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Control of microbial activity in aquaculture systems: active suspension ponds

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The control of aquaculture systems through the manipulation of microbial activity became an important and commonly discussed technology arising from the development of intensive aquaculture. Aquaculture depended, traditionally, on algal activity. Growing fish, including crustaceans or other cultured aquatic animals in water bodies depends, in traditional aquaculture, on algae generating the base for the food chain or food web, to feed the fish. Basic properties related to algae control are listed in Table 1. Algal productivity in non-fed ponds depends on the level of inorganic nutrients, mostly phosphorus (P) and nitrogen (N). Thus, one of the first stages of intensification was to add inorganic fertilizers to the pond. Following the elimination of nutrients as a limiting factor, as is common in well fertilized or fed ponds, productivity becomes limited by solar radiation reaching the algae. With optimal solar radiation

and algal activity, primary productivity (PP) can reach a maximal daily level of 8–10 g carbon (C) per m² (Boyd and Tucker 1998). However, normal high values are in the range of about 4 g C/m² day (Wetzel 1975). The assimilated organic carbon is the base of the food web. Only part of it may be consumed directly by fish, inasmuch as there is no control over the species of algae that grow in the pond.

Algae supply other services to the pond. A product associated with carbon

assimilation is the production of oxygen: $6\text{CO}_2 + 6\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$ (1)

Primary production of 4 g C/m² day adds 10.7 g/m² of oxygen; yet, only a fraction of that is a net addition to the pond. In the long run, all the organic carbon is respired and all of the oxygen that is produced is then consumed. The benefit to the pond stems from the fact that part of the organic matter settles as dead

algae or other particulate organic matter, and its microbial degradation takes place only later. This is the reason for oxygen problems in old ponds, where the sediment oxygen consumption becomes an important fraction of the pond oxygen balance. This is also the reason for the recommended drying of the pond bottom to oxidize the organic matter by exposing it to the air. An additional reason leading to inefficient use of the produced oxygen by algae is that oxygen is produced mostly in the top water layer, leading to oxygen supersaturation in that layer and to its release to the atmosphere.

One of the problems associated with the intensification of pond aquaculture is the accumulation of ammonia in the water. This accumulation places the fish at risk from elevated levels of the toxic ammonia (NH₃) and nitrite (NO₂⁻) species. Algae help to alleviate this problem because they take up nitrogen from the

Table 1. Comparison of algae and bacterial controlled systems.

Property	Algae Control	Bacteria Control
Energy source	Solar radiation	Primarily 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 to low water exchange.
Sensitivity to environmental variables	Light is essential (activity is decreased on 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 and consumed at night.	Oxygen is consumed.
Relevant activities	Primary production: produces organic matter and oxygen. Ammonia uptake.	Degradation of organic matter. Nitrification. Production of microbial protein.
Inorganic nitrogen control	Uptake driven by primary production Maximum Capacity ca. 0.7 g NH ₄ ⁺ /m ² /day.	Uptake of nitrogen affected by the C:N ratio in organic matter. Practically unlimited capacity.
Potential capacity	Normally, daily primary production does not exceed 4 g O ₂ /m ² .	Limited by substrate concentration and rate constant of degradation.

water as a part of their metabolism. As shown in equation (1), the basic activity of the algae leads to the production of glucose. However, a basic component of algal cells is a protein that is synthesized from glucose using nitrogen that is adsorbed from the water. Nitrogen constitutes about 1/6 of the carbon in algal cells (Wetzel 1975), thus the ammonia uptake potential of a pond with PP of 4 g C/m² day, is 0.66 g of ammonia nitrogen per day. A pond carrying 10 tons of fish/ha and fed with 40 percent protein feed at a rate of 2 percent bodyweight, gets 1.24 g N/m² day, and roughly 75 percent of it, or 0.93 g N/m², is released into the water. Thus, the algae that can serve as a good buffer against ammonia toxicity cannot function when pond intensification rises. Moreover, algal control suffers from inherent instability. One factor is the strict demand for light. A series of cloudy days, very common in the tropics, leads to severe light limitations and to drastic reduction of primary productivity. Oxygen concentration can be drastically reduced and ammonia concentration increased following a series of cloudy days.

To raise yields and to get better control of oxygen in ponds, fish farmers introduce aerators into the pond system. Aerators provide oxygen and, at the same time, mix the water. Intensive aeration provides optimal conditions for aerobic bacteria and is a feature typically found in bio-technological microbial-based industries. An additional factor leading to the microbial dominance in intensive ponds is the limitation on water exchange.

Water exchange is one of the few controls in algae dominated systems. When the water contains a surplus of algae, or a surplus of organic materials, oxygen consumption exceeds supply. One means of overcoming this is to exchange the organic rich water with cleaner water. However, this practice is limited because of environmental concerns and regulations, the risk of disease infestation resulting from the un-controlled introduction of water and, in part, the expense of pumping. When water exchange is limited, as in the case of zero exchange or minimal exchange regimes, organic matter builds up in the water. 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 lead to the development of microbial dominance in the pond. In intensive fish culture systems using heterotrophic microbial control, active suspension ponds (ASP) were studied and implemented in the last two decades (Avnimelech *et al.* 1992, Avnimelech *et al.* 1994, McIntosh 2000, Chamberlain *et al.* 2001).

