AN AQUACULTURE TECHNICAL BRIEF

Water Quality and Its Relationship to Freshwater Recirculating Aquaculture Systems

CARMEN AGUILAR, PH.D., SENIOR SCIENTIST AND RUSSELL CUHEL, PH.D., SENIOR SCIENTIST

University of Wisconsin-Milwaukee School of Freshwater Sciences

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WATER QUALITY AND ITS RELATIONSHIP TO FRESHWATER RECIRCULATING AQUACULTURE SYSTEMS

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INTRODUCTION

IN A FRESHWATER RECIRCULATING AQUACULTURE SYSTEM (FRAS), THE SYSTEM CHEMISTRY DEPENDS GREATLY UPON THE COMPOSITION OF THE INITIAL SOURCE WATER.

Aquaculture of vertebrate animals, usually fish, and invertebrates, including shrimp, requires daily attention for periods up to a year. Upon harvest, this effort can be well rewarded by selling a high-quality food product.

A recirculating aquaculture system is by far the most feasible approach for land-based aquaculture because it can simulate the significant continuous flow of water required for water quality safety. This continuous flow requires both source water availability and wastewater disposal. In a freshwater recirculating aquaculture system (FRAS), the system chemistry depends greatly upon the composition of the initial source water (i.e., groundwater, city water, river water or lake water). Each of these sources has distinct chemical characteristics that must be considered to suit the health needs of fish in the FRAS. With careful consideration of these and other chemistry aspects, FRAS can be linked directly to aquaponics to grow marketable vegetable crops.

There is great value in studying and understanding biological and chemical processes occurring within your particular system. Some of these are generalizable across all FRAS, and some are very specific to individual systems. A primary goal is to prevent development of adverse chemical conditions that reduce water quality. Toxicity and insufficient oxygenation can result from metabolism of critically necessary bacteria and the fish themselves. Poor conditions stress the fish and can lead to outbreaks of disease or direct mortality. These conditions are kept in check by bacteria that are cultivated in a biofilter component of the system. The biofilter is one of many components of a FRAS that affect its water quality. Regular testing of water from various components will serve as an early warning to adverse conditions.

Take-Home Point: A thorough understanding of FRAS components — from the source water in a new system to the biofilter of a mature system — and their individual contributions to water quality is crucial to success.

The first part of this technical brief, Basics of Water Chemistry, addresses the chemistry components of FRAS operation. The second part, Case Study: Science-Based Descriptions Critical to FRAS Operation, puts the basic chemistry into practice. We've added italicized take-home points that give you a quick look at a basic concept.

BASICS OF WATER CHEMISTRY

All living organisms have ranges of tolerance for environmental variables that differ not only among animals, plants and bacteria but also within those groups. Figure <u>1</u> illustrates a comparison of chemistry requirements between humans, fish, plants and bacteria. One to three pluses indicate mild to high requirements, and one to three minuses indicate minimal to no requirement. In this section, we will discuss each of the chemistry elements and a few additional non-chemistry considerations in detail.

A Note About Equilibria Equations

In this technical brief, we use equilibria equations to illustrate chemical changes that affect water quality. As an example, the below equation is a carbonate equilibrium.

$H_{2}O + CO_{2} \leftrightarrow H_{2}CO_{3} \leftrightarrow H^{+} + [HCO_{3}^{-} \leftrightarrow H^{+} + CO_{3}^{-}]$

In equations, the double-headed arrow (\leftrightarrow) indicates that the two sides are in equilibrium or balanced, and can readily change depending on conditions in the environment. If an element on one side of the equation increases (either by natural occurrence or by human intervention), the other side of the equilibrium will produce more of what it needs to maintain balance on both sides. If something is reduced on one side, the other side will try to replace it. In this case of carbonate equilibrium, if you add acid (H⁺) to the system, it will

	Ť			
	HUMAN	FISH	PLANT	BACTERIA
Oxygen	+++	+++	++/-	+/-
Carbon dioxide			+++	++/
Protein	+++	+++		++/
Nitrogen			+++	+++
рН	5-9	6-9	5-9	1-13
Surfaces	-	-	+/-	+++/-
Water	+	+++	++	+/-
Hardness/Alkalinity	low	range	wide	extra-wide

Fig. 1. Chemistry requirements of humans, fish, plants and bacteria.

