pond area (work by Wahab et al., 1999). Not all fish species are able to utilize periphyton. For instance, Gonia (L. gonius) in ponds increased shrimp growth, raised periphyton production as well as heterotrophic bacterial production in both water and soil and lowered inorganic nitrogen in the water.

These findings may open new opportunities for the use of BFT and C/N control rational toward higher productivity and better feed utilization in extensive ponds. Different types of substrate materials and geometry will probably be developed to achieve an efficient trapping and aerobic metabolism of most excessive organic residues. An interesting and advanced BFT farm, Hitide Sea Farm, exists in Tamil Nadu state in the south-most part of India. The farm owner, Mr. Suryakumar, is a mechanical engineer that got interested in aquaculture, learned about biofloc technology and decided to practice and develop a BFT shrimp farm. The farm has 24 ha of grow-out ponds and 12 ha reservoirs. Learning from a number of failures, the farm was converted to a zero water exchange with bioflocs, 2010 onward. The rational toward this change was to minimize risk of crop failure by diseases, poor performance of shrimp (Rate and extent of growth, FCR) and to ensure sustainable aquaculture practices. Water source is infested with pathogens, thus water is sanitized before use. An interesting modification of biofloc technology is the inclusion of vertical substrates in the ponds. Conventional Plastic shade nets are utilized as substrates. The nets (20 m long, 0.5 m deep) are kept in a vertical position using empty drinking water bottles as floats and sand filled bottles as anchors (Fig 9.1). The nets are maintained at a depth of 10 cm below water surface and about 50 cm above the bottom, placed in front of the paddle wheel aerators, to provide ample supply of oxygen (Fig 9.2).

Soon after placement, the substrates are covered with a layer of organic matrix made of detritus and periphyton population. The ponds are operated as biofloc ponds, using molasses to increase C/N ratio.

Figure 9.1: Shade net used as a vertical substrate in Hitide Seafarms, India

Figure 9.2: Placement of vertical substrates



* Vertical substrates are placed in front of paddle wheel aerators (noticed by the presence of floats), to maximize water and oxygen movement through the substrates

The ponds are presently operated at lower intensity as compared to intensive BFT ponds (see Chap. 17) and represent a good transition from extensive ponds to the intensive BFT ponds. Ponds are stocked with 50 PL/m^2 of *Penaeus monodon* with an FCR of 2. Average shrimp weight at harvest is 42 g, Ponds are equipped with paddle wheel aerators. The ponds have an earthen bottom with slopes stabilized by slabs, while some of the ponds are plastic lined.

We believe that the Hitide Seafarm technology is a very good example of a possible gradual transition from conventional aquaculture systems toward the potential intensification using BFT principles. It is anticipated that more experience in this direction will be obtained in the next few years.

Further development of the different approaches toward the utilization of BFT principles in extensive ponds can be a break-through in low input aquaculture systems.

One of the important conclusions drawn is the ability for a gradual change from conventional extensive aquaculture toward intensive BFT systems. The change from traditional aquaculture to sophisticated intensive bio-technology pond control requires a substantial capital investment,

Practical Implications and Tips

1. *There are ways to gradually change conventional systems into more intensive systems, yet, more research and farm scale experience are needed.*

2. *Drying and oxidation of pond bottom between production cycles is not a waste of effort. It effectively adds oxygen to the pond and enables at least partially aerobic processes to take place on the pond bottom.*

3. *Reducing the organic carbon in a 10 cm deep pond bottom by 0.1% is equivalent to the reduction of 10,000 kg sediment oxygen demand per hectare in the next production cycle.*

4. *Modern means of application of vertical substrates have to be further developed with the goal of efficient deployment of inexpensive substrate.*

Further Research Needs

Intensive aquaculture is an important technology to raise future aquaculture production. Yet, presently, most aquaculture production takes place in conventional extensive ponds. Thus, any research and development relevant to improving and cutting costs of extensive systems is very important.

Results presented in this chapter, demonstrating the possibility of improved production using BFT principles are interesting and exciting.

The hypothesis brought in this chapter is that positive response to the C/N control in extensive ponds depends on the existence of aerobic condition in the pond bottom or on vertical surfaces where much of the organic matter is adsorbed and metabolized. We need more data to support this hypothesis and to show a consistent and reliable way to achieve optimal results. This research is of high priority. The development of this technology would enable the gradual advancement of conventional aquaculture toward intensification.

Semi-intensive BFT farms should be used as training sites for farmers. A success of using such systems by farmers can lead to a sustainable development of intensive aquaculture systems and sustainable development and increased production in conservative farming systems.

Recommended Reading and Cited Literature

1. *Azim, M.E., Verdegem, M.C.J., van Dam, A.A., Beveridge, M.C.M (Eds.). 2005. Periphyton: ecology, exploitation and management, CAB International. Cambridge 352 pp.*

2. *Hari B., Kurup, M.B., Varghese, J.T., Scharma, J.W., Verdegem, M.C.J. 2004. Effects of carbohydrate addition on production in extensive shrimp culture systems. Aquaculture 241:179-194.*

3. *Hari, B., Kurup, M.B., Varghese, J.T., Schrama, J.W., Verdegem, M. 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252 (2-4): 248-263.*

4. *Kurup, B.M., Varagese, J. T. 2007. The potential of periphyton-based farming of Panaeus Monodon (Fabricius) with the control of Carbon/Nitrogen ratio. Abstracts P 488. WAS Aquaculture 2007 San Antonio.*

5. *Van Dam, A.A., Beveridge, M.C.M., Azim, M.E., Verdegem, M.C.J. 2002. The potential of fish production based on periphyton. Reviews in Fish Biology and Fisheries12: 1-31.*

6. *Verdegem, M.C.J., Eding, E.H., Sereti, V., Munubi, R.N., Santacruz-Reyes, R.A., Van Dam, A.A. 2005. Similarities between microbial and periphytic biofilms in aquaculture systems. PP 191-205. In: Azim, M.E., Verdegem, M.C.J., Van Dam, A.A., Beveridge, M.C.M, (Eds.). Periphyton: Ecology, Exploitation and Management, CAB International, UK, 352 pp.*

7. *Wahab, M.A., Azim, M.E., Ali, M.H., Bevereidge M.C.M., Khan. S. 1999. The potential of periphyton-based culture of the native major carp Calbaush, Labeo Calbasu (Hamilton). Aquaculture Research 30:409-419.*

Chapter 10

Aeration, Aerators and Aerator Deployment

A. Oxygen and basics of aeration

In Brief

Aeration is a basic means of management and control in BFT ponds. A proper aeration system is essential for the success of these ponds. The aeration system is needed in order to supply oxygen, to mix the water and to control bottom sludge accumulation.

The amount of oxygen that is required is determined by fish consumption (200–400 $mgO_2/kg\times hr$) and by the consumptive use of other in the pond. Oxygen added by algae is important during daytime, yet is of limited significance and reliability in intensive systems. Aerators of different types are used to cover oxygen deficits. Number, capacity and deployment of aerators are essential for proper management.

Low oxygen conditions are common in pond bottoms. These conditions can be defined quantitatively as the redox potential values. Different chemicals are produced and prevail at different redox potentials. The development and extent of low redox conditions has to be minimized in order to maintain a healthy and productive fish (shrimp) community. The deployment of aerators is an essential means to minimize the coverage of the pond bottom by anaerobic sludge.

Ponds are always in contact with the overlying air, containing 21% oxygen. Yet, the diffusion of oxygen into the water (and through the air-water interface) is very slow, and provides only a very small fraction of oxygen requirement. Aeration systems are thus needed to increase oxygen diffusion into the pond water. Pioneering quantitative research on aeration systems were published by Boyd (1987, 1988) A very brief description and characteristics of aerators used in different aquaculture systems will be given in section B of this chapter. However, most aerators in the market, consume energy inefficiently, do not provide the oxygenated water to the pond bottom, where it is most needed and do not mix the pond in an efficient way. Discussion as to the features of needed aerators and proper aerator deployment are brought here, hopefully leading to the production of better aerators.

Aeration is an essential means to achieve higher yields in ponds. Aeration systems are designed to achieve several goals:

g. **Supply oxygen to cover oxygen consumption and to overcome oxygen limitations and thus enable higher stocking growth and yields.**

h. **Distribute the oxygen in the pond, (1) horizontally and (2) vertically.**

i. **Mix water and sediment – water interface.**

j. **Control sludge coverage, location and drainage.**

It is important that aeration system will be designed, deployed and operated so as to achieve all the goals mentioned above as much as possible, in addition to merely supplying oxygen.

Approaching the achievement of all of these goals depends upon proper selection and planning of aeration capacity, aerator type, aerator location and operation mode as well as the appropriate design of aerated ponds.

- ***Supply and Demand of Oxygen***

Terrestrial life is not normally limited by the availability of oxygen. Oxygen makes about 21% of the air mass and there is normally plenty of air. However, oxygen is a limiting factor in aquatic systems. Solubility in water is very low, about 8 mg/l in fresh water, yet less, about 6 mg/l in saline water (See Table 10.1). As shown later, oxygen holding capacity in the water is too low to support fish life and pond respiration, unless oxygen is efficiently replenished.

Table 10.1: Oxygen saturation as a function of Salinity and temperature (Colt 2012)

Temperature (C)	Salinity (g/kg)		
	0	15	35
10	11.29	10.26	9.02
20	9.09	8.32	7.40
30	7.56	6.96	6.27

on conditions at the bottom of the pond. Oxygen concentration in saline water is lower than that of fresh water (Boyd and Tucker, 1998; Fast and Boyd, 1987). In addition, oxygen saturation is lowered with the increase in temperature. Thus, oxygen saturation for fresh water at 20°C, common for many fish ponds, is 9.08 mg/l, but in 30 ppt salinity, at 30°C, as common in shrimp culture, oxygen saturation is just 6.39 mg/l, i.e. 70% of the previous level. Maintaining appropriate oxygen levels is critical in intensive BFT ponds used to hold high fish density. A very short disturbance of aeration (minutes to hours) may be critical in such systems.

As a very first approximation, oxygen balance can be estimated by knowing the feed addition to the pond. Daily feed addition in grow-out ponds is usually 2-3% of fish body weight. Assuming a 2.5% daily ration and a fish biomass of 2,000 Kg/ha, the addition is 50 kg/ha x day. Feed materials contain about 50% carbon, i.e. a daily addition of 25 kg C/ha.

On average, only about 13% of the added carbon is assimilated by the fish, and we can assume that the total assimilation by fish and other organisms in the water adds up to 20%. The rest is respired, consuming oxygen, at an amount of 2.67 g (32/12) O_2 for 1 g carbon:

(10.1) $$C + O_2 \rightarrow CO_2$$

The amount of oxygen consumed through carbon potentially respired in the pond is thus:

(10.2) Daily O_2 requirement = Feed application * 50% * 80% * 2.67

~ 1.07 * feed application

In general, the ultimate oxygen consumption can be approximated as one kg oxygen for each kg feed. This is a simple and important first approximation. For the pond under consideration, the ultimate daily oxygen consumption will be 53.4 kg O_2/ha (5.3 mg/l for a 1m deep pond).

The estimate above does not take into account rates of the different processes involved. Some of the non-utilized organic carbon is rather stable and is respired way after the feed addition. Another neglected process is the sedimentation of organic residues to the pond bottom, where it undergoes a slow degradation, often along future cycles of culture. This residual effect can be minimized if pond bottom is either drained out or dried, allowing a fast degradation and removal of stored residues between crops when it is exposed to atmospheric oxygen. In addition, organic matter (=oxygen consumption) can be removed by draining the sludge, as commonly done, especially in tilapia BFT ponds.

Another important process affecting oxygen levels in the pond is algal photosynthesis (PP):

ment of ponds is complicated, require more refinements.

The estimates for oxygen demand, as given above, are the ultimate long range demand and, as such, are typically higher than one would expect from real-world experience. The actual dynamics of oxygen demand in ponds is complex, depending on several physical, chemical and biological factors (Pierdrahita, 1991; Smith, 1991). Due to these complexities, the extent of artificial aeration is not usually based upon comprehensive modeling, but rather based upon empirical approaches. Boyd and Tucker (1998) evaluated the extent of needed aeration based upon information on oxygen demand of shrimp or fish, by approximating an added biomass of about 500 kg shrimp/kw aeration. Common oxygen demand of shrimp or fish is 200-400 mg/ (kg fish x hr), or an average of 3.6 g O_2/kg fish per night. An oxygen supply of 1800 g O_2 by 1 kW aerator will suffice to add the oxygen needed for 500 kg shrimp. This approximation does not consider the addition of organic matter nor the change of pond's oxygen demand with the rise of shrimp biomass and feeding. In addition, it has been shown that when conditions in ponds improve, the efficiency of oxygen increases. In commercial ponds in Indonesia (discussed in detail in chapter 13), it was shown that by improving conditions and management in BFT ponds, power efficiency (kg shrimp/ kW) rose from about 500 kg/ kW up to 1,000 kg/ kW. However, the 500 kg/kW estimate seems to be a good initial estimate.

An important issue is the determination of oxygen supply by different aerators. Extensive work aimed at evaluating oxygen supply by a variety of aerators in fresh water ponds was performed and published by Boyd and Ahmed (1987) Three terms are used to define oxygen supply capacity: (1) Standard Oxygen Transfer Rate (SOTR - kg O_2/hr), defines the amount of oxygen transferred by an aerator to the water when the temperature is 20^{0}C, and oxygen concentration in the water is zero; (2) Standard Aeration Efficiency (SAE - Kg O_2/kW/hr), is equal to SOTR divided by the power applied to the aerator shaft (again, for zero oxygen concentration);

(3) Actual Oxygen Transferred (AOT) to any given pond at a given or average oxygen content. A very important factor affecting the transition between SAE and AOT, the actual transfer, is the oxygen deficit driving the process. Oxygen transfer is considered to be:

(10.4) $$dO_2/dt = Kl_a (C_s - C_m)$$

Where dO_2/dt is the rate of oxygen transfer to a unit area of pond, Kl_a a constant specific for a given system (aerator, water) and $(C_s - C_m)$, the oxygen deficit, the difference in concentration between the saturation level C_s (for the given conditions, temperature and salinity) and the concentration in the pond, C_m.

To clarify this point, let us compare oxygen transfer in say waste water treatment plant where O2 concentration is 1mg/l and fish pond where an O2 level of 4 mg/l is maintained. The temperature

Under standard conditions, 20^{0}C and **no salts**, water is **saturated in respect to oxygen at a point of** 9.1 mgO_2/l. However, for typical shrimp ponds, or other warm water ponds, at T=30^{0}C and salinity of about 30 ppt, oxygen at the saturation is 6.4 mg/l. The oxygen deficit as used to compute SAE for a fresh cold water pond is 9.1 - 0, = 9.1. Assuming we want to maintain a concentration of 4 mgO_2/l, the deficit in a standard fresh water pond (20^{0}C) will be 9.1 - 4, = 5.1. However, for a typical shrimp pond, the deficit will be 6.4-4 = 2.4 mg/l. The deficit in the case of the shrimp pond is accordingly about half that of a standard fish pond. Since oxygen transfer is supposed to be a direct function of the deficit, oxygen transfer is also supposed to be half. If we want to maintain a higher oxygen concentration (e.g. 5 mg/l) oxygen transfer efficiency is also lowered. Lowering oxygen transfer efficiency due to salinity is compensated to some extent by the effect of salinity on water drops or bubble size, due to its effects on surface tension. Oxygen transfer is enhanced due to the lowering of drops and bubbles size. Unlike the very detailed study of oxygen transfer in a fresh water system, there is not enough information enabling prediction of the oxygen enrichment by different types of aerators in salt water systems. Such research is needed.

The common practical way to determine needed aeration capacity is through trial and error, experiencing the minimal aeration needed to maintain a sufficient oxygen level, usually a level higher than 4 mg/l. The common aeration applied to shrimp ponds in South East Asia (Boyd and Tucker, 1998) is 5-10 kW/ha (3.7-7.4 hp/ha). Common aeration in semi intensive ponds in Brazil is in the range of 8-12 hp/ha. Higher aeration rates are applied in intensive shrimp ponds, from 20 to more than 60 hp/ha (McIntosh, 2000). Intensive fish ponds, carrying a much higher biomass (100-300 ton/ha) are aerated at a capacity of more than 100 hp/ha. A commonly used approximation (Boyd and Tucker, 1998) is that 1 kW supports an increased yield of about 500 kg shrimp (370 kg/hp).

- ***Oxygen Activity- Aerobic and Anaerobic Conditions***

Oxygen is an essential component affecting fish life as well as a variety of biological and chemical processes in the pond.

The extreme situations, of totally oxygenated or non-oxygenated conditions are very often considered (i.e. systems with ample oxygen supply as compared to no oxygen, when fish suffocate). However, there is a continuum of oxygenation levels, from fully oxygenated systems down to highly reduced systems with zero, or close to zero oxygen.

By chemically defining the oxidation – reduction system, oxygen is defined as an electron acceptor while reduced components are the electron donors. If we observe the process:

(10.5) $$H_2 + 1/2\ O_2 \rightarrow H_2O$$

Each hydrogen atom donates an electron to the oxygen atom, the electron acceptor. Systems that system. A water sample that contains oxygen at saturation or close to it will have a redox potential of about 500 mv, while a system with absolutely no oxygen and a presence of highly reduced hydrogen sulfide will have a redox potential of about -100 mv.

A series of metabolites are formed in the anoxic or anaerobic bottom water and sludge (Table 10.2).

Table 10.2: Redox reactions in pond bottom

Electron acceptor (oxidizing system)	Process	Approximate redox potential (mV)
Oxygen- O_2	Aerobic respiration ($C + O_2 \rightarrow CO_2$)	500-600
Nitrate- NO_3^-	Denitrification	300-400
Organic components	Fermentation	< 400
Fe^{+3}, Mn^{+4}	Reduction	200
SO_4, S	Sulfur reduction	-100
CO_2	Methane fermentation	-200

* Adapted following Reddy et al., 1986.

When oxygen concentration drops, anoxic conditions develop and nitrates are reduced (denitrification). (The term anoxic conditions describes a situation when oxygen concentration is low but not absolutely zero) Denitrification is performed by bacteria that are essentially aerobic, but can adapt to low oxygen (anoxic) conditions and can use nitrate as an oxygen source to decompose organic matter (schematically, CH_2O in equation 10.6):

$$4\,NO_3 + 5\,CH_2O + 4\,H^+ \rightarrow 2\,N_2 + 5\,CO_2 + 7\,H_2O \quad (10.6)$$

Denitrification takes place at a redox of 300-350 mv. Moreover, as long as nitrate is present, it poises (buffer, stabilize) the water and the redox does not further decline. This is an important feature of the redox system in ponds, to be further discussed. Subsequently, when nitrate is reduced and the redox conditions are severe, anaerobic metabolites are generated, such as a variety found in fish pond sediments at levels of 1,000-5,000 ppm (Kochba & Avnimelech, 1995), are also toxic. Butyric and lactic acids are toxic to fish and Daphnia at concentrations above 100-200 ppm (Richardson & Gangolli, 1994).

The bottom sludge is defined as an organically-enriched, anaerobic, soft and normally black layer that develops at the bottom of ponds. The sludge develops when organic matter is metabolized at the pond bottom under low oxygen conditions. This is commonly the case, due to the limited supply of oxygen to the pond bottom and to the high oxygen demand induced by the accumulation of organic residues. Sludge accumulation increases with an increase in stocking and feeding rates. The accumulation of sludge and the development of anaerobic conditions in the sediment were shown to be a factor limiting fish growth (Avnimelech and Zohar, 1986). The effect of sludge on shrimp growth may be especially hazardous, since shrimp live and eat at the pond bottom. Hopkins and co-workers (1994) grew shrimp at high density in zero water exchange lined ponds using three treatments: (a) control, no sludge treatment, (b) sludge mixed periodically and (c) sludge removed periodically (Table 10.3). Shrimp in treatment (a) hardly survived (survival = 0.2%) as compared to a reasonable survival and growth in the two other treatments. This was a non-replicated experiment in small ponds, so results could be different from those found in commercial shrimp ponds, yet the trend is clearly demonstrated. In another experiment (growing shrimp in concrete raceways, Avnimelech), it was found that sludge removal raised shrimp feed consumption, to 136 ± 11% as compared to that before removal. It can be anticipated that the environmental stress induced by the sludge limits growth and is conducive to disease outbreaks.

Table 10.3: Effect of sludge on survival and growth of shrimp in zero water exchange pond

Sludge Treatment	Remain	Remove	Re-Suspend
Survival (%)	0.2	32.8	54.1
Production (Kg/Ha)	18	2406	3474

* Adapted from Hopkins and co-workers, 1994.

Anaerobic sludge may accumulates in specific locations even in highly aerated and mixed intensive ponds, including BFT ponds. The local accumulation of the sludge may be due to imperfect mixing of the water, e.g. in corners, in the center of radially mixed ponds, in low lying locations and very often under and beyond the paddle wheel aerators. Though the pond is highly aerated, these anaerobic pockets may inflict damage to fish and to reactions in the pond. One of the most essential pond management practices is to minimize the extent of sludge accumulation sites. This may be done by proper design of the pond, proper location and selection of aerators and if needed, mechanical removal of the accumulated sludge, as discussed later.

corrective action. On the other hand, the presence of nitrates in the pond prevents lowering redox and buildup of sulphides. Thus, the effects of low oxygen may be counteracted by adding nitrate containing fertilizers. To some extent, the presence of nitrates in the water replaces oxygen: It does not supply oxygen to the fish, but it keeps the oxidizing potential of the water at an intermediate stage and can be looked upon as a means to slowing down the damage from low oxygen.

- ***Oxygen Distribution in the Pond***

Aerators are operated to provide other services in addition to oxygen replenishment. An essential additional service is the mixing of the water. As in most bio-technological reactors, microbial processes are more effective in mixed water, mostly due to the enhanced diffusion of substrates in microbial cells and the outward diffusion of metabolites. Mixing of aerated water in ponds is, in a way, a transition toward a semi-industrial biotechnological system. It was shown that organic degradation and nitrogen transformation in mixed fish ponds are more effective than in stagnant water (Avnimelech et al., 1992).

Mixing prevents water stratification, i.e. development of an oxygen rich, warm layer at the surface of the pond and an oxygen poor layer at the bottom. Another important service of water mixing is the control, or at least the partial control, of sludge accumulation at the bottom of the pond.

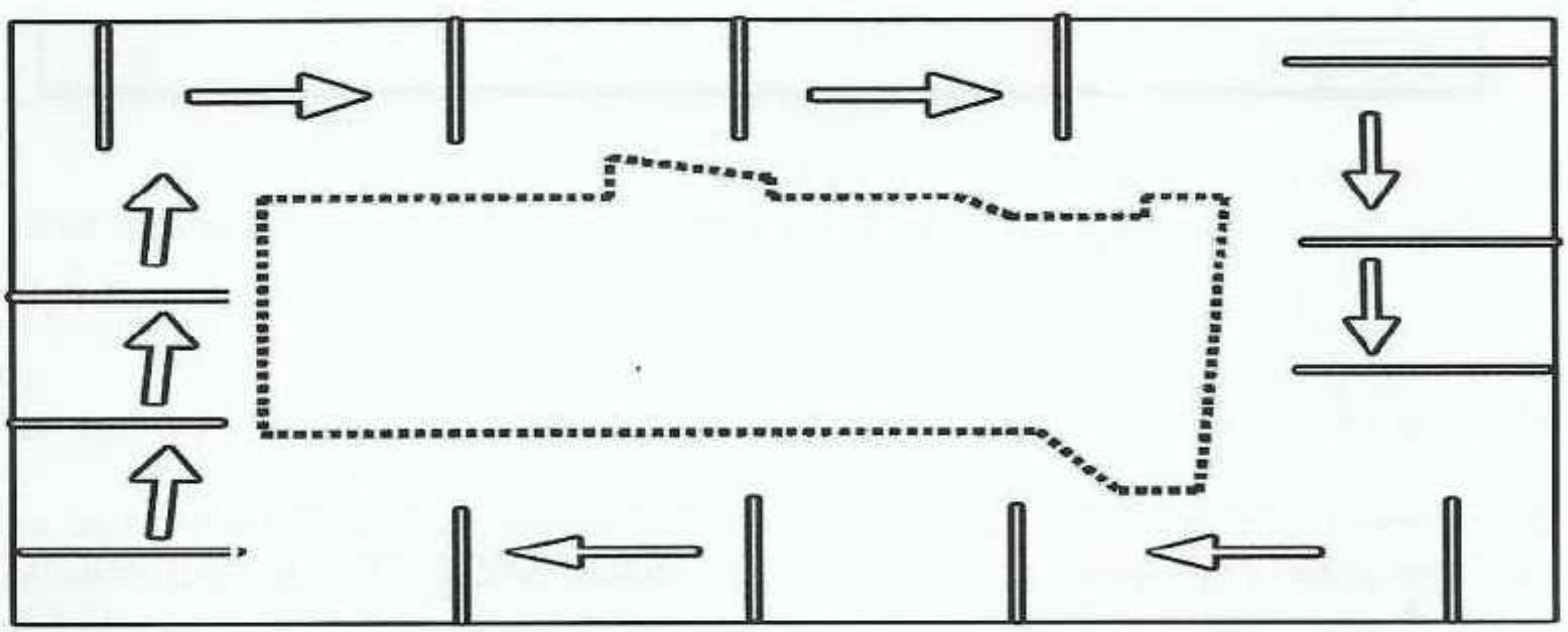
Aeration in semi-intensive fish ponds is often applied only during night time. Moreover, night time or emergency aeration is applied usually at a single point in the pond, creating an aerated zone that can be used as an oxidized shelter for fish during periods when oxygen is low. It is assumed that fish swim to that region and thus the deleterious effect of low oxygen can be avoided. In such ponds, there is no effort to aerate the whole pond (see Figure 10.1).

Figure 10.1: Aerators placed in one location in the pond[1]

Avnimelech and Ritvo (Un-published) tried a different approach, based upon the aeration and mixing of as much of the pond area as possible. This was done by placement of paddle wheel aerators and water circulators (see section B of this chapter) in the pond, using a total energy input of 6-12 hp/ha. In addition, unlike the conventional approach of activating aeration only during night time, aerators were used during most of the day, except for hours with strong winds. This approach led to a consistent 150% rise of tilapia yields in a series of commercial pond experiments in several farms in Israel.

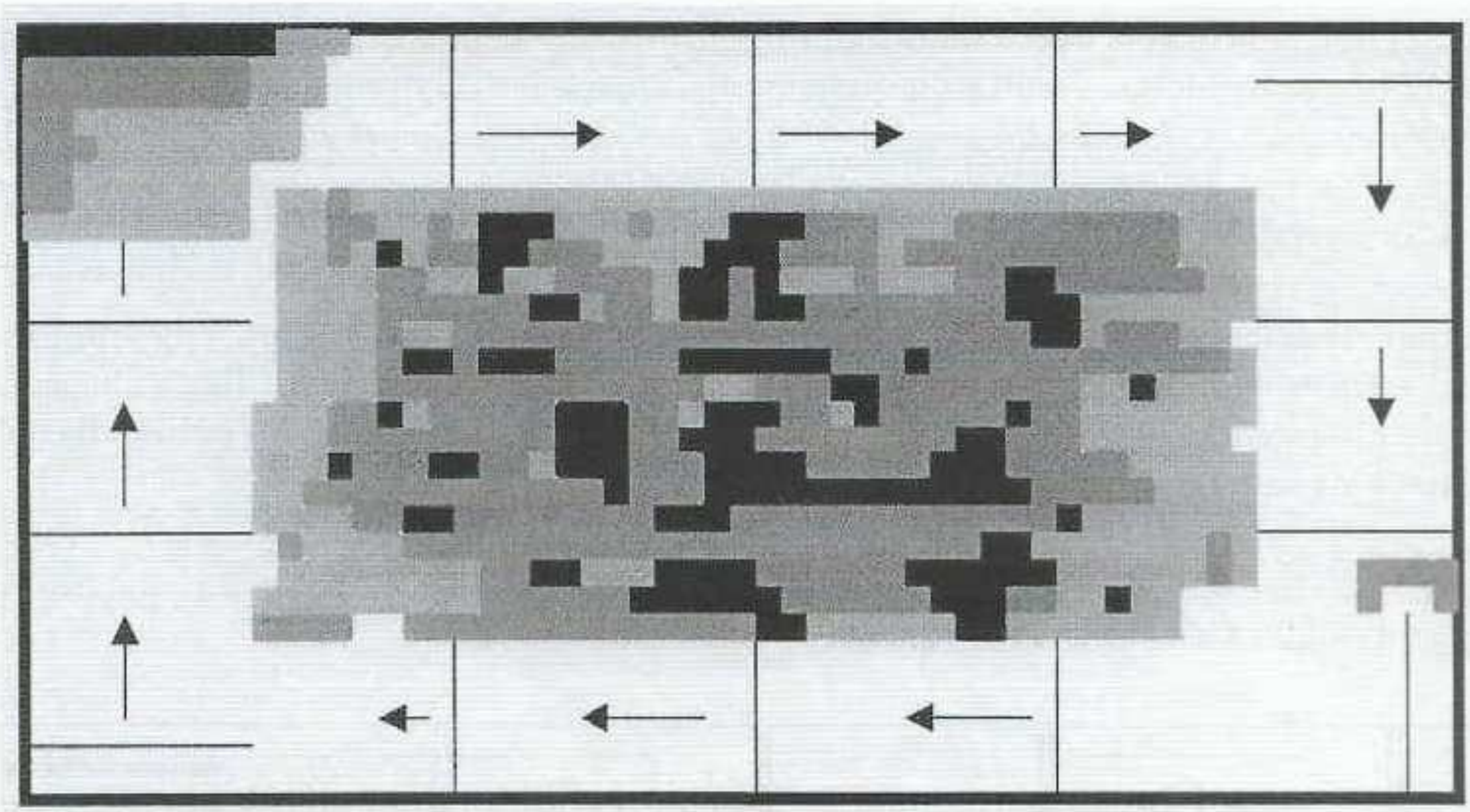
Common systems of shrimp pond aeration developed in South East Asia are long arm paddle wheel aerators with motors (diesel or electrical) located on the pond dykes. This configuration dictates placement of aerators parallel to the dykes, at a distance of at most 10 m from the dyke. The resulting water movement is elliptical (Figure 10.2(a)).

Figure 10.2 (a): Stagnant area (Water velocity < 1 cm/sec) in 1.2 ha shrimp pond equipped with long arm paddle wheel aerators (2 hp each)



Other types of paddle wheel aerators used elsewhere are tied to poles on the dykes and are thus usually close to the dykes, creating a similar water flow pattern. This very common mode of aerator placement creates a peripheral flow near the dykes and an area with no flow, or very limited flow, in the center of the pond. The physical aspects of this radial or elliptical flow were thoroughly analyzed and reported by Peterson and co-workers (2001). Suspended particles settling down in regions with fast water movement are re-suspended back into the water. However, this is not the case in the central area of radial flow. In this area, water velocity is very low, even zero, and settled material cannot be re-suspended and thus accumulates. This phenomenon is described in physics as the "tea cup effect". You may place tea leaves in a glass, stir it to generate a radial flow

Figure 10.2 (b): Sludge coverage in a 1.2 ha plastic lined shrimp pond 1.2

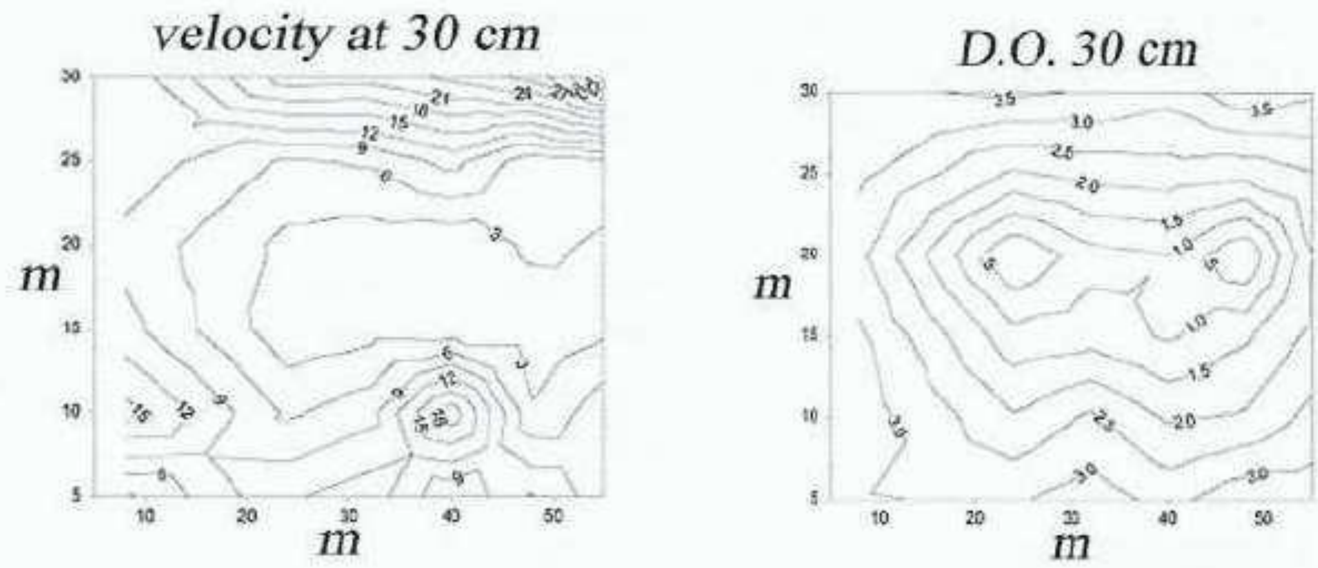


(1) Same as in Figure 10.2 (a)
(2) Black color >10 cm deep; gray 5-10 cm; light gray <5 cm and white color practically none

Sludge at different depths covered about 40% of the pond's area.

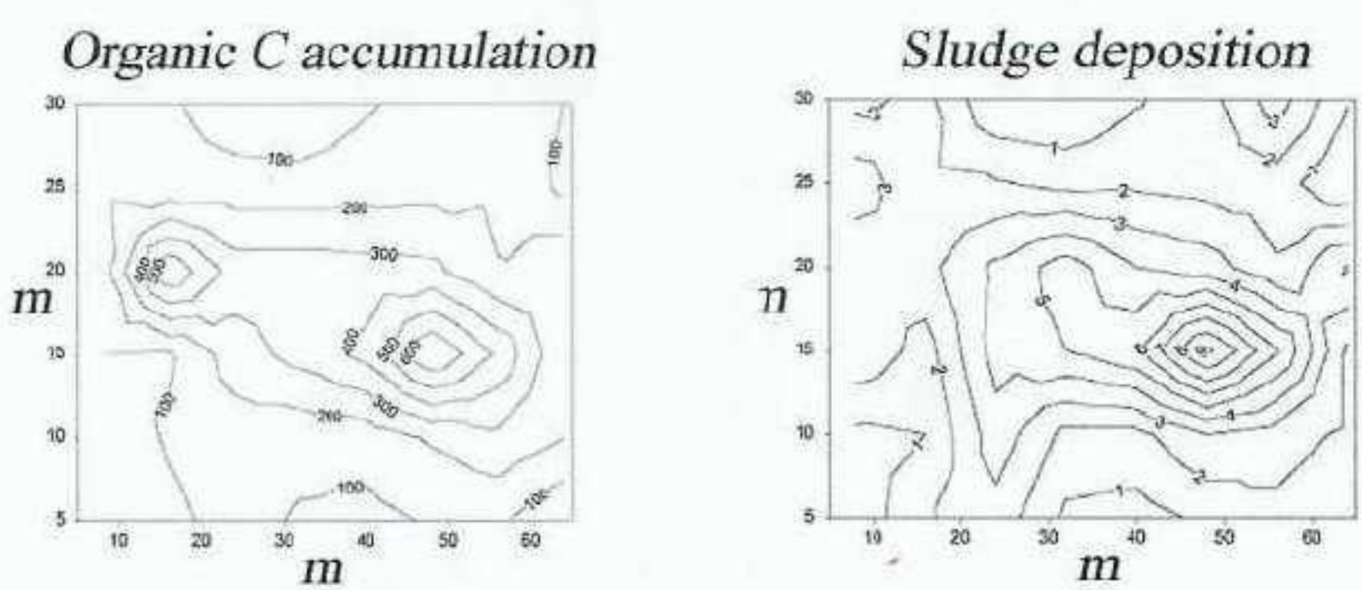
The effects of radial flow in a relatively small pond (0.25ha) equipped with 3 paddle wheel aerators (total power 5 hp, equivalent to 20 hp/ha) were studied by Calle -Delgado and co-workers (2003). A central stagnant region having a flow rate of less than 2 cm/sec covered about 25% of the pond area (Figure 10.3). Early morning oxygen concentrations in this region were very low (0-1 mg/l). Sludge accumulation paralleled the water velocity pattern (Figure 10.4).

the pond bottom



* Calle Delgado et al, 2003.