Typical features of microbial dominant systems are presented in Table 1. The size of the microbial population depends on the supply of organic matter and the stability of the aerobic community

depends on an ample supply of oxygen, though their sensitivity toward low oxygen is far below that of fish. The number of bacteria in zero exchange intensive ponds was found to be in the order of 10⁷-10⁸ cells/ml (McIntosh 2000). The level of organic matter in ponds tends to reach a steady state as a result of a balance between the addition of organic matter and its microbial degradation.

Continual aeration is conducive to the development of nitrifying bacteria that oxidize the excreted ammonia, first to nitrite and then to nitrate (NO₃⁻). Nitrate is not toxic to fish at the levels found

in ponds and it serves as an oxidizing agent, preventing the development of anaerobic conditions which can lead to several fermentation processes and to the build up of toxic reduced compounds. Nitrifying bacterial development is rather slow. It may take 2-3 weeks from the day the pond is filled until the full oxidation of ammonia to nitrate, with a possible short period of nitrite accumulation when the first stage of ammonia oxidation has developed while the second is still evolving (Avnimelech *et al.* 1986).

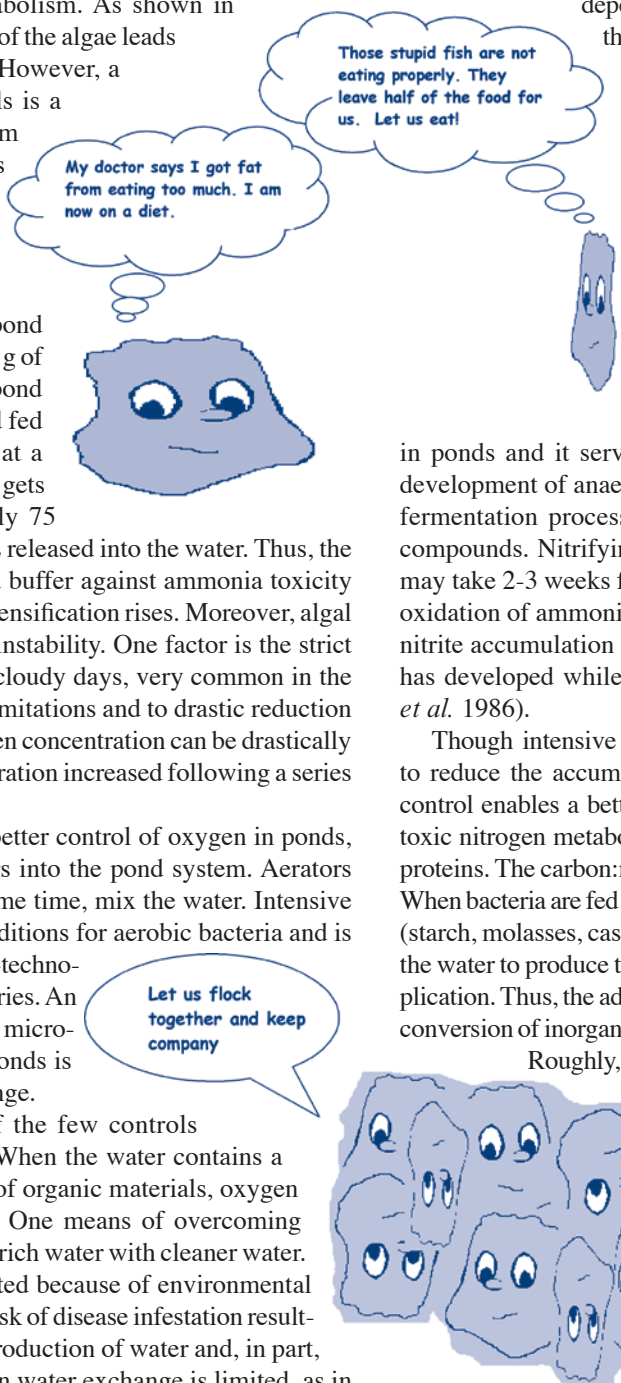
Though intensive nitrification is a very efficient mechanism to reduce the accumulation of ammonia and nitrite, microbial control enables a better and more efficient methods of reducing toxic nitrogen metabolites. Bacterial cells are made primarily of proteins. The carbon:nitrogen ratio of most microbes is about 4:5. When bacteria are fed organic substrates that contain mostly carbon (starch, molasses, cassava meal), they must take up nitrogen from the water to produce the protein needed for cell growth and multiplication. Thus, the addition of carbonaceous materials leads to the conversion of inorganic deleterious nitrogen to microbial proteins.