Key

- Required
- +++ Highly required
 - Not required, but used
- --- Never used
- +/- Different species use differently; or required but can survive without for a limited amount of time

combine with carbonate $(CO_3^{=})$ and bicarbonate (HCO_3^{-}) to ultimately produce carbon dioxide gas and water (left side). The gas can then vent out of the system, and two acids are consumed.

A Note About Chemical Measurements

Throughout this technical brief, you will read about measurements of the various chemicals in a FRAS. To measure your system, you will need some basic test equipment, including rapid tests for nitrite, ammonia and pH. Choose kits that can be used easily on-site, perhaps like those from a chemical analysis provider like Hach[®] (hach.com), which include ammonia and nitrite tests and the simple pH paper. Some chemicals are measured using a hand-held probe, while others are chemical tests. You will also need a plan to assess the function of each component of the system by testing and comparing the records you keep. For more details, see Testing and Recording Your Components' Functions.



Fig. 2. Oxygen concentrations change with water temperature.

OXYGEN

One of the most important chemicals in any aquaculture system is oxygen. Just as we need oxygen to breathe (air consists of 20% oxygen, 0.04% carbon dioxide and 78% nitrogen), so do fish. Fish consume oxygen that is dissolved in the water and need about five or more milligrams of oxygen per liter to be healthy. If there is less than that, they will not grow as well. If there is no oxygen, they will die.

Oxygen solubility changes with water temperature, as shown in figure 2. At near-freezing temperatures, there will be almost 15 milligrams of oxygen per liter. Very warm water, too warm for most fish, contains only half that much. It is easier to provide appropriate amounts of oxygen when you are working with cold-water fish than when you are working with warm-water fish. By changing the temperature of the water, you change how much oxygen is dissolved in the water in equilibrium. The maximum amount of oxygen that can be dissolved in water under normal conditions is known as the saturation concentration. At any temperature, oxygen concentration can range from zero to full saturation (or maximum concentration) depending on consumption and access.

Just as humans constantly breathe air, fish are constantly consuming oxygen, so a steady oxygen supply is crucial to fish growth. Low oxygen concentrations negatively affect fish, especially in tanks, recirculating systems and ponds with high stock densities of fish. Therefore, oxygen concentration must be measured regularly and kept at the highest level that works for your system. There are hand-held probes made specifically to measure oxygen in water.

Your goal is to find the critical threshold to avoid fish mortality. In terms of oxygen saturation, 80 percent is recommended. In terms of oxygen concentration, a desirable range of oxygen is 5 milligrams per liter (mg/L) and above. Fish can survive with an oxygen range from 3-4 mg/L, but growth is slow when the exposure is prolonged. At concentrations less than 3 mg/L, fish will survive with a short exposure, but prolonged exposure is lethal. Figure 3 illustrates how the fish decrease food consumption and experience decreased growth and increased mortality with decreasing oxygen in the system.

Plants produce oxygen in the light, but they require it in the dark. This is a challenge for submerged plants and algae, but above-ground plants have





plenty of oxygen from the atmosphere day and night. Aquaponics usually involves above-ground plants.

Bacteria needs for oxygen vary widely, and the bacteria we cultivate in RAS biofilters use dissolved oxygen in different ways. The bacteria that eat ammonia use the oxygen atom directly and attach it onto the ammonia molecule. For others, such as the bacteria that use nitrite for energy and produce beneficial benign nitrate, oxygen is required for respiration.

Take-Home Point: Oxygen is vital in aquaponics systems and is affected by many elements including temperature and aeration. Constantly monitor oxygen in your system — the most important number to know is the oxygen concentration.



Aeration

Aeration is a non-chemical component of aguaculture that is critical to oxygen and carbon dioxide gases. To make oxygen available for fish to consume, it must first pass through the air-water interface, which is a physical barrier for oxygen diffusion. The best way to do this is with an aeration technique. Aeration is a physical system that moves gases, including oxygen (into water) and carbon dioxide (out of water) across the barrier. Oxygen is dissolved into the water and is then consumed by fish, plants and bacteria. As you increase the number of fish in your system, you will need to increase the amount of oxygen. Likewise, fish and bacterial respiration produce harmful CO₂, and the aeration step equilibrates dissolved CO₂ with the much lower atmospheric concentration. (See Gas Exchanger and Turbulence section.)