Figure 10.4: Deposition of sludge (kg/m²) and organic carbon (g/m²) along one cycle of shrimp culture



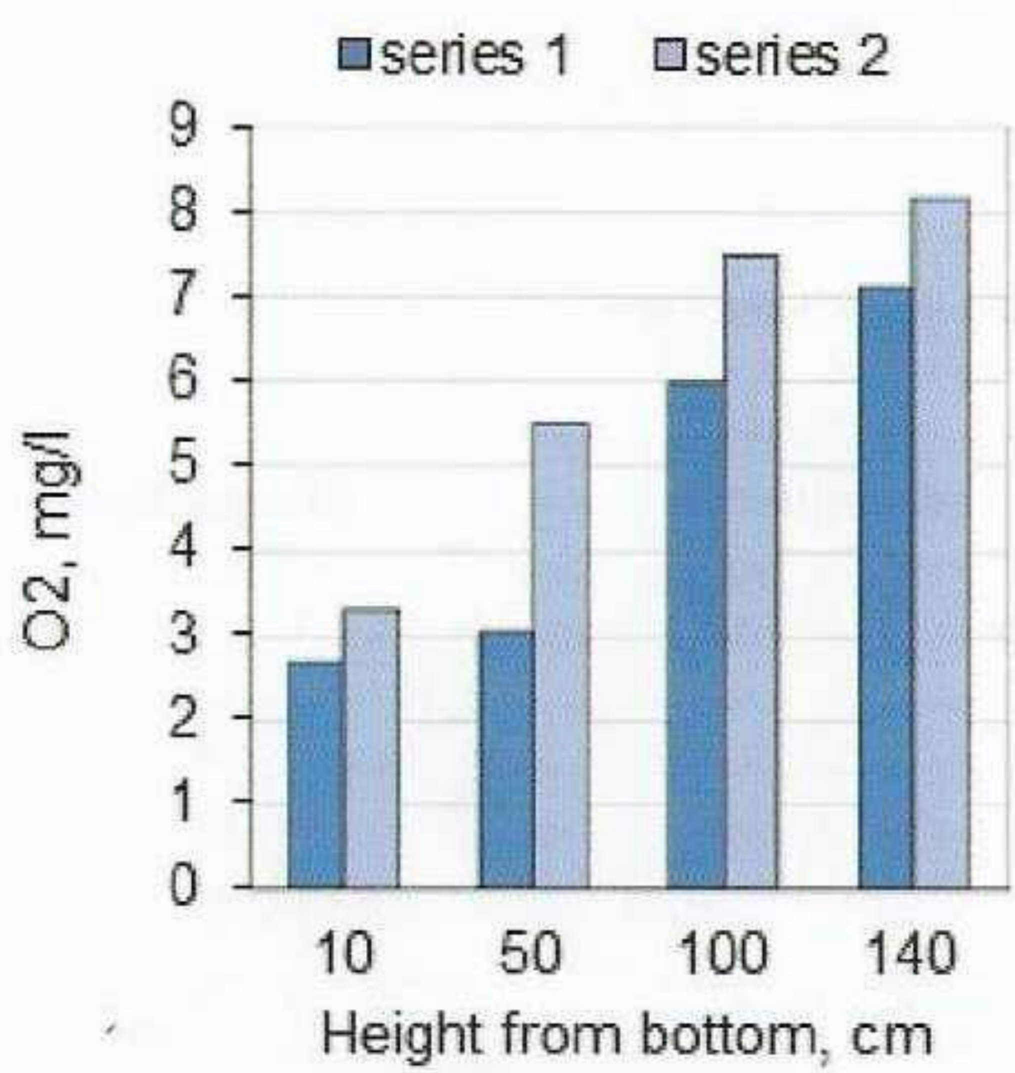
* Calle Delgado et al, 2003.

It can be seen that sludge deposition was less than 1-3 kg/m² in the outer shell but 4-9 kg/m² in the central zone, comprising about 30% of the pond area. Organic carbon accumulation in the could not survive due to the prolonged exposure to the bottom sludge.

Vertical distribution patterns of oxygen were also demonstrated. Oxygen concentration was uniform throughout the water column in the mixed peripheral region, yet clear oxygen stratification was obvious in the central stagnant zone: Oxygen was lower at the bottom during early morning hours as well as during the afternoon hours, when the surface layer was enriched to a level of super-saturation with oxygen.

Similar results were obtained in a commercial shrimp pond in Colombia, pond containing plentiful algae coverage. Oxygen profiles were monitored across the pond at about 11:00, on a sunny day, when oxygen supply is likely to be optimal. Oxygen concentrations in the surface layers were indeed very high, in fact, slightly super-saturated (Figure 10.5).

Figure 10.5: Oxygen profile in a commercial shrimp pond, 11 AM



* Avnimelech and Serna, unpublished.

water by wind, some aerators have to be kept on even during the daytime, otherwise, shrimp may suffer from anoxia even during active photosynthesis daylight periods.

B. Aerators, Required Development of Suitable Aerators and Aerators Deployment

Comments:

1. Drs. John Colt and Ronald Malone helped tremendously, by discussing the ideas raised here.

2 The proper scientific term to define power for aerators is Kilo-watts (kW). However, the more popular term used in the field is horse-power (hp). (You buy an aerator defined by its hp). The term used here is hp. The conversion factor relating the two terms is: 1 hp = 0.746 kW.

- ***Common presently used aeration systems***

The most common aerator used is the **paddle wheel aerator,** produced using different sizes (1-15hp) and shapes as long arm paddle wheels or smaller electrically powered units (e.g. figures 10.1, 13.1. 13.2 or 18.2). The action of the paddle wheel aerator is based upon splashing large volumes of water, in small droplets, into the air. The "cloud" of splashed droplets generates a large air to water surface area, an interface across which oxygen is diffusing from the air into the water side of the interface (as well as CO_2 diffusing out from the water droplets to the air). The water is splashed upward, as well as horizontally, and thus it applies a momentum to the pond water and generates a horizontal water current. This water flow helps in distributing the aerated water across the pond, in a pattern as discussed in the previous section, and helps in mixing the pond water.

A somewhat different type of aerators are the vertical aerators (Fig 10.6). Here, water is sprayed upward to the air by an impeller held by floats just underneath the water surface. In contrast to the paddle wheel aerator, the vertical aerators do not generate any directional vertical water flow.

Different aeration systems, are the **Diffused air systems.** These systems, used mainly in small tanks and ponds, are based on the release of small air bubbles to the water. The basic operative units are diffusers, made of ceramic air stones, porous rubber tubes and others, connected to a blower releasing a stream of small air bubbles rising in the water column. The principle of the diffused air systems operation is the same as that of the paddle wheel aerator, generating a large air-water interface. Yet, in this case it is the sum of air bubbles surface rather that water droplets ones. The efficiency of the diffused air systems rise with the decrease of the bubble size, yet, the smaller mostly by increasing the contact time but in part by raising the air pressure at the point of release.

A special case of diffuse air systems is the **air lift.** If we inject air bubbles into a vertical tube submersed in the water, the apparent density of the water in the tube (i.e. the density of a unit volume of water + air in the tube), is lower than that of the water in the pond outside this tube. As a result, outside pressure will lead to a rise in the elevation of the water, or to a flow of water from the pond through the tube. (e.g. If 30% of the volume is occupied by air, the apparent density of the water will not be 1, but 0.7 g/ml and water elevation at equilibrium will be 43 cm above water level in the pond. (See Figure 10.7.a)

An additional group of aerators are **aspirators**, based upon the Venturi principle (Fig 10.8). The **Venturi effect** is the reduction in fluid pressure resulting when a fluid flows through a constricted section of pipe and released. In an aspirator, water flows through a tube which then narrows. When the tube narrows, the fluid's speed increases, and because of the Venturi effect, its pressure decreases. Vacuum is created at this point and if connected to the atmosphere, air is sucked in and mixed with water out flow. A group of aspirators, propeller-aspirators are made of a unit with a built in electrical motor and a propeller generating a strong water jet. The water jet passing through a restriction pulls air into the water jet. The jet, containing very fine air bubbles is directed diagonally toward the pond bottom. Another type of Venturi aerators, used in small ponds is made of units having a diameter restriction and connection to the outside air. Fast water flow is generated by connection to a source of pressurized water.

A unit that may help aeration though rarely used in aquaculture is the "**pond water circulator**". The water circulator consists usually of a propeller, usually operated by a submersible electrical pump. The circulator is pushing water horizontally, in most cases to one direction. A very low pressure is needed to push water horizontally (no pressure gradient). Thus, high volume of water can be pushed, using a relatively low pressure. It has to be noticed that circulators as such just mix the water and do not bring oxygen to the pond. These circulators are used mostly in lakes in order to de-stratify the lake, i.e., to mix the water in the lake and disturb the development of stratified lake, having a relatively cold and oxygen poor bottom water layer. Avnimelech & Ritvo placed a 2.5 hp circulator in a commercial fish pond (about 8 ha in area and ~ 2 m deep). The effect of the water circulator was evaluated by monitoring sulfide concentrations in the topmost pond soil layer. Soil sulfide concentrations were close to zero in the direction of the circulator water jet up to a distance of 65 m.

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Do we need better/different aeration systems?

Reviewing the technical publications as well as field practice, one can be astonished by the fact that practically the same aerators are being used for the last decades, this in a period when is highest and diffusion of oxygen from the air is highest. (e.g., data in Fig. 10.5 show that water in this layer were over-saturated while water in the bottom layers had about 50% oxygen saturation). As shown in Eq. 10.4, oxygen transfer is a function of the difference between the oxygen saturation value for the given water (as depending on temperature, salinity and pressure) and the actual concentration of oxygen in the water. The efficiency of aerating the oxygen rich surface water is very low as compared to the possible aeration of the oxygen poor bottom water. As a demonstration we can assume fresh water pond where oxygen in the surface water is 5 mg/l and that of bottom water is 2 mg/l. The Aeration efficiency (values from Boyd 1998) for the surface water will be about 45% of standard efficiency (SAE) while for aerating the bottom water efficiency will be about 70%. An aeration device that will aerate the bottom water will probably be almost twice as efficient, supplying almost twice as much oxygen for the same power consumption. A simple suggestion made by Dr. John Colt (personal communication) is to modify the paddle wheel aerator to a Skirted paddlewheel. By inserting a barrier (plastic or metal sheet) on upstream side of a paddlewheel, the water would be pulled from the bottom (it has to come from somewhere). This would have to be strong enough to withstand water currents, but there are no hydrostatic loads.

The paddle wheel aerator favors oxygen replenishment of the surface layer, while oxygen is badly needed by the bottom water layers, and by the water soil interface. The paddle wheel aerator is doing a poor job in respect to it. The paddle wheel provides momentum to the water, pushing it horizontally across the pond. Yet, this momentum is given to the surface water, only partially used to move and mix the bottom water layers.

Vertical aerators pull water from deeper layers, though you need a powerful suction so as to pull bottom water. The oxidized water is released at the surface of the pond.

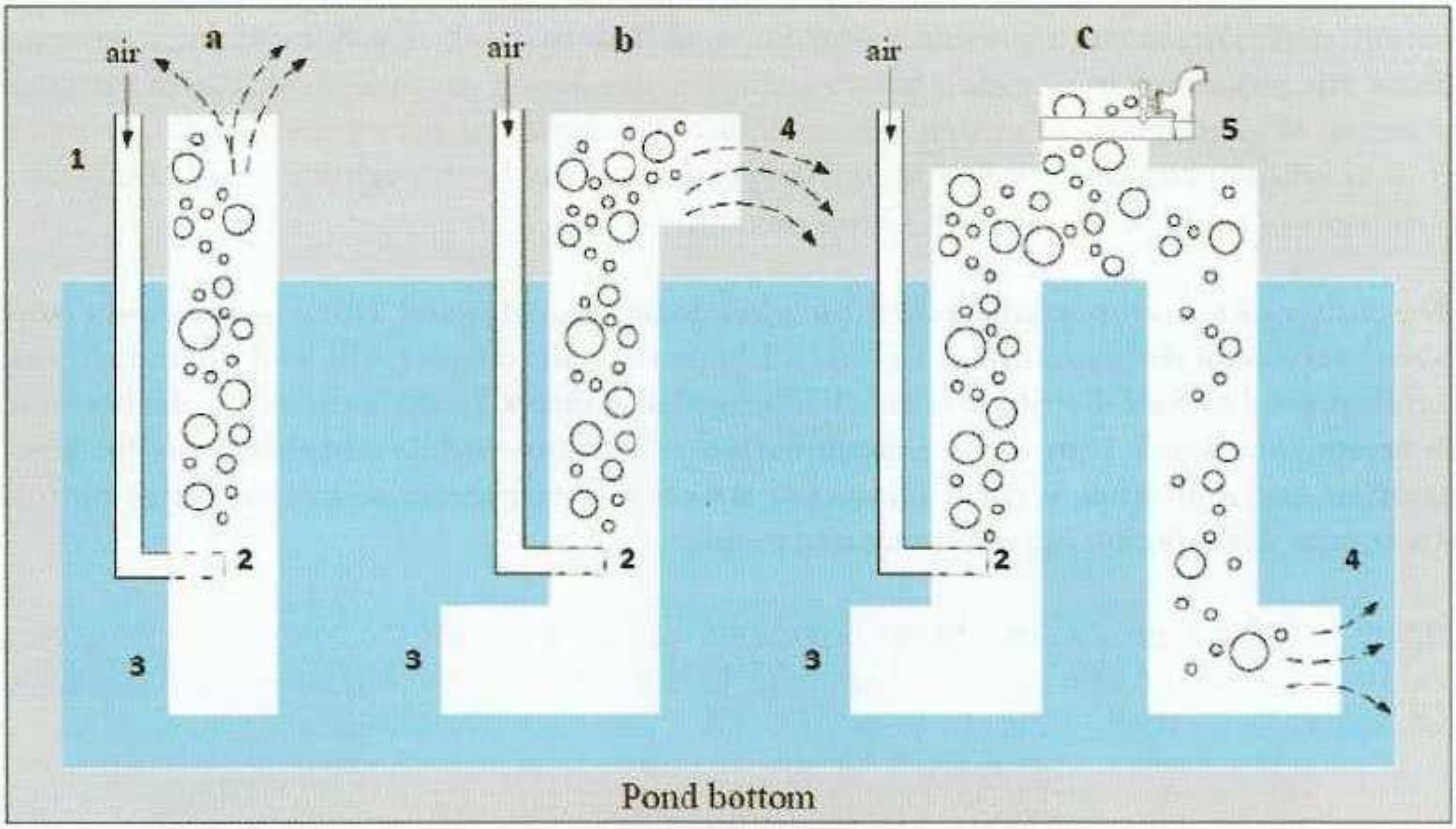
Figure 10.6 Vertical pump - Water is pumped upward with a submersible propeller and splashed onto the air.

Aspirators apply the oxygen to water of the topmost water layers, yet have the advantage of sending the aerated water jet downward. Yet, the effect of the jet is limited to a point of the pond bottom.

Diffuse air systems as such do not have an efficient mixing potential and unless placed in deep parts of the pond loose efficiency due to rapid rise of bubbles. It seems possible to use airlift technology to improve aeration systems.

Air-lifts are made by injecting air bubbles into tubes inserted in the water. The bulk density of the water with air bubbles inclusion is lower than that of the pond water, thus water outside flows into the lift and pumps the water upward. (Fig 10.7 a). The airlift unit pumps water from the pond bottom, an important advantage. If 90^0 inlet and out let tubes are placed, the same unit induces horizontal water movement in both bottom and surface layer (Fig 10.7b). These units were found to be very useful in aerating and mixing relatively small intensive tanks.

Figure 10.7 : Standard Air lift and proposed modifications



(1) Air source (blower); (2) Air inlet, fine bubbles; (3) Water inlet; (4) Water outlet; (5) Valve controlling return air distribution

William A, Wurts (2012) suggested the use of modified air lifts units including an attached blower, units that can be used for commercial scale ponds.

The airlift design may be further developed by us, so as to pump the water from the bottom layer, release the aerated water into the bottom layer and to generate a horizontal flow at the bottom of the pond. (Fig 10.7 c). The unit is made of an intake arm, roughly an ordinary air lift. A controlled air valve is placed in the horizontal part and an outlet arm follows. The air valve releases part of the rising air bubbles and thus, the water in the outlet arm is heavier than that in the inlet arm. If the air release valve is fully open and releases most of the air bubbles, water flow through the system will be maximal. On the other-hand, in case of limited release, flow will be lowered, but the down going bubbles will further release oxygen to the water. The Omega (Ω) unit can effectively act as a double length air lift and thus almost double the oxygen uptake efficiency.

As mentioned above (See Figure 10.5) aerators should be operated in ponds (especially shrimp ponds) also during day light, in order to mix the pond water and supply oxygenated water to the bottom water layers so as to provide reasonable conditions to the shrimp. Some farmers are practicing this, by keeping some paddle wheels aerators active during day time. As discussed here, this is not an efficient means of mixing the pond, since you aerate an already aerated surface water. A more efficient and effective way to mix the pond water can be through the use of pond water circulators or through the use of an Omega aerator.

Presently, most aerators available in the market do not provide good, efficient and power conscious service to the aquaculture industry. I hope that this chapter will lead the industry to develop, produce and distribute better, badly needed aerators. In the meanwhile, the best way to aerate your ponds is by using a combination of aerators, paddle wheel aerators for mass aeration, and aspirators, vertical aerators or airlifts to better aerate corners or better control the central drain region in radially aerated ponds.

Figure 10.8 Aspirator aerator - Water is pumped diagonally toward the bottom (see the motor and propeller rod) and sucks in small air droplets. See in front of the aerators a patch of bubble rich water.

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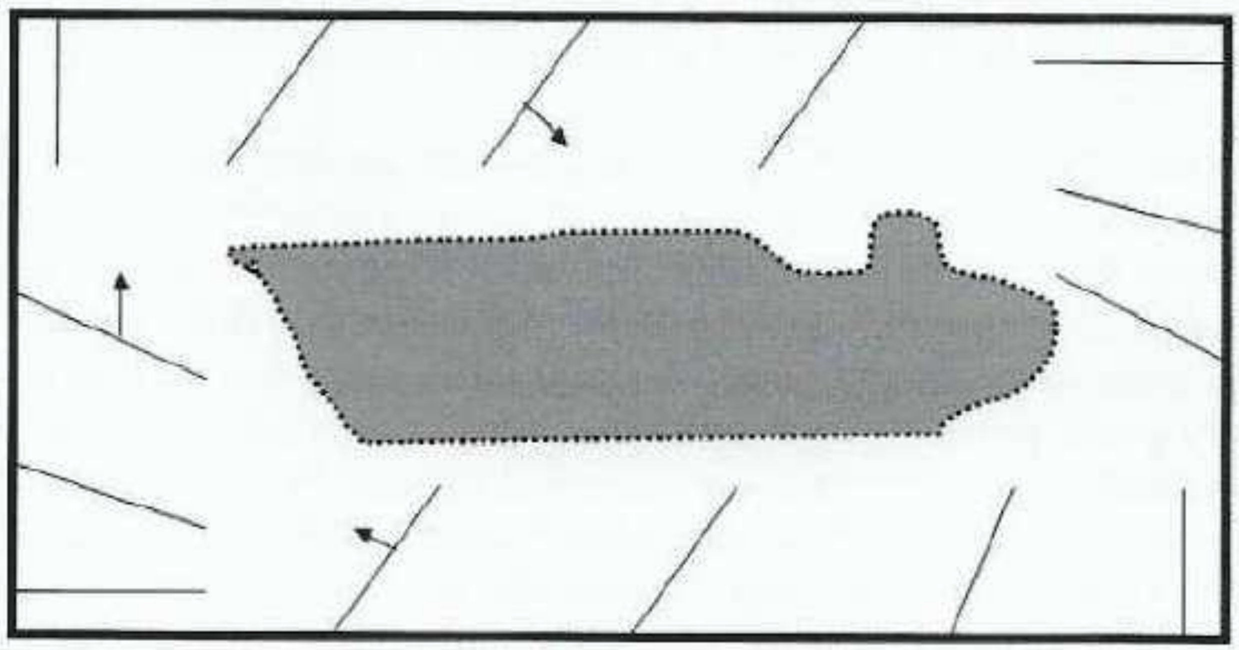
• *Aerator Deployment and Management Implementations* model. However, as discussed above, the creation of a relatively large stagnant region is a serious drawback to this method. Several possibilities are available to overcome, or minimize this problem. One example, as demonstrated in a commercial shrimp pond in Thailand, is shown in Figure 10.6.

Figure 10.9: Stagnant area (water velocity <1 cm/sec) following redirection of long arm paddle wheel aerators



The long arm paddle wheel aerators that are traditionally placed perpendicular to the pond dykes (see Fig 10.2.a) were turned and directed at about 30⁰ toward the center. A different water flow pattern was achieved, and the stagnant fraction of the pond was reduced from 25% to 18%. Another solution was demonstrated in Belize Aquaculture ponds. The paddle wheel aerators that were initially placed close to the sides of the pond, creating a narrow ring of flowing water and a large stagnant central region, were moved toward the center (McIntosh, 2000). This change led to a wider ring of intensive water movement, a better coverage of the pond area and a smaller sludge accumulation zone. The same principle was used in relatively large ponds in Colombia (8 ha ponds aerated with 8 hp/ha). The stagnant region in these ponds was found to be relatively small, about 10-15% of the pond area. It should be stressed, that due to the centrifugal forces, radial flow generated further away from the dykes, still induces a significant flow along the pond sides. The application of this method, especially in large ponds, demands that the anchoring system for the aerators be located close to the aerator (e.g., by anchoring the aerators to poles or weights on the pond bottom) and not by cables tied to positions in the dyke.

A very effective solution, applicable mostly in relatively small ponds or in ponds equipped with high aeration capacity, is to install a secondary system aimed at the sites of sludge accumulation, to facilitate sludge particle re-suspension. Such an arrangement has been used in small (50-1000 m^2) tilapia ponds (Crab and et al., 2009). The main aeration system in these ponds was based upon a number of paddle wheel aerators, creating a radial flow pattern. Sludge was thus accuor water circulators with a paddle wheel aeration system, in order to better mix the whole pond.

A very important point is the need to properly place the aerators prior to the accumulation of significant sludge piles. Mixing the pond bottom after the buildup of large sludge piles may lead to a collapse of the pond: The anaerobic particles are dispersed, oxygen concentration in the water drops quickly and anaerobic products are mixed in the water; a sequence of events that may be lethal to fish or shrimp. Treating anaerobic sludge piles, in cases where preventive steps were not taken, should be done gradually, in tandem with sufficient aeration and followed by careful monitoring.

A proper aeration system involves designing the pond for aeration and designing the specific aerator placement. To assist concentrating sludge in a small part of the pond, pond bottom topography has to be designed to have a sludge disposal pit at the same location where the aerators deposit the sludge. Thus, an elliptical flow pattern calls for a central drainage canal in the pond, rather than the common two lateral canals. In ponds with a radial flow pattern, it is advisable to dig a central sludge pit, preferably connected to a drainage outlet. Efficient sludge drainage is an essential requirement in intensive ponds carrying a high biomass. With high biomass, the organic suspended load cannot be effectively mixed with the water and deleterious sedimentation takes place. To overcome this and facilitate high biomass, effective drainage is needed.

Practical Implications and Tips

1. *Selection and placement of aerators is a critical step in managing ponds. Most failures in BFT ponds are due to wrong selection and placement of aerators.*

2. *Ample aeration is essential. Do not let oxygen go below 4 mg/l (certainly not below 3 mg/l).*

3. *Electricity is a major expense. Use aerators in a rational way. Start the season by operating ~ 25% of maximal capacity and raise aeration with the growth of fish and rise of feeding ration.*

4. *However, use aerators also during daytime in order to mix the water column. You may stop all aerators during windy hours.*

5. *Place the aerators in a way as to minimize formation of sludge piles. Place air-lifts or aspirators in places where the paddle wheels do not mix the sludge.*

6. *Drain sludge as needed.*

7. *Unfortunately there are no clear and simple rules as to the placement of aerators. Conditions are different for different locations, fish and ponds. Nothing can replace the experience of the farm manager.*

8. *One has to get into the pond, measure oxygen in different sites and depths, monitor NO_2, and detect sludge accumulation locations.*

9. *Be careful of re-suspending large anaerobic sludge piles.*

Further Research Needs

A large part of the work reported here is based upon the rather qualitative use of the physical principles involved and on accumulated experience.

The use of different aerators was studied extensively in fresh water ponds. The data on aerators efficiency in brackish and sea water ponds is insufficient. Further work is needed. In addition, the existing data is based mostly on tests of aerators in very small experimental ponds. The effects of different aerators on water movement and the up-scaling of the results to large ponds has to be worked out.

Placement of aerators in ponds is based to a large extent upon the farmer's experience, which means quite simply that you need to adapt to specific conditions. One important local parameter is the common wind pattern, a factor that clearly affects water movement, de-stratification and re-suspension of sediments. An important pioneering work on the modeling of water and sludge movement in aerated shrimp ponds has been done in recent years by Peterson (2001). Yet there is a need to continue in developing this work to a point that it may be used routinely for the design of ponds and pond aeration.

The industry is called upon to give the proper priority to R&D aimed at the production of more efficient and suitable aeration systems. Power usage in intensive aquaculture is the second high expense after feed. With the increasing cost of electricity and the environmental call to minimize power consumption, developing efficient aeration systems should be done soon.

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Zohar, G. 1986. The effect of local anaerobic conditions on growth retardation in aquaculture system. Aquaculture 58: 167–174.*

2. *Avnimelech, Y., Mozes, N., Weber, B. 1992. Effects of aeration and mixing on nitrogen and organic matter transformations in simulated fish ponds. Aquacultural Engineering 11: 157- 169.*

3. *Avnimelech, Y., Kochva, M., Diab, S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israeli Journal of Aquaculture- Bamidgeh 46: 119–131.*

4. *Avnimelech, Y., Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture. 220(1-4): 549–567.*

5. *Boyd, C.E. 1998 Pond water aeration systems. Aquaculture Engineering, 18:9–40*

6. *Boyd, C.E., Ahmad, T. 1987. Evaluation of aerators for channel catfish farming. Bulletin No. 584. Auburn AL: Auburn University/Alabama Agricultural Expt. Sta.*

7. *Boyd, C.E., Tucker, C.S. 1998. Pond aquaculture water quality management. Kluwer Academic Publication. Boston, MA, USA.*

8. *Calle-Delgado, P., Avnimelech, Y., McNeil, R., Bratvold, D., Browdy, C.L., Sandifer, P. 2003. Physical, chemical and biological characteristics of distinctive regions in paddlewheel aerated shrimp ponds. Aquaculture 217: 235–248.*

9. *Colt, J. 2012. Dissolved gas concentration in water: computation as function of temperature, salinity and pressure. Second Edition, Elsevier, Amsterdam.*

10. *Crab, R., Kochba, M., Verstraete, W., Avnimelech, Y. 2009. Bioflocs technology application in over-wintering of tilapia. Aquacultural Engineering 40: 105–112.*

11. *Fast, A.W., Boyd, C.E. 1992. Water circulation, aeration and other management practices. In Fast, A.W., Lester, L.J., (Eds.). Marine Shrimp Culture: Principles and Practice, Elsevier Science, Publication, Amsterdam.*

12. *Hopkins, J.S., Sandifer, P.A., Browdy, C.L. 1994. Sludge management in intensive pond culture of shrimp-effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. Aquacultural Engineering 13:11–30.*

13. *Kochba, M., Avnimelech, Y. 1995. Use of nitrate to increase production potential in fish ponds. Research report. Technion, Haifa, Israel.*

15. *Peterson, E.L. 2000. Observations of pond hydrodynamics. Aquacultural Engineering. 21: 247-269.*

16. *Peterson, E.L., Wadhwa L.C., Harris, J.A. 2001. Arrangement of aerators in an intensive shrimp growout pond having a rectangular shape. Aquaculture Engineering 25: 51-65.*

17. *Piedrahita, R.H. 1991. Modeling water quality in aquaculture systems. In: Brune, D.E., and Tomasso, J.R., (Eds.). Aquaculture and Water Quality. WAS, Baton Rouge, LA, USA.*

18. *Reddy, K.R., Feijtel, T.C., Patrick Jr., W.H. 1986. Effect of soil redox conditions on microbial oxidation of organic matter 117-156 pp. In: Chen, Y. and Avnimelech, Y. (Eds.). The Role of Organic Matter in Modern Agriculture, Martinus Nijhoff Publication, Dordrecht, The Netherlands.*

19. *Richardson, M.L, Gangolli, S. (Eds.). 1994. The dictionary of substances and their effects. Royal Society of Chemistry, Cambridge UK.*

20. *Smith, D.W. 1991. Mechanistic simulation modeling of phytoplankton-oxygen dynamics in aquaculture ponds. In: Brune, D.E., Tomasso, J.R. (Eds.). Aquaculture and Water Quality, WAS, Baton Rouge, LA, USA.*

21. *Wurts, W.A. 2012. Rectangular airlifts pump design outperforms cylindrical units. Global Aquaculture Alliance Advocate, 77-78.*

Chapter 11

BFT Effects on Fish and Shrimp Disease Control

In Brief

A citation from a paper by Loc Tran, Kevin Fitzsimmons and Donald Lightner, published a paper entitled: AHPND/EMS: From the academic science perspective to the production point of view. (Aqua Culture Asia Pacific, 2014, 10:14-18). Opens the present chapter:

- ***Potential of biofloc farming technology (BFT)***

I think BFT is a great technology with promising potential. However, it requires high investment, reliable power supplies, and knowledge. If it is successfully applied, the benefits can be enormous. However, not many people are able to apply this technology in shrimp farming. In addition, BFT requires technicians to have skills and experience."

Information on existing experience and information on the effects of bioflocs in controlling EMS and other diseases is given in this chapter. Moreover, the objectives of this chapter and of this book is to help farmers, scientists, students, technicians and farmers to be able to understand and master biofloc technology. We believe it is not so complicated.

Exciting possibilities for controlling diseases of fish and shrimp through the use of biofloc technology have been raised, studied and revealed in the last few years.

Practicing BFT helps to control the intrusion of diseases with the major infection vector, water. We grow shrimp or fish using zero or minimal water exchange, and thus we facilitate the achievement of biosecurity.

In addition, there are indications, for both shrimp and fish, that in the presence of a biofloc system, the animals are more immune and less susceptible to the spreading and occurrence of diseases.

Recent research shows the existence of some specific mechanisms, such as the production of short chain fatty acids or Poly-ß-hydroxy butyrate (PHB) by biofloc, components that may serve as an antiseptic mechanism. Biofloc's potential activity seems to overcome some of the drawbacks of conventional drugs used against disease in aquaculture.

Maintaining a dense fish or shrimp biomass level unavoidably includes risk of disease outbreaks. The animals are crowded, thus interaction with infected individuals and spreading of pathogens may occur very quickly. Pathogens are often preserved in the pond system and in the sediment for a rather long duration and may induce repeated infections. And, on top of this, the dense and intensive culture conditions may invoke situations of stressful circumstances, be it night-time low oxygen, buildup of metabolites, social stress, etc., all raising the probability of disease outbreak.

During the last few decades, the shrimp industry experienced events of viral disease outbreak, events that led to the collapse, or near collapse, of the flourishing shrimp industries in many countries. A disease named firstly as Early Mortality Syndrome (EMS), later defined as acute hepatopancreatic necrosis disease (AHPND), was detected in China, 2009. And later spread to other countries such as Vietnam, Thailand, and Malaysia, then spreading to South and Central America. The disease led to huge damages to the industry. The annual income loss in Vietnam was about 1 billion US$, similar loses in other affected countries.

The spread of diseases was promoted by the practice of producing shrimp in an open systems. To control water quality, farmers used to exchange water intensively. The common practice was to continuously drain some of the pond water to a nearby estuary or other water body and replace it with "fresh" water pumped back out of the estuary. Quite often, others used the same estuary, thus, the "fresh" water introduced into the ponds was actually a cocktail of all disease vectors and pathogens existing in the area. A disease infecting one pond would quickly spread to the whole area. The concept and practice of *Biosecurity*, restrains the effects of viral disease outbreaks on the shrimp industry.

The term *biosecurity* is rather new to aquaculture. It is defined as "the practice of exclusion of specific pathogens from cultured aquatic stocks in broodstock facilities, hatcheries and farms, or from entire regions or countries for the purpose of disease prevention" (Lightner, 2003). More and Frelier (2003) in a summary of means to prevent disease stated: "Water exchange bestows a high risk for disease outbreak when virus diseases are present. Water should not be exchanged if white spot syndrome virus (WSSV) is present and if disease probability is high. The practice of zero water exchange for the first 60 days is recommended when possible and open water flow through the pond system is discouraged".

The practice of biofloc technology ponds is one of the practical and available means of growing shrimp or fish in a biosecure method without exchange of water or by using minimal exchange in a way that either takes the water from within the farm (e.g. a recirculation reservoir) and/or carefully filtrates incoming water. The water introduced into the pond at the start of the cycle can be sterilized, within the pond or in a water supply reservoir, in all cases when disease input is suspected.

feed, enabled the operation of the farm with no outbreak of disease for extended periods of time.

There are indications that in addition to biosecurity, BFT raise the immunity of shrimp or fish toward diseases. An example of ponds constructed and operated in a region inflicted with viral disease was given by Manuel Grillo and co-workers in Panama (2000). The shrimp industry in Panama had been severely affected by WSSV, leading to a collapse of farms and low survival rates of shrimp. A different approach, using zero exchange system, was designed. Water was pumped and screened into a series of reservoirs. There were 20 production ponds, 4000 m^2 each, aerated at a rate of 20 hp/ha. Local feed pellets (25% protein) were used. At harvest, following an average 118 day culture period, it was found that survival was about 80%, and average production was 4,941 kg/ha which was considered excellent compared to other farms in the area. The conclusion of the authors was that "it is possible to grow shrimp commercially in areas of Panama where WSSV is endemic."

An example of achieving good survival and production in an area inflicted by viral diseases and poor environmental conditions is given by correspondence and reports of Ms. Ninuk Sri Maharti, a shrimp farm manager from Indonesia. The message related to shrimp survival was: "survival rates are 75% and improving with a feed conversion of 1.4. Although we have not fully succeeded in maintaining biofloc in grow-out ponds due to low C/N ratio, overall I think the yields (11-19 ton/ha) were not too bad considering that most shrimp farmers around have failed in shrimp culture under present conditions in our country due to some viral disease outbreaks, deteriorating water quality and environmental quality, etc. From here I conclude that closed system (with BFT as a part of its implementation) is one real promising option for shrimp culture now in my country".

More evidences are reported in several papers of Nyan Taw, related to long term results obtained in a large shrimp farm in Malaysia (See Chapter 13 and http://www.aesweb.org/shrimp_health.php for more details).

A thorough review and discussion on the effects of biofloc and similar system on curbing shrimp and fish diseases was conducted in a workshop held in Ho Chi Minh City, Viet Nam 9-10 December 2013 (Organizers: Yoram Avnimelech, Hoang Tung, Craig Browdy, John Hargreaves). The workshop was motivated by the large economic losses inflicted on the commercial shrimp aquaculture sector by EMS and by the evidences that this and other diseases can be controlled by using biofloc technology. Most of the workshop presentations are available from the web page of the Biofloc Technology Working Group of the Aquacultural Engineering Society (http://www.aesweb.org/shrimp_health.php).

- ***Effects of bioflocs on the immune mechanism***

A number of empirical observation were made, demonstrating the effects of biofloc systems on nate response. Here, the immune system adapts its response during an infection to improve its recognition of the pathogen. This improved response is then retained after the pathogen has been eliminated, in the form of an immunological memory, and allows the adaptive immune system to mount faster and stronger attacks each time this pathogen is encountered. **Shrimp do have an innate immune mechanism, but do not have an adaptive mechanism and immunological memory.**

A critical step in any immune response is the recognition of invading organisms. This is mediated by a group of proteins, called pattern recognition proteins or PRPs, which recognize and bind to molecules present on the surface of microorganisms. Because crustaceans lack antibodies, studying their innate immunity may help identify potential tool to avoid disease outbreaks.

Research done in the National Fisheries Research & Development Institute, Incheon, Republic of Korea compared immune components in shrimp PL's growing in clear water or in a biofloc suspension (Kim Su-Yong and co-workers, 2014). Some genes including prophenoloxidase1 (proPO1), prophenoloxidase 2 (proPO2), prophenoloxidase activating enzyme (PPAE), serin proteinase1 (SP1), masquerade-like serine proteinase (mas), and ras-related nuclear protein (Ran) related to shrimp immunity or response to pathogens were found in high levels in shrimp PL's held in a biofloc suspension

Shrimp growing in BFT ponds are constantly in contact with a large variety of micro-organisms in the system. The number of bacteria in BFT ponds is in the range of 10^6 to 10^9 cells/ml. The bioflocs contain a very large variety of organisms, bacteria, algae, protozoa, zooplankton etc. It was found, using molecular pyrosequencing technology that a biofloc system contains 1000-2000 different species (Jang 2011). The continuous contact of shrimp with this large variety of organisms, is supposed to constantly maintain high level of activity within the innate immune system of the shrimp and is thus supposed to raise the protection of shrimp population against disease out-breaks. **Bioflocs can be viewed as a mechanism that provides shrimp with pattern recognition and other molecules that lead to stimulation and maintenance of the non-specific immune system.** These molecules are provided to shrimp constantly and help in raising the immune potential of shrimp.

Gene expression was found to be greater in *L.vannamei* than in other shrimp species (*F. chinensis, Metapenaeus japonicus*), possibly related to better uptake of bioflocs by *L.vannamei* due to differences in morphology of the third maxilliped, which affects the ability of shrimp to capture and use bioflocs as food. Julie Ekasari and co-workers (http://www.aesweb.org/shrimp_health.php)

reported that phenoloxidase activity increases in response to organic carbon loading from ecology considerations was brought recently by De Schryver and coworkers (2014).

The causative agent of EMS/AHPND is probably a bacterium—a pathogenic Vibrio belonging to the Harveyi clade, presumably Vibrio parahaemolyticus. Luminescent vibriosis, as well as EMS/AHPND occur mostly during the first 10–45 days after stocking of shrimp post-larvae in the grow-out ponds.

These agents represent a group of opportunistic bacteria that develop fast in nutrient rich systems, lacking competition. Accordingly, they proliferate in newly stocked ponds or in ponds that were sterilized in order to seemingly destroy pathogens. However, these are the ideal conditions for the opportunistic bacteria population to develop. They lose this advantage in mature water. The microbial maturity of water can be described based on the ecological theory of r/K selection. Biologically matured water, characterized by a limited nutrient supply per bacterium are typified by the dominance of slow-growing bacteria, that can extract nutrients regardless on the low levels and compete under such conditions, the K - strategists. By utilizing the nutrients and maintaining low concentrations of nutrients, they eliminate the niches for fast growing bacteria, the r strategists, rapid growers that require high nutrient concentrations, a group including many disease-causing Vibrio spp. As such, K selective pressure in shrimp culture systems may avoid proliferation of the vibrio causing EMS/ AHPND.

It was reported that EMS/AHPND is less prevalent in ponds colonized by Copepods (small crustaceans used as live feed for the larvae of aquaculture animals). Copepod presence is an indicator of a naturally mature/stable ecosystem, as it requires constant amounts of phytoplankton and bacteria as feed. The same is true in biofloc systems (containing 10^6 and more heterotrophic bacteria inducing a tight competition for feed), green water or co-culture with tilapia.

- ***Stability of water quality parameters***

It is assumed that changes, and especially abrupt changes in water quality parameters are potentially inducing stress in shrimp and fish population. To prevent such effects, we do not transfer shrimp from saline water to fresh water without a gradual, slow adaptation process, the same is true to the need for a slow adaptation to other variables. Stress is a precursor of disease outbreak, by affecting the resistance of organisms to disease agents. (e.g. People are affected by flu when exposed to abrupt temperature change).

The stability of water quality is considered to be an important factor lowering the danger of disease out-breaks.