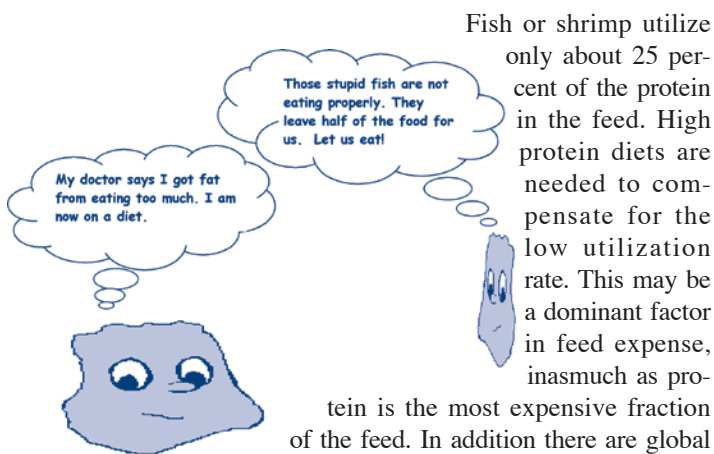
Roughly, 20-25 g of carbonaceous material are needed

to convert 1 g of ammonia nitrogen into microbial protein (Avnimelech 1999).

This process is relatively fast and it is possible to reduce an elevated level of ammonia to any desired level within a period of 1-3 days. One feature of this mechanism is the possibility of using the microbial protein as a source of protein for fish.

The availability of microbial protein is not trivial nor is harvesting. Individual bacteria are too small to be harvested physically by fish. In addition, the digestibility of the bacterial protein must be determined. In a series of tank and commercial size pond experiments it was found that tilapia ingest and digest the microbial protein (Avnimelech *et al.* 1989). Similar results were found with shrimp (McIntosh 2000) and bass (Milstein *et al.* 2001). The harvesting of the bacteria were possible because in dense microbial suspensions the organisms tend to form flocs that were visible, having a size of a few tenths of a millimeter. These flocs can be directly harvested or filtered. MacKintosh (2001) reported that in the Belize zero exchange shrimp ponds flocculation was enhanced through the re-use of water from ponds that had a good flocculation, seemingly by selection of bacteria with high flocculation potential.





Fish or shrimp utilize only about 25 percent of the protein in the feed. High protein diets are needed to compensate for the low utilization rate. This may be a dominant factor in feed expense, inasmuch as protein is the most expensive fraction of the feed. In addition there are global fluctuations in the quantities of animal protein sources available that can be problematic. The utilization of protein in active suspension ponds is doubled. The fish eat and digest the feed-originated protein and use it again by ingesting and digesting the microbial protein, actually a recycling of the non-utilized feed protein. A demonstration of this effect, obtained in a field trial, is presented in Table 2. Very similar results were obtained in commercial shrimp ponds (McIntosh 2000). The higher utilization of feed leads to significantly lower feed costs (Table 2).

Preliminary results indicate that microbial dominance may help induce disease resistance in fish. Several observations as well as a controlled experiment (Avnimelech and Ritvo 2001) indicated that a dense heterotrophic population can act as an antagonist against pathogens. This effect, a probiotic control mechanism, deserves further study.

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Conclusions

Microbial controlled ponds, (ASP), provide a stable control over processes in the pond. The microbial control is stable, does not depend on light intensity and is not sensitive to population crashes. Microbial control leads to efficient degradation of waste materials, efficient nitrification and, through the manipulation of the C:N ratio, facilitates the control and recycling of nitrogen and doubles the protein utilization.

The basic demands to establish and maintain microbial dominance are:

- The pond gas must be well aerated and mixed.
- Concentration of organic substrates has to be high to support the heterotrophic population. This is achieved in intensive systems with zero or restricted water exchange. An addition of organic substrates in the beginning of the culture cycle may help promote the development of the microbial community.
- Microbial controlled ponds rely on the development of natural microbial populations. Presently, there is no microbial inoculum proven to be effective in promoting the development of microbial controlled ponds.
- The efficiency of microbial controlled ponds must be evaluated for the given organism to be cultured.

Notes

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Table 2. Effect of added carbon substrate (wheat flour) on yield, feed conversion ratio, protein conversion ratio (protein in feed/protein gain in fish) and feed cost in intensive tilapia ponds. Averages based on three replicates in commercial scale ponds. (Adapted from Avnimelech *et al.* 1994.)

Protein level in feed percent	30 percent	20 percent
Experiment 1 (51 days)		
Feed C:N ratio	11.1	16.6
Daily gain (% body weight)	1.59	2.0
Food conversion ratio	2.62	2.17
Protein conversion ratio	4.38	2.42
Feed cost (US\$/kg fish)	0.85	0.58
Experiment 2 (30 days)		
Feed C:N ratio	16.6	11.1
Daily gain (% body weight)	1.63	2.22
Food conversion ratio	2.62	2.02
Protein conversion ratio	4.35	2.18

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