Recirculating Aquaculture System Conditioning

Ritesh Ranjan, Sampath Kumar, G.,
Chinnibabu Bathina and Ravi K Avadhanula

A newly established recirculating aquaculture system should be conditioned before stocking of the live animal into the RAS tanks for the culture. It is necessary because fishes produce waste from the day of stocking as a result of the nutrition they receive. It is not only the waste produced from the fish but also from the waste generated due to feed and feeding activity. These wastes, mainly ammonia and nitrite are very toxic to the fish and are an environmental stressor that causes reduced appetite, reduced growth rate and death at high concentrations. These toxic waste needs to be removed from the system from the date of stocking otherwise will create problem to the stocked animals. To remove these wastes, the biofilter or biological filter, which form a key component in the filtration system of a recirculating aquaculture system (RAS) needs to be activated. The biofilter houses the nitrifying bacteria and is the primary site where biological nitrification occurs. Nitrifying bacteria converts toxic nitrogenous waste produced or excreted by the aquatic organisms into the simpler form, which is less toxic to the fishes.

Naturally occurring ammonia oxidizing bacteria (AOB) grow on ammonia by consuming it and converting it to nitrite (NO_2^-). The bacteria that convert ammonia to nitrite are known collectively by their genus name *Nitrosomonas*. Like ammonia, the nitrite produced by the *Nitrosomonas* bacteria is toxic to aquatic organisms and must be oxidized further to a less toxic form of nitrogen. This is accomplished by another naturally occurring genus of bacteria referred to as *Nitrobacter*. These bacteria metabolize and oxidize the nitrite (NO_2^-) produced by Nitrosomonas and convert it to nitrate (NO_3^-). Nitrate is the end product of the conversion of ammonia and nitrite and is non-toxic at the levels around < 200 mg/L.

Biofilter activation

Biofilter activation is the controlled management of colonizing the nitrifying bacteria cells in a biological filter. A biological filter consists of various material such as plastic beads known as bioball, net fiber, molluscan shells, fiberglass, ceramic or rock that has large amounts of surface area for colonizing the nitrifying bacteria cells. Generally, nitrifying bacterial cells grow on all surfaces of the biological filter media and not only the biofilter tanks but also on all wet surfaces of the system, such as the insides of pipes, drum filter, tank walls, etc. Bacteria follow a continual cycle of growing and multiplying, maturing and dying, sloughing off of the media, and being replaced by new cells.

A biofilter is activated by adding bacteria to the system, which can be done in several ways. It can be introduced either with water or bits of biofilter media from an already operating system, with pond sediment or pure culture of nitrification bacteria or with small numbers of the fish going to be cultured. The fish stocked in the tank will have to survive in the presence of elevated ammonia and nitrite concentrations whereas bacterial cells reproduce and colonize the biofilter media. Whichever method is used to introduce bacteria to a system to colonize, there is always a chance of introducing pathogens as well in the system. The choice of a method of introducing bacteria in biofilter should depend upon overall facility management and biosecurity plan.

Generally, two strategies are followed to activate the biofilter. The first strategy, sometimes called the cold start method, involves stocking the fish without having an activated biofilter. In this method, the technician will be prepared to deal with the rapid increase in ammonia concentrations by exchanging water to keep ammonia level in controlled conditions so that the fish should survive. In this system, feeding rates must be reduced or suspended until biofilter activation occurs. This method has the advantage of using the bacteria that entered the system with the first animals introduced, bacteria that must have been well-suited to the conditions from which those animals came. This passive biofilter activation is a slow and stressful process for the animals as well as the system operator.

The second strategy is more aggressive and fast method for biofilter activation. In this method, the nitrifying bacteria will be well developed and colonized in the biofilter tanks before stocking the culture tank with the animals. This system will help in reducing stress on the newly introduced stock and shortening the growing cycle with higher feed rates from the first day of stocking and creating better water quality, which improves health, growth rates, and survival of the cultured fish. Generally, a mixed approach of two strategies can be used to introduce nitrifying microbes in the system.

Steps in RAS conditioning

1. Prepare the system and water filling

The component of the system should be connected and installed as per the plan. The tank should be filled with ozonated or chlorinated sea water when the system is used first time to sanitize the whole system. The system should be run for 1-2 days and water should be checked for the presence or absence of ozone or chlorine halides and their byproducts. The water quality parameters of the water filled in the tank such as pH, salinity and alkalinity should be checked and if at all it should be adjusted as per the requirement of the system as well as the fish going to be stocked for the culture. Aeration should be provided in the tank to provide oxygen for the bacteria as it is required by them in oxidation process.