Data presented in Figures 4.1 and 4.2 a (Nyan Taw, 2005) demonstrate the stability of water quality in biofloc shrimp ponds as compared to conventional algae dominated ponds. Oxygen concentration (DO) in the biofloc systems is rather constant, day and night as well as along the days of culture. Oxygen concentrations in conventional ponds vary drastically in a range of 4 to

The pH in the conventional ponds diurnal variation reached 1 pH unit (10 fold difference in H^+ activity) as compared to a much lower variability in the biofloc ponds.

The stability of water quality stability is probably contributing to lower stress and disease outbreaks in biofloc systems.

- ***Effects of specific bio-components on disease repression***

Predicting and controlling the effects of BFT on disease prevention in aquaculture is a research topic that has started just recently. De Schryver and co-workers (2008) reviewed several basic feature of biofloc technology (see also Chapter 15). They stated that bioflocs may be instrumental to aquaculture in other aspects in addition to water quality control and supply of feed, but may act as bio-control agents against pathogenic diseases. One mechanism toward the control of diseases is the production of short chain fatty acids (SCFA). Adding such acids (butyric, formic, acetic, propionic or valeric acids) to the culture water of *artemia franciscana* resulted in the protection of these organisms against pathogenic Vibrio campbellii (Defroit et al., 2006). Poly-β-hydroxy butyrate (PHB) has a similar effect acting against Vibrio infections. The accumulation of PHB in bioflocs can be induced under conditions of excess carbon (high C/N ratio) and thus offer a probiotic advantage to aquaculture.

Sinha and co-workers (2008) further review the potential use of bioflocs as an anti-inflammatory agents in aquaculture and state "these indicate that biofloc can be a novel strategy for disease management on a long term basis in contrast to conventional approaches such as antibiotic, probiotic and prebiotic applications".

One has to realize that these works are just the beginning of the exciting field of using bioflocs to abate diseases in aquaculture.

- ***Effects of Bioflocs on Streptococcus Disease of Tilapia***

One of the global problems of tilapia culturing is the infection of the fish by *Streptococcus iniae* (and other) leading to slow growth and to eventually to death of fish, often leading to 30% mortality. Losses due to *S. iniae* infections in Israel range between 30% (tilapia) and 50% (trout) of total predicted crops.

Biofloc technology can be used to support intensive production of tilapia as discussed in previous chapters. Several field observations indicated that infection with streptococcus and fish mortality are low, almost negligible, in biofloc ponds.

The effect of the biofloc technology on infection of tilapia by streptococci was studied (Avnimelech and Bejerano) in the Genosar Intensive Fish-Culture Experimental Station, located by the Sea of Galilee, Israel. The experiment was conducted in 2 m^3 tanks. Aeration and mixing of the

Two treatments were tested. Conventional high water exchange was practiced in the control treatment, using a water exchange of 0.5 l min^{-1} per kg fish (i.e. about 700% daily water exchange). The biofloc treatment had a limited daily water exchange of 10%. Each treatment had 4 replicates. A pre-treatment period of 3 weeks was used forthe development of dense biofloc populations, as evident by the high turbidity and presence of visible bioflocs. Total bacterial counts were in the order of 10^5/ml in the control tanks and $10^6 - 10^7$/ml in the biofloc treatment. Fish were added following the pre-treatment period to cover for accidental fish mortality and to maintain the pre-determined fish population of 200 per tank.

Fifty fish were challenged by injecting 0.2 ml of streptococcus suspension (5×10^4 bacteria /ml) at the end of the pre-treatment period. The challenged fish were returned to the tanks for an experimental period of 20 days. **Two types of infections could take place: A direct infection of the challenged fish and an indirect one, induced by bacteria released from the challenged fish, infecting the non-challenged ones.**

Challenged fish, recognized by a blue dot and cut tail, were counted separately as healthy, sick or dead. Out of the 50 challenged fish 19 ± 4 and 13 ± 3 healthy fish were found in the control and the biofloc treatments, respectively, at the end of the 20 days experimental period with no statistically significant difference between treatments.

Table 11.1: Infection of non-challenged fish after 20 days co-habitation with fish challenged by Streptococcus iniae

Treatment, Tank #	Dead fish	Sick fish	Total infected
Control, High water exchange			
1	3	3	6
2	6	6	12
3	14	3	17
4	4	5	9
Average (± SD)	6.8 (5.0)	4.3 (1.5)	11 (4.7)
Bioflocs			
1	2	2	4

Treatment, Tank #	Dead fish	Sick fish	Total infected
4	0	1	1
Average (± SD)	1.8 (1.7)	1.3 (1.0)	3 (1.0)
T-test significance for difference between treatment	0.107 N.S	0.015	0.017

Non-challenged fish were counted at the end of the experimental period and sorted to dead, sick and healthy ones (Table 11.1). The average number of sick and dead fish in the biofloc treatment was 3±1, as compared to 11±5 in the control treatment, a highly significant difference. It was clearly shown that indirect infection was lower in the case of the biofloc system as compared to the clear water system. Intuitively, it may be expected that less infection will be found in tanks with intensive water exchange and flushing out of the pathogens (with the water exchanged at a rate of 7 times a day), in comparison with the bioflocs tanks, where only 10% of the water was exchanged. The results were clearly different from this intuitive guess. The number of dead and sick fish in the biofloc treatment was just about one quarter of that found in the control, clear water tanks.

Probiotics are conventionally defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance (Fuller, 1989). This definition, that seems relevant for terrestrial animals, appears to be non-satisfactory in aquatic systems. The water body in aquaculture systems serves partly as a nutrient recycling process of food, in a way similar to the rumen in ruminants. A dense population of bacteria (in the order of 10^6-10^9/ml) surrounds the fish and potentially interacts with the fish.

The results of this experiment demonstrate that naturally occurring bacteria, rather than specially selected bacteria introduced into the system, may have a beneficiary effect on the health of the cultured animal and its resistance toward disease (though it is possible that specialized microbial cultures may be superior). It was shown that the biofloc systems provide a protective shell for the fish, through a probiotic effect.

Different modes of action of probiotics are proposed in the literature (Verschure et al., 2000; Farazanfar, 2006). Among possible mechanisms is the effect of probiotics on water quality, antagonism with the pathogens, competition on adhesion sites within the host or a positive effect on host physiological conditions. The effect demonstrated here could be due to several mecha-

Practical Implications and Tips

Farmers noticed that biofloc systems prevent or ease disease outbreaks of shrimp and fish. Controlled scientific studies confirmed such observations and suggest mechanisms affecting diseases. The following mechanisms were identified:

1. *Minimizing and if possible avoiding water exchange is an important bio-security element.*
2. *Immune mechanism of shrimp is enhanced in the presence of the biofloc diverse microbial community.*
3. *Diverse and stable community in the pond helps to control the development of pathogens.*
4. *Biofloc systems are characterized by stable water quality parameters, a feature preventing stress of fish and shrimp.*
5. *Several specific biological agents have been identified as preventing or easing diseases.*

Recommended Reading and Cited Literature

1. *Defroit, T., Halet, D., Sorgeloos, P., Bosier, P., Verstraete, W. 2006. Short-chain fatty acids protect gnotobiotic Artemia franciscana from pathogenic Vibrio Campbellii. Aquaculture 261: 804–808.*

2. *De Schryver P., Crab, R., Defroit, T., Boon, N., Verstraete, W. 2008. The basics of bio-floc technology: The added value for aquaculture. Aquaculture 277:125–137.*

3. *Grillo, M., Dugger, D. M., Jory, D.E. 2000. Zero exchange shrimp production success in WSSV infected Panama. Global Aquaculture Advocate V3 (6):55–56.*

4. *Jang, I.K. 2011 Super intensive shrimp production in commercial scale greenhouse systems using no-water exchange. World Aquaculture Soc conference, Natal, Brazil, June 6-10, 2011*

5. *Lightner D.V. 2003. Exclusion of specific pathogens for disease prevention in a panaeid shrimp biosecurity program. PP 81–115. In: Lee, C.S. and O'Bryen, P. (Eds.). Biosecurity in Aquaculture Production Systems: Exclusion of pathogens and other undesirables. World Aquaculture Society. Baton Rouge, LA, USA.*

6. *More, W.R., Frelier, P. 2003. Shrimp diseases: prevention and control. PP 167–178. In: Jory, D.E. (Eds.). Responsible Aquaculture for a Secure Future. World Aquaculture Society. Baton Rouge LA. USA.*

7. *Farazanfar, A. 2006. The use of probiotics in shrimp aquaculture FEMS Immunol. Medical Microbiology 48:149–158.*

8. *Fuller, R. 1989. A Review: probiotics in man and animals. Journal of Applied Microbiology 66: 365–378.*

9. *Kim, Su-Kyong, Pang, Zhenguo, Chei, Hyung-Seu, Samocha Tzachi, Jang In-Kwon. 2014. Effect of Bioflocs on growth and immune activity of Pacific white shrimp Litopenaeus vannamei post larvae.Aqua. Res. 45:362–371*

10. *Sinha, A.K., Baruah, K., Bossier, P. 2008. Horizon scanning: The potential use of biofloc as an anti-infective strategy in aquaculture– An overview. Aquaculture Health 13: 8–10.*

11. *Taoka, Y., Maeda, H., Jo, J.Y., Kim, S. M., Park, S.L., Yoshikawa, T., Sakata T. 2006. Use of live and dead Probiotic cells in tilapia Oreochromis Niloticus. Fisheries Science 72: 755–766.*

12. *Verschuere, L., Rombaut, G., Sorgeloos, P., Verstraete, W., 2000. Probiotic bacteria as biological control agents. Aquaculture Microbiology and Molecular Biology Reviews 64: 655–671.*

Chapter 12
Pond Construction

In Brief
BFT ponds should be designed and constructed to enable aeration and mixing of the whole pond area/depth. The classical design is based upon a round pond concept with aerators inducing radial water flow, or otherwise square or rectangular ponds where water flow is sort of radial, mostly in parallel to the pond dykes. In such cases, corners are rounded or cut, to minimize stagnant areas. A different approach is to construct the pond as a closed raceway, where water flow is directed by a solid or loose partition along and around the pond.

Ponds should be constructed to prevent dyke erosion, to enable complete drainage of water, to minimize area of sludge accumulation and to facilitate ease of draining of sludge. Lining the pond is highly recommended. Ponds should be lined with plastic or hard solid materials. Drainage and harvesting openings should be constructed in low- lying locations and coordinated with water flow regime.

An intensive BFT pond has to be planned bearing in mind the need to provide proper aeration to all parts of the pond, mixing the water to minimize anaerobic sludge accumulation and to enable periodic drainage of the sludge both during the crop and between crops. Additionally, designs should facilitate efficient harvest and easy feeding. General rules and demands of pond design should be followed in designing BFT systems (e.g. Boyd 1955; Fast and Lester, 1999).

Intensive ponds should not be too large. The biomass in the ponds is high, thus controlling large volumes of water is difficult, harvesting of too high biomass is complicated and the risk of holding dense fish or shrimp populations in very large reservoirs may be too high. The risk of losses if something goes wrong in large intensive ponds is very high. The typical size range of intensive ponds is normally in the range of 100-20,000 m^2 (0.01-2 ha). The common size of intensive BFT fish ponds is 100 – 1,000 m^2 while the typical size of intensive BFT shrimp ponds is 1,000 – 20,000 m^2 (0.1-2 ha). The depth of ponds is in the range of 1-2 m. The advantage of deep ponds is their high heat buffering capacity, which helps to avoid over-heating or over-cooling during the diurnal cycle. In addition, the deeper water column minimizes contact of the surface water to pond bottom anaerobic conditions and allows a deeper water column for feeding and biological processes. However, constructing deeper ponds demands a higher investment and, in cases of ties in pond maintenance. Avnimelech and co-workers (1986) found that a soft clay dominated pond bottom becomes highly anaerobic due to the mixing of organic matter with the clay and the very limited oxygen diffusion into the deep bottom layer. Additional advantages of lining are the ease of cleaning pond bottom in between cycles and possibly more efficient mixing and utilization of feed residues sinking to the bottom. It is interesting and important to note that **the nature of organic matter accumulating on the pond bottom differs between lined and earthen bottoms. In earthen ponds, the organic residues mix with the soil, forming a rather stable complex, in comparison to highly degradable, unstable and bio-reactive organic residues** that accumulate on the lining. This difference affects pond management: in earthen ponds, organic matter accumulates over a period covering several cycles and has to be periodically removed. In the case of lined ponds, organic deposits should not accumulate, and due to the high reactivity they vigorously affect chemical and biological processes in the pond and may cause a real problem. A very clear example of the difference between earthen and lined ponds is demonstrated by following phosphorus interactions. In earthen ponds, the soluble phosphorus interacts with soil components and is, to a large extent adsorbed. In lined ponds, such interaction does not take place and excessive phosphorus remains, mostly as soluble phosphorus in the water (Avnimelech and Ritvo, 2003).

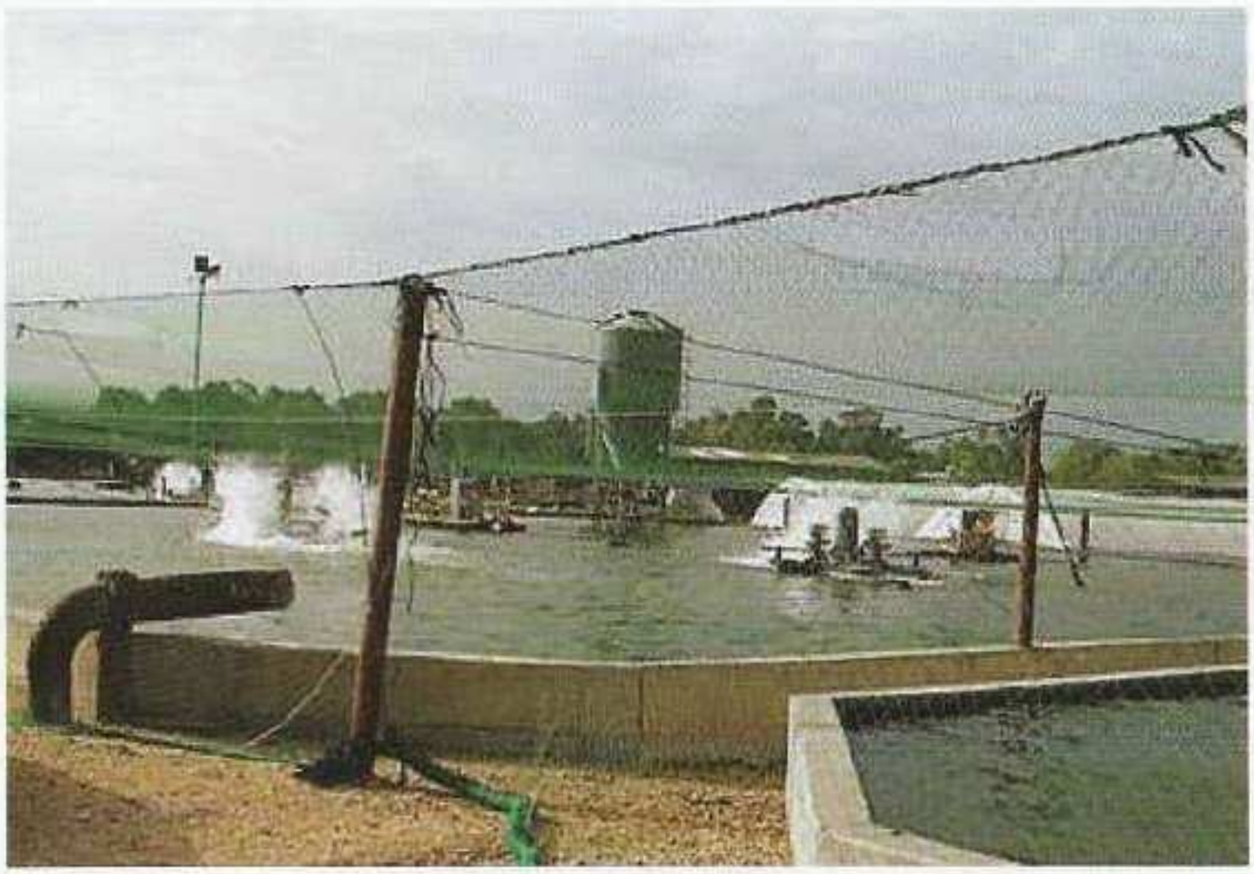
Lining of ponds is usually done with High Density Polyethylene (HDPE) sheets of about 1 mm (30-40 mil). Cheaper alternatives may be constructing a pond bottom with compacted laterite soil (or laterite crushed stones). Laterite, the red soil commonly found in tropical regions, makes a stable pond bottom upon compaction. However, in this case, the banks should be covered with plastic sheets to prevent erosion. Another possibility is to line the pond with a soil cement mixture. One can mix cement with a top layer of soil and obtain a stable lining. The bottom should be smooth to ease draining and cleaning.

Most BFT ponds design is based upon radial aeration of the pond. In most cases round ponds are managed, with aerators placed parallel to the dykes, leading to a radial water flow pattern. Round ponds are the most common design for small ponds, used in hatcheries and some production units (Figure 12.1).

Figure 12.1: Round BFT pond used for tilapia production

Building larger round ponds is more difficult (digging, utilizing land, lining) and rectangular or similar design is more common. In many cases the corners in these types of ponds are rounded or smoothed, in order to enable undisturbed flow of water and to minimize the area where flow and aeration are limited (Figure 12.2).

Figure 12.2: Square pond with rounded corners



The placement of aerators is similar to that used in round ponds, aimed at creating mostly radial or elliptical flow patterns. It is advantageous to place some aerators with their flow vector tilted toward the corners and others directed toward the center. A different approach is to place the aerators deviating from the classical radial flow, having a water flow vector toward the center and generating a somewhat turbulent flow pattern (see extended discussion in Chapter 10).

Another BFT system design is the closed raceway approach. The closed raceway (Figures 12.3 (a), 12.3 (b)) is based upon a linear rather than a radial water movement pattern.

Figure 12.3 (a): Closed raceway ponds

Closed raceway within a green-house used for tilapia production

Figure 12.3: (b) Closed raceway ponds

Closed raceway units can be constructed as such, when all walls and flow partitions are built as an integral part of the system (e.g. walls and partitions built of concrete). A cheaper and easier mode enabling conversion of existing rectangular ponds is to place longitudal partitions, dividing the ponds to closed raceways. It is important to note that the flow partition is separating two sides having the same water head and that the separation does not have to be tight. Thus, a partition made of simple plastic sheets placed in position supported by poles are sufficient. Aerator placement is generally parallel to the raceways. Sludge tends to accumulate in closed raceways at the ends of the flow partitions where water flows around the baffle and relatively stagnant regions are created. The linear flow mode of the closed raceways seems to enable the operation of long ponds. Pond length can be as long as there are enough aerators along the pond (and as long as you can have all pond length sloping toward the outlet). The width of the raceway should be such that enable smooth water flow and easy access. A width of about 10-30 m seems to be appropriate.

Mr. Adam Body in the Northern Territory, Australia, operated a shrimp farm (Figure 12.3 b) that included four 2.5 ha ponds, 500 m long and 50 m wide (Chamberlain, 2000). The ponds were divided into 2 raceways, about 25 m wide, by earthen baffles. The ponds were equipped with 4 long arm paddle wheel aerators each. Such ponds seem to have many advantages, being relatively inexpensive and simple. Yet, more experience in planning and operating closed BFT raceways is required.

A new and interesting approach is the installation of pre-fabricated ponds, as shown in Figure 12.4. Such ponds are produced presently in both Mexico and Colombia (possibly else-where as well), are relatively inexpensive and can be installed within a few days. The ponds (tanks) are relatively small (up to about 150 m^2) and can be used as a startup technology for individual farmers. Such ponds are suitable for the production of dense fish biomass (shrimp in special cases, to provide fresh shrimp) and can easily be placed in green-houses.

Organic residues are always produced and their accumulation as bottom sludge is an un-avoidable problem. The basic solution to this problem is to concentrate the sludge in limited points in the pond (sludge traps) and design for the capacity to drain out the sludge, during the production cycle and between cycles.

Radial flow ponds (round, square, rectangular) tend to collect the sludge at the center of the pond (see Chapter 10). To help concentrate the sludge at the center, the center of the pond should be the deepest part of the pond and the pond should slope toward the center. Operating a BFT pond, two opposing goals should be kept in mind. On the one hand, it is desirable to trap the sludge in a small drainable area. However, we need to keep high enough active suspended matter in the pond (in the order of a few hundred mg/l). In order to keep from getting too much organic residues in the center and to achieve enough active suspension, it is necessary to get some

Figure 12.4: Pre-fabricated pond within a green house, Mexico

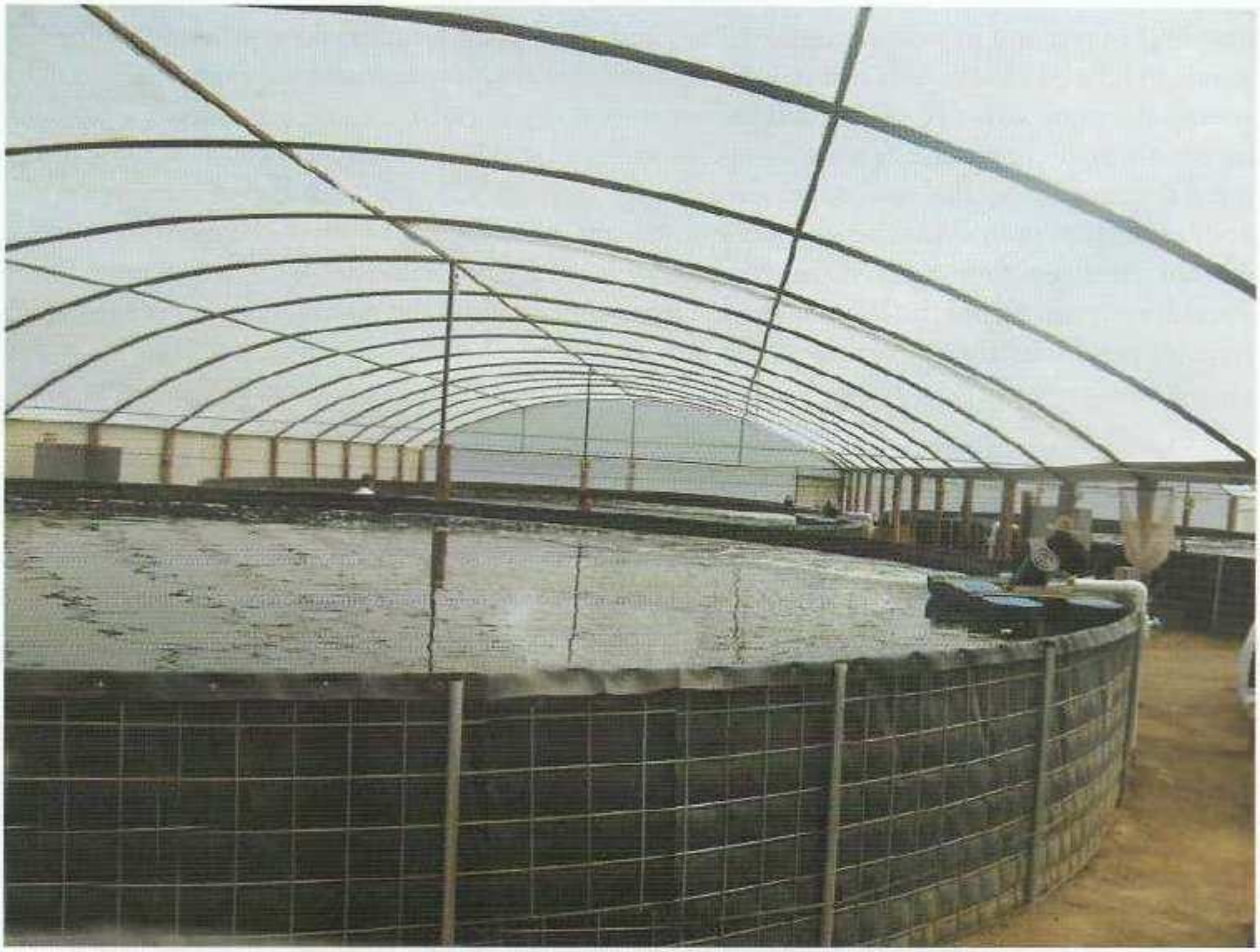


Figure 12.5: Scheme of central sludge collection and drainage system in circular pond

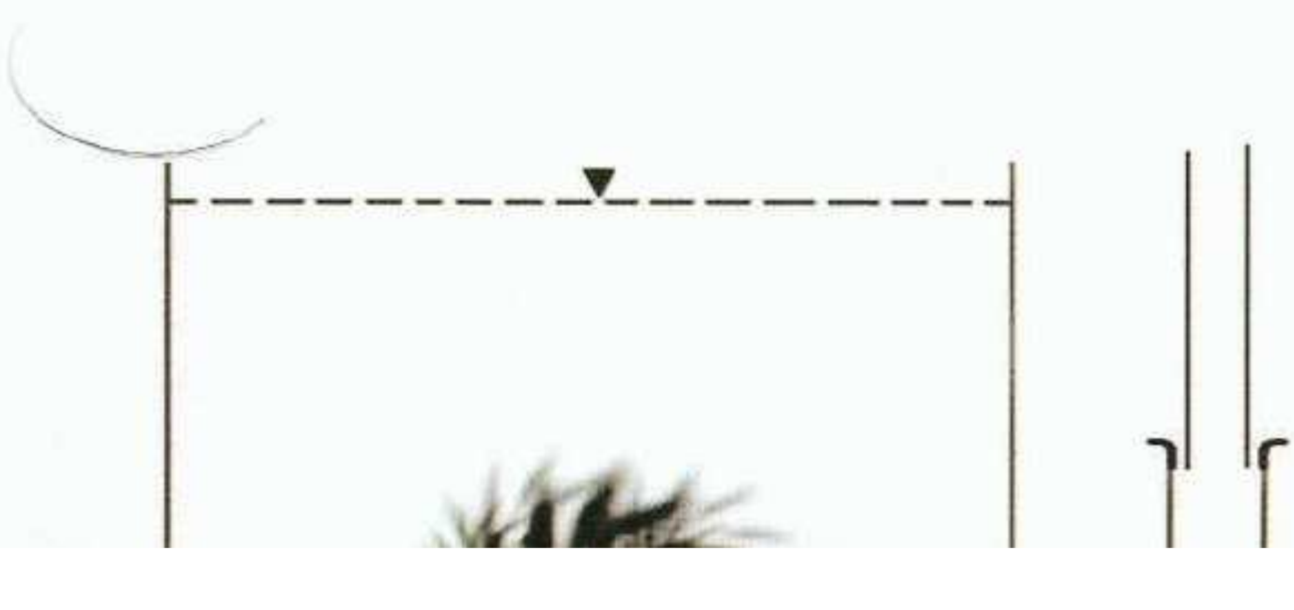


Figure 12.6: Pond slopping toward the drainage point

*Circle inserted using Photoshop, to emphasize slopping in of pond bottom

Unlike radial flow ponds with their self-cleaning mechanisms, the flow in raceways does not direct the sludge to a certain point in the pond. Controlling the accumulation of sludge in closed raceways can be achieved by constructing depressions in the pond, which will serve as sludge removal pits. One possibility is to place such a pit at the edge of the raceway. The pond should slope toward this depression, to facilitate harvesting and pond bottom cleaning. More experience and better design parameters are needed.

A number of accessories are essential or beneficial for the successful operation of intensive BFT ponds. Intensive ponds carry a high biomass and strongly depend upon aeration. A fault in the aeration system may be critical and may lead to a complete failure of a pond or even of the whole cess. It is advisable to have a fish/shrimp collection pit with proper lifts, pumps or other means to transfer the animals as quickly as possible to the processing site. The proper devices and procedures are needed for fast shrimp cooling and processing, live fish transfer etc. All these arrangements are an important part of planning and design of the farm.

Drainage and sludge collection are important parts of pond operation. Drainage of ponds before and during harvest should be done quickly. There is a need for an effective hydraulic gradient and proper pipes / canals to get the water out without obstruction. In many farms you see that the water level in drainage canals is too high, especially so during fast drainage of ponds. Ensuring proper slope and water discharge capacity of the drainage canals toward the final outlet should be included in the planning process. It has to be emphasized that for efficient pond operation, drainage has to be completed in 1-2 days at most and **all** the water in the pond has to be drained, without leaving any remnants such as wet puddles. The achievement of thorough drainage is very important in extensive BFT ponds, where a very good exposure to the air and drying are essential.

The location of final water and sludge discharge is not a trivial question. The environmental issues of this will be discussed separately (Chapter 17). Transfer of discharged water into a recycling reservoir located in the farm is an advantage, yet other options are possible and commonly used, such as use of effluents for irrigation or hydroponics (Diver, 2006, Bernstein 2012). The disposal, treatment and re-use of the disposed sludge are very important problems that require more research and field experience. Possible solutions based upon utilizing the nutritive value of sludge include transfer of sludge from intensive ponds to extensive ones, where it supplements feeding (Diab and co-workers, 1992), treatment of the sludge so as to prepare feed material out of it (Chapter 8; Schneider et al., 2006). Sludge can be used to build dikes for ponds or used as an agricultural soil amendment. The last proposed usage is feasible when fresh water is used in the ponds. Land application of saline sludge is a problem. Sludge collected in waste water treatment plants is incinerated or fermented anaerobically to produce methane. Such solutions are quite costly, leading to a challenge in operating the ponds in a way as to minimize sludge accumulation. As discussed in Chapter 17, environmental regulatory demands and the costs associated with following those demands are increasing with time. Thus, developing ponds that produce minimal amounts of pollutants – of which sludge is a heavy component -- is a critical challenge we are faced with.

The construction and operation of intensive ponds is a costly operation, even in the case of BFT ponds, which are relatively less expensive. To justify the investment, the ponds should be utilized at peak capacity or close to it, as much as possible. The conventional operation of ponds starts with stocking of small fish/shrimp and subsequent culture up to market size. Ponds reach their full carrying capacity only when the target crop reaches market size. However, most of the time, the pond carries less, sometimes much less, than its carrying capacity. Ideally, one can work with a series of ponds of different sizes, stocking the small animals in the smaller ponds, at a biomass nursery tanks are kept in green house structures, one can extend the growing season by starting the nurseries in the cool spring). The exact usage of the above mentioned approach depends on local conditions and on business planning of the farm. More imagination, research and experience are needed to bring these ideas to common practice. Partial harvesting (See Chapter 13) is also a means to better utilize the pond.

In temperate regions, where cold temperature during the colder seasons may limit production, putting a plastic cover may improve profitability. Different greenhouse designs are available, ranging from small green houses (a few hundred m.), up to structures covering large ponds. Stability of these structures during strong winds may be a problem, calling for a solid and expensive structure. In all cases, ventilation is needed, so as to enable release of CO_2 and allow better temperature control.

One of the issues raised in relation to intensive BFT ponds is the need of shading to reduce or eliminate algae growth. Algae growth is considered by some as an element of instability in the pond. Shading of the pond with opaque (green) plastic sheets is possible in the case of relatively small intensive ponds. This demands a solid structure and thus adds significantly to a pond's expenses. There are cases, in temperate zones, where the cover is needed to maintain high temperature in cold seasons. Experience shows that when a dense biofloc community develops, algal activity is lowered, due to intense shading of the microbial suspension. Some observations hint that in the presence of algae, larger flocs are formed. Presently, there are no clear indications as to the advantage of pond opaque covers to reduce algal growth.

Practical Implications and Tips

1. *We are only beginning to use BFT ponds. Try to follow proven pond design, yet, there is a lot of room for new ideas.*

2. *Planning of BFT ponds must consider all management options, especially intensity levels, aeration devices, drainage, harvesting and sludge control.*

3. *Choose the pattern of ponds to be constructed based upon experience available in your region.*

4. *Having a reasonable gradient to the drainage location is essential. Insufficient gradients or high water level in drainage canals is a cause of failure for many pond systems. Coastal shrimp ponds should be constructed high enough (>2 m) above mean sea level.*

5. *Sludge collection and reuse are important problems. Plan ahead! Do not delay them to a later stage of operation.*

Further Research Needs

Design and construction of BFT ponds are still, to a large extent, intuitive processes rather than rigorous engineering blueprints. The further development of BFT ponds requires the development of such rigorous planning.

Certainly, there is no one type of pond covering different fish, different climatic conditions etc. Yet, there should be a few tested, performances proven and economically sound models.

1. *There is a need to develop applicable models to optimize aeration, water mixing and sludge control. Pioneering work was done and published by John Peterson (2000, 2001); however, more work should follow.*

2. *Different design considerations should be given to the harvesting site and mechanism, which are different for fish and shrimp.*

3. *Sludge control is a very important design issue. Better sludge draining, concentrating processes and disposal should be developed.*

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Weber, B., Hepher, B., Milstein, A., Zorn, M. 1986. Studies in circulated fish ponds: Organic matter recycling and nitrogen transformation. Aquaculture and Fisheries Management 17: 231-242.*

2. *Avnimelech, Y., Ritvo, G. 2003. Shrimp and fish pond soils: Processes and nt. Aquaculture 220: 549-567.*

3. *Bernstein S, 2012 Aquaponic Gardening: A Step by Step Guide to Growing Fish and Vegetables Together*

4. *Boyd, C.E. (Eds.). 1995. Bottom soils, sediments and pond aquaculture. Chapman & Hall, N.Y.*

5. *Chamberlain, G.W. 2000. Family shrimp farm in Australia: Novel approach could be a model for the future. Global aquaculture Advocate V 3 (6):48-51.*

6. *Diab, S., Cochaba, M., Mires, D., Avnimelech,Y. 1992. Combined intensive- extensive (CIE) pond system. A: Inorganic nitrogen transformations. Aquaculture 101: 33-39.*

7. *Diver, S. 2006. Aquaponics- Integration of hydroponics with aquaculture. ATTRA Publication, at: http://attra.cnet.org*

8. *Fast, A.O., Lester, J. L. 1999. Marine shrimp culture: Principles and practices. PP. 862. Elsevier, Amsterdam.*

9. *Kuhn d.d., Boardman, G.D., Craig. S.r., Flick, J.G., McLean, E. 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, Litopenaeus Vannamei, in recirculating aquaculture systems. Journal of the World Aquaculture Society 39: 72-82.*

10. *Peterson, E.L. 2000. Observations of pond hydrodynamics. Aquacultural Engineering. 21: 247-269.*

11. *Peterson, E.L., Wadhwa L.C., Harris, J.A. 2001. Arrangement of aerators in an intensive shrimp grow-out pond having a rectangular shape. Aquaculture Engineering 25: 51-65.*

12. *Schneider, O., Sereti, V., Eping, Ep.H., Verreth, J.A.J. 2006. Molasses as C source for heterotrophic bacteria production on solid fish waste. Aquaculture 261:1239-1248.*

Chapter 13

Field Experience

Written in cooperation with Dr. Nyan Taw

In Brief
Is BFT just science fiction?

BFT technology has been adopted by growers in many parts of the world as it offers a system which is on one side bio-secure and more environmentally friendly, while allowing for the intensive stocking levels necessary for financial sustainability. Although much practical experience has been accumulated, much of it is unpublished or proprietary. The present chapter reviews some practical commercial scale experience with tilapia and shrimp production. Some detailed know-how and economical information is provided. Although further development of these technologies on a commercial scale offers potential for improvement, it can be concluded that the system is viable and practical from both technological and economic points of view. Moreover, there are many farms, each developing some changes and introducing modification providing examples to follow if one want to start a BFT based farm.

In a way of a personal confession I admit that the major drive to write this book (quite a lot of work) were the many letters I got in my e-mail box, letters from farmers from all over, asking how to practice BFT ponds. It is difficult to give good advice by e-mail. I hope this book will help.

One of the people that kept asking questions for about 2 years was Ms. Ninuk Sri Maharti, working for *Suri Tani Pemuka shrimp farm in Indonesia. Ms. Ninuk Sri Maharti kept asking questions, followed advice (and added much of her experience). It was nice to get a letter saying that though she still has problems, things are going well. I got her permission to add her last letter and a short report on the farm operation to this chapter.*

These types of contacts are an important factor helping us advance aquaculture together.

Use of BFT For Tilapia Production

Avnimelech and co-workers (1994) ran triplicated pond scale experiments evaluating feed uptake and response to addition of carbohydrates with tilapia. Tilapia hybrids (*Oreochromis niloticus* x *Oreochromis aureus*) were grown in circular $50 m^2$ ponds at a density of about $10 kg/m^2$. Fish of 20% protein, (C/N = 16.7, the BFT treatment). Daily feed addition was 2% body weight in the conventional control treatment and 2.6 BW in the BFT treatment, to cover carbohydrates needed for the microbial ammonium conversion. The results of this pond scale experiment are given in Table 13.1.

Figure 13.1: Dense production of Red tilapia

Table 13.1: Fish growth and yield coefficients of tilapia fed with conventional Pellets[1], in 2 pond experiments

	Treatment	
	Conventional control (30% protein)	BFT, C enriched (20% protein)
Feed C/N Ratio	11.1	16.6
Experiment #1: 51 days, averages of 3 replicates		
Fish weight (g/fish)		
Initial weight	112	112
Final weight	193	218
Daily gain*	1.59[a]	2.0[b]
Mortality (%)	14.6	10.3
Feed conversion coefficient	2.62	2.17
Protein conversion coefficient	4.38	2.42
Feed cost coefficient (US$/kg fish)	0.848	0.583
Experiment #2: 30 days, averages of 3 replicates		
Fish weight (g/fish)		
Initial weight	205	205
Final weight	254	272
Daily gain[2]	1.63[a]	2.22[b]
Mortality (%)	3.4	0
[illegible]	[illegible]	[illegible]

It can be seen that fish growth was better in the 20% protein BFT treatment, most likely due to the lower toxic inorganic nitrogen species concentrations. In addition to a lower feed conversion ratio (FCR), the protein conversion ratio (PCR =, Protein in feed/protein in fish harvested) was markedly reduced in the 20% protein treatment. The PCR in the conventional 30% protein feed treatment was 4.35-4.38, meaning that only 23% of the feed protein was recovered by the fish, as reported for conventional systems by numerous researchers. The PCR in the tested BFT treatment was 2.2-2.4, i.e. protein utilization was twice as high. The increased protein utilization is due to its recycling by the microorganisms. It may be said that the proteins are eaten by the fish twice, firstly in the feed and then again as microbial proteins. It is possible that protein recycling and utilization can be further increased. Due to the fact that proteins are the expensive component of the feed, its reduction was reflected in the feed price which decreased from 0.85 US$ needed to produce 1 kg fish in the control to about 0.55 US$/kg fish in the BFT treatment. This is a very significant reduction of production costs. Similar results were obtained in the desert aquaculture farms in Israel.