Temperature

Another important non-chemistry component is temperature because it affects rates of various aquaculture and aquaponic processes. Temperature influences dissolved oxygen in water, fish growth and chemical rates. Different fish species have ranges of optimum temperatures for growth, depending on whether they are cold-water, cool-water or warm-water species. Because fish cannot regulate their own temperature they depend on the water of the system. For cold-water fish, such as salmon and trout, the optimal temperature range for growth is between 9 and 18 degrees Celsius. Cool-water species, like yellow perch, prefer water between 16 and 29C, and warm-water fish, like catfish and tilapia, prefer water between 24 and 32C.

CARBON DIOXIDE (CO₂)

Carbon dioxide is a gaseous waste product of animal metabolism and is not required by humans or fish. Most green plants require carbon dioxide, as it is the source of carbon for their major structural and metabolic content. Some bacteria require carbon dioxide just like plants, especially critical bacteria such as nitrosofiers and nitrifiers that live in a FRAS biofilter. Bacteria that use carbon dioxide can usually get it from the carbonate equilibrium which always maintains some dissolved CO_2 gas. The total carbon dioxide can get very low because of acid production that results from nitrogen oxidation in the biofilter, and so the small amount that is present needs to be available. (See The Biofilter and its Components section.)



Fig. 4. Assorted sources of ammonia in aquaponics systems.

PROTEIN

Protein is a primary nutrient for all animal growth including humans and fish. When fish eat protein-rich food, much of it goes to making fish tissue and what isn't used is expelled as waste, releasing ammonia and other breakdown products containing nitrogen. Protein must be supplied, but overfeeding produces excess waste that requires removal. The feed rate will increase as the fish grow, and balancing supply and demand is essential in reducing waste. (See <u>Solids Removal</u> Filter section.)

NITROGEN (N)

Nitrogen, primarily from feed protein, is the basis of the main waste cycle in aquaculture and includes inorganic forms, N_2 gas, ammonia, nitrite, nitrate, urea and other rarer compounds. While plants and bacteria require mineral N, humans and fish do not. Several forms can be toxic to fish, so careful monitoring is crucial. Almost all plants will take up many forms of mineral N, but most prefer nitrate (NO_3^-), the end-product of nitrification created by biofilter activity and a basis of aquaponic system function.

In aquaculture systems, the three most important components of nitrogen that need to be monitored are ammonia, nitrite and nitrate. (See the <u>The Nitrogen</u> <u>Transformation Series</u> section.)

Ammonia (NH₃)

Fish waste and decomposition of uneaten food and other solids in a RAS produce a stream of inorganic nitrogen, mostly as ammonia, which can be managed by a biofilter, aquaponic plants or both. See <u>figure 4</u>. Ammonia is toxic to the fish, so careful monitoring of its concentration in the system is important. Different species of fish can tolerate different levels of ammonia. For example, salmon, trout and perch tolerate only low levels of ammonia (<1 mg/L), while bass, carp, catfish, tilapia and bluegill can withstand higher levels of ammonia (1-3 mg/L).

Ammonia occurs in two forms in water: ionized ammonium (NH_4^+) and un-ionized ammonia (NH_3) . Un-ionized ammonia, also called free ammonia, is toxic to fish. As a gas dissolves in the water, like oxygen, it readily crosses the gills into the bloodstream of fish.



Total Ammonia Nitrogen (TAN) = $NH_3 + NH_4^+$

When ammonia combines with water that is a near neutral pH of seven (see <u>pH</u> — Acidity vs. Alkalinity section), it becomes ionized ammonia. Ionized ammonia is not toxic to fish. The amount of ionized and free ammonia depends on pH and temperature of the water. At lower pH, the hydrogen ions (H⁺) in the water drive the balance to ammonium. As pH increases, conditions favor the un-ionized form.