2. Nitrifying bacteria introduction

Introducing nitrifying bacteria accelerates the acclimation process. It can be added from any source as mentioned in the biofilter activation. However, if using commercial preparations of bacteria, follow the manufacturer's recommendations during the previous steps and in adding the bacteria. Apply as per the suggestion of manufacturer. The most effective way to introduce bacteria is to move biofilter substrate like bioball from an acclimated system operating under similar culture conditions. A number of prepared mixes of cultured nitrifying bacteria are available from suppliers, for both freshwater and marine applications. These are available as dry powders or concentrated liquid suspensions. Although they are not essential, these bacterial preparations can shorten the time that it takes to establish the biofilter.

3. Ammonia and nitrite supplementation

Ammonium hydroxide, or unscented household ammonia used for cleaning, which is an aqueous solution of ammonia can be used for providing a source of ammonia. Ammonium chloride and ammonium nitrite are commonly used. Add sufficient ammonium hydroxide to raise the total ammonia level to between 3 and 5 mg/L. Start with a minimum amount, allow enough time for thorough mixing through the system, and then test for the ammonia concentration. If more is needed to increase the concentration in the system to between 3 and 5 mg/L, add more incrementally.

4. Water quality parameters monitoring

Water quality parameters should be monitored regularly during the start-up of a biofilter. Ammonia, nitrite, pH, temperature and alkalinity are the primary water quality parameters to be tested. Reliable, accurate and repeatable methods for measuring ammonia and nitrite concentrations in the system should be established. Testing can be done with test kits and color comparators, colorimeters, or spectrophotometers. Operators should be able to compare changes in water quality from one day to the next with confidence that the test results are representative of changing water quality conditions that the biofilter is causing. Water samples should be collected from the culture tank, since that is where the animals will be. Samples should be collected from the same place in the tank and analyzed promptly. They should be taken at the same time each day, and persons conducting tests should follow the same procedures, using the same accurate measuring equipment. It is good to plot graph of both ammonia and nitrite concentrations, adding each day's readings over time. This gives us a visual concept of biofilter development. The characteristic curves associated with biofilter start-up have been shown in figure 1. The data may vary between the different cycles as well as in different RAS system and biofilters, however the shape and relationship of the curves will remain almost the same. Initially, ammonia concentration in the system will increase to a peak and then declines and after a lag period, nitrite concentration begins to increase to a point and then declines and finally the nitrate will increase then follow the same trend like the other parameters.