Production of tilapia in BFT ponds is practiced in other farms. Pacific Aqua farms in Southern California (Farrell, 2006) used concrete cement ponds (see Figure 12.1) to grow Tilapia (Mozambique) using either high exchange or BFT technology ponds using a daily water exchange rate of up to 5% with the daily sludge drainage. Tilapia biomass in the BFT ponds was around 20 kg/m3. BFT ponds were fed with 20% protein pellets. In cases when TAN levels rose, corn starch was applied to raise the C/N ratio and control inorganic nitrogen concentrations. Average daily fish growth was 2.7 g (2005 data). The production expenses in the BFT ponds were lower than those for the conventional high water exchange ponds by 0.4/kg US$. About 50% of this due to lower feed cost and the other half due to saving of acid addition needed to neutralize the rather basic local ground water. The BFT ponds had a limitation during the cold season, when the thermal ground water exchange served as a heat source.

The principles of bioflocs technology are similar for tilapia and shrimp culture Yet, shrimp biomass in BFT ponds is in the range of about 10 – 30 ton/ha (1 – 3 kg/m^3). Though higher shrimp biomass can be achieved in BFT raceways or by using partial harvests. Tilapia biomass in BFT ponds is 100-500 ton/ha (10 – 50 kg/m^3) about 10 times higher than common shrimp BFT ponds. As a result, feeding rates for fish and shrimp are different: Daily feed application to shrimp ponds is about 20-60 g/m^3 as compared to about 200 – 1,000 g/m^3 in the case of BFT fish ponds. Such huge differences make an important difference among the different subsystems. The feeding load in the fish system is huge, way above the carrying capacity of the pond. Thus, daily and often twice daily drainage of excessive sludge is needed. The feed load and the overall organic residue accumulation in shrimp ponds is much lower so residues can be drained only at the end of the season (though periodic drainage toward the end of the season is important and frequent drainage is practiced in raceways).

ponds is derived out of the experience gained in BAL, led by Dr. Robins McIntosh.

Ponds in BAL are 0.065 to 1.6 ha in size, the commercial ones are 1.6 ha. No negative effect of size was found. Depth of ponds was above the 1 meter common depth in shrimp ponds: 1.4 m at the edges and 2.3 m at the point of drainage (average depth of 1.8 m). Deepening the ponds seemed to have some advantages particularly in restraining temperature changes, both daily, and to some extent seasonal. Deeper ponds allowed for larger oxygen reservoir and the water volume for organic matter assimilation was increased. In addition, in case of anaerobic sites development at the bottom, shrimp are less exposed to the anaerobic products. However, pond construction is more expensive. Deep ponds cannot be operated in places where drainage basis is shallow. This is not the case in BAL where ponds are constructed 6-7 meters above sea level.

Water is pumped from the sea, about 3 km away from the farm into a receiving reservoir. The farm system is practically closed and there is no drainage released to the environment. Recirculation, settling reservoirs take water drained from the ponds. Water is stored in the reservoirs and conditioned before they are returned into the ponds. Water conditioning includes sedimentation of sand and suspended matter and addition of fertilizers as needed.

Ponds were lined with 30 to 40 mil HDPE. (i.e. 0.76-1.0 mm thick) Aeration capacity is 30 – 60 hp/ha, with about 30 hp/ha in the larger ponds. Paddle wheel aerators are placed in the periphery of the ponds at a distance of about 30% the width of the pond from the pond edges. The resultant water flow regime is of about 23 cm/sec at the outside of the pond and 5 cm toward the center. Aspirator type aerators are placed toward the center to induce resuspension and add to the oxygen in the central area. Positioning of the aerators has been very important in maximizing stable TSS concentration, minimizing sludge accumulation and promoting effective heterotrophic activity. Resultant oxygen concentration is above 4 mg/l in most of the pond volume. Aerator operation varies with time. As long as shrimp biomass is less than 12,000 kg/ha only 50% of aerators are turned on during day time. McIntosh reported aeration requirements for BFT shrimp ponds in the range of one hp for the production of 300 to 550 kg in zero water exchange systems producing 11 – 26 mt/ha/cycle. The upper end of this range of production per hp of aeration was reached by periodically draining concentrated organic sludge from a point of accumulation near the center drain.

Ponds were stocked with white shrimp (*L.vannamei*) at a rate of 125 – 140 PL/m^2. It was found that when stocking rates falls below 100 PL/m^2, it becames more difficult to establish the heterotrophic community because there were not enough organic substrates to support the microbial growth.

A process of community succession occurs over time within BFT ponds in response to the in-

3. Large amount of foam on the surface of ponds, due to accumulating dissolved organic material and inadequate bacterial community (see Figure 13.2);

4. Change to brown water color, disappearance of foam, and generation of flocculated masses of bacterial cells, organic detritus, and adsorbed colloids;

The transition toward heterotrophic dominance takes place after 4-8 weeks. Yet, application of molasses can speed up the transition and an application of 50 kg/ha, twice weekly; reduced the transition period to about 2 weeks. The need to shorten the transition period is not trivial. If shrimp grow well with minimal addition of molasses you do not need to add more.

Floc particles are agglutinated by a polysaccharide slime produced by the bacteria. Suspended materials are adsorbed onto the floc where they are hydrolyzed by extracellular bacterial enzymes. Older ponds are characterized by large floc masses that rapidly settle out of suspension if water agitation is stopped.

Feeding is an essential feature of managing the ponds. In the beginning, feed pellets with 30% protein (C/N = 11) were used. This resulted in excessive nitrogen. Raising the C/N ratio of feed to 16, (protein level of 22%) has resulted in balanced inorganic nitrogen levels and a very healthy heterotrophic community. Feeding was done with a combination of 31 - 24% protein pellets and grain-based pellets made of ground wheat, corn and soy, with a protein level of 18.5%. The two feeds were given separately. The higher protein feed is given during the first 7 weeks (prior to the proper bioflocs development), followed by 24% protein feed pellets plus the grain based pellets. Reducing the protein levels in the feed to 20% raised nitrogen retention (protein conversion factor) to 48%. McIntosh (2000b) compared the cost of using feed with reduced protein levels to that of conventional high protein feeding. The cost of feed with the low protein level was 0.85/kg US$ harvested shrimp, compared to a cost of 1.7/kg US$ shrimp for the conventional diet. It has to be kept in mind that these costs are site and time specific and are different in different specific cases.

There are still different views as to the proper control of the C/N ratio in production ponds. High C/N ratio (~>15) helps to control build-up of inorganic nitrogen and induce an effective recycling of protein. An alternative strategy used in a number of farms is to use the added carbohydrates as a means to control nitrogen until the development of effective nitrification and then to rely mostly on nitrification as a means of controlling inorganic nitrogen. At that point, high protein feed is given. There are conflicting results as to the need to supply shrimp with high protein feed. It was reported (Wasielesky and co-workers, 2006) that shrimp growth in experimental tanks was higher with high protein feed (35% as compared with 25% CP), even when water rich in bioflocs or algae was supplied, conditioned by a full control of TAN and nitrite. The strategy practiced in Belize was probably based on the assumption that at the last stages of shrimp production, high protein feed is needed. Different results were reported by Decamp and co-workers (2002) indicating that in their BFT system, there were no significant differences in the perfor-
mance of shrimp fed with 25% or 35% protein pellets. Lawrence and co-workers (2002) as well

more experience is accumulated.

When shrimp biomass exceeds 10,000 kg/ha, normally at the 10th week, it is beneficial to remove sludge that accumulates in the center of the ponds. Sludge is drained through 20 m radius concrete sumps at the bottom center, or using centrifugal pumps.

Feed conversion ratio (FCR) should be controlled targeting a level under 2:1 using 24% protein diet. Energy cost per unit production is only slightly higher than semi-intensive culture. Once it is established, the management of this system is easier and more forgiving than management of a well-run semi-intensive farm. The system is forgiving when it comes to over-feeding (feed is assimilated into bioflocs) or under-feeding (shrimp are fed by biofloc harvesting).

S.E Asia Active practice and development of biofloc based shrimp culture takes place in S.E. Asia. A number of companies operating in Indonesia and in Maleysia are producing shrimp on a very large scale using BFT systems. Reports on these operations were given by Dr. Nyan Taw in World Aquaculture Society meetings and published in the Global Aquaculture Advocate (e.g. Taw, 2005, 2007, 2008, 2010).

Biofloc technology has been applied in Indonesia since 2003 in Lampung, South Sumatra. It spread to Medan, to the North and to Center and South Sumatra shrimp farms. The technology was later applied in East Java and Bali Island. Initially the technology started in Lampung (CP Indonesia) shrimp farm successfully based on Belize technology. Later-on, new technologies and approaches were introduced.

The scale of these operations is immense, covering thousands of hectares. Figure 13.3 gives some idea of the scale of that operation.

Ponds are either completely lined with HDPE or at least partially. Aeration capacity is 28 hp/ha or more. Paddle wheel aerators are placed in two rings parallel to dykes and one at the center zone (See Figures 13.4 and 13.5). Stocking rates are 130 PL/m^2 or more.

Figure 13.2: Aerated pond (Paddle wheels and long arm paddle wheel)

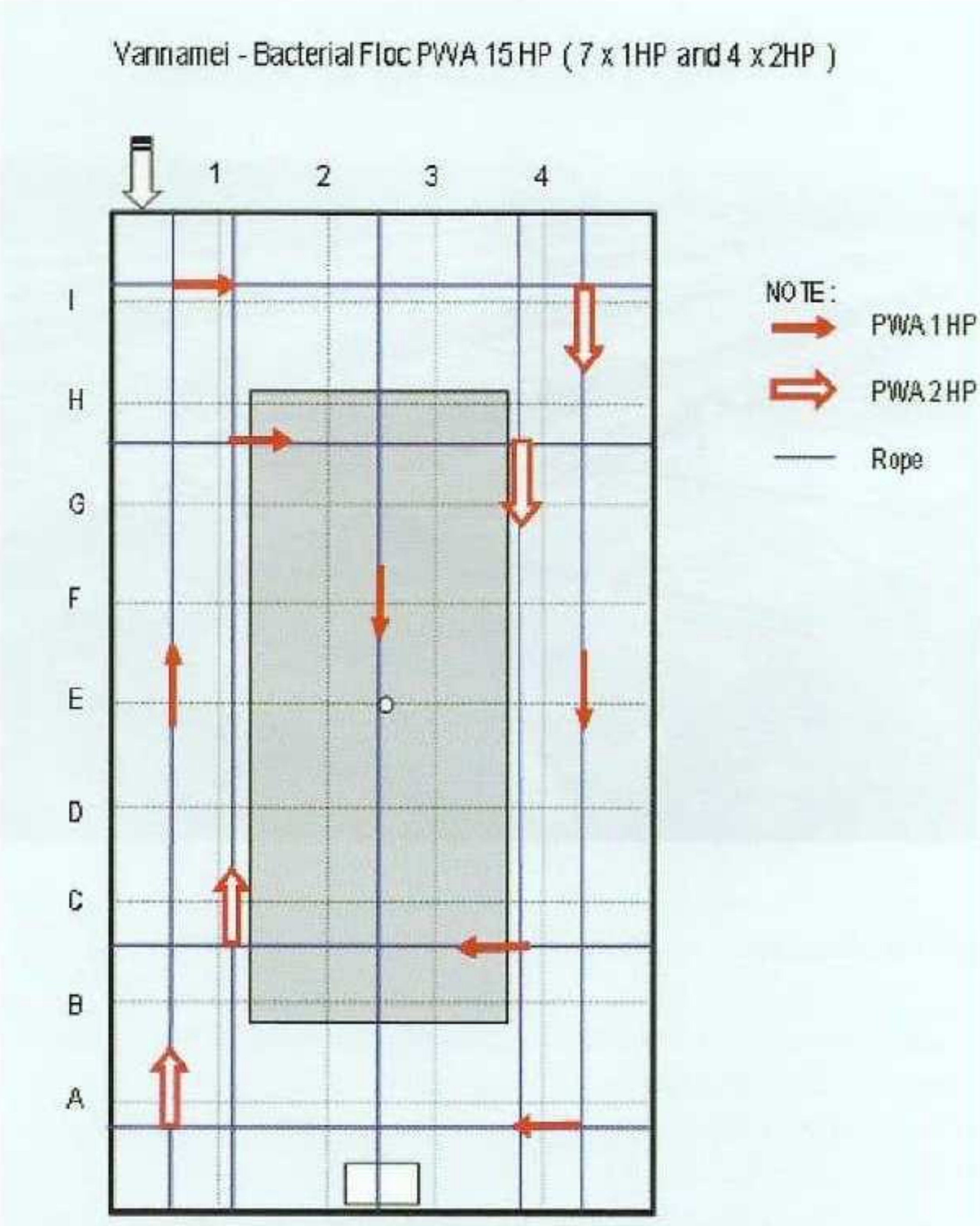
* Courtesy of Dr Nyan Taw

Pay attention to coverage of the pond with foam, indicative of the transition from algal to bacterial control.

Figure 13.3: Shrimp production farm using biofloc technology in Indonesia

* Courtesy of Dr Nyan Taw.

Figure 13.4: Aeration layout in vannamei BFT pond in Indonesia



* Courtesy of Dr Nyan Taw.

Figure 13.5: Aeration using two rings of aerators and aerators aimed at the center zone of the pond

* Courtesy of Dr Nyan Taw

Table 13.2: Water preparation routine, following transfer from treatment reservoirs

Time	Activity (application in kg/ha)
Day 1	16 Urea, 2 TSP, 100 dolomite & 60 grain based pellets
Day 2	Tea seed cake 15 ppm
Day 4	100 dolomite, 60 grain based pellets
Day 6	100 dolomite, 60 grain based pellets
Day 8	100 Kaolin, 100 grain based pellets, 16 kg molasses
Day 10	100 Grain based pellets
Day 12	100 Kaolin

Water quality is built along a period of about 2 weeks prior to stocking to build a proper biofloc pond. Water treatment includes addition of fertilizers (urea and TSP, triple super phosphate, water conditioners, dolomite and tea seed cake as well as organic matter needed for the biofloc about 1 to 1.7 when both formulated feed and grain based pellets are considered. An interesting achievement is the high energy efficiency. The power efficiency is about 600 kg shrimp/kW, appreciably more than the conventional 500 kg/kW.

Yields and efficiency were shown to increase by using partial harvests. Ponds are stocked at 100-280 PL/m². Harvesting is performed 2-6 times starting about 90 days after stocking. Yields are drastically raised, and power efficiency has risen up to more than 1,000 kg shrimp/kW as seen in Table 13.3, adapted from Taw et al., 2008.

Detailed results of a control pond (Pond # 1, with no addition of carbohydrates) with ponds No 2-7, fed with 34% protein pellets + grain pellets were all managed using seversal partial harvests, from 2 in ponds 1-3 up to 5 partial harvests in ponds 4 & 5. Shrimp production increased by partial harvesting, up to almost 50 ton/ha. The more bsalanced biomass load along the growing season, enabled to reduce power consumption up to more than 1000 kg shrimp/hp.

Table 13.3: Partial Harvest Performance with Bio Floc Technology (Medan, Indonesia, Taw, et al 2008)

Power input[1] Hp/ha	Stocking Density $1/m^2$	Harvest Number	Days of Culture	Yield Kg/ha	Mean body weight g	Total Production Kg/Ha	FCR [2]
			Pond 1 – 5,896 m²				
27	100	1	118	736	21.28		
		Final	127	18,703	23.26	19,439	1.60
			Pond 2 – 5,896 m²				
31	145	1	108	3,548	16.95		
		2	121	1,723	18.18		
		Final	131	17,629	19.23	22,910	1.38
			Pond 3 – 5,940 m²				

Power input[1] Hp/ha	Stocking Density $1/m^2$	Harvest Number	Days of Culture	Yield Kg/ha	Mean body weight g	Total Production Kg/Ha	FCR [2]
Pond 4 – 4,704 m^2							
34	257	1	85	4,171	10.75		
		2	99	4,031	13.33		
		3	113	3,977	16.13		
		4	127	5,500	17.86		
		5	134	5,261	18.87		
		Final	155	15,289	21.28	38,229	1.29
Pond 5 – 2,500 m^2							
48	280	1	84	3,696	11.63		
		2	99	5.820	13.51		
		3	113	5,296	16.39		
		4	127	5,792	17.54		
		5	134	4,172	18.52		
		Final	155	24,708	20.00	49,484	1.25
Pond 6 – 2,500 m^2							
38	145	1	110	6,664	19.61		
		2	124	1,468	20.41		

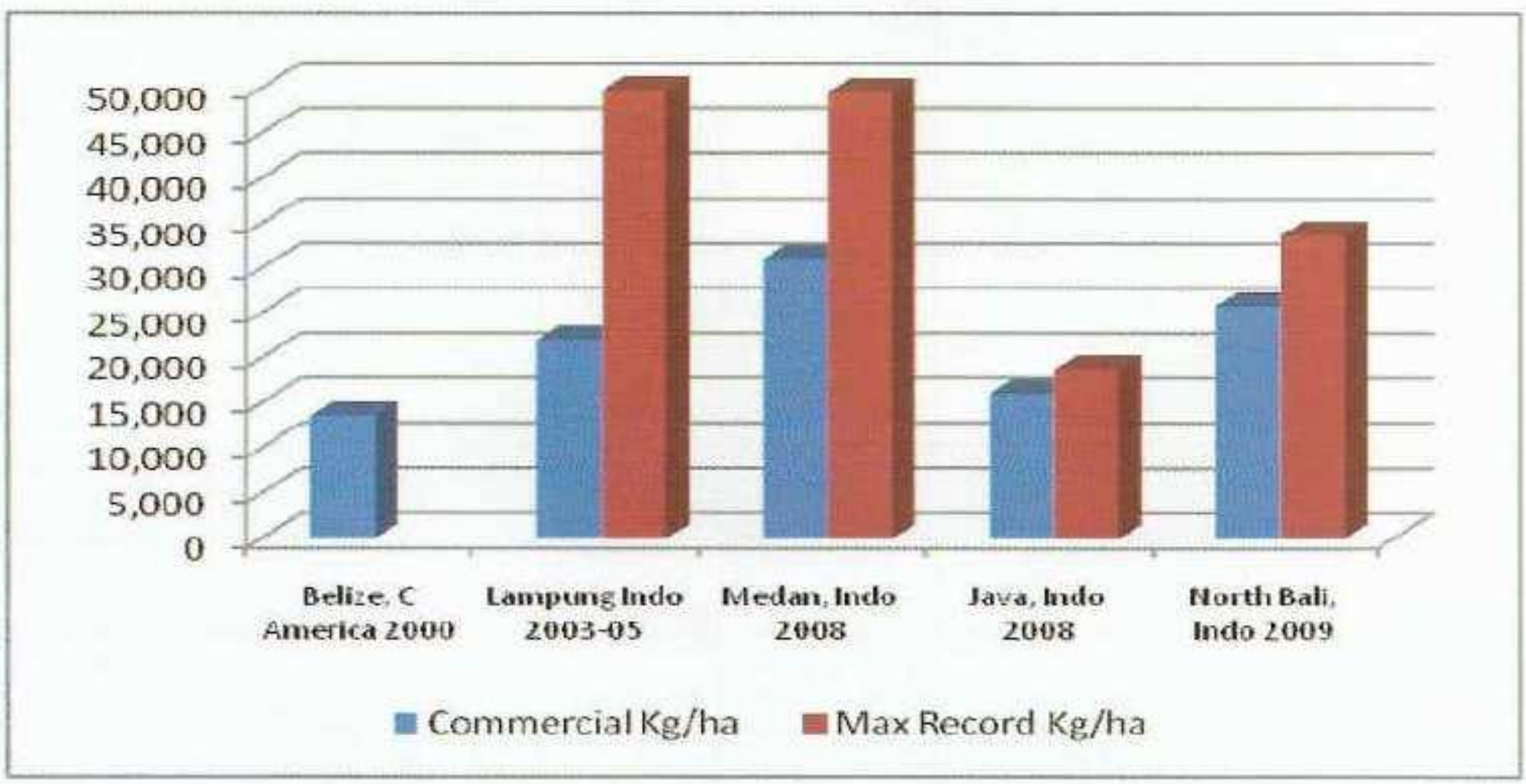
1. Aeration was by paddle wheel aerators. Ponds 5, 6, 7 had air diffusers in parallel to aerator lines, powered by a 12 hp blower.

2. FCR is calculated taking in account total feed supply, i.e. regular feed pellets + wheat grain pellets. Yet, since the cost of grain pellets was 30% of feed pellets, the sum was normalized according to cost.

The developed technology lead to yields and performance improvements as compared to those developed before in Belize, as shown in Fig. 13.6. Yields of shrimp of above 20 ton/ha per cycle, with an FCR of 1.25 and power yield of up to 1000 kg shrimp/hp.

The use of biofloc technology in Indonesia and in Malaysia started through the adoption of the system by large companies. Later on, individual farmers followed (in cases, technicians that learned the technology while working in BFT commercial farms). A number of successful individually owned and operated farms exist in both Indonesia and Malaysia.

Figure 13.6: Performance of biofloc technology – Belize and Indonesia



D. Taw's summary statement on BFT practical experience is as follows:

Advantages:

1. Very good bio-security minimized WSSV occurrence.
2. Zero water exchange.
3. Production (carrying capacity) 5-10% better than normal systems.
4. Shrimp size, about 2.0 g higher than normal systems.
5. Low FCR, 1.0-1.3 (Without grain based pellets).
6. Production costs lower by 15-20% as compared to conventional systems.

Disadvantages:

1. High energy input, 28 hp/ha
2. Power failure for more than 1 hour is critical. Need for back up.
3. Need for full HDPE lining (minimum partial coverage)
4. Technology is more advanced than conventional. Need to train technicians.

Coverage of practical field experience is far from complete, both due to limited dissemination of information and due to the dynamic nature of field experience which is improving and developing very rapidly. However, the fact that BFT ponds cover many thousands of hectares and that coverage is expanding, is a strong real world indication that the system is viable.

It seems that besides good and efficient usage of land, water and feed, the system saves production expenses and is economically viable. The economic advantages of BFT may be increasingly important in the future as feed and energy costs are on the rise while decreasing shrimp prices are increasing pressure to reduce production costs to achieve economic sustainability

It is interesting to compare the BFT system with the efforts to produce synthetic microbial protein for feed materials. Over the years, a number of attempts have been made to commercially produce bacteria for sale as "single cell protein" (SCP). A typical process was the Pruteen

E-mail correspondence of Ms. Ninuk Sri Maharti, Indonesia

One of the people that kept asking questions for about 2 years is Ms. Ninuk Sri Maharti, working for Suri Tani Pemuka shrimp farming in Indonesia. Ms. Ninuk Sri Maharti kept asking questions, followed advice (and added much through his experience). It was nice to recently receive a letter saying that though he still has problems, things are going well. I got his permission to add his last letter and short report on the farm operation to this chapter. The letter and brief report are given with minimal editing. It did not need much and I felt that editing would reduce the value of Ms. Ninuk Sri Maharti's contribution.

Dear Mr Yoram,
Do you remember our problematic biofloc ponds that I told you about last time?
We have harvested 5 of 6 shrimp ponds that we have. Average stocking density is 140 per square meter. Grow-out lasts 110 days. Survival is 75% and improving with a feed conversion of 1.4. Although we have not fully succeeded in maintaining biofloc in grow-out ponds due to low C/N ratio, overall I think the yields were not too bad considering most shrimp farmers have failure in shrimp culture at present condition in our country due to some viral disease outbreaks, deteriorating water quality and environmental quality, etc.
From here I conclude that a closed system (with BFT as a part of its implementation) is one real promising option on shrimp culture now in my country.
We realized that establishing biofloc in our facility was an accidental discovery as a secondary effect of increasing stocking density and the numbers of aerators we used and by applying closed system/no water exchange. As I said before, we are not ready with this technology in relation to how to maintain the flocs alongside a cycle including C-organic supplementation. So for the next period I hope we will focus on management of bioflocs within systems.
By the way, for the future, we plan to establish and develop bioflocs of tilapia in some reservoir ponds that will be used to inoculate bioflocs in shrimp ponds; we call them biofloc libraries. We can pump this biofloc water to all shrimp ponds to facilitate establishing bioflocs at initial and or for water addition due to evaporated or siphoning. So shrimp ponds flocculation was enhanced through inoculated water from tilapia ponds that had a good and high flocculation potential.
Is it a good idea?
But, what is an ideal stocking density of tilapia to get a good biofloc established (quick and easy) in such reservoirs?
Please send any relevant info on biofloc technology of tilapia used as a biofloc donor/reservoir.
Thanks for your help, as soon answer would be appreciated.

Short report of the BFT pond operation

Review : Suri Tani Pemuka, Pond Management

Pond Description

All of the ponds we have are square, varying in size averaged from 4,000 – 5,000 m2. The depth of all ponds bottoms slope from 1.0 meter at the water inlet to 1.4 meters at the point of harvest. From 53 grow-out ponds we began with BFT commercial trials in 6 shrimp grow-out ponds. All of the 6 ponds are HDPE lined because of high water-seepage problems.

Pond Preparation

The day following harvest, the pond is cleaned by brush and washing any accumulated sludge. The pond is sun-dried for a few days and then filled with the water pumped from reservoir-2 pond. Initially new seawater was pumped to reservoir-1 as sterilizing pond. In this pond, we treated the water with 20-30 mg/l of calcium hypochlorite. We must do this because of outbreaks of some viral diseases (TSV, IMNV and WSSV) around our pond region. As soon as neutralized, the water can be pumped into reservoir 2 that has been stocked with a number of tilapia and milkfish inside. This water is ready for use for initial filling or water addition for shrimp ponds.

Stocking Rate

Once the grow-out ponds have been filled, we treat the pond by applying 3-5 ppm of sugar cane molasses and inoculate with commercial bacterial products due to the sterilized water we used. We don't require pond fertilizer. After we got colored water, we stocked directly Penaeus vannamei post larvae (SPF) from shrimp hatchery at 114 – 156 PL/m2. There is no water exchange along the cycle.

Aeration

In grow-out ponds, we use about 44 HP of Taiwanese paddlewheel aeration per hectare, all of the paddlewheel are turned on along the cycle. These aerators are directed to create a clockwise circular motion. We don't use aspirator-type aerator devices. With such aerator type and numbers, we can keep solids in suspension. We remove the accumulated sludge by siphoning and sludge removal from a central drain pipe.

Feeding, CN Ratio and pond dynamics

force them to do emergency harvesting. I would like to say something about the disease prevention benefits of using this system. We are able to maintain this condition until day 65, everything is OK. But , when feed amount is gradually increased to up to 220 kg/pond/day at 70 days old and above, molasses and rice bran addition were not enough to meet organic C requirements for bacteria to work. C/N ratio was still low (± 10). With such C/N ratio, carbon becomes the limiting nutrient and bacterial populations were dropped due to not enough energy sources. I know that diets with such a protein level as 35% has C/N ratio of less than 10:1. and feeding such diets in high density, zero water exchange systems slows the rate of waste decomposition and results in accumulation of inorganic nitrogen (and that is true and occurs in our system, the ammonia-nitrogen starts to built up). We should add more carbohydrate to increase C/N ratio according to the amount of feed. But in reality, C-organic supplementation is insufficient, although we have increased the molasses amount.

At this point, we have started to face water quality problems (pH swing, bacterial counts were dropped). Finally, we combine aquaculture diets with lower protein level (32 %) and exchange water for a few days to overcome and repair this condition and go back to zero water exchange once the water parameters are in stable condition.

Community Succession

It takes about 30 days for flocs to develop with an average floc volume of 0.1 – 0.5 ml/l. We go through an algal bloom, massive foam at the water surface appears and by week 6-7 of culture, these blooms are replaced by microbial floc. Almost magically, the dense phytoplankton change to thin phytoplankton. The cell numbers of phytoplankton per ml is going down (less than 10,000 unit/ml and at the same time floc volume and bacterial counts rapidly increase). As floc volume increases to 7 ml/l, the total bacterial count increases from 106 to over 109. That was our first miracle experience with flocs in grow-out pond, although we have often seen this paradigm in our raceways or small concrete tanks.

The transparency readings are 30 – 35 cm using secchi disk, if we compare with typical autotrophic system at the same cultured period, the transparency can get 20-25 cm with dense phytoplankton.

But, when entering day 70 and up, floc volume dropped initially as we had a power failure for more than one hour, and then the suspension rapidly settled out, also resulted in insufficient c-organic supplementation while increasing the feed amount at the near end of the culture period.

Results

Table 13.4: Harvest results of BFT commercial ponds-based trial for the production of Litopenaeus vannamei

Pond	Density	Days of culture	Biomass (kg)	Avg. weight (gr)	Prod. level (mT / Ha)	Survival (%)	FCR
D – 6	115	113	8,214	16.70	16.3	85	1.37
D – 5	115	121	7,374	15.36	18.7	106	1.60
D – 8	141	118	8,566	17.30	18.5	77	1.51
D – 7	172	121	6,739	17.89	14.6	79	1.75
D – 9	176	121	5,256	20.08	11.4	53	2.0
D – 4*	139	108	7,533	15.50	16.4	75	1.65

*Just an estimation. This pond hasn't been harvested yet.

Practical Implications and Tips

In the synopsis of this chapter we asked if BFT is just a science fiction. I hope this demonstrates that BFT is alive (and kicking). A lot already studied and known, and much more to be further done. However, when we sum up practical experience, the major question many readers will ask (as many people talking or writing to me have already done): "Is BFT a method for making a business? Can I make money, or lose money?".

These are difficult questions. BFT farms exist in practice for just a few years, along a period when aquaculture as a whole went through very difficult times. We do not have enough open data enabling the preparation of a solid balance sheet. Prices and costs vary from place to place and from time to time.

It does seem that BFT is a way to have intensive shrimp or tilapia production systems cheaper than the alternative intensive (or super intensive) systems. It seems that the recycling of feed and the apparent effects on health are very important features in constructing the business plan. However, experience shows that BFT farms planned and run by knowledgeable people do succeed, while most failures (and there were some), were caused because the managers did not take the right technical decisions.

An important question is related to the possibility of changing conventional, traditional small farms to the sophisticated biofloc technology system. This is a crucial question related to the further introduction of BFT to wider rings of the aquaculture community. Encouraging approaches and even field experience (see Chapter 9) indicate the possibility of gradual transition, in tandem with development in understanding the system.

My personal hope is that this book will help people understand the system, help them make the right decisions and succeed in conducting profitable aquaculture.

Exchange of information is the key for progress. Most everyone gains by exchanging information, discussing it, improving ideas and learning from others. Write us about new developments and experience (both achievements and problems).

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Diab S., Kochva M. 1992. Control and utilization of inorganic nitrogen in intensive fish culture ponds. Aquaculture and Fisheries Management 23: 421–430.*

2. *Avnimelech, Y., Kochva, M., Diab, S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio, Israeli Journal of Aquaculture- Bamidgeh 46: 119–131.*

3. *Azim, M.E., Little, D.C. 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile Tilapia (Oreochromis niloticus). Aquaculture 283 (1-4) 29–35.*

4. *Browdy, C.L., Bratvold, D., Stokes, A.D., McIntosh, R.P. 2001. Perspectives on the application of closed shrimp culture systems. PP 20–34. In: Browdy, C.L., Jory, D.E (Eds.). The New Wave, Proc. Special Session on sustainable shrimp farming. World Aquaculture Society. Baton Rough. LA. USA.*

5. *Burford, M.A., Thompson, P.J., McIntosh,P.R, Bauman, R.H., Pearson, D.C. 2003. Nutrient and microbial dynamics in high-intensity, zero-exchange shrimp ponds in Belize. Aquaculture 219: 393–411.*

6. *Chamberlain, G.W., Hopkins. S.J. 1994. Reducing water use and feed cost in intensive ponds. World aquaculture 25: 29–32.*

7. *Chamberlain, G., Avnimelech, Y., McIntosh, R. P., Velasco, M. 2001. Advantages of aerated microbial reuse systems with balanced C/N. II: composition and nutritional value of organic detritus. Global Aquaculture Advocate 4: 22-24.*

8. *Decamp, O., Conquest, L., Forster, I., Tacon, A.G.J. 2002. The nutrition and feeding of marine shrimp within zero-exchange aquaculture production systems: Role of eukaryotic microorganisms. PP 79-86. In: Cheng-Seng, L. and O'Bryen, P. (Eds.). Microbial approaches to aquatic nutrition within environmentally sound aquaculture production systems. World Aquaculture Society. Baton Rouge, LA USA.*

9. *Farrell D. 2006. Commercial tilapia microbial controlled systems in California. Abstracts. WAS Aquaculture America. Las Vegas.*

10. *Hopkins S.J., Hamilton, R.D., Sandifer, P.A., Browdy, C.L., Stokes, A.D. 1993. Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. Journal of the World Aquaculture Society 24: 304–320.*

12. *McIntosh, R.P., Series of reports published in the Global Aquaculture Advocate, e.g.:*

13. *Changing paradigms in shrimp farming: 1-General description. Global Aquaculture Advocate 2 (4/5): 42-45. 1999.*

14. *Changing paradigms in shrimp farming: 3- Pond design and operation considerations. Global Aquaculture Advocate 3 (1): 42-45. 2000a.*

15. *Changing paradigms in shrimp farming: 4- Low protein feeds and feeding strategies. Global aquaculture Advocate 3 (2): 44-50. 2000b.*

16. *Changing paradigms in shrimp farming: 5. Establishment of heterotrophic bacterial communities. Global Aquaculture Advocate 3 (6)52-54. 2000c.*

17. *Rosenberry, B. (Eds.). 2006. World shrimp farming. Shrimp News International. San Diego. CA USA 272 PP.*

18. *Serfling, S.A. 2000. Closed-cycle, controlled environment systems: the solar aquafarms story. Global Aquaculture Advocate 3 (3):48-51.*

19. *Taw, N. 2005. Shrimp farming in Indonesia, evolving industry responds to varied issues. Global Aquaculture Advocate 8 (4): 65-67.*

20. *Taw, N., Wartono, C.R.R., Slamet, Y., Tomo, P. 2007. Reengineering dipasena: huge plasma shrimp farm adopts biosecure modules. Global Aquaculture Advocate 10 (3): 42-45.*

21. *Taw, N., Fuat, H., Tarigan, N., Sidabutar, K. 2008. Partial harvest/biofloc system promising for pacific white shrimp. Global aquaculture Advocate. pp 84-87.*

22. *Taw, N. 2010, Biofloc technology expending at white shrimp farms. Biofloc systems deliver high productivity with sustainability. Global Aquaculture Advocate May/June pp 20-23*

23. *Wasielesky W. (Jr), Atwood, H., Stokes A., Browdy, L.C. 2006. Effect of natural production in a zero water exchange suspended microbial floc based super-intensive culture system for white shrimp Litpenaeus vennamei. Aquaculture 258: 396-403.*

Chapter 14

Biofloc Technology for Super-Intensive Shrimp Culture

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In Brief

Super-intensive shrimp culture technology has progressed significantly in the last decade. We define super-intensive shrimp BFT systems as those which are stocked at 250 shrimp/m^3 or more and typically yield 3-6 kg/m^3, though higher yields are reported. With high biomass and feeding, the organic load is substantial, thereby necessitating careful monitoring, informed management, and removal of a portion of the suspended matter. Super-intensive shrimp production allows the use of smaller systems, some of which are contained in greenhouses or insulated buildings allowing year-round production. The technology is more expensive than traditional extensive styles; however, consistent production, versatile location options, and high product quality allow for unique product marketing opportunities.

Interest in closed aquaculture systems for the production of shrimp and fish has increased during recent years, mostly due to some key environmental and marketing advantages that such systems have over extensive systems. When water is reused, the risk of discharging pollution is reduced. This is a benefit for protecting natural resources. Furthermore, environmental regulations and discharge fees are inhibitive in most regions. Another advantage is biosecurity: Introduction of contaminants and pathogens from the environment to cultured animals is unlikely, especially when measures such as source water disinfection are employed. Using closed systems limits the chance of animal escapement, helping to prevent exotic species and disease leakage to the natural environment. Because of reduced water use, marine animals can be cultured at inland locations. This can allow producers to market fresh, never frozen marine shrimp or fish to inland metropolitan locations and can help to reduce land cost and the dangers associated with coastal extreme weather events. Furthermore, the fact that heat is contained in closed systems allows tropical animals to be cultured in cool climates year-round.

Several technologies exist for the closed-system culture of marine shrimp. Biofloc technology is discussed in detail in this book. In discussing these systems we have typically been referring to ponds, normally rather large (in the range of 100-20,000 m^2), usually, but not exclusively lined, aerated (usually without the use of pure oxygen), and thoroughly mixed. Yields of shrimp in such systems are in the range of 10-50 ton/ha. Fish yields reach a range of 100-300 ton/ha.

To increase shrimp yields and reduce the spatial footprint, higher intensity is required, by using strict suspended matter control in smaller tanks or raceways. Water quality control is systems are described in the present chapter.

One of the major advantages of culturing shrimp in biofloc systems is that multiple external filtration systems are not required as they are in clear water recirculating systems (RAS). This reduces the mechanical complexity, start up and operational expenses of biofloc systems. There is no need for external biological filtration because the microbial processes detoxifying nitrogen compounds are found within the water column; sterilization devices such as ozonation systems would only hinder these processes. Some level of control over the accumulation of biofloc particles is required, especially in the most intensive systems. However, this can typically be accomplished using a side arm settling system or simple foam fractionators.

As mentioned in Chapters 3 and 4, a combination of nitrogen pathways occur in biofloc systems. The three major aerobic processes are described in detail in this book and by Ebeling et al. (2006): Heterotrophic assimilation, chemoautotrophic nitrification, and photoautotrophic assimilation. Each of these processes has unique benefits for shrimp biofloc systems and management goals should be carefully evaluated to determine which process, or combination of processes should be encouraged. Some examples of microbial management include increasing the carbon: nitrogen ratio (C:N) to favor heterotrophic nitrogen assimilation (Chapter 4; Hari et al., 2006), adding bacterial supplements to facilitate nitrification (Kuhn and Drahos, 2011), and encouraging photoautotrophic production by removing particles from the water to increase light penetration (Ray et al., 2009).