Both types of ammonia exist in your system at the same time and cannot be measured separately. You can only measure the total amount, known as the total ammonia nitrogen or TAN (see <u>figure 5</u>). So how

Fig. 5. Total ammonia nitrogen or TAN.

	Aquaculture with Yellow Perch																		
	Percent Un-ionized Ammonia (NH $_3$) in Water for Ranges of Temperature and pH																		
	30	0	0.1	0.3	0.8	1.4	2.5	4.4	7.5	12.6	20.4	31.4	44.8	59.1	72.0	82.0	89.0	96.3	98.8
nre	25	0	0.1	0.2	0.6	1.0	1.8	3.1	5.4	9.3	15.4	24.4	36.5	50.6	64.5	76.4	85.2	94.8	98.3
Temperature	20	0	0	0.1	0.4	0.7	1.2	2.2	3.8	6.6	11.2	18.3	28.5	41.5	55.7	69.1	79.9	92.6	97.5
ube	15	0	0	0.1	0.3	0.5	0.9	1.5	2.7	4.7	8.0	13.4	21.6	32.9	46.5	60.8	73.4	89.7	96.5
Ten	10	0	0	0.1	0.2	0.3	0.6	1.0	1.8	3.2	5.6	9.5	15.7	24.9	37.1	51.1	65.1	85.5	94.9
	5	0	0	0	0.1	0.2	0.4	0.7	1.2	2.2	3.8	6.6	11.2	18.3	28.5	41.5	55.7	79.9	92.6
рΗ		5	6	6.5	7	7.25	7.5	7.75	8	8.25	8.5	8.75	9	9.25	9.5	9.75	10	10.5	11

Acceptable levels of NH₃: pH = 7.0, (NH₃) = 0.4%, NH₃ concentration = 0.05 mg/L

Critical levels of NH₃: pH = 9.0, (NH₃) = 28.5%, NH₃ concentration = 3.4 mg/L

Toxic levels of NH₃: pH = 9.5, (NH₃) = 55.7%, NH₃ concentration = 6.7 mg/L

will you know how much of the TAN is the toxic free ammonium? You will use a table like that in <u>figure 6</u>. Here's why.

The balance between the two types of ammonia depends greatly on the pH of the system. If the pH is on the low side, around six or seven, very little of the ammonia is in the toxic form. If you increase the pH to nine, which is an additional stress for fish, 60 to 70 percent of the ammonia is toxic. You didn't add any more ammonia; the equilibrium shifted because of the pH.

The tool you use to measure ammonia will have a table similar to that in figure 6. Let's look at yellow perch as an example. We need to keep the un-ion-ized (free) ammonia at less than a milligram per liter. In our green (safe) example, the TAN is 12 milligrams per liter, the temperature is 20C, and the pH is seven. Using the chart, select the temperature of 20C and the pH of seven. The number at the intersection is the

percentage of the total ammonia that is un-ionized – 0.4. Of the 12 milligrams per liter, only 0.4 percent is un-ionized, which is 0.05 mg/L, far less than the 1.0 milligram per liter maximum for yellow perch.

For another example, try the same tank, fish, total ammonia nitrogen measurement and temperature, but a higher pH, pH9. Using the table in <u>figure 6</u>, you can see that 28.5 percent of the ammonia in the system is toxic. Twenty eight percent of 12 milligrams per liter is 3.4 milligrams per liter, more than three times the maximum for yellow perch.

Nitrite (NO,-)

Nitrite is the product of the first step of ammonia transformation, or nitrification, in aquaculture systems. (See the <u>Nitrogen Transformation Series</u> section below for more detail.) The first step of nitrification is the oxidation of ammonia to nitrite by nitrosofying bacteria,

Fig. 6. Sample table used for measuring TAN.

Procedure

- 1) Measure temperature, pH and TAN (total ammonia nitrogen) of a system.
- 2) Calculate the amount of NH₃ (un-ionized ammonia) present in the system.

Note

Un-ionized ammonia can lead to a toxic environment to yellow perch at <1 mg/L. such as *Nitrosomonas* sp., which live on surfaces of the biofilter and derive energy for their metabolism during the process. One acid (H+) is produced at this step.