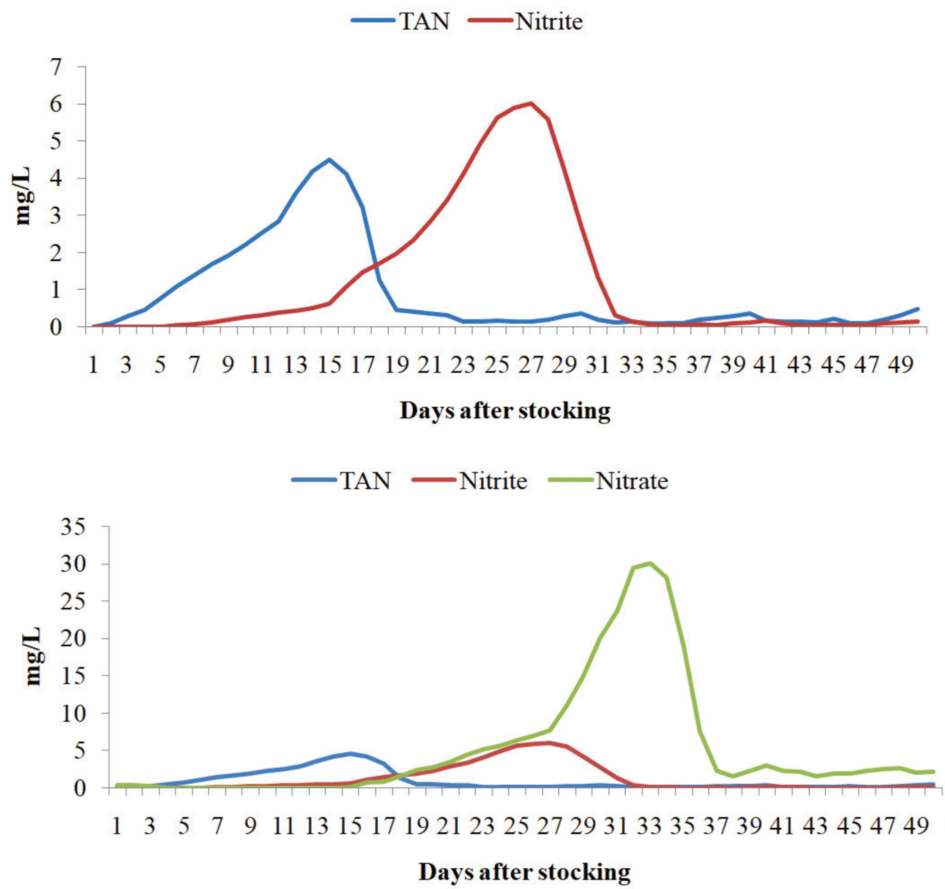

Fig.1. Water quality parameter from Recirculating aquaculture system start-up

5. Fry Stocking

Stocking of the tank with the Indian pompano fry will start once the ammonia and nitrite level is under controlled/tolerable level. The trend of the declining nitrite level should be noted. The top of the nitrite curve represents the point at which ammonia oxidizing bacteria have developed in numbers sufficient to consume more nitrite than is being produced or added to the system; thus, the nitrite concentration decreases. This trend will continue until nitrite concentration levels off. At this point of time, the tank should be stocked with animals to provide food for the micro-organisms; otherwise there may be chance of dying the developed micro-organism. The stocked animal should be fed at slightly reduced level for 4-5 days and slowly increasing the feed to the required level.

6. Water quality parameters monitoring

During stocking and afterward for one week, the water quality of the RAS should be monitored twice in a day to manage the undesirable changes in the water quality. Dissolved oxygen level needs to be monitored frequently and if required need to be adjusted as per the requirement.

7. Protein skimmer regulation

The protein skimmer works upon the load of dissolved organic load in the culture water. After stocking of the tank with fish, dissolved organic load will start producing protein that needs to be skimmed using protein skimmer. However, it should also be taken care water should not be wasted with the skimming process. Hence the protein skimmer-controlled valve needs to be adjusted as per requirement during the first week of start.

Some tips

Sometimes RAS conditioning is unnecessary when the culture cycle progress is one after another. If the system is being transitioned from one production cycle to the next, the system can be kept operating and the biofilter kept active between cycles, stocking the next batch of fry immediately after harvesting or transferring the previous one.

If a biofilter is kept alive and functioning between production cycles, some bacterial cells will be lost because the amount of ammonia and nitrite available to sustain the bacteria population will be decreased. Some cells may slow down their processes, and the biofilter's overall nitrification capacity will decrease slowly. Nitrifying bacteria activity will increase again as soon as animals are fed and waste production increases. However, feed rates should be increased slowly, as it will take some time for the bacterial biomass to return to pre-harvest levels. If there will be several days or weeks between cycles, doses of ammonia can be added to the system to keep the bacteria alive and functioning.

As a prophylactic measure to control the transmission of any disease if at all in commercial aquaculture production, it is often necessary to disinfect the system upon completion of a rearing cycle. Disinfection is normally accomplished by flooding the tanks, filtration components, and pipes of the entire system with a solution of water and bleach (chlorine) or other suitable disinfectant and allowing it to circulate, followed by draining and drying of the system. This process kills not only pathogens in the system but also the nitrifying bacteria in the biofilter, which must then be started anew for the next growth cycle.

It can be difficult to start a biofilter containing brand new media, possibly because the media may still have a slick, shiny surface to which it is more difficult for bacteria cells to attach. Once the first growth of bacteria is established, however, subsequent cycles usually start more quickly. Some polystyrene bead material has shown initial resistance to bacterial colonization. It has been suggested that additives such as flame retardants on the surface of the beads may inhibit the initial growth of bacteria. The resistance of the bead material to colonization by nitrifying bacteria appears to be short-lived, however, and most operators work through the problem relatively quickly.

With moving bed or mixed bed biofilters, bacteria may be slow to colonize because of excessive agitation or aeration of the media bed. This is essentially a mechanical problem caused by the abrasion of the surface of the biofilter elements, continually scraping off bacteria cells that are trying to adhere. Reducing the physical agitation of the media bed by reducing aeration and/or water flow through the bed can help in colonizing bacteria. As more bacteria cells become established on the media surfaces, as evidenced by daily decreases in ammonia and nitrite concentration, aeration and agitation can be gradually increased.

It is better to adopt the method that works best for your operation and your systems. If you are learning to start and operate systems, keep records and notes of your experiences so that you can determine what works best or not for your situation.

References and suggested readings

DeLong, D.P. and Losordo, T.M., 2012. How to start a biofilter. SRAC publication No. 4502, Southern Regional Aquaculture Center, Stoneville, MS.

Ranjan, R., Megarajan, S., Xavier, B., Raju, S.S., Ghosh, S., Gopalakrishnan, A., 2019. Design and performance of recirculating aquaculture system for marine finfish broodstock development. Aquaculture Engineering 85, 90-97.