An experiment was conducted at the University of Southern Mississippi's Gulf Coast Research Laboratory (GCRL) to directly compare heterotrophic-dominated and chemoautotrophic-dominated shrimp culture systems (Ray et al., 2011). Sucrose was added to four 500 liter tanks in addition to shrimp feed to create a combined C:N of 25:1 for both sucrose and feed, while no sucrose was added to another four experimental tanks (chemoautotrophic tanks). A high spike in nitrite concentration and a continual increase in nitrate concentration were observed in the chemoautotrophic tanks, while a much lower spike of nitrite and virtually no nitrate were seen in the heterotrophic tanks. A greater concentration of solids was generated in the heterotrophic tanks. However, there were no significant differences between the two treatments in terms of shrimp survival and growth rate. Further experiments such as this are needed to help refine management strategies for super intensive biofloc shrimp systems.

Another major benefit of growing shrimp in biofloc systems is that shrimp, like some fishes, are able to take advantage of the microbial community in the water column. Numerous studies have demonstrated benefits of culturing shrimp in biofloc-rich water compared to clear water; those benefits include improved growth rate, improved feed conversion ratio, and the contribu-

Super-intensive Shrimp Culture Systems

When the biofloc microbial community is managed properly, microbial processes can assimilate and recycle large quantities of nitrogen. Although chemoautotrophic nitrification are usually important, we often find that all three major nitrogen pathways are functioning. The nitrogen pathways in these systems may therefore referred to as mixotrophic. The term super-intensive arises from high animal stocking densities, the large inputs of nutrients that are added to support those animals, and very low rates of water exchange. Although shrimp biofloc systems can be operated at varying levels of intensity, the focus of this chapter is specifically on super-intensive systems.

Density

The optimal shrimp density for a super-intensive system will depend on management and production goals. Higher shrimp density leads to a greater concentration of microbes in response to more nutrients. This will increase the oxygen demand of the system (by both shrimp and bioflocs) and augment the generation of solids. Also, higher density can slow shrimp growth rates, although a greater overall biomass may be produced.

In super-intensive biofloc shrimp culture systems it seems appropriate to refer to shrimp or shrimp biomass density in relation to volume (ex. shrimp/m^3 or kg shrimp/ m^3). This is a departure from much of the literature on pond culture which refers to density or biomass per area with units such as shrimp/m^2, shrimp/acre, and shrimp/hectare. In super-intensive biofloc systems the microbial community is predominantly suspended in the water column. Therefore, the level of biological filtration is dependent on water volume.

Shrimp density has a great deal to do with which nitrogen pathway dominates. Generally, lower density intensive biofloc systems that are exposed to consistent light will be dominated by photoautotrophic assimilation. Heterotrophic-favored systems in which the C:N ratio is elevated through feed formulation, supplemental carbon addition, or both can be thought of as mid-level density systems. Heterotrophic shrimp systems perform well at stocking densities between about 150 and 350 shrimp/m^3. On the high end of the scale are the chemoautotrophic-favored systems in which nitrifying bacteria convert ammonia to nitrite, and then convert nitrite to nitrate. Super-intensive levels of production, stocked at 300 shrimp/m^3 or more can be achieved by relying primarily on the heterotrophic or chemoautotrophic processes. We almost always find that these two processes occur simultaneously; which process is favored depends largely on intensity and management, but both are very important for intensive and super-intensive culture. When abundant light is available algae will also be found in the culture water, although they play a much smaller role in more intensive systems than compared to bacteria.

The Waddell Mariculture Center (WMC) in Bluffton, South Carolina, USA has been conduct

Wasielesky et al. (2010) conducted a study to help determine maximum theoretical shrimp stocking density independent of water quality factors by growing shrimp in small containers receiving flow through water from an adjoining large tank which contained shrimp stocked at 300/m^3. Water quality in the large tank remained satisfactory and maintained that of the smaller containers. Shrimp were stocked into the smaller containers at varying densities and sizes over the course of four experiments. The authors found that as shrimp grew, the containers could support fewer shrimp, but an increasing overall biomass per container volume. Survival was 96% and shrimp grew from 0.003 g to 0.30 g in 30 days at a stocking density of 13,200 shrimp/m^3. Survival was 90% or more when shrimp were cultured for 40 days and stocked at 1,760 shrimp/ m^3, growing from 1.2 g to 6.7 g; likewise when shrimp were stocked at 1,180 shrimp/ m^3, growing from 6.3 g to 10.6 g; and when shrimp were stocked at 880 shrimp/ m^3, growing from 11.9 g to 15.7 g. This study indicates that high densities of shrimp can be cultured provided that good water quality is maintained. Although the overall system volume was larger than what was reported in the containers, the study indicates that shrimp could be grown in relatively compact culture units. Additional work may need to focus on improving growth rates at these densities.

Shrimp Production

During the period between 2000 and 2005, shrimp production at WMC ranged from approximately 2.3-6.8 kg/m^3 (Browdy et al., 2006). The upper end of this level of production can generally be repeated presently at WMC. Otoshi et al. (2007) reported three super-intensive shrimp production trials at the Oceanic Institute in Hawaii, USA. These trials were conducted during the years 2006 and 2007 and resulted in production of 5.7, 7.6, and 10.3 kg shrimp/m^3. Typical production levels range from approximately 3-6 kg/m^3; however, recent high production values are an encouraging indicator of the progress being made in super-intensive shrimp culture research.

A different aspect is the water consumption needed to produce shrimp along the cycle, this criterion includes water exchanged. According to Otoshi et al, (2007) the highest production values were obtained during a trial in which over 400 liters of water were used per kilogram of shrimp produced. Samocha et al. (2010) reported super-intensive shrimp production ranging from 9.3-9.8 kg/m^3 in three raceways, Using less than 130 L water/kg of shrimp. This level of production and low rate of water use are unusual among most super-intensive shrimp culture systems.

Systems

Super-intensive biofloc shrimp culture systems, are commonly contained in tanks or lined raceways. Because shrimp are grown at high density, the size of these systems can be significantly smaller than ponds. This allows super- intensive systems to be operated in a closed building or in a greenhouse. An insulated building may allow for rigid temperature control, but if animals indoors, biosecurity and anti-predation efforts can be facilitated more effectively.

Round tanks and small round ponds have proven effective for culturing shrimp in super- intensive biofloc systems. Circular water movement can be achieved with relatively low energy input and water homogeneity is facilitated. Round ponds with circular flow can have a drain at the center to remove settled solids, facilitating efficient solids management (Chapter 12). However, it is difficult to utilize farm space effectively with round systems. Many super-intensive shrimp biofloc systems are contained in raceways: long, narrow tanks. Raceways are typically rectangular in shape, but with rounded corners. A central wall or baffle spans the majority of the longer dimension (Figs. 13.1, 13.2). Water can be propelled around this wall to encourage thorough mixing of the system. Raceways are usually sloped towards one end to facilitate proper draining during harvest, and they utilize space more effectively when contained within buildings.

Figure 14.1: A 282 m^3 raceway at The Waddell Mariculture Center (WMC) in Bluffton, South Carolina, USA

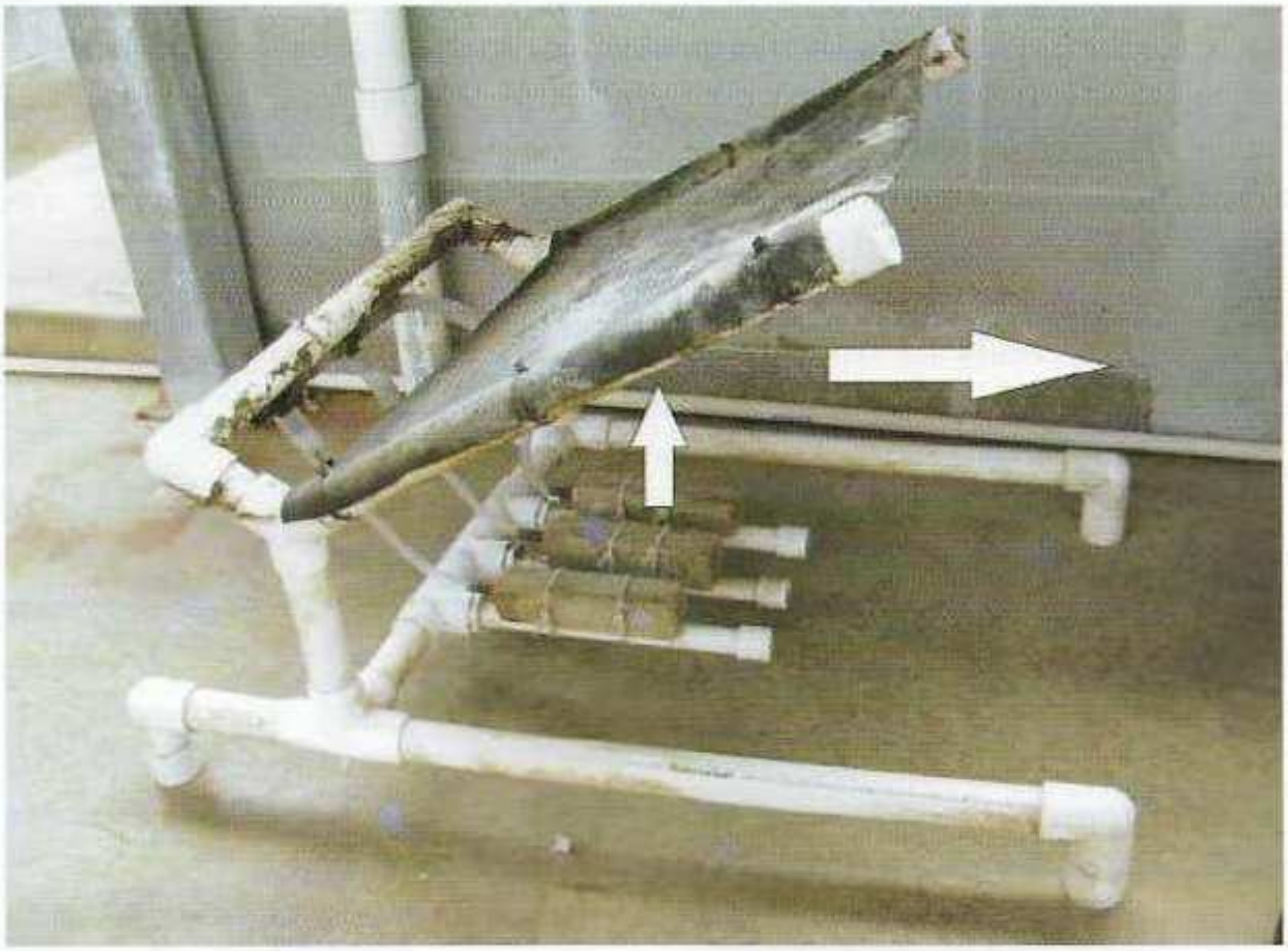
Water Movement and Oxygenation

Water movement and oxygenation are imperative issues in super-intensive biofloc shrimp culture systems. As with any biofloc system, water movement is necessary to keep particles in suspension. If the biofloc particles settle, pockets of anaerobic sludge often form, releasing ammonia or sulfides; sulfide can be extremely toxic to animals and can inhibit nitrification, thereby causing a further increase of toxic nitrogen compounds (Avnimelech and Ritvo, 2003). Maintaining adequate dissolved oxygen concentration is also of paramount importance and can be the most critical factor to successful super-intensive operation.

Large aerators used in ponds are typically not appropriate for super-intensive shrimp systems due to the relatively small size of these culture units. When selecting aeration devices it is important to take into account system size as well the density of animals, as some mechanisms can cause injury to densely stocked shrimp. Air blowers are commonly used in super-intensive systems. Super-intensive systems, 6.2 m^3 each, exposed to sunlight with 3.2 kg shrimp/ m^3 and adequate dissolved oxygen concentrations that exclusively used blown air delivered through ceramic diffusers were described by Ray et al. (2010). This may be an appropriate strategy for small, super-intensive systems.

In many biofloc systems water movement and oxygenation are achieved using the same equipment. Air lift mechanisms are commonly used to move and aerate water (see chapter 10). Generally, an air lift is an apparatus that uses blown air, usually passed through a diffuser. That blown air is then guided with physical structures so that it moves in a predictable direction. The density of water with air bubbles is lower, and thus an upward movement is generated. These mechanisms typically pull water near the bottom of a culture system and discharge it at or above the surface, thereby helping to alleviate stratification. The low cost, low energy input, dual purpose (propulsion and aeration), and effectiveness of air lifts make them popular in biofloc systems, although designs vary. Air lifts can move water through PVC piping, which help to move water around a shrimp raceway. Another design is seen in Fig. 13.4; rather than moving air and water through pipes, this airlift has a deflector. When air and water contact the deflector, they are propelled forward, thereby helping to circulate water around a tank. Although airlifts are useful for moving and aerating water, in super-intensive systems they may not be able to meet the oxygen demands on their own.

Figure 14.3: Air lift mechanism used at the Gulf Coast Research Laboratory in Ocean Springs, Mississippi, USA



Water pumps are commonly used to propel water and supply aeration and oxygenation in super-intensive systems. Pumps can simply be used to move water around a tank or raceway, keeping particles in suspension and homogenizing the water column. However, a more effective strategy is to attach nozzles in the water line that help to inject air and/or oxygen into the water. Venturi-style nozzles are commonly used. These nozzles are typically the same diameter as the pipe through which water is moving, except in one small area of the nozzle where the diameter is substantially restricted; in this area water velocity increases. Water pressure decreases as the flow is released to the wide outlet. The small diameter region has an opening and, due to the low pressure, gases are drawn in through this opening and injected into the water. This can be an effective means of injecting ambient air for aeration or injecting pure oxygen for oxygenation. Venturi nozzles are usually placed in a pipe line behind the point that water exits the line. This helps to ensure that there is ample distance (water area) for the injected gases to transfer into the water. A newly available type of nozzle known as an a³® nozzle (All Aqua Aeration, Titusville, Florida, USA) has shown great success in super-intensive biofloc culture systems (Fig. 13.5). Samocha et al. (2010) described using a³ nozzles to maintain high dissolved oxygen concentrations while eliminating the use of pure oxygen injection and

Figure 14.4: An a^3 nozzle. As water is propelled through the nozzle air is drawn in through the vertical pipe. This nozzle requires a high-powered pump, but is highly effective in oxygenating water using only ambient air.

It is recommended to have back up pure oxygen on hand when operating super-intensive systems. Dissolved oxygen fluctuations can be unexpected, and unwelcome, occurrences; typically occurring in conjunction with high temperatures, feedings, and carbohydrate additions. Nozzles are an effective way to supply pure oxygen; diffusers are also available for this purpose. Such diffusers have finer pore sizes than aeration diffusers. The use of oxygen diffusers is typically an efficient method of injecting pure oxygen; however, to adequately disperse the oxygen either a water pump or an airlift mechanism is recommended to circulate water around the tank.

Super-intensive Indoor Systems

As mentioned above, super-intensive biofloc systems can be constructed within closed build-

This strategy is gaining popularity in the United States. Dozens of relatively small-scale indoor biofloc operations have been opened in the last ten years, and several large entities have been developed as well. Although this move to indoor biofloc systems holds tremendous opportunity, there are technical challenges associated with the strategy. As interest in indoor shrimp farming continues to grow, unforeseen challenges will surely arise. Two of the issues specific to indoor biofloc systems that should be considered include venting gases while retaining heat, and how much and what type of lighting to use.

Super-intensive biofloc systems can produce substantial quantities of carbon dioxide through microbial and animal respiration; this gas must be vented for the safety of the culture animals and that of farm workers. It is important to note that CO_2 is heavier than air and so it tends to sink. This means that CO_2 will tend to sit on top of a tank that has excessive free-board (space between the water surface and that of the top of the tank wall). Many shrimp culture systems have some free-board to help prevent shrimp from jumping out of the tank onto the floor. However, netting or some other containment method may be more appropriate so that CO_2 may escape from the tank. If the gas becomes concentrated on the water surface it will tend to diffuse back into the water. Excessive CO_2 dissolved in the water will form carbonic acid and drive down pH, forming an acidic and potentially dangerous environment for shrimp. Not only can CO_2 lower pH, but it can be directly toxic to shrimp, and it can be toxic to humans.

Care should be taken to remove CO_2 from the rooms in which culture tanks are held, keeping in mind that the gas will accumulate low to the ground. Exhaust systems should be considered that pull gas from the lowest parts of a room to remove CO_2 and prevent the removal of warmer air that rises higher in the room, especially in cool climates where heat conservation is important. However, another gas that can be problematic is water vapor. In indoor, aquaculture operations there are often high concentrations of water vapor in rooms that house tanks. The vapor condenses on surfaces, including the ceiling and overhanging structures after which it drips back into culture tanks. This can be beneficial for the sake of water conservation; however, microorganisms such as molds and fungi tend to develop on surfaces that are constantly wet. Water that drips from surfaces into tanks can then be a potential source of contamination. Furthermore, it is generally difficult to properly sanitize rooms and equipment that do not have an opportunity to dry completely. Engineering concepts should be developed that consider water and heat conservation, while minimizing the potential negative impacts of CO_2 and water vapor. It seems that fresh air must be brought into rooms with shrimp culture tanks and CO_2 should be flushed from a low point in the rooms. If heat can be retained or even captured from another source during this process profitability and sustainability can be enhanced while helping to achieve a more consistent production environment. Some engineers have considered pulling air from warm areas such as the top of a room or through metal coils inside the exhaust stack of a boiler. The air drawn in through blowers or nozzles can be sourced from such warm locations, thereby reclaiming otherwise lost heat.

Libes (2009) indicated that chlorophyll-a concentrations greater than 1 μg/L were relatively high for natural seawater systems. There is evidence that shrimp gain nutritional benefits from algae that are present in biofloc-based aquaculture production systems (Ju et al., 2009; Kent et al., 2011). However, culture vessels exposed to sunlight with abundant algae can offer challenges to system managers. Challenges include unreliable nitrogen cycling, shifts in algal composition related to sunlight availability (Ray et al., 2009; Sookying et al., 2011), and the proliferation of harmful algal taxa (Alonso-Rodriguez and Paez-Osuna, 2003; Hargreaves, 2006). Therefore the exclusion of light and consequential elimination of algae may lead to greater environmental consistency; however, some of the beneficial aspects of algae may be lost.

The amount and the source of light may have significant impacts on biofloc systems and shrimp performance. In 3.8 m^3 heterotrophic biofloc shrimp (*L.* vannamei) systems with sucrose additions, Neal et al. (2010) compared biofloc tanks that were exposed to natural sunlight and those that were continuously exposed to a single 60-W, incandescent light bulb. The authors found that shrimp in the incandescent lighted tanks had significantly lower growth rates and survival, and higher feed conversion ratios. These authors attributed the relatively poor shrimp production to the proliferation of *Leucothrix mucor*, a bacterium that can clog the gills of shrimp and cause suffocation (McKee and Lightner, 1982).

Cyanobacteria do well in poorly lighted environments and can be detrimental to shrimp aquaculture (Alonso-Rodriguez and Paez-Osuna, 2003). To prevent the occurrence of cyanobacteria in an insulated building, system engineers may consider excluding light altogether. Wang et al. (2004) conducted an experiment evaluating the effects of five light intensities, including the absence of light, on the growth rate and feed conversion efficiency of Chinese shrimp (*Fenneropenaeus chinensis*). These authors found that there were no significant differences between shrimp grown in the dark and those grown in the best performing light intensity (300 Lux), however, the systems used by Wang et al. (2004) were not biofloc systems.

Aside from affecting the biological function of biofloc systems, light is also needed in shrimp aquaculture facilities so that staff can work safely. It may be possible that light fixtures can be situated near the floor of culture facilities such that only limited amounts of light reach the culture water. Otherwise, abruptly turning lights on and off can startle shrimp, causing them to jump. When shrimp at high density jump they can escape the tanks and they often puncture one another with the sharp rostrums on their heads. Puncture wounds can become infected and lead to mortality, disease, and low quality shrimp at harvest.

Nursery Systems

In super-intensive nurseries, shrimp can be stocked at high densities up to about 3,000 shrimp/m^3. At facilities with the capacity to operate year-round these high densities allow better utilization of tank space. Shrimp can be placed into relatively small nurseries near the end of a grow-out phase for immediate stocking when the grow-out phase shrimp are harvested. This ensures that there is a steady rotation of shrimp through the grow-out phase tanks which are up to 10 times larger than the nursery tanks.

Even system managers who do not operate biofloc-based grow-out systems are benefiting from biofloc nursery systems. Some farmers who grow shrimp in extensive ponds have constructed small greenhouses near those ponds in which they operate biofloc nurseries. Indoor biofloc systems in insulated buildings are another option for application of this technique. Farmers are able to start growing shrimp in such nurseries largely independent of the weather which in many climates can give them a head start on the growing season. There is also anecdotal evidence that growing shrimp in biofloc nurseries prior to pond stocking can help reduce the effects of diseases such as Early Mortality Syndrome.

Establishing a biofloc microbial community for any super-intensive systems can be challenging. The function of nitrifying bacteria can be slow to develop. This may be more problematic in nursery systems where shrimp typically receive higher protein diets that contribute more nitrogen to the water. As ammonia and nitrite concentrations become elevated, carful additions of carbohydrates can be made to bring down those concentrations. However, some ammonia and nitrite is needed to ensure the establishment of nitrifying bacteria, so complete heterotrophic assimilation is not desired in this case. The use of probiotic nitrifying bacteria products may be considered. There are a variety of these products readily available, although many are costly and seem to function inconsistently. A potential problem with adding such products to biofloc systems is that the high concentrations of microbes already present may not allow new probiotic additives to become established, following this logic, it seems that guiding the composition of the microbial community through careful management practices is a more effective strategy to achieve desired outcomes.

During the establishment period ammonia concentration should be monitored very closely. Once ammonia is detected, the concentration of nitrite should then be measured routinely as well. In addition to adding carbohydrates during this period, feed rations should be adjusted such that only moderate amounts of nitrogen are added until the microbial community is able to accommodate larger feed inputs. Lower protein diets may be substituted at least partially during this time. Also, if temperature can be controlled, lower temperature will generally reduce the rate of metabolite excretion allowing bacteria populations time to process nitrogenous waste.

Once nitrifying bacteria populations are established they should be retained. At least part of that water should be transferred to the grow-out phase; ideally all of the nursery phase water is reused. If established biofloc water needs to be saved for future use it should be aerated, biofloc

Biofloc Assessment

The benefits of operating biofloc culture systems are well documented in this book and in an increasing number of scientific and technical papers. In biofloc systems, controlling the abundance of particles has proven to be an essential management step in optimizing the function of super-intensive shrimp culture systems. The terms particles, solids, suspended solids, and biofloc are all used interchangeably in this section. Ideally all particles are in suspension unless there is a specially designed chamber or area of the tank in which settling is encouraged.

In less intensive biofloc systems particle management may not be an important issue, although monitoring of concentration is advised. In more intensive culture systems particles can rapidly become concentrated due to greater feed inputs. Carbohydrate addition also contributes to greater particle concentration and bacterial biomass; if carbohydrates are used as a management strategy, a plan to manage particles should also be considered. There are three commonly used methods of inferring the concentration or abundance of particles: total suspended solids (TSS), turbidity, and floc volume (FV, or settleable solids). Further details regarding the methodology and monitoring of suspended matter are given in Chapter 18.

Biofloc Concentration Management

External settling chambers are one mechanism used to control suspended solids concentration in super-intensive shrimp culture systems. Ray et al. (2010) found that settling chambers (Fig. 13.4) placed adjacent to super-intensive shrimp culture systems reduced TSS by 59%. The authors also found that nitrate and phosphate were reduced by about 60% and alkalinity was increased by 33% compared to systems without settling chambers. These could have been indications that denitrification was occurring in the settling chambers. Furthermore, Ray et al. (2010) found that shrimp biomass production was increased by 41% through the use of settling chambers. This study helped to demonstrate the benefits of solids management in these systems and showed that management may be achieved using simple, cost-effective settling chambers. A quantitative control of biofloc concentrations can be obtained by varying the rate of water cycling through the settler.

Figure 14.5: A settling chamber adjacent to a larger shrimp culture tank. Water is moved to the settling chamber using an air lift mechanism*

*A ceramic air diffuser located in the PVC pipe submerged in the shrimp culture tank moves water up the pipe and into the settling chamber. Water then enters a larger diameter pipe, causing velocity to slow. Particles settle on the bottom and clarified water at the surface of the settling chamber returns back to the shrimp culture tank.

External settling chambers are convenient because particles settle outside the culture unit. Shrimp are not directly exposed to settled material. However, such chambers can, at times, return ammonia to the culture system, likely due to the decomposition of organic matter. Settling particles from the water column is a low-energy, efficient way to control biofloc concentration. Settling is usually most effective at removing large particles above approximately 100 μm in diameter. Settling chambers with cone bottoms are preferred, as the cone shape helps with the are that fractionators can be inconsistent in what and how much they remove and they can use a larger amount of water than settling systems. Samocha et al. (2010) compared the use of external settling chambers to the use of foam fractionators in super-intensive shrimp raceway systems. They found that nitrate was lower in the systems with settling chambers, likely due to denitrification, but that there were no significant differences in shrimp production between raceways with either type of solids management device. Further research may be needed to help determine what solids filtration is best for super-intensive shrimp systems. It is possible that foam fractionators would better facilitate long term water reuse, as they may remove dissolved contaminants that can otherwise accumulate over time.

Several other types of solids management filters are available, such as bead filters. However, for super-intensive shrimp culture systems in which a goal is to use the lowest volume of water possible, settling systems and fractionators are currently the most popular devices.

A critical issue facing biofloc and other RAS systems is the disposal, or preferably the reuse, of removed material. Chapter 8 of this book addresses using this material as a source of nutrition for aquatic animals, thereby recycling otherwise wasted nutrients. Other options include using the material as a fertilizer for plants. Use of material from saltwater shrimp operations can be problematic due to the low tolerance of many plants to salt. However, one option that is being explored is using the waste as a fertilizer for halophytes, such as those used in salt marsh or dune restoration projects (Ray et al., 2011).

The Effects of TSS Concentration

Control over the concentration of TSS is important for super-intensive biofloc systems. It is unclear exactly how particle concentration management leads to improved shrimp production, although some possibilities include reduced gill clogging, promotion of a younger and potentially healthier microbial community, possible removal of nuisance organisms, or reduced biochemical oxygen demand which may lead to increased oxygen availability for culture animals.

Ray et al. (2009) found that by reducing particulate concentration in super-intensive shrimp systems, photosynthetically active radiation was significantly increased in the water column, as was primary productivity. Ray et al. (2010) reported that the removal of particles reduced the overall abundance of bacteria, cyanobacteria, nematodes, and rotifers. These studies help to demonstrate that simple particle management can cause substantial changes in the microbial communities of super-intensive systems. More research is needed to understand whether such changes may affect animal production.

In a recent super-intensive biofloc study Brunson et al. (2011) operated five experimental treatments: in one treatment solids were not removed, and in the remaining four treatments

FCR were about the same for shrimp grown at TSS of 100, 200 or 400 mg/l. Shrimp performance was significantly lower when TSS was 800 mg/l.

Studies have indicated that, in general, a lowering TSS concentration above a set value can improve shrimp production. However, Ray et al. (In Press) found that maintaining TSS concentrations below approximately 200 mg/L may have reduced the nitrification process in commercial scale, 50 m^3 shrimp raceways, whereas in raceways with a TSS concentration of approximately 300 mg/L nitrification proceeded.

More research is needed to determine the optimal TSS concentration range for super- intensive systems. Production goals, waste remediation techniques, and nutrient cycling strategies should be considered in determining the level at which TSS is managed.

Economic Considerations

Currently there are not many commercial super-intensive biofloc systems and lack of data on the economy of the systems, as information open to the public. The cost of production needs to be lowered substantially to enhance profitability. Also, special marketing efforts should be made to help augment the sale price of animals that are cultured in these systems.

An overview of economic factors influencing the profitability of super-intensive biofloc systems was provided by Hanson et al. (2009). These authors created a model with which economically important production variables could be analyzed in detail. Total baseline cost of production in the southeastern United States was estimated to be 5.40 USD per kilogram. The authors found that the biological improvement that could lower production costs the most is shrimp survival. A 20% increase in survival was capable of decreasing production costs by 0.80 USD per kg.

Another important objective that should be met to make these systems profitable is an improvement in the consistency of production. Currently, final production values are often variable among the research institutions that study these systems in the US, due to both biological and mechanical problems. Some of the problematic biological issues include poor growth rate, an inability to maintain adequate pH, likely due to high rates of microbial respiration and high CO_2 concentration, and bacterial infections in animals. Some of the mechanical issues include pump failures and oxygen supply system failures. Research is ongoing to solve biological problems; mechanical problems will likely be solved with redundancy in important life support systems.

be substantial at inland locations. For this reason, research on super-intensive biofloc systems is typically centered around strict water use limitations.

Typically the most expensive variable cost for super-intensive systems, and many aquaculture systems, is feed. Hanson et al. (2009) estimated that feed costs represent 37% of the variable costs of operating super-intensive biofloc systems. Much work is being conducted to help remedy this problem. Feeds that would not be nutritionally complete for other aquaculture systems are being explored, with the idea that the dense microbial community in super-intensive systems can help to supplement some important feed components. For instance, low protein feeds and feeds with protein sources other than traditional marine fish products have been used with encouraging success (Azim et al., 2008; Ray et al., 2010a).

The fact that super-intensive biofloc systems can incorporate environmentally responsible aquaculture practices and the animals can be fed specialty diets, some of which are almost entirely plant-based, can lead to marketing opportunities. Marketing products as environmentally friendly or nutritionally exceptional can enhance the potential profitability of these systems. Producers should carefully market their shrimp by clearly distinguishing them from other products. If competition becomes problematic in a particular market there are options available to further enhance the attractiveness of products. These options include exploring feeds to alter the texture, flavor, or appearance of shrimp, changing the size of shrimp produced, and using brine dips to alter flavor. Such options are partially the result of having greater control over super-intensive production systems than what is typically found in large ponds.

Conclusion

Biofloc shrimp culture systems are a viable alternative to traditional shrimp aquaculture. When the microbial communities in these systems are managed appropriately, according to the goals of the shrimp producer, they can be operated as super-intensive systems. Although this brings greater risk of mechanical or biological complications, such as dissolved oxygen depletion, substantially larger shrimp crops can be produced using very little land or water.

Super-intensive shrimp culture systems require a somewhat unique set of engineering and management criteria. Many of these issues are still being explored by the scientific community as well as by members of the aquaculture industry. Important concerns include water propulsion, oxygenation, appropriate shrimp density, solids management, and waste reuse to name a few. The future surely holds a place for super-intensive biofloc systems or some adaptation of this technology if responsible aquaculture development is to progress.

Practical Implications and Tips

1. *Very dense biomass of L. Vannamei (300-500 shrimp/m^3) can be controlled. We do not know yet if these values hold for other shrimp species.*

2. *Super-intensive systems have clear advantages when production is near to metropolitan areas and/or in cases where temperature control in the cold season is essential. Due to higher cost of production these systems are most financially attractive for niche markets where fresh, high-quality, local shrimp are desired.*

3. *The very dense biomass and high feeding rates require strict control of suspended matter.*

Further Research Needs

The development of super-intensive capacity and its viability are very dramatic. Yet, there are many variables that produces must consider. The density of shrimp and level of control over environmental parameters are two important issues that dictate how these systems function. Further work is needed to evaluate control and monitoring systems, examine the effects of various lighting regimes for indoor systems, and overcome inconsistencies.

Improving feed utilization, energy conservation and reducing infra-structure cost seem to be of high priority. Specially designed diets that take into account the low water exchange rates, dense microbial community, and the nutritional needs of shrimp should be created. Diets are not only important to the economics and animal performance but may also help to drive microbial function.

Recommended Reading and Cited Literature

1. *Almeida, M., Gaona, C., Poersch, L., Wasielesky, W. 2011. Effect of different levels of total suspended solid in water quality and production of Litopenaeus vannamei in BFT system. World Aquaculture 2011 Book of Abstracts, The World Aquaculture Society, BatonRouge, Louisiana, USA.*

2. *Alonso-Rodriguez, R., Paez-Osuna, F., 2003. Nutrients, phytoplankton and harmful algal blooms in shrimp ponds: A review with special reference to the situation in the Gulf of California. Aquaculture 219: 317–336.*

3. *APHA (American Public Health Association), American Water Works Association, and Water Pollution Control Association, 2005. Standard Methods for the Examination of Water and Wastewater, 21st edition. American Public Health Association, Washington, D.C., USA.*

4. *Avnimelech, Y., Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. Aquaculture 220: 549-567.*

5. *Azim, M.E., Little, D.C., Bron, J.E. 2008. Microbial protein production in activated suspension tanks manipulating the C:N ratio in feed and the implications for fish culture. Bioresource Technology 99: 3590–3599.*

6. *Browdy, C.L., Stokes, A.D., McAbee, B., Atwood, H., Wasielesky, W., Leffler, J. 2006. Insights into the functional roles of major components of microbial communities in zero exchange superintensive shrimp systems. Aquaculture America 2006 Book of Abstracts. The World Aquaculture Society, Baton Rouge, Louisiana, USA.*

7. *Brunson, J., Haveman, J., DuRant, E., Weldon, D., Leffler J. 2011. Effect of solids removal on production of Pacific White Shrimp Litopenaeus vannamei in a minimal exchange, biofloc-based system. Aquaculture America 2011 Book of Abstracts. The World Aquaculture Society, Baton Rouge, Louisiana, USA.*

8. *Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C. 2004. The contribution of flocculated material to shrimp (Litopenaeus vannamei) nutrition in a high intensity, zero-exchange system. Aquaculture 232:525–537.*

9. *Crab, R., Chielens, B., Wille, M., Bossier, P., Verstraete, W. 2010. The effect of different carbon sources on the nutritional value of bioflocs, a feed for Macrobrachium rosenbergii postlarvae. Aquaculture Research 41: 559–567.*

10. *Hanson, T.R., Posadas, B.C., Samocha, T.M., Stokes, A.D., Losordo, T.M., Browdy, C.L.*

11. *Hargreaves, J.A., 2006. Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering 34: 344–363.*

12. *Hari, B., Madhusoodana K.B., Varghese, J.T., Schrama, J.W., Verdegem, M.C.J. 2006. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252: 248-263.*

13. *Ju, Z.Y., Forster, I.P., Dominy, W.G. 2009. Effects of supplementing two species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (Litopenaeus vannamei). Aquaculture 292: 237-243.*

14. *Kent, M., Browdy, C.L., Leffler, J.W. 2011. Consumption and digestion of suspended microbes by juvenile Pacific white shrimp Litopenaeus vannamei. Aquaculture 319(3-4): 363-368.*

15. *Libes, S.M., 2009. Introduction to marine biogeochemistry, 2nd Ed. Academic Press, Elsevier Burlington, MA, USA.*

16. *McKee, C., Lightner, D.V., 1982. Effect of several algicides and surfactants on the filamentous bacterium Leucothrix mucor Oersted. Applied and Environmental Microbiology 43: 715–718.*

17. *Moss, S.M. 1995. Production of growth-enhancing particles in a plastic-lined shrimp pond. Aquaculture 132:253-260.*

18. *Moss, S.M., Forster, I.P., Tacon, A.G.J. 2006. Sparing effect of pond water on vitamins in shrimp diets. Aquaculture 258:388-395.*

19. *Neal, R.S., Coyle, S.D., Tidwell, J.H., Boudreau, B.M., 2010. Evaluation of stocking density and light level on the growth and survival of the Pacific white shrimp, Litopenaeus vannamei, reared in zero-exchange systems. Journal of the World Aquaculture Society 41(4): 533-544.*

20. *Otoshi, C.A., Naguwa, S.S., Falesch, F.C., Moss, S.M. 2007. Commercial-scale RAS trial yields record shrimp production for Oceanic Institute. The Global Aquaculture Advocate 10(6): 74-76.*

21. *Ray, A.J., Shuler, A.J., Leffler, J.W., Browdy, C.L. 2009a. Microbial ecology and management of biofloc systems. Pages 255-266 in C.L. Browdy and D.E. Jory, eds. The Rising Tide, Proceedings of the Special Session on Sustainable Shrimp Farming, World Aquaculture 2009. World Aquaculture Society, Baton Rouge, Louisiana.*

prove shrimp (Litopenaeus vannamei) production and an evaluation of a plant- based feed in minimal-exchange, superintensive culture systems. Aquaculture 299: 89–98.

24. *Ray, A.J., Seaborn, G., Wilde, S.B., Leffler, J.W., Lawson, A., Browdy, C.L., 2010b. Characterization of microbial communities in minimal-exchange, intensive aquaculture systems and the effects of suspended solids management. Aquaculture 310, 130–138.*

25. *Ray A.J., Farno, C.C., Breland, V.M., Dillon, K.S., Lotz., J.M. 2011a. Differences in chemical dynamics between chemoautotrophic and three different heterotrophic biofloc-based shrimp (Litopenaeus vannamei) culture systems. The National Shellfisheries Association Annual Meeting Abstract Book, Baltimore, Maryland, USA.*

26. *Ray, A.J., Farno, C.C., Lotz, J.M. 2011b. Use of settled solids from intensive shrimp culture as a fertilizer alternative for Bitter Panicum Panicum amarum var. amarum. Aquaculture America 2011 Meeting Book of Abstracts. The World Aquaculture Society, Baton Rouge, Louisiana, USA.*

27. *Ray, A.J., Dillon, K.S., Lotz, J.M. 2011. Water quality dynamics and shrimp (Litopenaeus vannamei) production in intensive, mesohaline culture systems with two levels of biofloc management. Aquacultural Engineering 45: 127–136.*

28. *Ray, A.J., Seaborn, G., Vinatea, L., Browdy, C.L., Leffler, J.W., 2012. Effects of biofloc reduction on microbial dynamics in minimal-exchange, superintensive shrimp (Litopenaeus vannamei) culture systems. Journal of the World Aquaculture Society 43(6): 790–801.*

29. *Samocha, T.M., Advent, B., Correia, E.S., Morris, T.C., Wilkenfeld, J.S. 2010. Growth performance of Litopenaeus vannamei in superintensive mixotrophic raceway culture with zero discharge using Taeration® technology for aeration and extended CO2 degassing. Aquaculture 2010 Meeting Book of Abstracts. The World Aquaculture Society, Baton Rouge, Louisiana, USA.*

30. *Samocha, T.M., Wilkenfeld, J.S., Morris, T.C., Correia, E.S., Hanson, T. 2010. Intensive raceways without water exchange analyzed for white shrimp culture. The Global Aquaculture Advocate 13(4): 22-24.*

31. *Sookying, D., Soller, F., Silva, D., Davis, D.A., Hanson, T.R., 2011. Effects of stockingdensity on the performance of Pacific white shrimp Litopenaeus vannamei cultured under pond and outdoor tank conditions using a high soybean meal diet. Aquaculture 319, 232-239.*

32. *Venero, J.A., McAbee, B., Lawson, A., Lewis, B.L., Stokes, A.D., Leffler, J.W., Browdy, C.L., 2009. Greenhouse-enclosed superintensive shrimp production: Alternative to traditional*

34. *Wasielesky Jr., W., Fróes, C., Silva, A., Krummenauer, D., Foes, G., Poersch, L.H. 2010. Effect of high stocking density on growth and survival in different life stages of Litopenaeus vannamei in BFT culture system. Aquaculture 2010 Meeting Book of Abstracts. The World Aquaculture Society, Baton Rouge, Louisiana, USA.*

Chapter 15

The biology and biotechnology behind bioflocs

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In Brief

Biofloc technology (BFT) becomes more and more established in the field of aquaculture but remains often practiced in a non engineered approach. A better understanding of the biological and biotechnological basics of bioflocculation can lead to a further optimization of BFT practice. Using this knowledge, the formation of bioflocs within the ponds can be better managed and thus be performed ad hoc. Not only floc morphology, but also floc (nutritional) composition and floc production can be adjusted to a certain degree by the farmer's choice of BFT parameters. For such control of BFT systems, valuable lessons can be learned from the established field of domestic wastewater treatment systems, of course translated to aquaculture environments. In this chapter, the methodology behind biofloc aggregation within the ponds is examined, and an overview of different pond operational parameters and their influence on floc morphology and floc composition is provided. The relationship between the different parameters is described and also suggestions for additional research are made. From the practical point of view for aquaculture, it is of interest to have microbial flocs that have a high nutritional value. In this respect, the strategies that lead to bioflocs that can easily be taken up by the aquaculture animals and that are rich in energy or added value products are of particular interest. In addition, the management of bio-floc production can be a key parameter to control disease outbreaks in culture ponds.