It is important to understand the full nitrogen transformation process, because nitrite (NO_2^{-}) can become toxic and possibly lethal for fish. This can happen if the bacteria in the biofilter are not established, that is, not working at full capacity, resulting in a significant accumulation of nitrite. (See the <u>Biofilter and its Components</u> section.)

Nitrate (NO₃⁻)

Nitrate is the product of the second step of nitrification. The second step is the oxidation of nitrite to nitrate by nitrifying bacteria such as Nitrobacter sp. and Nitrospira sp. Nitrification removes toxic nitrites and produces one acid.

STEP	ONE	тwo
PROCESS	Nitrosification	Nitrification
WHAT HAPPENS	Nitrosifying bacteria oxidize ammonia into nitrite.	Nitrifying bacteria oxidize nitrite into nitrate.
RESULTS	Toxic Water	Benign Water

Fig. 7. Nitrogen transformation series as a two-step process.

THE NITROGEN TRANSFORMATION SERIES

The nitrogen transformation series is arguably the most important aspect of water chemistry in aquaponics. It is a series of three naturally occurring chemical changes, influenced by the system operator, which critically affects the nitrogen in the system. The transformation of nitrogen is represented as:

$NH_4^+ \leftrightarrow NH_3 + H^+ \rightarrow [NH_2OH] \rightarrow HNO_2 = H^+ + NO_2^- \rightarrow HNO_3 = H^+ + NO_3^-$

Because this series of chemical reactions is in equilibrium, you can increase or decrease chemicals on one side of the equation to change the other side, including moving from toxic to non-toxic conditions. See this series represented in figures $\frac{7}{2}$ and $\frac{8}{2}$. Let's take each step one at a time.

NH_4^+	\leftrightarrow	NH₃ + H⁺
ionized ammonium pH<8	freely reversible	un-ionized ammonia pH>9.3
safe		toxic



When organic matter and fish waste decompose, they create dissolved nitrogen as ammonia. In biochemistry, it is usually in the form of ionized ammonium (NH_4^+) because most cells are nearly neutral in pH, near seven. When the water has a high pH, the ammonia is in an un-ionized form that is toxic to fish.

Here, if you add acid (H⁺) to the system, it will keep un-ionized ammonia down by converting it to ammonium, which is not toxic. On the other hand, if you take away the acid, it will pull ionized ammonium into the un-ionized side and increase its toxicity. The best practice is to get rid of the ammonia altogether, as in step two.

In step one, you call upon the nitrosofying bacteria that convert ammonia to nitrous acid to get energy from those ammonia electrons. Simply put, if a molecule has a lot of oxygen atoms, like nitrate (NO_3^-) , it does not contain energy itself for bacteria to use; one with a lot of hydrogen, like ammonia (NH_3) , is rich with energy for bacteria. So, the nitrosofying bacteria (which are named for their function, such as *Nitrosomonas* sp.) have enzymes that can get their energy from ammonia. They do this:

 $NH_3 \rightarrow [NH_2OH] \rightarrow HNO_2 \rightarrow H^+ + NO_2^$ ammonia ½ O_2 nitrite

The arrow in one direction means that it goes that way only, and the reaction also requires whatever is under the arrow in this discussion. An interesting aspect of this reaction is that the bacteria use un-ionized ammonia directly, and they add an oxygen atom to it. So, even if the equilibrium is far toward ionized ammonium, there is always one molecule for the bacteria to use, and when they do, a new one takes its place. At this point, the nitrite is very toxic, much more than un-ionized ammonia. You need to get rid of it right away.

In step two, fortunately, there are bacteria that eliminate nitrite. They are the nitrifying bacteria with names like *Nitrobacter* or *Nitrospira*:

$$NO_2^- + H_2O \rightarrow H^+ + HNO_3 \rightarrow H^+ + NO_3^-$$

nitrite acid nitric acid nitrate acid

Nitrite is biochemically oxidized to nitric acid (HNO₃), also a powerful mineral acid, which immediately dissociates to acid plus nitrate, a benign substance to fish. The nitric acid is an additional element to keep ammonia in line. But this only works if the nitrosofying and nitrifying bacteria are in balance.