The Biological Selection Criteria Driving Biofloc Formation

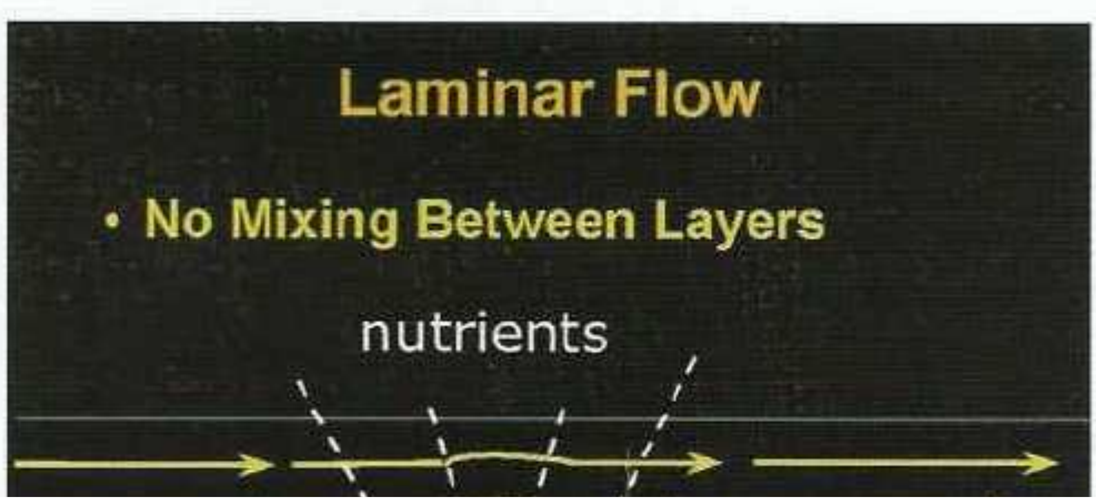
Microbial flocs consist of a heterogeneous mixture of microorganisms (floc-formers and filamentous bacteria), particles, colloids, organic polymers, cations and dead cells and can reach more than 1000 μm in size. Typical flocs are irregular by shape, have a broad distribution of particle sizes, are fine, are easily compressible, and highly porous (up to 99% open pores) and thus permeable. The aggregation into flocs offers microorganisms the capacity to settle. Settling can conavoid grazing by the higher organisms feeding in the top layer or acquire more food. Stokes' law describes settling of impermeable particles in aqueous suspensions with laminar flow and indicates that the settling speed is proportional to the size of the particle according to the formula:

$$V = \frac{1}{18}\frac{g}{v}\frac{\left(\rho_s - \rho_l\right)}{\rho_l} d^2$$

Where V is the sedimentation speed of the particle, d is the diameter of the particle, g is the gravity, ρ_s is het density of the particle and ρ_l is the density of the liquid (if ρ_s is smaller than ρ_l the particle will float) and ρ is the kinematic viscosity of the fluid. Due to the presence of pores and water flow passing through microbial aggregates, the actual drag is lower resulting in faster settling than predicted by Stokes' law. Efforts were made to model the settling of microbial aggregates (Johnsson et al., 1996), and it was found that these settle at slightly higher rates than predicted by Stokes' law (Li and Yuan, 2002). Good settling flocs are not necessarily lost as a feed source for the cultured animals, because the energy input at densities of about 10 W m^{-3} by mixing as applied in culturing basins keeps them in suspension.

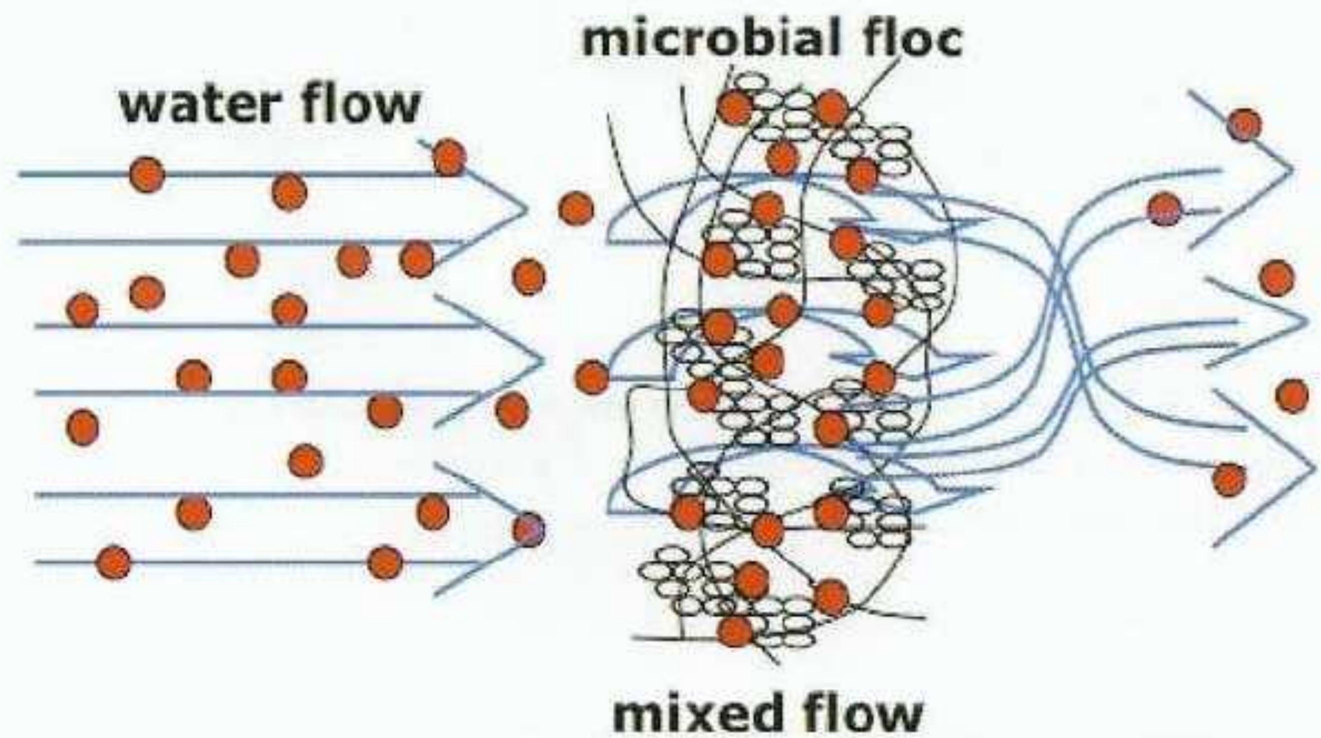
Individual bacterial cells are generally sized in the order of 1 μm. This implies that these organisms are surrounded by a small layer of liquid that hampers the mass transfer of nutrients and waste products. Calculation of the Reynolds number (Re = a dimensionless parameters that indicates whether a fluid flow in a particular situation will be laminar or turbulent) for bacterial cells, even for freely dispersed ones, will result in a value far below 2300 which is the upper limit for laminar flow. In other words a laminar regime is always present around bacteria smaller than 100 μm, and interferes with nutrient mass transfer as they move through the water column (Figure 1). This may result in mass transfer limitations when the rate of substrate consumption exceeds the rate of substrate supply.

Figure 15.1: Mass transfer of nutrients to a bacterial cell occurs mainly by molecular diffusion through a laminar water layer surrounding the cell



Microorganisms are considered to counter the nutrient diffusion problem by growing in flocs, as is the case in BFT. The aggregation of cells is a mechanism resulting in a nutritious advantage for which energy may be invested in order to sustain this floc formation. The reason can be found within the highly porous internal structure of aggregated microbial communities. The permeability of the flocs allows advective flow to pass through the pores since the water tends to follow the path of least resistance (Li and Ganczarczyk, 1992). As a result, the amount of nutrients supplied to the microorganisms in the flocs by mixed flow is considered to be higher as compared to the amount supplied by laminar flow to an individual cell (Figure 2). The substrate availability can thus increase up to a factor two.

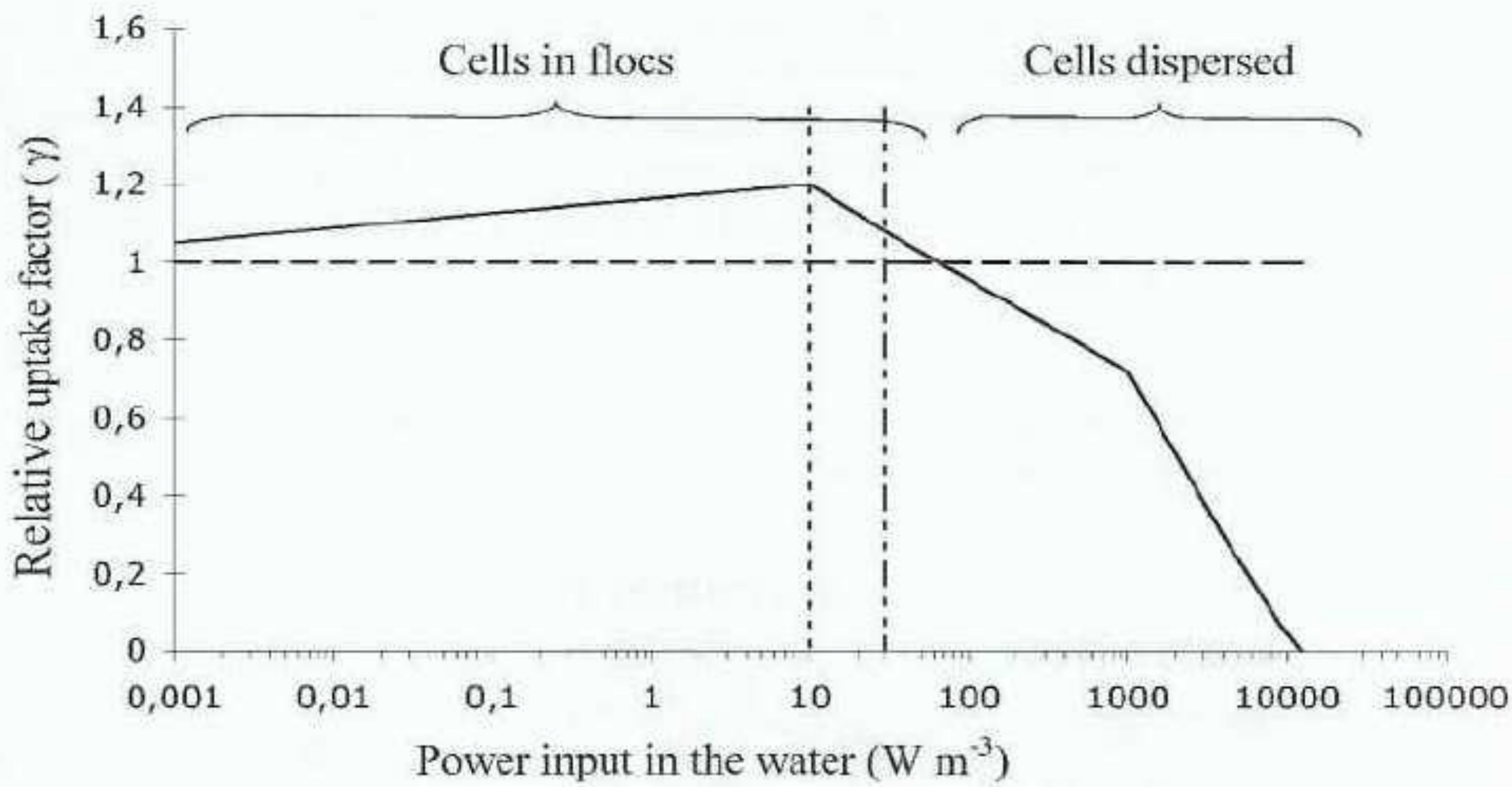
Figure 15.2: Schematic representation of the mixed flow resulting in high nutrient supply to bacterial cells living in floc structures (red rounds = nutrients)



The advantage due to bioflocculation can be represented by comparing the mass transfer rate towards cells inside flocs with the mass transfer rate towards dispersed cells. This is expressed by the relative uptake (γ) that is defined as the ratio of the uptake rate by cells growing in flocs over the uptake rate by cells dispersed in the fluid. Here, both the floc and dispersed cells are in the same fluid mechanical environment. If the relative uptake factor is larger than one, living in flocs is advantageous and microorganisms will organize themselves into aggregates.

The relative uptake factor is function of the power input into the water for aeration and mixing. As a consequence, the power input into the water is one factor that can be managed towards floc formation and floc morphology. In general, $0.1 - 10\ W\ m^{-3}$ is applied in aquaculture. When the relative uptake factor is calculated according to Logan and Hunt (1988) in function of the

Figure 15.3: Predicted relative uptake for microbial cells within permeable flocs (Logan and Hunt, 1988).



A shear rate of 10 W m-3 represents the mixing of the sea (-------); a shear rate of about 30 W m-3 represents the mixing in aerated activated sludge (—·—) and a shear rate of 0.1-10 W m-3 corresponds to the mixing in most aquaculture systems

For aquaculture ponds, applied values for the shear rate (0.1 – 10 W m^{-3}) generally result in flow regimes in which natural floc formation will have a selective advantage. Yet, it must be noted that these considerations are based on approximate unit values and theoretical calculations. They can however certainly be used in the design of BFT aquaculture units. Indeed, up to date the approach has mainly been to replace the conventional recirculation systems in existing culture systems by BFT. As such, the degree of variation to manage biofloc production was limited. The availability of mathematical background offers the opportunity to switch towards an approach where biofloc production is designed as the preferred water treatment technique and thus is an integral part of the design of the aquaculture system. The design leads to a starting point but the optimal power input will have to be adjusted for each individual culture unit. Assessing floc formation at varying power inputs will allow to determine a range for optimal biofloc growth and each power input within the range will result in a different floc size distribution. The required floc size (distribution) will mainly depend on the cultured species. Adult animals will be able to feed on larger flocs whereas these organisms in a juvenile life stadium will prefer/need smaller

The knowledge on how to promote floc formation in activated sludge systems can be used for application in BFT. The parameters listed in Table 1 can be adjusted to obtain good aggregation and high quality bioflocs together with optimal growth conditions for the aquaculture organisms. Since most of them are strongly interrelated, in many cases it is not easy to predict a certain outcome due to changing parameters. As far as known, almost no research has been performed on the relation between the operational parameters discussed further and the functioning of the BFT systems or biofloc quality. The BFT farmer must be provided with a set of factors that influence the floc structure and floc composition and the physical and/or biological mechanisms supporting it. Without the need to know all details, the farmer can thus have an idea on the possible implications of these manipulations on the BFT system and can work accordingly in a more considered way towards a desired result.

Table 15.1: Overview of the main operational parameters for biofloc technology based aquaculture, the floc parameters they influence and how these can be manipulated. The interrelation between the parameters is indicated

Parameter	Floc parameters influenced	Manipulation possibilities	Related to
Mixing intensity/ shear rate (W m^{-3})	- Floc structure and floc size	- Level of power input - Mixing device	- Dissolved oxygen
Dissolved oxygen (DO) (mg L^{-1})	- Microbial floc composition (filamentous vs. zoogloeal bacteria) - Floc structure and floc volume index	- Level of power input (W m^{-3}) - Kind of aeration device	- Mixing intensity - Organic carbon source - Organic loading rate
Organic carbon source (e.g. glucose, acetate, starch, glycerol)	- Chemical floc composition (fatty acids, lipids, protein, polyhydroxyalkanoates)	- Type of organic carbon source	- Organic loading rate - Dissolved oxygen
Organic loading rate (kg m^{-3} day^{-1})	- Microbial floc composition (filamentous vs. zoogloeal bacteria) - Chemical floc composition (cellular reserves, e.g. polyhydroxyalkanoates)	- Feeding strategy (continuous feeding or interval feeding)	- Dissolved oxygen
Temperature (°C)	- Floc structure and	- Addition of heat	- Dissolved oxygen

2.1 Mixing intensity

The mixing intensity imposed by a chosen aeration device at a certain power input will determine the steady-state floc size, this is the equilibrium between the rate of aggregation, the rate of breakage and the rate of reaggregation, as well as the floc size distribution. As mentioned before, energy dissipation in general is in the range of 0.1 – 10 W m^{-3} in aquaculture systems. However, in highly intensive water treatment systems, values can reach up to 100 W m^{-3}. At higher mixing intensities and thus higher shear rates, the average floc size decreases due to increased floc breakage. Biggs and Lant (2000) showed in case of an activated sludge that for an average velocity gradient (or G-value) of 19.4 s^{-1} (≈ 0.5 W m^{-3}), the stable aggregated cell size was ca. 130 ∝m whereas this was decreased to ca. 20 ∝m for a velocity gradient of 346 s^{-1} (≈ 125 W m^{-3}).

The relationship between floc size and mixing intensity has been represented by Parker et al. (1972) with the power law relationship $d = CxG^{-x}$, where d is the maximum stable floc size, G is the average velocity gradient, C is the floc strength component and *x* is the stable floc size component. For BFT, the steady-state floc size is an important feature as the quality of feed for different aquaculture species is also dependent on the feed size. BFT operation can thus be adjusted to balance floc size with for example the life stage of the cultured animals.

2.2 Dissolved oxygen

A change in mixing intensity, either by the use of an alternative aeration device or a different power input, will directly influence the dissolved oxygen (DO) concentration in the water. The DO level is not only essential for the metabolic activity of cells within aerobic flocs but it can also influence floc structure. There seems to be a trend towards larger and more compact flocs exists at higher DO concentrations (Wilen and Balmer, 1999). Poorer settling properties seem to occur at low DO values (0.5 – 2.0 mg L^{-1}) compared to settling at higher DO values (2.0 – 5.0 mg L^{-1}). This can be ascribed to the presence of a higher amount of filamentous bacteria compared to the zoogloeal bacteria at the lower DO-range. As filaments have a higher affinity towards oxygen, they are able to outcompete their zoogloeal counterparts at periods of oxygen limitation and thus tend to dominate the microbial flocs. From the previous, it can be expected that bioflocs with a higher floc volume index (FVI) are produced at lower DO-levels in the bioflocs ponds. The FVI is defined as the volume that a certain mass of flocs in the water phase represents (in mL g^{-1}). The smaller the FVI, the better the settleability of the flocs. Flocs with a higher FVI give the aquaculture organisms more opportunity to filter the flocs from suspension before they finally sediment to the bottom of the ponds and are lost as feed. Negative impacts of a higher FVI, however, like e.g. possible clogging of fish gills, have to be taken into account as well. As the required FVI will depend on the animal under culture, further experimenting should be performed to yield information on this parameter.

2.3 Organic carbon source

The dosing of an organic carbon source to the culture water in biofloc ponds is sometimes a prerequisite to stimulate adequate biofloc production. The organic carbon can either be supplied as additional organic carbon source (e.g. sugar, acetate, molasses, glycerol, etc.) or by changing the feed composition thus increasing its organic carbon content. It is possible to theoretically calculate the amount of organic matter needed for an intensive pond, based on the amount of nitrogen excreted by the aquaculture species (Figure 4).

Figure 15.4: Schematic calculation of the daily amount of organic carbon needed by bioflocs to remove the nitrogen excreted in an intensive aquaculture pond of 50 kg fish live weight m^{-3}

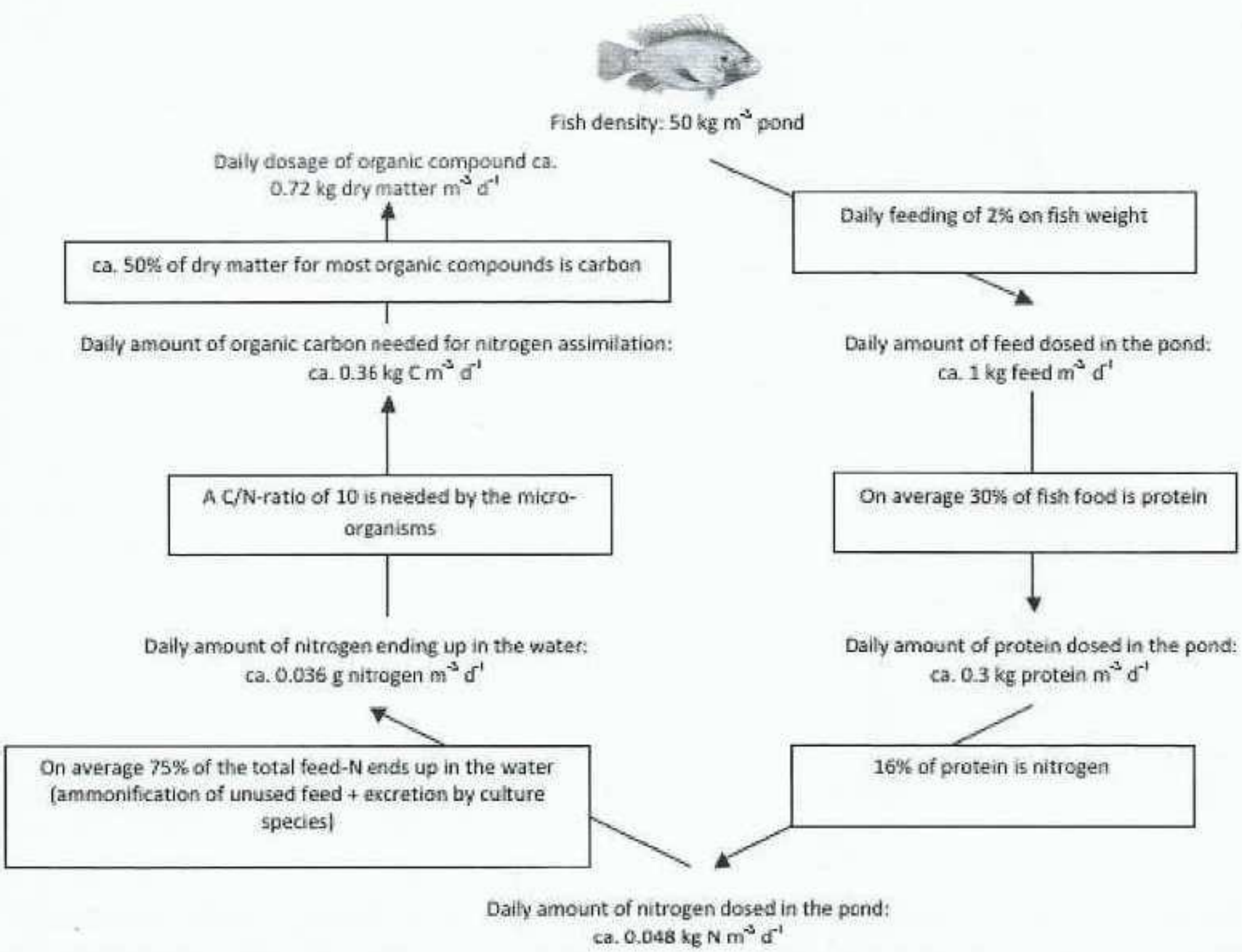


Table 15.2: Overview of the nutritional value of bioflocs produced with different types of carbon sources

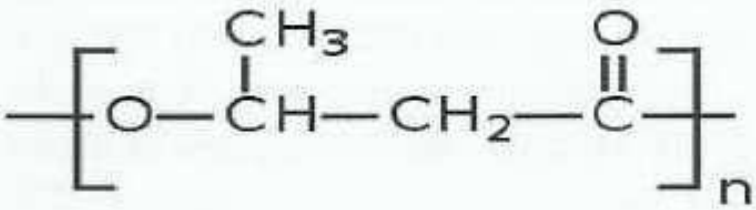
Study by:	Kuhn et al. (2009)	Kuhn et al. (2010)	Crab et al. (2010)			Emerenciano et al. (2011)	Azim et al. (2008)
Carbon source:	Sugar	Shrimp feed	Acetate	Glycerol	Glu-cose	Sugarcane molasses (90%) and wheat bran (10%)	Composition of a commercial shrimp diet
Floc composition:							
Crude protein*	49.0	38.8	42	43	28	30.4	35.1
Crude lipid*	1.13	< 0.1	2.3	2.9	5.4	0.47	11.9
Crude fibre*	12.6	16.2	---	---	---	0.8	2.8
Carbohydrate *	36.4	25.3	29	34	50	29.4	45.0
Ash*	13.4	24.7	27	20	17	39.2	2.8
Energy content**	18	17.8	15.5	16.9	17.0	2.2	20.1

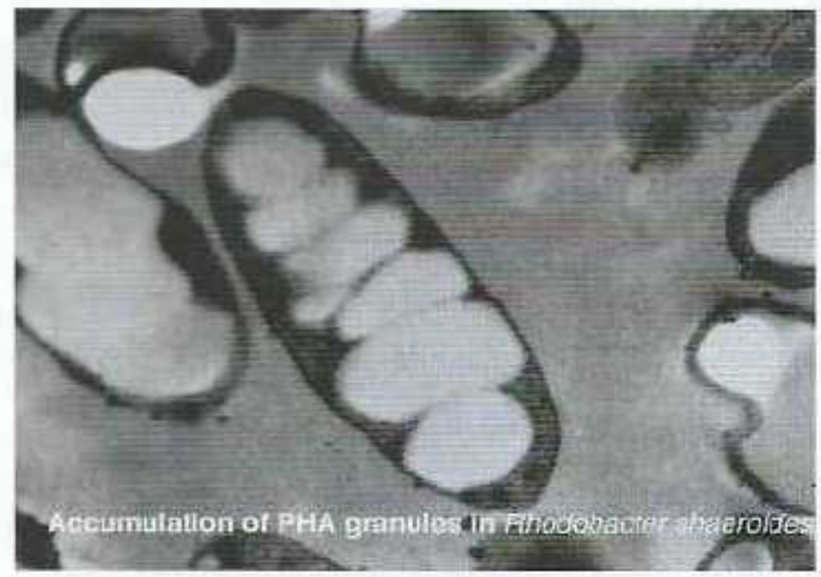
* % of dry weight

** kJ g^{-1} dry weight

As can be seen from the studies in Table 2, the composition of the flocs can differ considerably depending on the carbon substrate used to grow the flocs. This implies that the composition can be optimized by the choice of the organic carbon source. However, up to date, studies on the effect of the organic carbon source on the floc nutritional composition are almost non existing. Performing such studies focusing primarily on maximizing floc nutritional value is indispensable lular biodegradable polymer produced by a wide variety of microorganisms from soluble organic carbon. It is involved in bacterial carbon and energy storage and can even be build up to 80% of the cell dry matter (Figure 5).

Figure 15.5: The chemical structure of PHB (left) and the visualization of PHB accumulated in a bacterial cell (right)





PHB is considered to be depolymerised in the gut of higher organisms and has also been shown to act as a preventive or curative protector of *Artemia franciscana* against *Vibrio* infections, and to stimulate growth and survival of shrimp and fish larvae (De Schryver et al., 2009). Upon release from the bacterial cell, e.g. in the case of cell death and lyses, degradation of PHB is performed by the activity of extracellular PHB depolymerase enzymes which are widely distributed among bacteria and fungi. This can result in the release of 3-hydroxybutyrate into the surrounding intestinal environment and act inhibitory against pathogenic species.

The accumulation of PHB in bioflocs is a natural and easily to induce process. PHB contents of up to 16 % on biofloc dry weight have already been measured (De Schryver and Verstraete, 2009). But PHB is only one compound of a whole family of polyhydroxyalkanoates. Different structures of the carbon substrate will result in varying types of polyhydroxyalkanoates. Each pathway employs different enzymes that are encoded for by different genes. While the presence of acetate mainly results in poly-b-hydroxybutyrate as storage polymer, these are 3-hydroxy-2-methylvalerate and polyhydroxyvalerate in case of propionate dosing. Although there is considerable scientific knowledge available on the accumulation of PHB in bacterial cells and mixed microbial cultures, the efforts of maximizing PHB content in bioflocs for aquaculture is largely unexplored.

2.4 Organic loading rate

Theloading rate or the organic carbon dosing strategy can also be important for BFT. It represents the amount of carbon that is dosed per volume of pond water and this over a certain time period (kg organic carbon m^{-3} day^{-1}). The organic carbon can be added in small amounts and thus almost continuous mode. It results in a continuous availability of organic carbon for the microorganisms in the bioflocs. Alternatively, the carbon can be added in larger doses but only at regular time intervals. The second type of application is also known as a feast and famine regime, or alternating periods of carbon excess and carbon shortage, and thus results in transient conditions of substrate availability. Such regime is the trigger for extra storage of reserves in the form of poly-b-hydroxyalkanoates and thus also PHB. The storage under conditions of excess nutrient availability allows the microorganisms to bridge the periods of nutrient shortage. The PHB accumulation approach is mainly valid in case additional organic carbon is dosed rather than the use of feed with a lower protein content. The use of feed as organic carbon supply will not as much result in a feed and famine regime due to the lag effect of the organic carbon release resulting from feed digestion.

Occasionally, the production of bioflocs in external flocculation units located next to the culture ponds may be worth concidering. This approach offers the advantages of an even higher level of control over the production of the bioflocs as well as an easier floc harvest. Moreover, as the cultured animals are physically separated from the flocs production units, the manipulations towards optimized floc formation do not affect the animals, e.g. in terms of dissolved oxygen level, mixing etc. Also for the accumulation of PHB the production of flocs in external flocculator units certainly offers more flexibility. A downside, however, are extra investment costs.

2.5 Temperature and pH

The influence of temperature is complex. The temperature is of major importance for not only the microbial metabolism, but also the amount of dissolved oxygen in the water. The culture species will thus not only be influenced by the chosen temperature (changes in growth rates, food conversion efficiencies), but also by the associated dissolved oxygen level. The water temperature in BFT ponds is not a factor that can be easily adjusted without imposing considerable additional operating costs, especially in outdoor ponds. In most cases, the climatic conditions determine the operation temperature and thus the species that can be cultured.

Changes in pH may determine the stability of the bioflocs present in the pond. But similar to temperature, it is not an easy factor to control and will even fluctuate highly in relation to the diurnal pattern. Moreover, as the pH can have a major influence on the culture animals, possible changes in pH would be limited to the optimal range for the cultured animals to avoid mortality and disfunctioning.

feed source. Currently, most of the need for the essential compounds in fish food is fulfilled in the form of fish meal and fish oil, due to their optimal nutritional quality. It is common practice that 1.0 - 5.0 kg of fish live weight has to be caught in the oceans to be able to produce 1.0 kg of live aquaculture fish. It represents a non-sustainable way of producing food products that can be solved by the production of new biomass in the form of bioflocs grown on the nutrient waste streams of the aquaculture systems. As such, the amount of "unsustainable" feed can be decreased. Bioflocs do not allow for a complete replacement of the traditional feed but can bring about a substantial decrease of the processing costs since the food represents 40-50% of the total production costs (see box: the feed economics of aquaculture with and without BFT).

The Feed Ecoomics of Aquaculture With And Without Bft

The potential savings on feed that can be obtained by BFT can be theoretically calculated. Taking as an example tilapia produced with food at a 30% protein content and at an average food conversion ratio of 2.2:

For a Tilapia culture unit without application of bioflocs technology, the feed conversion ratio can be taken 2.2 with 30% protein feed

Without flocs 2.2 kg feed is dosed kg^{-1} fish live weight produced (feed conversion ratio of 2.2)

→ 0.3 x 2.2 = 0.66 kg protein is dosed kg^{-1} fish live weight produced (30% protein content in feed)

→ 0.25 x 0.66 = 0.17 kg protein is taken up kg^{-1} fish live weight produced (25% of the feed is taken up by the fish).

In a system with bioflocs, part of the feed will be recycled into flocs, which can also be used by the animals as feed source. Therefore, less conventional feed needs to be dosed to the water. Take F the amount of conventional feed added to the system if BFT is applied

With flocs, F kg feed is dosed kg^{-1} fish live weight produced

→ (0.3 x F) kg protein is dosed kg^{-1} fish live weight produced

→ 0.25 x (0.3 x F) = 0.075 x F kg protein is taken up kg^{-1} fish live weight produced

Assume that the fish also take in only 25% of the flocs

→ 0.25 x (0.23 x *F*) = 0,06 x *F* kg protein is taken up out of the flocs per kg fish live weight produced

<u>Calculation of the amount of external feed needed when BFT is applied</u>

The total protein requirement by the fish is 0.17 kg protein kg^{-1} fish live weight produced

→ Total protein requirement = protein obtained from feed + protein obtained from the flocs = 0.17

→ Total protein requirement = (0.075 x *F* + 0.06 x *F*) = 0.17

→ The amount of feed that still needs to be applied (F) is ca. 1.3 kg

<u>Calculation of the amount of organic carbon needed to grow the flocs</u>

→ 0.75 x 1.3 = 1.0 kg of the decreased feed amount (at 1,5 kg feed kg^{-1} fish produced) is unused by the fish (75% of the feed for fish is unused)

→ 0.25 x 1.0 = 0.25 kg protein is unused kg^{-1} fish live weight produced (assumed protein content in flocs is 25%)

→ 0.16 x 0.25 = 0.04 kg nitrogen is unused kg^{-1} fish live weight produced (16% nitrogen content in protein) and is recycled into floc biomass

The flocs have a C/N-ratio of 4 (Avnimelech, 1999)

→ 4 x 0.04 = 0.16 kg C in floc biomass is produced kg^{-1} fish live weight produced since all the excess nitrogen should be assimilated in the bioflocs

The yield of bacterial biomass can be taken to be 0.5 (Avnimelech, 1999)

→ 0.16/0.5 = 0.32 kg C needs to be added in the water for the flocs to be able to assimilate the excess nitrogen kg^{-1} fish live weight produced

In case acetate (40% C) is used as organic carbon source

<u>Calculation of the cost saving by the application of BFT</u>

The costs for the production of 1 kg fish live weight without BFT

→ 2.2 x 0.6€ kg^{-1} feed (in Belgium) = 1.3€ kg^{-1} fish live weight produced

The costs for the production of 1 kg fish live weight with BFT

→ (1.3 kg feed kg^{-1} fish live weight produced x 0.6€ kg^{-1} feed) + (0.8 kg acetate kg^{-1} fish live weight produced x 0.43€ kg^{-1} acetate) = 1.12€ kg^{-1} fish live weight produced

The gain thus appears to be in the order of 14% in terms of feed costs kg^{-1} fish live weight produced. For an intensive culture system producing at e.g. 500 ton fish live weight yr^{-1}, this represents a gain of 90000€ yr^{-1}. Clearly, these economics are only indicative and depend largely on the price of organic carbon source added.

The Biological Value of Biofloc Technology For Aquaculture

Maintaining nitrogenous waste below toxic levels and improving the feed nutrient utilization efficiency of the cultured animals are not the only positive features that are associated with the application of BFT. Animals cultured in BFT systems also seem to be less susceptible to the outbreak of diseases and recent research has started to provide explanation for this. The natural microbes and microbe associated molecular patterns (MAMPs), such as lipopolysaccharides, peptidoglycans and β-1,3-glucans, seem to enhance the non-specific immune system and the antioxidant status of animals cultured in BFT systems. Increases of total haemocyte count, phagocytic activity of the haemocytes, superoxide dismutase activity, prophenoloxidase (proPO) activity, in respiratory burst activity, and in survival after challenge with infectious myonecrosis virus (IMNV) have been ascribed to the uptake of bioflocs (Ekasari et al., 2014; Xu and Pan, 2013). In addition, the expression of six genes involved in a series of responses known as the proPO cascade, one of the major innate immune responses in crustaceans, was described to be up regulated in shrimp cultured in BFT systems (Kim et al., 2014).

Alternatively, the decrease in disease outbreaks in ponds operated in biofloc mode may be due to the establishment of a mature microbial community in the water. Disinfection of ponds -

ponds too soon after pond filling and feeding is commenced. The maturation of the pond water by the culture of bioflocs prior to stocking will control the number of opportunistic pathogens in the water and thus their chance to cause infection in the stocked animals. This disease controlling aspect of BFT recently became very topical because of the outbreaks of the early mortality syndrome (EMS), also known as acute hepatopancreatic necrosis disease (APHNS), in the shrimp industry. Anecdotal evidence suggests that the emergence of EMS is much less in systems that apply BFT. De Schryver et al. (2014) suggested this may be due to the mature water effect of BFT.

Conclusions

The benefits that BFT brings to the aquaculture farmer are undeniable. Besides water quality control, it may lead to the saving of about 15% on the feed costs and a decrease in the risk for disease outbreaks. Understanding the concepts supporting BFT, however, will provide a considerable advance in the farmer's abilities to maximize the advantages resulting from this clean-tech type of recycling. While pond operational parameters are currently mainly adjusted on a trial-and-error basis, there is a need to shift towards a more scientific based approach. Without the need to know the details, the farmer can make more considered choices based on this knowledge in varying the BFT operational parameters to meet certain requirements (e.g. floc size depending on animal life stage, floc composition, etc.).

In this chapter, an overview was given of the biotechnology behind different parameters and how the latter can influence biofloc characteristics. This information remains however far from complete, as currently research is mainly focusing on the nutrient removal from the water, and not so much on the steering and optimization of the morphological and compositional aspects (protein, lipids, poly-b-hydroxybutyrate,...) of the bioflocs. The nutritional value of the bioflocs, as well as their morphological characteristics, are dependent on a large set of operational parameters currently under development in BFT aquaculture systems. There is an urgent need for research focusing on the optimal way to manage the BFT aquaculture ponds with respect to optimal floc morphology and compositional and nutritional value of the flocs so that indeed it can not only replace water treatment but also protein supply based on fishery products.

Acknowledgements

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Recommended Reading and Cited Literature

1. *Azim, M.E., Little, D.C., and Bron, J.E. (2008) Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. Bioresource Technology, 99, 3590–3599.*

2. *Biggs, C.A., and Lant, P.A., 2000. Activated sludge flocculation: on-line determination of floc size and the effect of shear. Water Research, 34, 2542–2550.*

3. *Crab, R., Avnimelech, Y., Defoirdt, T., Bossier, P., and Verstraete, W. (2007) Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture, 270, 1-14.*

4. *Crab, R., Chielens, B., Wille, M., Bossier, P., and Verstraete, W. (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for Macrobrachium rosenbergii post-larvae, Aquaculture Research, 41, 559–567.*

5. *De Schryver, P., Defoirdt, T., Sorgeloos, P. (2014) Early mortality syndrome outbreaks: a microbial management issue in shrimp farming? PlosPathogens, 10, e1003919.*

6. *De Schryver, P., Sinha, A.K., Kunwar, P.S., Baruah, K., Verstraete, W., Boon, N.,De Boeck, G., and Bossier, P. (2009). The bacterial storage compound poly-⊠-hydroxybutyrate (PHB) increases growth performance and intestinal bacterial range-weighted richness in juvenile European sea bass. Applied Microbiology and Biotechnology, 86, 1535-1541.*

7. *De Schryver, P., and Verstraete, W. (2009). Nitrogen removal from aquaculture pond water by heterotrophic nitrogen assimilation in lab-scale sequencing batch reactors. Bioresource Technology, 100, 1162–1167.*

8. *Ekasari, J., Azhar, M.H., Surawidiaia, E.H., Nurvati, S., De Schryver, P., and Bossier, P. (2014) Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. Fish and Shellfish Immunology, submitted.*

9. *Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., and Wasielesky, W. (2011) Biofloc technology application as a food source in a limited water exchange nursery system for pink shrimp Farfantepenaeus brasiliensis (Latreille, 1817). Aquaculture Research, 43, 447, 457.*

10. *Johnson, C.P., Li, X., and Logan, B.E. (1996) Settling velocities of fractal aggregates. Environmental Science and Technology, 30, 1911–1918.*

13. *Kuhn, D.D., Lawrence, A.L., Boardman, G.D, Patnaik, S., Marsh, L., and Flick, G.J. Jr. (2010) Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, Litopenaeus vannamei. Aquaculture, 303, 28-33.*

14. *Li DH, Ganczarczyk JJ (1992) Advective transport in activated sludge flocs. Water Environment Research, 64, 236-240.*

15. *Li, X., and Yuan, Y. (2002) Settling velocities and permeabilities of microbial aggregates. Water Research, 36, 3110-3120.*

16. *Logan, B.E., and Hunt, J.R., 1988. Bioflocculation as a microbial response to substrate limitations. Biotechnology and Bioengineering, 31, 91–101.*

17. *Parker, D.S., Asce, A.M., Kaufman, W.J., and Jenkins, D., 1972. Floc breakup in turbulent flocculation processes. Journal of the Sanitary Engineering Division-ASCE 98 (NSA1), 79.*

18. *Wilen, B.M., Balmer, P., 1999. The effect of dissolved oxygen concentration on the structure, size and size distribution of activated sludge flocs. Water Research, 33, 391–400.*

19. *Xu, W.J., and Pan, L.Q., 2013. Enhancement of immune response and antioxidant status of Litopenaeus vannamei juvenile in biofloc-based culture tanks manipulating high C/N ratio of feed input. Aquaculture, 412 – 413, 117 – 124.*

Chapter 16

Biofloc Technology Applied To Shrimp Broodstock

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In Brief
Biofloc technology (BFT) has become a popular grow-out technology in shrimp farming, however little is known about its use for husbandry of penaeid broodstock. With the global spread of viruses, getting biosecure broodstock appears as a priority to avoid vertical infections. Post larvae suppliers have to ensure high-quality and guarantee superior genetics that will result in better performance in grow-out ponds. BFT can improve biosecurity and provide in situ nutrients such as "native protein", amino acids, fatty acids and vitamins; nutrients stored in digestive gland and utilized for first stages of broodstock's gonad formation and ovary development. Protocols for breeders production include the adjustment of stocking density, control of total suspended solids, supply of fresh food such as squid, blood worms, mussels and also control of pathogen bacteria bloom such as cyanobacteria or Vibrio sp. that can lower survival. Moreover, BFT is also becoming common worldwide in nursery phase, mainly by the optimization of farm land, increase in survival and enhanced immunological and growth performance in grow-out phase.

Considering the global spread of viruses, closed-life cycle broodstock appears as a priority to biosecurity and to avoid vertical transmissions of diseases. The shrimp industry places a considerable interest on penaeid breeding program, often performed in closed facilities, controlling the production with successive generations. However, nutritional problems in domesticated broodstock remained unresolved (Wouters et al., 2001) and alternatives should be evaluated.

In the 70's, Ifremer-COP Tahiti started R&D with enclosed limited exchange biosecure system with different penaeid species including *Penaeus monodon, Fenneropenaeus merguiensis, Litopenaeus vannamei and L. stylirostris.* In connection with Aquacop and Ralston Purina, biofloc technology (BFT) was applied to grow-out *L. stylirostris* and *L. vannamei* both in Crystal River (USA) and Tahiti; and first considerations on benefit of such system for shrimp culture emerged. The program called "Ecotron" was initiated in the 80's in Tahiti and several studies including juveniles and broodstock enabled a comprehensive approach to biofloc and explained interrelationships between different compartments (water, phytoplankton, bacteria and shrimp) and aspects of nuand ovary development. Biofloc as a rich protein-lipid natural food is mainly composed of phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates, flagellates and protozoa. Currently, BFT have being applied successfully in large-scale shrimp farming in Asia, Central and South America as well as small-scale greenhouse system in Europe, USA, South Korea, Brazil and others. However little is known about BFT benefits on penaeid broodstock.

Broodstock used to be produced in large ponds at low density with an aim to provide nutritional-rich natural biota to obtain large and healthy breeders. On the other hand, accumulation of organic matter, cyanobacteria blooms and fluctuations of some water quality parameters such as temperature, DO, pH and N-compounds, could affect the shrimp health in outdoor systems. In this context, Monroy-Dosta et al (2013) demonstrated the ecological succession of microorganisms during 14 weeks in a BFT culture. The authors observed that during this period beneficial microorganism present in bioflocs such as *Bacillus sp.* acted positively against *Aeromonas sp.* and *Vibrio sp.*, bacteria with pathogen potential. This result suggested a "natural probiotic effect" in BFT. This effect might be interesting to prevent EMS/AHPNS.

Production of broodstock in BFT could be located in small areas close to hatchery facilities, preventing spread of diseases caused by shrimp transportation. Moreover, once the system is stable (sufficient particulate microbial biomass as measured in Imhoff cones), BFT provides stabilized parameters of water quality when performed in indoor facilities such as greenhouses.

Broodstock nutrition and biofloc composition

Understanding the effect of different food sources such as industrial pellets vs. live or fresh food contribute to improvement of broodstock performance. Differences in quality food items ingested prior to ablation could affect physiological processes and may potentially reduce reproductive output. In the wild, mature animals can ingest a large range of food items such as meiofauna, rotifers, copepods, polychaetes, bivalves and other small crustaceans; but in captivity the ingestion is limited to feed pellets and some added fresh food items such as squid, fish, Artemia biomass, worms and mussels (Browdy 1998; Marsden et al., 1992). However, seasonal and/or storage factors could potentially limit dietary nutrients supplied in captivity.

Nutritional composition of biofloc differs according to environmental conditions, applied carbon source, TSS level, salinity, stocking density, light intensity, phytoplankton and bacteria communities, etc. Protein, lipid and ash content could vary in bioflocs (12 to 49, 0.5 to 12.5 and 13 to 46%, respectively; Table 16.1) as well as its fatty acids profile (Table 16.2). Lipid content in shrimp

Table 16.1: Proximate analysis of biofloc particles in different studies.

Reference	Crude protein (%)	Carbohydrates (%)	Lipids (%)	Cruce fiber (%)	Ash (%)
McIntosh et al (2000)	43.0	-	12.5	-	26.5
Tacon et al (2002)	31.2	-	2.6	-	28.2
Soares (2004)	12.0 - 42.0	-	2.0 - 8.0	-	22.0 - 46.0
Wasielesky et al (2006)	31.1	23.6	0.5	-	44.8
Ju et al (2008a)	26.0 - 41.9	-	1.2 - 2.3	-	18.3 - 40.7
Ju et al (2008b)	30.4	-	1.9	12.4*	38.9
Kuhn et al (2009)	49.0	36.4	1.13	12.6	13.4
Maica et al (2012)	28.8 - 43.1	-	2.1 - 3.6	8.7 - 10.4	22.1 - 42.9
Emerenciano et al (2012b)	30.4	29.1	0.5	0.8	39.2

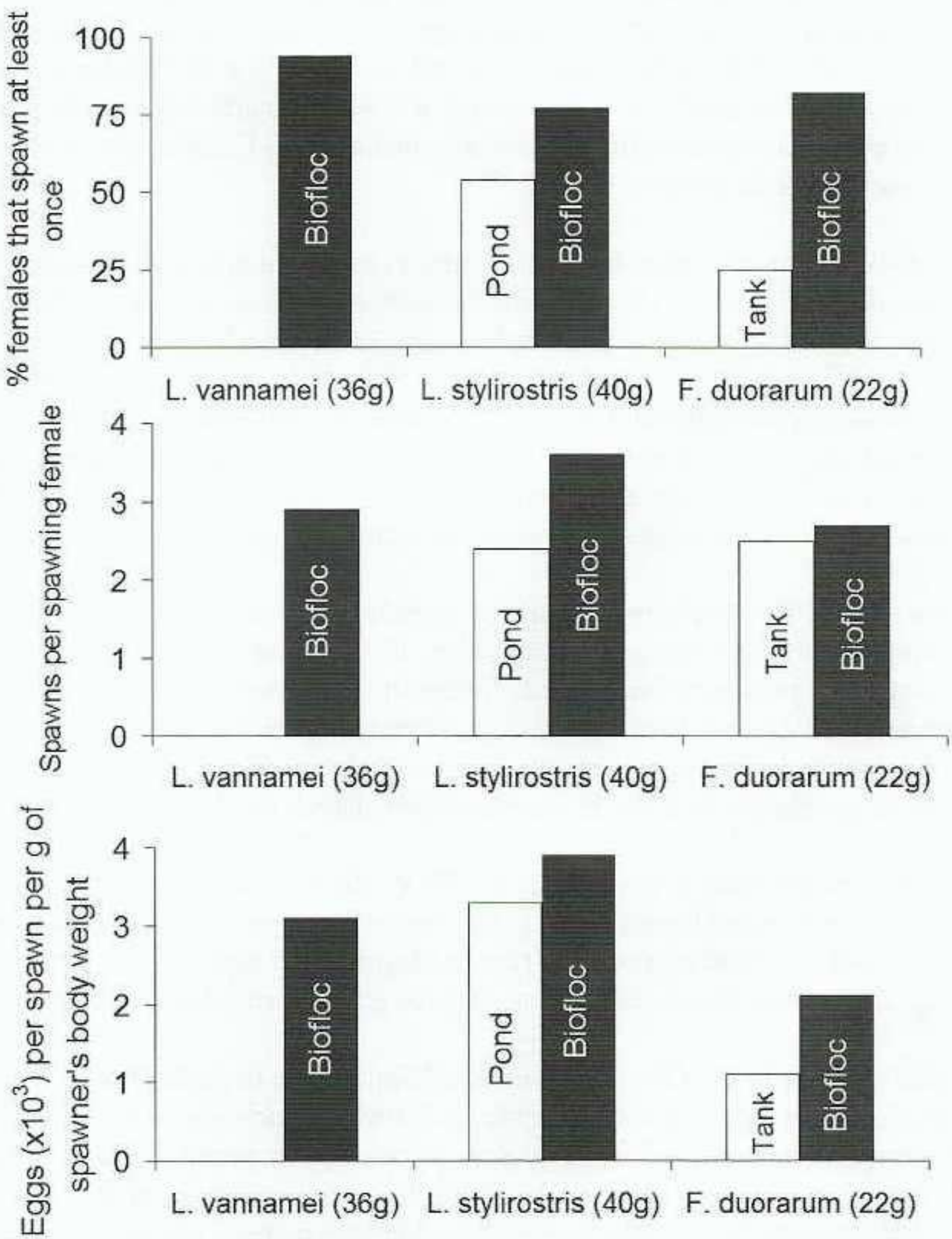
*Lignin+cellulose

Table 16.2: Fatty acid profile of biofloc collected from bioreactors using different carbon source in marine water (Ekasari et al. 2010)

Fatty Acid	% of total fatty acid	
Carbon source	Glucose	Glycerol
C14:0	0.61	0.43
C15:0	0.17	0.26
C16:0	6.34	8.86
C16:1	1.61	1.54
C17:0	0.14	0.68
C18:0	3.94	6.27
C18:1 n-7	2.71	4.19
C18:1 n-9	8.12	12.05
C18:2 n-6	11.95	21.87
C18:3 n-3	0.20	0.21
C20:0	0.33	0.49
C20:1 n-9	0.06	0.02
C20:3 n-6	0.15	0.04
C20:4 n-6 (ARA)	0.17	0.06
C20:3n3	0.03	-
C20:5 n-3 (EPA)	0.19	0.12
C22:6 n-3 (DHA)	0.18	0.10
Σ Saturated	11.53	16.99
Σ Monounsaturated	12.5	17.8
Σ n-3	0.60	0.43
Σ n-6	12.27	21.97

Studies were performed in Mexico and Tahiti with Pacific white shrimp *L.vannamei*, pink shrimp *Farfantepenaeus duorarum* and the blue shrimp *L. stylirostris* broodstock using BFT. A summary of these works is presented in Figure16.1. It is shown that BFT can enhance reproductive per-

Figure 16.1 : reproductive performance trials of *L.vannamei* (biofloc), *F. duorarum* (tank-reared vs bifloc) and *L. stylirostris* (pond reared vs biofloc) performed in 40, 45 and 30 days after ablation, respectively



the reduction in dietary protein levels and the maintenance of spermatophore and sperm quality compared with the conventional system.

For *F. brasiliensis*, Lopes et al (2012) evaluated males during 45 days in terms of water quality, growth and sperm quality in two systems: clear-water (100% daily water exchange and diet based on commercial broodstock feed, fish, squid and crab, composing a 68.9% dietary protein) and biofloc (38.0% commercial feed). Growth, survival and water quality was maintained and males of *F. brasiliensis* were maintained with low water exchange rates (biofloc) and low protein content, without loses in sperm quality.

BFT seemed to be effective to other broodstock species such as tilapia and *Macrobrachium rosenbergii*. In tilapia, Ekasari et al (2013) evaluated *Oreochromis niloticus* breeders (85±5 g), acclimatized for 7 days, stocked at a density of 20 fish m^{-3} at a male:female ratio of 1:4. Using outdoor concrete tanks, two treatments were applied: control (30% crude protein diet) and BFT (30% CP, molasses and carbon to nitrogen ratio of 15). At the end, tilapia breeders from biofloc presented higher final weight, fry production and fecundity. The results of this experiment suggested that the application of BFT effectively enhanced tilapia reproductive performance and therefore *in situ* biofloc production can be suggested as a way to increase tilapia seed production.

Perez-Fuentes et al (2013) evaluated during six months two rearing systems of *M.rosenbergii*: biofloc and traditional water-exchange cultivation. Rectangular lined ponds (20 m3) with a stocking density of 37 prawns m^{-2} were used. The results suggested that survival rate was similar in both treatments (>85%), but final size was significantly higher in BFT. Protein (51.19%) and lipid (13.84%) content in harvested prawns was also higher in BFT. With this result in mind, BFT seems to be an efficient tool for *M.rosenbergii* broodstock production and maintenance.

It is important to know what is the reason for BFT effects on improvement of reproductive performance. There are several hypotheses: (1) Better control of water quality parameters; and (2) continuous availability of food in a form of fatty acids protected against oxidation, vitamins and highly diverse "native protein" sources for first stages of gonad formation and ovary development.

In conventional systems, "young" breeders were food limited due to pellets feeding without some nutritional requirements that could not be achieved. Nutrients such as essentials amino acids, fatty acids and phospholipids required for early gonad formation in young breeders and subsequent ovary development are continuously available in BFT systems in a form of diverse microorganisms. This could promote high nutrient storage in hepatopancreas, transferred to hemolymph and directed to ovary. As a result a better sexual tissue formation and reproduction activity are achieved. These aspects seem to be primarily factors for the superiority of BFT as compared to conventional systems.

Microorganisms in biofloc could partially replace fresh food provided to breeders before eyestalk ablation. Fresh food represents a high cost for shrimp maintenance in broodstock facilities. The about how microorganisms profile and its nutritional composition could impact shrimp growth and reproductive performance. Further research in this field is encouraged.

Management of biofloc in shrimp broodstock production

One of the most important management procedures in BFT management of broodstock preparation is related to stocking density. Stocking density has to be carefully managed, mainly with shrimp of above 15g. High density will lead to an increase in organic matter in tanks, and consequently increase TSS levels and high levels of N-compounds (Vinatea et al., 2010). Moreover, physical damages are prevented at low density, improving breeder's health. Suggested stocking density is presented in Table 16.3. The logic is simple: increase shrimp weight with lowering stocking density. Also, guarantee of essentials nutrients supply and increase of shrimp weight could be obtained by fresh food supplements (squid, mussels, etc) provided to breeders at least once a week.

Table 16.3: Recommended protocols of broodstock production under BFT conditions

Shrimp weight (g)	Recommended stocking density (per m^2)	Final estimated biomass[1] (kg/m^2)	Suggested food protocol
1-10	100-300	0.8-2.5	Pelletized feed
10-20	50	≈0.8	Pelletized feed + fresh food[2] (at least once a week)
20-35	less than 20	≈0.6	Pelletized feed + fresh food (three times per week)
more than 35	less than 15	0.4-0.6	Pelletized feed + fresh food (once a day)

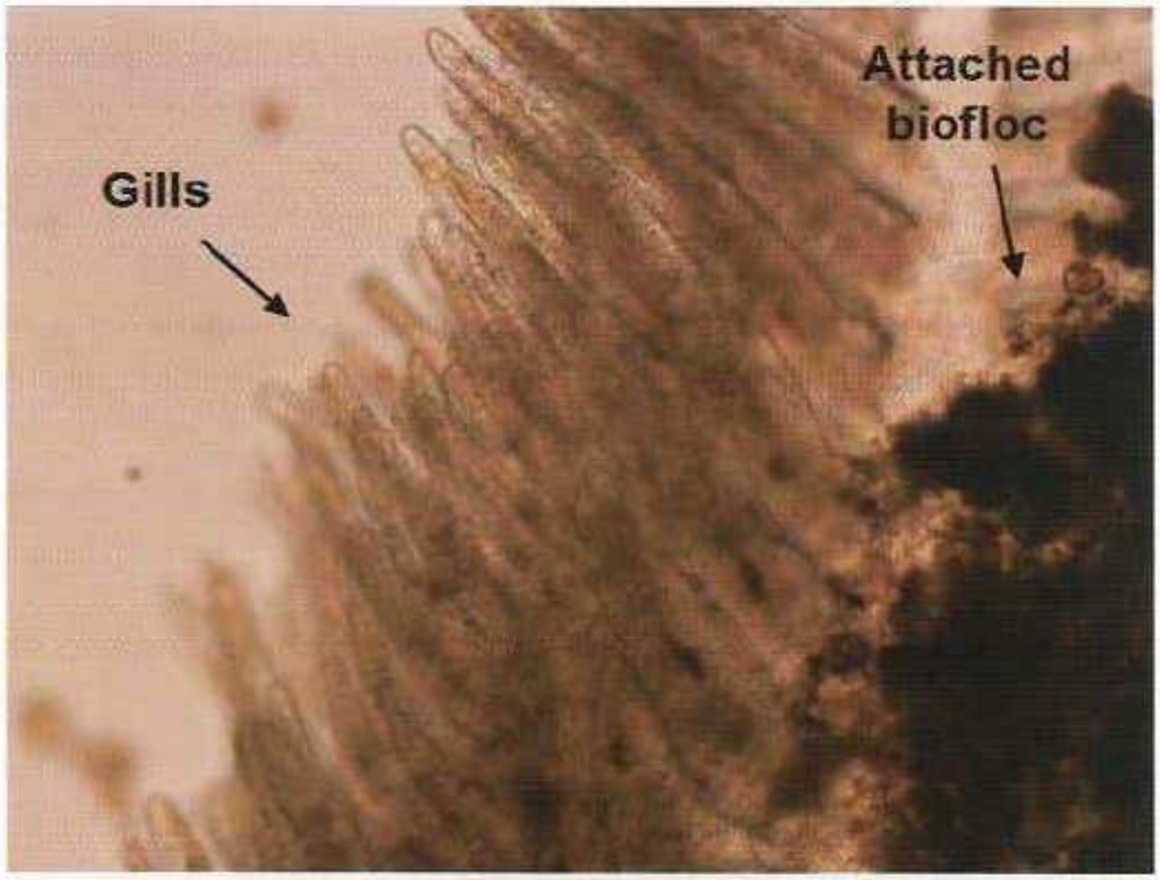
[1]Estimated survival of 85%
[2]Shrimp's weight above than 15g

Floc volume (FV) is another important parameter in BFT broodstock management. In field observations in Mexico and using Imhoff cones, it seemed that FV higher than 15ml/L negatively affect health of shrimp broodstock above 15g. The particulate organic matter covered breeder's gills and could limit oxygen exchange. Shrimp were susceptible to handling resulting in mortalities. Animals were sampled and gills covered by excess of biofloc concentration (Figure16.2). One postulation is that both suspended (TSS) and volatile solids (VSS) affect shrimp health; to avoid this problem, excessive solid in the systems was removed. Low-cost devices are available and more research is necessary to detect optimal level of solids. Such level tolerance can vary according shrimp size.

BFT large-scale ponds have an area of 0.1-2 ha per pond. For broodstock production, shrimp could grow in ponds until 10-15g and then transferred to more controlled facilities as enclosed greenhouses. In greenhouse, the shape of BFT tanks could be round or rectangular, less than 200m^2 to guarantee better water quality parameters control. Modules with small tanks, i.e. 20-40m^3 are recommended. The number of tanks and its layout will depend on PL's production plan and space available. Round tanks seem to be easier to manage in terms of water movement and sludge removal. On the other hand, rectangular tanks are more efficient in terms of space usage.

The depth of tanks should be between 1.2-1.5m to avoid temperature fluctuations and to guarantee enough depth for shrimp swimming (important issue in shrimp reproduction) and good mixing of water. High temperature is a well-known factor that affects shrimp health. Previous studies carried out in Tahiti detected that males cultured in ponds had more frequent melanized spermatophores caused by high temperatures than males growing in biofloc tanks (Emerenciano et al., 2012a). Abiotic factors are better controlled in BFT by tank shading and low or zero water renewal providing a more stable medium for broodstock. Heterotrophic bacteria might control phytoplankton blooms as well as reduce pH-dissolved oxygen fluctuations typically observed in ponds. Moreover, N-compounds such as ammonia is constantly recycled and taken up by heterotrophic bacteria. Thus, water movement (no more than 30 cm/s), dissolved oxygen (minimum of 5mg/L), N- compounds (TAN and NO_2, less than 3.0 and 1.0 mg/L, respectively) and temperature (26-28°C) must be continuously controlled and stabilized.

Figure16.2: Gills and attached biofloc in F. duorarum broodstock as a result of excess of TSS (10x magnitude) (Photo: Maurício Emerenciano)



Bioflocs in Nursery Management

Current spread of diseases such as EMS have directed farmers to enclose their systems, in the nursery phase. Nursery phase is defined as an intermediate step between hatchery-reared early post-larvae and grow-out phase. Such phase presents several benefits such as optimization of farm land, increase in survival and enhanced growth performance in grow-out ponds. BFT has been applied successfully in nursery phase in different shrimp species such as *L.vannamei, L.stylirostris*, *P.monodon*, *F.paulensis*, *F.brasiliensis* and *F.setiferus*. The primary advantage observed is related to a better nutrition by continuous consumption of biofloc, which might positively influence in immunological system and grow-out performance (Aquacop et al. 1991). In addition, biosecurity and optimization of farm facilities provided by the high stocking densities in BFT nursery phase seems to be an important advantage to achieve profitability in small farms, mainly when farmers are operating indoor facilities.

Wasielelesky et al (2013) evaluated the effect of compensatory growth in BFT *L.vannamei* nursery. During a first stage, shrimp postlarvae were reared at densities of 1,500, 3,000, 4,500, were achieved at the higher density. Souza et al (2012) evaluating the effect of commercial probiotics in *F. brasiliensis* postlarvae observed that shrimp reared in the probiotic treatments showed higher final weight, specific growth rate, as well as higher levels of total protein and granular haemocyte. The bacteriological analysis showed that the concentration of *Vibrio spp.* measured in probiotic treatment tanks was lower than that recorded in the control tanks. Further research is needed in this field.

Emerenciano et al (2011) observed that presence of bioflocs resulted in increases of 50% in weight and almost 80% in final biomass in *F. paulensis* early postlarval stage when compared to conventional clear-water system. This trend was observed even when post-larvae were not fed with a commercial feed (biofloc without commercial feed). Furthermore, Emerenciano et al (2012b) found that *F. brasiliensis* post-larvae grow similarly with or without pelletized feed in biofloc systems during 30-d nursery phase, in both cases 40% more than conventional clear-water continuous exchange system.

Different managements in nursery phase have been applied. Some alternative protocols are:

1. Start biofloc procedures from clear-water together with postlarvae stocking, maintaining a C:N ratio of 20:1 until floc volume reach at least 5ml L^{-1}. This management could be more risky (water quality fluctuations) and will get more time to reached a mature system;
2. Start biofloc procedures at least two weeks before post-larvae stocking, feed the water based on a C:N ratio of 20:1, using rations equivalent to that expected to be given to 300g PL per m^2;
3. Inoculate biofloc water from a mature production tank/pond at least two days before stocking. The proportion recommended is 1/3 of mature water and 2/3 of clear-water. Application a C:N ratio of 20:1 until floc volume reach at least 5ml L^{-1}.

In all cases, we might divide the process to two phases: (1) Initial bioflocs build up phase till achieving floc volume of at least 5ml L^{-1}. Organic feeding (carbon source application) can be reduced at that point. (2) In the maintenance phase organic carbon feeding is controlled according to ammonium (TAN) level. When TAN concentration reaches 1 mg L^{-1} or higher, external carbon sources such as molasses, dextrose, etc., should be added. This might be done, i.e., according to equations proposed by Avnimelech (1999), assuming that 20 g of carbohydrates is needed to convert 1 g of TAN to microbial protein.

Further research development is encouraged to utilize BFT more efficiently in nursery and broodstock production as its optimal stocking density, TSS control, nutrition supplements, and use of commercial probiotics and facilities lay-outs. This technology is still under investigation

Practical implications and Tips:

1. *Intensive control of total solids in BFT aiming to produce shrimp broodstock is not waste of effort. It must be often controlled.*

2. *Nutrition supplements as fresh food item (squid, blood worms, mussels, etc.) at least once a week before eyestalk ablation will enhance reproductive outputs. In BFT, recent research showed that those food items also improved reproductive performance. However, optimal protocols are still under investigation.*

Further research needs:

1. *More knowledge on optimum stocking density and effect of vertical substrates aiming to improve shrimp integrity and health*

2. *Optimal levels of solids for different penaeid species according to different shrimp size.*

3. *Use of probiotics before transfer to the maturation room and its effects.*

4. *Following the current trend, "biofloc meal" must be evaluated in breeders diets Protocols of fresh food supplementation (squid, mussels, etc.) are still under investigation and optimal amount and food type need further research in BFT system.*

Recommended reading and cited literature:

1. *AQUACOP, Le Moullac G., Damez D. (1991) Modélisation de la résistance au chocs de salinité des postlarves de Penaeus vannamei. Aquatic Living Resources 4:169–173*

2. *Arnold S.J., Coman F.E., Jackson C.J., Groves S.A. (2009) High-intensity, zero water- exchange production of juvenile tiger shrimp, Penaeus monodon: An evaluation of artificial substrates and stocking density. Aquaculture 293:42–48.*

3. *Avnimelech, Y. (1999) C/N ratio as a control element in aquaculture systems. Aquaculture 176, 227–235.*

4. *Braga A., Lopes D.L.A., Magalhães V., Poersch L.H., Wasielesky W., 2013. Use of biofloc technology during the pre-maturation period of Litopenaeus vannamei males: effect of feeds with different protein levels on the spermatophore and sperm quality. Aquaculture Research (article first published online: DOI: 10.1111/are.12352)*

5. *Browdy C.L., 1998. Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. Aquaculture 164, 3–21*

6. *Cahu C., Guillaume J.C., Stéphan G, Chim L., 1994. Influence of phospholipid and highly unsaturated fatty acids on spawning rate and egg and tissue composition in Penaeus vannamei fed semi-purified diets. Aquaculture 126, 159-170*

7. *Coman G.J., Arnold S.J., Barclay M., Smith D.M., 2011. Effect of arachidonic acid supplementation on reproductive performance of tank-domesticated Penaeus monodon. Aquaculture Nutrition 17, 141–151*

8. *Cruz-Ricque L.E., Guillaume J., Cuzon G., 1987. Squid protein effect on growth of four penaeid shrimp. Journal of the World Aquaculture Society 18, 209-217.*

9. *Ekasari J., Crab R., Verstraete W., 2010. Primary Nutritional Content of Bio-flocs Cultured with Different Organic Carbon Sources and Salinity. HAYATI Journal of Biosciences 17,125-130.*

10. *Ekasari J., Zairin-Jr M., Putri D.U., Sari N.P., Surawidjaja E.H., Bossier P. 2013. Biofloc-based reproductive performance of Nile tilapia Oreochromis niloticus L. broodstock. Aquaculture Research (article first published online: DOI: 10.1111/are.12185)*

11. *Emerenciano M, Ballester ELC, Cavalli RO, Wasielesky W (2011) Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp Farfantepenaeus paulensis: growth performance, floc composition and salinity stress tolerance. Aquaculture International 19, 891- 901.*

13. *Emerenciano M., Gaxiola G., Cuzon G., 2013a. Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. In: Miodrag Darko Matovic. (Org.). Biomass Now – Cultivation and Utilization. 1st ed., InTech, p. 301-328*

14. *Emerenciano M.G.C., Cuzon G., Arevalo M., Mascaró M, Gaxiola G., 2013b. Effect of short-term fresh food supplementation on reproductive performance, biochemical composition, and fatty acid profile of Litopenaeus vannamei (Boone) reared under biofloc conditions. Aquaculture International 21, 987-1007*

15. *Glencross B.D., 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture 1, 71-124*

16. *Huang J.H., Jiang S.G., Lin H.Z., Zhou F.L., Ye L., 2008. Effects of dietary highly unsaturated fatty acids and astaxanthin on the fecundity and lipid content of pond-reared Penaeus monodon (Fabricius) broodstock. Aquaculture Research 39, 240–251.*

17. *Ju Z.Y., Forster I., Conquest L., Dominy W., 2008. Enhanced growth effects on shrimp (Litopenaeus vannamei) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquaculture Nutrition 14, 533–543*

18. *Ju Z.Y., Forster I., Conquest L., Dominy W., Kuo W.C., Horgen F.D., 2008. Determination of microbial community structures of shrimp floc cultures by biomarkers and analysis of floc amino acid profiles. Aquaculture Research 39, 118-133*

19. *Kuhn D., G. Boardman, A. Lawrence, L. Marsh, G. Flick., 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 296, 51-57.*

20. *Maicá P.F., Borba M.R., Wasielesky W., 2012. Effect of low salinity on microbial floc composition and performance of Litopenaeus vannamei (Boone) juveniles reared in a zero-water-exchange super-intensive system. Aquaculture Research 43, 361–370*

21. *Mishra J.K., Samocha T.M., Patnaik S., Speed M., Gandy R.L., Ali A. (2008) Performance of an intensive nursery system for the Pacific white shrimp, Litopenaeus vannamei, under limited discharge condition. Aquacultural Engineering 38, 2–15.*

22. *Pérez-Fuentes JA., Pérez-Rostro CI., Hernández-Vergara M.P., 2013. Pond-reared Malaysian prawn Macrobrachium rosenbergii with the biofloc system. Aquaculture 400-401:105-110.*

23. *Soares R., Jackson C., Coman F., Preston N., 2004. Nutritional composition of flocculated*

25. *Wasielesky W, Froes C, Fóes G., Krummenauer D.,, Lara G., Poersch L., 2013. Nursery of Litopenaeus vannamei reared in a biofloc system: the effect of stocking densities and compensatory growth. Journal of Shellfish Research, 32, 799–806*

26. *Wouters R., Lavens P., Nieto J., Sorgeloos P., 2001. Penaeid shrimp broodstock nutrition: an updated review on research and development. Aquaculture 202, 1–21*

Chapter 17

Bioflocs Technology and Sustainable Aquaculture Development

In Brief

Aquaculture is obliged to grow 5 fold until 2050, in order to supply enough healthy food for the growing world population. This huge development has to be done respecting environmental and social sustainability guidelines.

BFT systems have an obvious advantage of minimizing release of water containing nutrients, organic matter and pathogens to the environment.

Sludge minimization and proper recycling or disposals are major issues for the sustainability of aquaculture development tand need to be further studied.

BFT systems may contribute to social sustainability conditioned by the proper development of services and backup systems. BFT is strongly dependent on dissemination of information and ensuring high level professionalism. As such, it may advance education and learning among aquaculterists.

Sustainable development requires consideration of environmental resource management, social factors and economic factors. In planning for future sustainable aquaculture development, continued growth and expansion of the sector must be taken into account. As discussed in Chapter 1 there is a need to increase aquaculture production by 5 fold by the year 2050. This huge expansion must be accomplished in a sustainable way.

Aquaculture production has increased more than 40 times during the last 50 years and is expected to additionally expand by 5 times in the coming 50 years. Such rapid growth of aquaculture production must be planned keeping in mind the need to minimize environmental impacts while optimizing resource utilization. Until recently, aquaculture development planning did not include enough sustainability considerations, especially during the period of 1980-2000 when shrimp best management practices to control aquaculture activities.

Figure 17.1: Getting sludge out of a shrimp pond

Collection and disposal of sludge is not an easy matter!!!

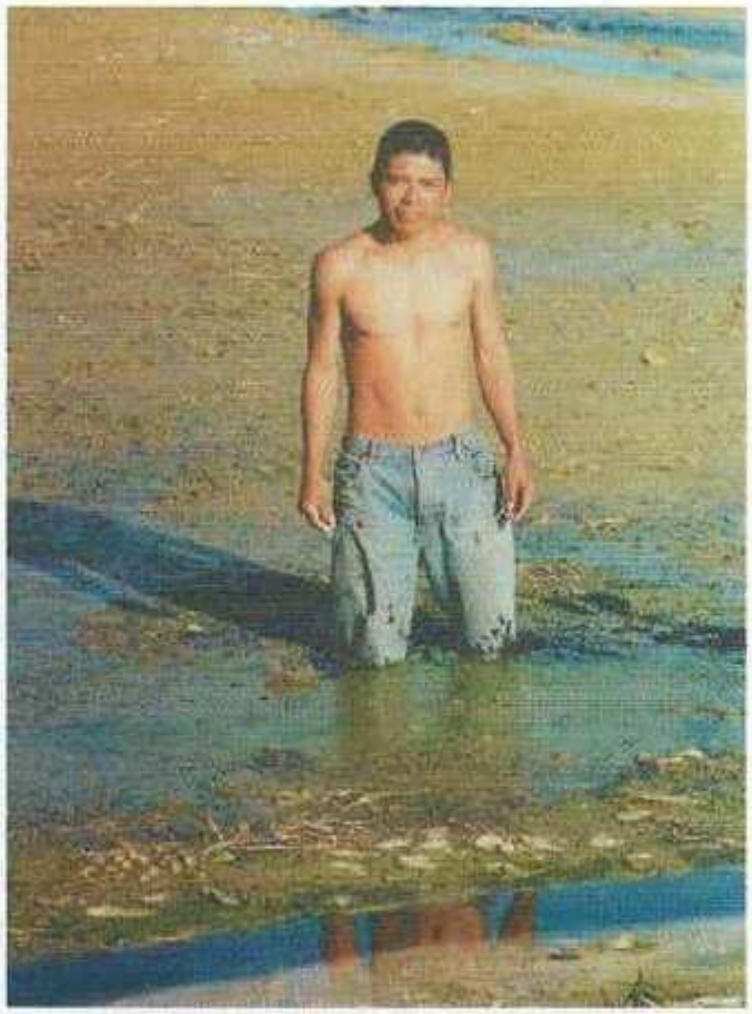
A major issue related to the effect of aquaculture on the environment is the emission of pollutants from drained water. Ponds are fed, often fertilized, leading to relatively high concentrations of nutrients such as nitrogen and phosphorus. Phosphorus is considered to be an algal growth limiting factor in many aquatic systems and its emission to rivers or lakes may induce eutrophication in these water bodies. Soluble phosphorus in "clean" natural water bodies is in the range of a few parts per trillion ($10^{-9} - 10^{-8}$), while soluble phosphorus concentrations in most aquaculture ponds is about 100-1000 times higher. Emissions of pond water into pure receiving water may raise phosphorus concentrations to levels that may endanger water quality. Similarly, nitrogen species concentration in ponds is higher than that found in marine and fresh water bodies. Most aquaculture ponds contain high concentrations of organic matter, both soluble and particulate. These compounds are biologically degradable, consuming oxygen as they break down. Emission of water rich in organic matter to receiving water bodies may lead to anaerobic conditions in the water and sediments around the effluent discharge. In addition to emission of chemicals, ment and are thus potentially endangering the surrounding environmental quality. A production system that does not release water to the environment is, in this respect, an environmentally friendly system that does not affect the environment by emission of pollutants.

Another aspect of aquaculture sustainability is the optimal utilization of natural resources, mostly land and water. The challenge for developing aquaculture to supply the demand for sufficient supply of healthy food to the growing world population was discussed in Chapter 1. There is just not enough water and available land to raise production 5 fold as demanded. A conventional pond, producing 2 tons of fish per ha and losing 35,000m^3 per year by evaporation and seepage and an additional 10,000 m^3 with the water drainage will consume 45 m^3 of water to produce 1 kg fish, and the production rate per unit area will be 0.2 kg/m^2. In contrast, the water consumption in intensive, zero or limited water exchange systems is less than 1 m^3 per kg fish production and the productivity of the pond area is in the range of 10-100 kg fish per 1m^2. Using such systems relieves water or land limitations to further production of fish making more optimal usage of land and water.

An additional environmental issue that led to a conflict between aquaculturists and environmentalists is the dependence of aquaculture on fish products originating in the marine environment. Fish meal and fish oils are common components of fish feed. Marine finfish feeds are made of about 50% fishmeal, marine shrimp feeds contain about 30% fishmeal and tilapia feeds about 15%. About 5 kg wild fish are needed to produce 1 kg marine fish in ponds (Naylor et al., 2000). Less (<1kg) is needed to produce carp. The dependence upon wild fish is a critical factor endangering marine ecology due to the degradation of fish populations in many marine regions. A replacement of this protein source by a more sustainable feeding program is vital for the development of aquaculture. Many efforts are made in order to develop fish feeds where plant proteins replace wild fish sources. The doubling of protein utilization demonstrated in BFT systems and potential for further increases in utilization efficiencies represents a major contribution towards the sustainability of aquaculture. Further improvement of feed quality in bioflocs as discussed in Chapter 7, is a topic that deserves further research.

An environmental issue common to all intensive aquaculture systems is the proper treatment and disposal of sludge. Sludge is accumulating in both recycling aquaculture systems (RAS) and BFT systems. Sludge is a concentrated source of reactive organic matter, rich in nutrients, similar to sludge produced in waste water treatment systems (though they normally do not contain human pathogens). Sludge originating from waste water treatment plants is treated by composting (with potential agriculture uses), incineration or by anaerobic fermentation (producing bio-gas). These treatments add significantly to the cost of waste water treatment. Presently, there are no well established methods of treating aquaculture sludge. There are some studies and practical experience of using such sludge as a source of feed for fish (Kuhn et al., 2008; Schneider et al., 2004), feed applied to adjacent extensive ponds or as soil amendment. An important point that needs further study and quantitative data analysis is the fact that in BFT systems, residues are suspended should be further studied and developed.

Figure 17.2 : If we will not solve the sludge disposal problems, we can get stuck in the mud



* Note the black mud, indicative of high sulphide concentrations

An important component of sustainability is the economy of operating a certain production system. A sustainable aquaculture production system has to provide a decent income to its owner(s) and workers. The economy of a given aquaculture production unit varies over time, sites and conditions. Quite obviously, when ponds already exist and no further investment is needed, there are good chances that continued operation of such ponds is a better choice as compared with the construction of new intensive ponds. Increasing production intensity can be made possible by retrofitting the existing ponds. A different set of considerations exists when one has to start building ponds, a set of conditions that may favor the construction of small intensive ponds. Though there is a lack of open economic data, existing analyses indicate that the use of BFT systems leads towards improved profitability when compared with both less intensive systems or with RAS ponds. Yet, it has to be remembered that BFT systems have been shown to provide better conditions for the particular production of shrimp, tilapia and possibly carp. Very little is known on its usage for other species. More experience, development and more data are needed to ited water supply. Can such a development sustainably support community development based upon small scale operation of farmers? The answer to this question is not trivial. Operating a BFT farm can be based upon a relatively small area. A one ha farm can produce a yield in the order of one hundred and more tons of fish or ten and more tons of shrimp, both having a substantial value. The investment needed is much lower than that needed for RAS or other highly intensive systems. Yet, such a farm depends on reliable power supply and backup systems, usually above the means of an individual farmer. There seems to be a possibility that the back-up system will be provided by the same company that provides the electricity. The public utility, private company or a farmers' cooperative will supply electricity and at the same time provide backup to cover the supply in case of power failure, a fault in the power conveyance system or even in the aeration supply within the farm. Without such regional support, it is difficult to envisage the development of small scale farms efficiently applying BFT systems. An important point discussed in chapter 9 is the possibility of a gradual transition from conventional farming systems toward the intensive BFT systems.

Another condition necessary for development of small scale individual farmer utilization of BFT is proper education and know-how. BFT is not drastically different than conventional aquaculture, yet, due to the intensity of the system there is a need to understand the system. Hopefully, this book will make a contribution in this regard.

Recommended Reading and Cited Literature

1. *Delgado, P.C., Avnimelech, Y., McNeil, R., Bratvold, D., Browdy, C.L., Sandifer, P., 2003. Physical, chemical and biological characteristics of distinctive regions in paddlewheel aerated shrimp ponds. Aquaculture 217: 235-248.*

2. *Hopkins, S.J., Sandifer, P.A., DeVoe, R.M., Holland, F.A., Browdy, C.L., Stokes, A.D 1995. Environmental impacts of shrimp farming with special reference to the situation in the continental United States. Estuaries and Coasts 19: 25-42.*

3. *Kuhn, D.D., Boardman, G.D., Craig, S.R., Flick, G.J., Mclean, E. 2008. Use of microbial flocs generated from tilapia effluent as a nutritional supplement for shrimp, Litopenaeus Vannamei in recirculating aquaculture systems. Journal of the World Aquaculture Society 39: 72-82.*

4. *Naylor, R.L., Goldburg, R.J., Primavera, J.h., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. 2000. Effects of aquaculture on world fish supplies. Nature 405 (29): 1017-1024.*

5. *Schneider, O., Amirkolaie, A.K., Vera-Cartas, J., Eding, E.H., Schrama, J.W., Verreth, J.A.J. 2004. Digestibility, faeces recovery, and related C, N, and P balances of five feed ingredients evaluated as fishmeal alternatives in Oreochromis niloticus L. Aquaculture Research 35: 1370-1379.*

Chapter 18

ABC of Pond Management

In Brief

This chapter, is edited in response to some readers of previous editions, saying that they read the book, enjoyed it, but finally, they need clear advice on how to start and to manage a biofloc system. In this short section, we try to give a concise road map, giving the beginner a guiding hand in the way to operate a biofloc system.

As a general introductory remark, it has to be stressed that biofloc systems are versatile, friendly and forgiving systems. They are versatile since you have a choice of how to develop your system. You can choose what degree of intensity suits your needs, what shape and size of pond you choose, what percentage of water exchange you want (or can afford), do you want to have higher or lower dominance of heterotrophic bacteria, etc. With time and experience, the pond manager can find his optimal route, all within the general principles of biofloc technology. The system is also forgiving. If you watch and understand your system, you can correct mistakes and solve problems. If you got an increase of TAN or nitrite, you can add carbohydrate, add nitrifying bacteria or carefully raise the water exchange rate a bit. You can control oxygen by adding aerators or increasing drainage of sludge or control biofloc density by increasing or decreasing carbohydrate feeding. Operating a biofloc system is, in a way, like driving a car, controlled by a combination of using the accelerator, the break, the steering (and in cases the horn). If you understand the basic processes, the biofloc system behaves in a friendly way.

Some of the points to be raised in this chapter were discussed, usually in a detailed way, in previous chapters. Yet, having most points related to pond management in one chapter may justify a brief repetition. Thus, if you read here something you remember reading in a previous chapter, it shows that you paid attention to whatever you read. You get a high grade. My compliments!

1. Starting the Pond

Biofloc systems will develop spontaneously in close (zero exchange), intensive ponds, when feed residues accumulate to support the buildup of a dense microbial community. In shrimp ponds, we often need to provide the pond with fertilizers that will support initial algal development (ca by having clear water; where algal blooms dominate within days to weeks. McIntosh (See Chamberlain et al., 2001a) reported that Belize Aquaculture ponds went through a transition period (often associated with an algal crash) wherein foam accumulated on the surface of the ponds, due to accumulating dissolved organic material and an inadequate bacterial community to degrade it. (See the foam coverage in Figure 13.2).

However, it is often desired to have a rapid development of the biofloc system (e.g., in nurseries). In such cases, we need to start early feeding of the pond system, and in some cases inoculation is favorable. This is especially important if you fill the pond with outside water of suspected quality, and sterilize the water, usually with different chlorine compounds.

Upon filling the pond, the addition of substrates is needed for microbial development. This is especially so in plastic lined ponds, where feed storage from previous cycles is minimal. Organic matter can be added as feed pellets (one can use old pellets that have lost their water stability or any other pellets that cannot be used for fish production, yet be careful not to add molds). The alternative is to add carbonaceous substrates, such as molasses, flour etc. In this case, there is a need to add nitrogen as fertilizer (urea, nitrate fertilizers, ammonium fertilizers) or as manure. Organic matter should be added at levels equivalent to about 20-100 mg/l (200-1000 kg/ha), distributed along a period of about 1 week. In case of nitrogen fertilization, the rate should be 0.5-2.5 mgN/l (5 – 25 kg N/ha). A better formulation of feed to be added can be obtained through experience accumulated for the specific farm conditions and availability of cheap feed.

In shrimp ponds, Algae will develop normally a few days following the addition of feed materials and the pond will be green. Later-on, a microbial, heterotrophic community will develop. Heterotrophic and nitrifying bacterial communities and biofloc development will be faster if inoculum is added.

The choice of inoculum and the need to add inoculum opens a large variety of possibilities. Firstly, adding inoculum is not a must since most systems contain a large variety of micro-organisms serving as a natural inoculum. However, this may be a long process, wasting some of the growing period and possibly enabling a high transient buildup of ammonium and nitrite (see detailed discussion below). A simple way to add inoculum is to add pond sediment or agriculture soil (ca 100 kg soil per ha, dispersed in water and well distributed) or add mature pond water. It is recommended to use water or sediments from ponds that had good target crop performance and robust biofloc (large particles, dispersed in the water). The stock inoculum can be pumped from an existing pond in the farm or may be stored in a reservoir for future applications. In hatcheries, where the relative volume of the inoculum is small, a stock suspension may be stored in a small aerated tank. Another type of inoculum can be made in the farm by mixing sediment from the pond with some kind of bran (rice bran, wheat bran or other): Add enough bran to the wet sediment so it becomes semi-dry and produces crumbs upon mixing. In this stage air can penetrate, and the bran-sludge mixture can be composted. Stir the mixture once a day and keep it in the sun (or in enclosed space if it may rain) till it is dry (about one week). At this stage, the mixture

Nitrogen immobilization into microbial cells may take place within the first few days of pond stocking. Yet, in the case of shrimp ponds, it may take a few weeks until enough organic matter as feed is applied to the pond. During this period, we have a limited activity of heterotrophic bacteria as well as slow development of nitrifying bacteria. We often face an increase of inorganic nitrogen in the water. This is not the case in intensive tilapia ponds where the initial biomass is in the range of a few kg/m^3.

When organic carbon assimilation is low, nitrification maybe the major control of inorganic nitrogen dynamics. However, newly stocked ponds start by having meager populations of nitrifying bacteria, both ammonium and nitrite oxidizers. As described in chapter 3, ammonium oxidation starts slowly when TAN concentration is built up. Nitrite oxidation starts later, when nitrite buildup is evident. Thus, we see a sequence of events: ammonium concentration rises up to a maximal value about 2-3 weeks after stocking. Subsequently, it declines with a parallel rise of nitrite. The subsequent decline of nitrite depends on the development of nitrite oxidizers, taking place only when nitrite starts to buildup. We can prepare the nitrite oxidizing bacteria, ahead of time, by fertilizing the pond, upon stocking with a nitrite salt ($NaNO_2$ or other) at a level of about 5 mg salt/l. Normally about 4-6 weeks after stocking, both ammonium and nitrite concentrations are lowered, replaced by increasing concentrations of nitrate (see Chapter 3 and Figure 3.2 for detailed discussion). This period may be critical to the stocked fish and shrimp. Inoculating the pond shortens this dangerous period. It is possible to inoculate using pond sediments or water, yet we should use these only from ponds of known safe history. An alternative approach is to inoculate the pond using commercial nitrification cocktails. There are a number of reliable laboratories that produce and sell stable mixtures of efficient nitrifying bacteria. The only drawback to this approach is the fact that the commercial nitrification inoculants cost money.

Development of bioflocs is helped by the presence of floc nuclei, particles to which microbes and feed residue will adhere and form a small biofloc. Different materials can be used, from inert kaolin (fine clay) as used in some shrimp ponds (Chap 13, Sec 2.2), to the application of fine bran (rice bran or others). The use of bran seems to be advantageous since it provides some nutrients to the attached bacteria. Algae can also serve as nuclei to the formation of bioflocs.

2. Feeding the Pond

Feeding the fish is an essential action in all aquaculture systems. However, both feeding practices and composition are different in biofloc systems, as compared to conventional ones. Unfortunately, there is not enough recorded experience and research, thus we can give here general outlines rather than accurate blue prints and instructions. **We strongly emphasize that present estimates as to feed rations and composition should be taken "with a grain of salt". Pond managers should follow fish response and develop their optimal feeding.**

and less water quality deterioration. (As a first approximation, we can estimate that feed protein can be lowered by 10%). Protein may be lowered by two ways. One is to apply feed with a lower protein concentration (e.g. 25% protein). The other option is to apply a portion of the feed as conventional high protein pellets and another portion, as feed containing mostly carbohydrates (such as grain pellets, see Chapter 13). Experience, (**though limited**), shows that total feed requirement may be lower than that needed for conventional production systems (about 20% less, as an estimate).

The control of C/N ratio in the pond was discussed in Chapters 6 and 7. Some practical aspects are mentioned here.

1. In most cases when the C/N ratio is mentioned, it refers to the **C/N ratio in the feed applied to the pond.** The C/N term used is an abbreviation: **It is actually the ratio of organic carbon to total nitrogen.** The total carbon (determined in the laboratory as COD), in most cases is equal to 50% of the dry feed. This approximation does not simply hold for molasses! Molasses contains 20-25% water, 75-80% dry matter. Thus the carbon content of molasses is about 40% of the feed weight. Total nitrogen in the feed can be approximated as the protein percentage times the percentage of nitrogen in the protein (~ 15.5%).

Thus C/N = feed * 50% /Protein% * 15.5%) (See examples in box)

Example of C/N calculations

a. Pond is fed by pellets having 40% protein.

For each 100 kg feed,

C= 50% * 100 = 50 kg C

N = 40% * 15.5% * 100 = 6.2 kg N

C/N = 50/6.2 = 8.06

b. Pond is fed with 100 Kg pellets, 40% protein, + 75 kg cassava meal.

C = 100 * 50% + 75 * 50% = 87.5 kg C

N = 40% * 15.5% * 100 = 6.2 kg N

C/N = 87.5/6.2 = 14.1

$$C = 100 * 50\% + 56 * 50\% = 78 \text{ kg C}$$

$$N = 35\% * 15.5\% * 100 = 5.4 \text{ kg N}$$

$$C/N = 78/5.4 = 14.4$$

2. As discussed in details in previous chapters, one way of controlling toxic nitrogen level in the pond, is by adding carbohydrates, controlling C/N ratio (See Chapters 5, 6). **Several processes participate in controlling nitrogen: Algae uptake of TAN, nitrification and N assimilation by heterotrophic bacteria.** Yet, the only process we can quantitatively control is the heterotrophic N assimilation. The computations made in Chapter 6 as to the C/N ratio of feed required to control TAN build up took only this process into account. The conclusions of the C/N ratio needed to control nitrogen in the pond are true only if the heterotrophic process is the only (or the major) process taking place. Experience shows that these computations lead to realistic results in many intensive systems, but these are just approximations. In practice, it is important to adjust the C/N ratios to specific conditions. For example, if we had significant nitrification in the pond, carbohydrates application can be significantly lowered. The best means to adjust the amount of needed carbohydrates is through the monitoring of TAN and total inorganic nitrogen. In most cases we will find that we can lower the application of carbon. If an increase of TAN or nitrite is found, it is advised to raise carbohydrate application, at a rate equivalent to about 20 times the required nitrogen reduction (i.e. 20 kg for each kg TAN we want to reduce).

We can adjust feeding so as to minimize inorganic nitrogen build up and wastage of feed protein. There are two possibilities of adding the carbohydrates: (a) as a supplement not included in the feed or (b) within the feed, by applying lower protein feed. The application of lower protein feed pellets (e.g. 20% protein instead of 30% ones to feed tilapia) has an advantage of convenience and saving labor, as compared to separate applications of feed pellets and carbohydrates. In one experiment Avnimelech and co-workers (1994) found that applying the carbohydrate with reduced protein pellets gave better results compared to addition of wheat flour to a separate application of high protein feeds. The pros and cons of separate or joint application need further research and consideration.

The feeding program has to be adjusted to the organism you grow and to the development of both the organism and the pond. Thus, **upon stocking the pond with shrimp PL's or fish fingerlings, biofloc population has not yet developed and cannot be considered as a significant source of protein, you have to supply feed with high protein levels.**

3. Sludge control

age. Sludge can be effectively drained if it accumulates on the pond bottom, in a location from which one can remove it using strong water current. In radially aerated ponds this location is in a drainage pit at the center of the pond. In raceways it will be in a pit at the exit (See Chapter 12). Efficient sludge removal demands a fast water flow that will effectively pull out the sludge from around the water outlet, a flow that can be obtained by lowering a stand pipe in the drainage canal. You drain the sludge as long as the outgoing flow is black-brown and you should stop the flow once you get clear water. Usually it takes 1-2 minutes. It is important to have a significant hydraulic drop from pond water level to the drainage base and wide enough tubes to drain out the sludge. Some farmers place perforated pipes on the pond bottom with the intention of collecting the sludge, out to the draining canal. It seems that this method is of limited efficiency since one needs vigorous water flow to pull the sludge out of the pond bottom, a demand that is hard to achieve with perforated pipes along the pond bottom. Drainage of sludge in intensive shrimp ponds is required toward the end of the cycle, when feeding is high. By that time, a weekly or bi-weekly drainage is recommended. With intensive fish ponds or very intensive shrimp ponds, the generation of bioflocs and waste is way too high to be suspended in the water, thus a large quantity of material settles and accumulates at the pond bottom. Daily or even twice daily sludge drainage is required. A different means to control sludge accumulation, applicable in small intensive systems, it to continually drain a fraction of the water to settling tanks and recycle the relatively clear water (see chapter 14).

4. Monitoring and responses

The importance of routine monitoring of BFT ponds cannot be over-estimated.

However, we do not monitor just to accumulate data in note-books or computer files. Managing the pond involves the control of a number of bio-reactions and the only means to optimize this control is by monitoring several parameters to be followed by rapid responses as necessary. Monitoring is not important as such, unless proper responses (often fast responses!) are taken following the examination of monitoring data.

Conventional aquaculture determinations, such as oxygen, pH and alkalinity as well of periodic sampling of your animals are needed also in BFT ponds

Oxygen and pond bottom conditions monitoring.

Oxygen should be monitored at least daily. If oxygen level is lower than the set value for the cultured animals, more aeration or longer duration of aeration should be supplied. Oxygen concentration may vary during the day. In extensive ponds, algae contribution is significant. Thus, high

and depths in the pond, to get an idea on the pond uniformity, existence of poorly aerated regions and to better know your pond. Looking on the water flow pattern in the pond, one can identify regions in the pond where water flow in limited. Oxygen supply in such regions may be limited! Additionally, sites where water is stagnant, are susceptible as sites where bottom sludge accumulate. Development of sludge accumulation sites may be causative to fish stress, disease development and poor growth. Farm operator should be aware of this and check accumulation of sludge in such sites. The best way to determine sludge accumulation in shallow ponds, is to walk periodically in the pond, especially in suspected sites.

Presence of anaerobic pockets may be critical. The response to poorly aerated sites or sites where sludge accumulates, is to re-locate aerators placement, adding aerators, targeted to sites where the existing units do not properly mix the water and the bottom. (Add aspirators, air lifts, vertical aerators). As discussed previously, **one has to be very cautious in dispersing piles of reduced (black) sludge** containing H_2S. A vigorous resuspension of this sludge in the water could be critical to your crop! Such operation should be gradual.

The pH and the alkalinity should be maintained at conventional levels. Alkalinity should be above 50-100 mg as $CaCO_3$/l and pH should be 7-9. Alkalinity and pH are usually stable in BFT ponds though there might be a need to add alkalinity in cases of high stocking density (Ray et al., 2009; Wasielesky et al., 2006). Nitrification is a major process leading to significant alkalinity consumption and to lowering of the pH. Systems, where the major inorganic nitrogen control is based upon nitrogen immobilization, are typically rather stable in respect to alkalinity and pH, in contrast to systems where intensive nitrification is taking place.

Monitoring and Control of Biofloc Density

The presence of flocs can be observed by taking a water sample in a transparent container and looking at the presence of suspended particles. Biofloc concentration can be simply evaluated using calibrated Imhoff cones (Figure 11.1).

Figure 18.1: Measuring floc volume using imhoff cones

The cone should be filled with 1 liter water (sampled in front of an aerator in the stream of moving water to ensure representative mixed water sample) and let to stand still for 15-20 minutes (a suitable stand can be used to hold the cones). The volume of the settled floc volume is read following this period. In many cases, flocs are small and hardly seen when the water is sampled, but develop to large flocs in a few minutes. Leaving the cone for time periods longer than about 20 minutes results in gas formation within the floc plug and re-suspension of the particles. Typical floc volumes are a 2-40 ml/l in shrimp ponds and up to 100 ml/l in fish ponds

An alternative, and possibly more accurate term to define and determine biofloc concentration is the Total Suspended Solids (TSS). Total suspended solids are determined by filtering a samples of pond water (normally 10-20 ml) through a pre-weighed filter paper (Whatman GFC or similar), evaporating the water in an oven (65-100°C) for a few hours and re-weighing.

Volatile Suspended Solids (VSS) is a conventional way of determining the organic fraction of the total solids, a common parameter in water technology. VSS is obtained by weighing the dry filter following TSS determination in a muffle at 550°C and weighing the residue, corresponding to the ash fraction. The VSS, the organic fraction is the TSS minus the ash. VSS determination demands laboratory equipment that is not commonly available in farms. Turbidity is defined as "an expression of the optical property that causes light to be scattered and absorbed rather than transmitted with no change in direction or flux level through the sample." This procedure produces a number, reported as Nephelometric Turbidity Units (NTU), in a short period of time and can be a reliable indicator of solids concentration. However, the color of a water sample can affect the results of this procedure. Water that is brown in color can produce a higher turbidity that is green in color, independent of the TSS concentration. If proper equipment exists, turbidity determinations are fast and easy (even enabling monitoring along time) and can give a good estimate of TSS.

Determination of FV is simple, does not require expensive equipment and can be easily done in any farm. Determinations of TSS and the other parameters mentioned demand better equipment and can be done periodically as a means of calibrating the FV determinations

It is advisable to correlate TSS with floc volume results. This correlation, possibly somewhat different for different farms and cropping system, enables estimation of TSS by the simple field determination of floc volume. Avnimelech (2007) found in tilapia ponds that biofloc plugs contained 1.4% TSS (as dry weight), yet this may not be the rule. **You can use the relation TSS = 10 x FV as a reasonable approximation.**

The values obtained in monitoring floc volume or the related terms are very important in conducting the pond. The presence of bioflocs in the pond is essential as means to control water slowed down with floc volume higher than about 10 ml/l. Normal TSS values in shrimp ponds water are in the range of about 50 – 300 mg/l, while those in fish ponds reach levels of up to 1,000 mg/l. It seems that TSS should be limited to about 200 and 400 mg/l in shrimp and fish ponds, respectively, though there are as yet no proven data, especially in case of tilapia. It should be noticed that the gross feed equivalent value of 100 mg TSS/l is about 1,000 kg Feed/ha. It is possible to determine gross protein in the bioflocs, an estimate of which can be obtained by determining total nitrogen in the water.

When floc volume is lower than 2 ml/l (shrimp) or 5 ml/l (fish) in advanced stages of culture, it is advisable to add organic matter (molasses or other). However, floc volume above ~15 ml (shrimp) or ~25 ml (fish) may be too high. Excessive floc volume leads to increased biological oxygen demand (BOD), demanding an un-needed increase of pond aeration. Heavy load of suspended matter can lead to clogging of gills. Excessive flocs should be drained and in some cases the water exchange rate should be raised.

Control of Toxic Nitrogen Species

An increase in the concentrations of ammonia (determined through TAN levels) and of nitrite can critically damage pond production. Inorganic nitrogen levels should be very closely monitored. The parameters to be followed are TAN (total ammonium nitrogen) and nitrite. It is advisable to determine also nitrate, (NO_3), concentrations from time to time. TAN concentrations should not be in the range that may endanger the well-being of the target crop (see Chapter 5). An increase of TAN concentration, or those of total inorganic nitrogen, indicates that C/N ratio in the added feed is too low. The response to an increase of TAN should be very fast, since ammonia toxicity is high. The common response is to raise carbohydrate addition. In critical situation you may exchange some of the pond water or carefully add acid to slightly reduce the pH of pond water (see chapter 5).

An increase of nitrite concentrations may be associated with different causes. An increase of nitrite concentration in the beginning of the production period is expected and is due to the known sequence of nitrification (Chapter 5). Later-on, such a rise may be due to a too low C/N ratio and increase of TAN that is nitrified. Another important reason for a rise in nitrite is incomplete oxidation processes as affected by redox conditions in the pond (See Chapter10). Thus, when a rise in nitrite concentration is measured in the middle of the grow-out season, it is often due to improper aeration or mixing and the resulting accumulation of sludge in the pond. Nitrite increase is a very subtle early warning of the creation of anaerobic pockets. One should react quickly. The best way to react is to get into the pond (bare foot or not is up to you), walk around and physically feel if there is sludge buildup somewhere in the pond. With all our sophisticated remote sensing instruments, walking around and feeling what you have down there is still the most sensitive tool. If you find sludge pile(s) you may change the position of aerators to better

Practical Implications and Tips

Developing a biofloc system can be done, in a closed intensive pond, by two ways: The first one is to stock the crop, let algae develop and subsequently microbial population grows and dominate. The second way is to enhance a rapid heterotrophic development by feeding the pond prior to stocking and also inoculate the pond. As usual in life, in between approaches are common.

1. *Bioflocs develop in ponds having a high fish (shrimp) biomass and high feed application. Often, especially in shrimp production systems, such conditions develop only after a few weeks, when the shrimp grow. In order to take advantage of the bioflocs and utilize the ponds, a high stocking rate is advantageous. Transfers or partial harvests can be planned so as to keep the pond at a high density that does not exceed carrying capacity of the system.*

2. *A strategy of recycling water from the good ponds in the farm to newly filled ponds will help in maintaining a well balanced microbial community adjusted to your farm's conditions.*

3. *Drainage of excessive sludge is important. For effective draining it is good to have a vigorous outflow of water from the pond bottom. The drainage system should be based upon a steep hydraulic gradient.*

4. *Carbohydrates added to the pond should be made of finely ground starch (cassava, wheat, corn, rice etc. or of soluble liquids (molasses). Depending on location, the available and inexpensive sources should be identified.*

5. *Computers are now common in practically all farms. Monitoring and recording data in computerized files and plotting the results in charts is a way to see trends, compare data and draw conclusions. It is recommended to process data into computerized format and to draw charts as soon as possible, in order to see changes as they occur in real time. Keeping records of past years also helps in detecting long range changes.*

6. *Sampling water from several points in the pond is a good practice to remain familiar with your pond. Find out how uniform the pond is and determine how best to adjust the aerators.*

Further Research Needs

The experience obtained on the management of BFT ponds is limited since we are dealing with a new technology. Moreover, there is a limited volume of controlled research. Very important experience ob-

Recommended Reading and Cited Literature

1. *Avnimelech, Y., Kochva, M., Diab, S. 1994. Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Israeli Journal of Aquaculture- Bamidgeh 46: 119-131.*

2. *Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. Aquaculture 264:140-147.*

3. *Chamberlain, G., Avnimelech, Y., McIntosh, R.P., Velasco, M. 2001 A. Advantages of aerated microbial reuse systems with balanced C/N I. Nutrienttrans formation and water quality benefits. Global Aquaculture Advocate 4 (2):53-56.*

4. *Chamberlain, G., Avnimelech, Y., McIntosh, R.P., Velasco, M. 2001 B. Advantages of aerated microbial reuse systems with balanced C/N. II. Composition and nutritional value of organic detritus. Global Aquaculture Advocate 4 (3): 22-24.*

5. *Ray, A.J., Shuler, A.J., Leffler, J.W., Browdy, C.L. 2009. Microbial ecology and management of biofloc systems In: C.L. Browdy and D.E. Jory (Eds.). The rising tide, proceedings of the special session on sustainable shrimp farming. World Aquaculture 2009. World Aquaculture Society, Baton Rouge Louisiana, USA.*

6. *Wasielesky, W (Jr.), Atwood, H., Stokes. A., Browdy C.L. 2006. Effect of natural production in brown water super-intensive culture system for white shrimp Litopenaeus Vannamei. Aquaculture 258:396-403.*

Chapter 19
Final Words

The Biofloc technology is an exciting and complex system involving different aspects and branches of science and engineering:

A. A combination and interaction of bacteria, algae, zooplankton and fish;

B. Control of complex ecosystem toward the control of water quality;

C. Recycling of waste material and their utilization by the fish;

D. Engineering controls, such as aerators, pond structure and bio-engineering.

The Biofloc technology is aimed at the development of a sustainable and profitable system to produce more healthy food for the global population. We are just starting our way to know, understand and master this technology. We need more multi-disciplinary research, far more farm scale experience and observations made by interested practitioners. We need cooperation, mutual learning processes and better understanding.

This book is just one step in this process. We can move forward by communicating, exchanging information so that future books will contain more information. I hope that the new information will be disseminated to help our industry. You are kindly requested to send your comments and suggestions to: biofloc@technion.ac.il.

List of Authors

Professor (emeritus) Yoram Avnimelech

Prof. Avnimelech, got an M.Sc. in Soil Science-Microbiology (1960) and Ph.D. in Soil Science-Physical Chemistry (1964) from the Hebrew University, is involved in a wide range of environmental research areas, including soil and water chemistry, wastewater treatment and reuse, soil fertility, aquaculture, composting, and environmental management and policy.

As a specialist in watershed management, he served as the head of the Sea of Galilee Watershed Research Unit, a unit responsible for water quality management in Lake Kinneret and the River Jordan tributaries. He was invited to serve as the chief scientist of the Israeli Ministry of the Environment (1989-94). At this position he was in charge of all research conducted by the Ministry and a variety of technical topics as well as contacts with Palestine, Jordan and Egypt.

His interest in the field of aquaculture stemmed from his work on the environmental effects of aquaculture, an approach that led to the development of zero, or minimal water exchange systems. He developed novel technologies of intensive fish and shrimp systems, based on the control of the microbial system in the pond (Bio Floc Technology). He served as a board member of AES, Aquaculture Engineering Soc. And heads the working group on Bio Floc Technology, BFT.

Professor Yoram Avnimelech was the Dean of the department of Agricultural Engineering (2001-2003).

He served as a consultant in Africa (Ivory coast, Malawi, South Africa), Thailand, Philippines, Colombia, Brazil, Panama, Australia and the USA in both aquaculture as well as soil and water management. Gave extended seminars and courses in Brazil, Ecuador, Belgium, Indonesia, Mexico, India, Australia and other .

Prof Avnimelech published more than 100 papers in refereed scientific journals, and had 34 graduate students toward the master and doctorate degrees.

GLOSSARY and ABBREVIATIONS

Aerobic (conditions, processes)	Processes taking place in the presence of oxygen
Anaerobic (conditions, processes)	Processes taking place when no oxygen is present and reducing compounds (e.g. sulfides) dominate
ATP	Adenosine Triphosphate. A molecule essential in energy transfer in living cells
Autotrophic nitrification	A two-step process in which ammonia is biologically oxidized to nitrite (nitritation) and then to nitrate (nitratation) with oxygen as terminal electron acceptor
Autotrophs	Autotrophs are the primary producers in a food chain, such as plants on land or algae in water. Bacteria which derive energy from oxidizing inorganic compounds such as ammonia, hydrogen sulfide or iron
BAL	Belize Aquaculture
BFT	Biofloc Technology
Biosecurity	The practice of exclusion of specific pathogens from cultured aquatic stocks in broodstock facilities, hatcheries and farms, or from entire regions or countries for the purpose of disease prevention" (Lightner, 2003)

BOD	Biological Oxygen Demand. A term used to measure or define availability to micro-organisms of organic substrates in water
Carrying capacity	A term used in ecology defining the ability of a system to raise load (biomass, feed) without deteriorating or collapsing
C/N Ratio	The ratio of carbon to nitrogen (g/g) in feed, total organic matter, etc
FCR	Feed Conversion Ratio. Weight of fish produced by adding a given weight of feed
FV	Floc Volume. The volume of flocs settling out of pond water in a settling cone
HDPE	High Density Polyethylene. Plastic used for pond lining
Heterotrophic	Organism requiring organic substrates (basically produced by the primary producers, autotrophs) to get its energy and organic carbon for growth and development. An heterotrophic organism derives energy from externally produced organic compounds
HR	Heterotrophic Ratio
Imhoff cone	A clear, cone-shaped container used to measure floc volume (FV) in a specific volume of water (See book cover)
Kjeldahl nitrogen	The total ammoniacal and organic nitrogen determined using the Kjeldahl method

NPP

Net Primary Production

PC

PC is the protein concentration in the feed

PCR

Protein Conversion Ratio: Amount of protein in the feed devided by added protein in the fish. A term equivalent to FCR

Periphyton

Periphyton is a complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus that is attached to submerged surfaces in most aquatic ecosystems. It may serve as an important food source for fish

PL

Post larvae

Probiotics

Live microbial feed supplement which beneficially affect the host animal by improving its intestinal balance

RAS

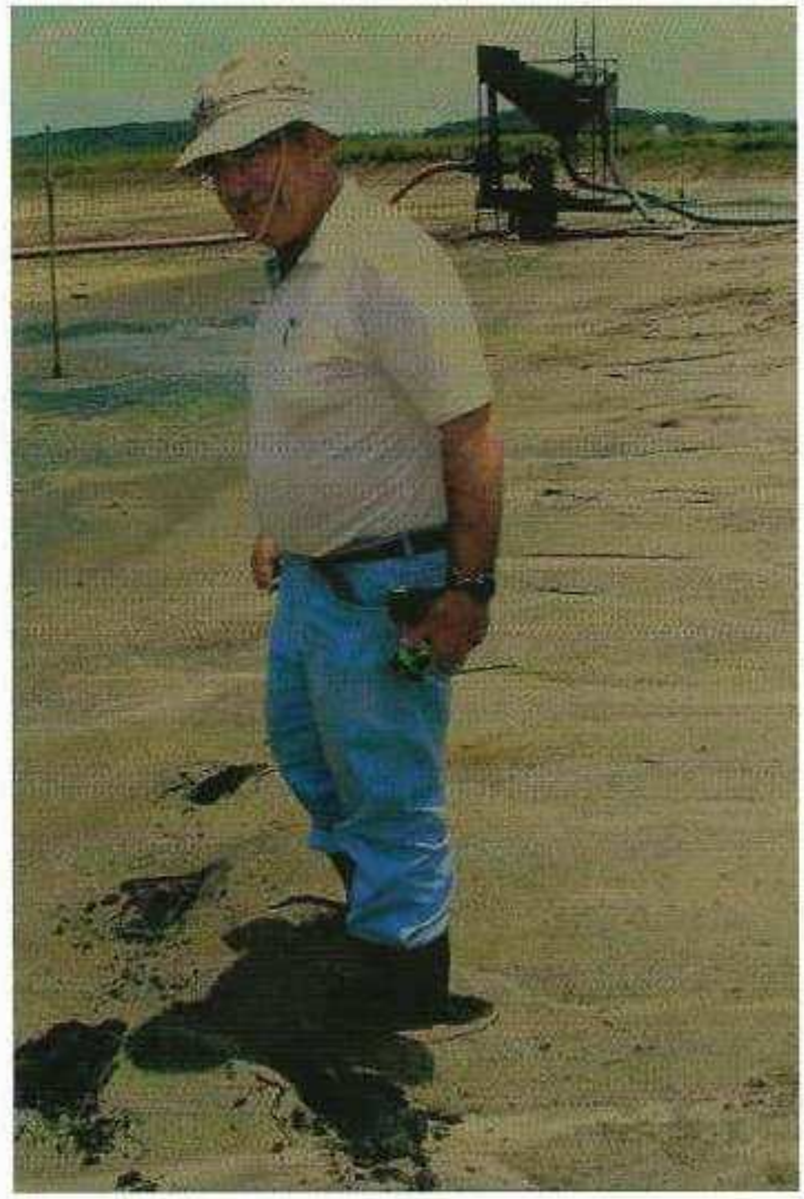
Recirculating Aquaculture Systems

Secchi disk

The Secchi disk, created in 1865 by Pietro Angelo Secchi, is a circular disk used to measure water transparency in oceans and lakes. The disc is mounted on a pole or line, and lowered slowly down in the water. The depth at which the pattern on the disk is no longer visible is taken as a measure of the transparency of the water. This measure is known as the Secchi depth and is related to water turbidity

Sedimentation	Settling of particles from the water onto pond bottom
TAN	Total Ammonium Nitrogen
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids, a means to determine and evaluate organic particular matter in the water
WSSV	White Spot Syndrome Virus

The picture, taken in a shrimp pond in Brazil, demonstrates the presence of sulfides in pond bottom. The black sludge is an indication of reduced soil with high sulfide concentrations. The top layer was oxidized and turned brown upon exposure to air and sun. Stepping in the mud reveals the reduced mud under the brown cover. **One learns a lot by getting into the pond.**



Professor (Emeritus) Yoram Avnimelech was the Dean of Faculty of Agricultural Engineering of the Technion (2001-2003). He served as the head of the Sea of Galilee Watershed Research Unit, responsible for water quality management in Lake Kinneret and the River Jordan tributaries.

When the Israeli government established the Ministry of Environmental Protection he was invited to serve as its Chief Scientist (1989- control of the microbial system in the pond. Yoram studied sediment water interaction in ponds and lakes, carbon/nitrogen control in intensive aquaculture and prevention of anaerobic conditions in ponds.

Prof. Avnimelech published more than 100 papers in refereed scientific journals, edited 4 books and had 34 graduate students toward the master and doctorate degrees.

Yoram is heading the International Working