There is another consequence of acid production during the nitrifying process that is important: both ammonia- and nitrite-consuming bacteria use carbon dioxide (CO₂) as their source of growth carbon to make new cell material. Previously you saw the carbonate equilibrium, which is very similar to the nitrogen reaction sequence here, except that the CO₂ sequence is reversible. But, if you pump up the system with acid you drive the system to gas-phase CO₂ that vents out. Hence with a lot of nitrification, the bacteria may lower the pH enough to starve for CO_2 . Therefore, some growers add sodium (bi)carbonate or marble chips to maintain near neutral pH. Regular monitoring of the pH gives early notice when an intervention is necessary. See the Testing and Recording Your Components' Functions section for details.

There are other types of bacterial communities that make these changes as well, but they are rare in functional systems. Your system will grow those bacteria



Fig. 9. The pH scale measures the amount of hydrogen ions (H⁺) in a substance.

that are right for its conditions, and you will keep the system running by careful attention to the chemistry that results.

Take-Home Point: Learn and understand the nitrification sequence, know where each component is active in your system, provide circumstances favorable to growth of those bacteria in action, and assess system response to your amendments on a regular basis by <u>testing and recording</u> your component's functions.

pH-ACIDITY VS. ALKALINITY

As noted earlier, knowing your water pH is critical to successful management of an aquaculture system. The acidity or alkalinity of a solution is measured by the pH scale, which ranges from zero to fourteen as seen in <u>figure 9</u> and is determined by the amount of hydrogen ion activity in a solution. Zero is very acidic, like battery acid; fourteen is very basic or alkaline, like drain cleaner. It is a logarithmic scale, which means each step is a 10-fold change in concentration. The numbers zero to fourteen represent an enormous range of concentration.

Putting this in perspective of common solutions, bleach has a pH of thirteen; it is very basic. Lake Michigan water is approximately eight. Fish and other vertebrates have an average blood pH of seven point four. This is important because blood vessels of gills are in contact with water as it goes through them. The saliva in your mouth is neutral at about seven. The contents of your stomach are very acidic with a pH of one to three, but your throat can only handle a pH of seven, which is why vomiting can burn your throat.

It is crucial that you know the pH of your source water before introducing fish into your system. Different fish have specific pH requirements. A healthy pH range for fish is between six and eight. Outside those parameters, their growth will be slowed, they may experience reproduction issues, and extremes can be lethal.

It is important to know that pH not only affects the processes going on in the system; it is also affected by those processes. For example, ammonia combines with water and produces hydroxide ions, which



calcium carbonate + carbon dioxide + water = calcium ion + bicarbonate

 $CaCO_{3} + CO_{2} + H_{2}O = Ca^{+2} + 2 HCO_{3}^{-1}$

change the pH. Ammonia and oxygen combine to produce hydrogen ions. If you change the pH, these processes will shift.

Take-Home Point: Maintain pH of your system in the six to eight range. Absolutely prevent swings of pH into alkaline territory at all times.

complexes. Water source chemistry also changes with seasons and with episodic events such as heavy rains or drought.

Take-Home Point: Know the characteristics of your water source before starting your system, and know what events, if any, cause it to change.

WATER

Water is critical for all life; most body tissue is 60-95% water by weight. More than the presence of water, it is the quality and chemical composition of the water that is critical to living organisms. Water characteristics vary tremendously depending on source, from dilute alpine groundwater to grossly contaminated CAFO (concentrated animal feeding operation) regional groundwater, and from spring-fed streams to large lakes to river waters downstream of large urban or industrial

SURFACES AND SUBSTRATES

Because of gravity, plants, humans and all other animals (except birds, flying insects and aquatic suspended organisms) are always in touch with some surface. Fish swim but can be damaged and stressed by bumping into irregularities in tank structure. Therefore, they are very aware of swimming restrictions and many do not fare well in closed systems. In contrast, some bacteria benefit from surfaces on which to grow in communities. In a tank, surface films containing many kinds of bacteria will form on the walls and bottom, though the growth area per volume of water is small compared to that of the biofilter. Beads or other solid materials known as solid phase are placed in the biofilter specifically to cultivate these bacteria communities. See <u>The Biofilter and Its</u> <u>Components</u> section for more details.)

Take-Home Point: Choose the best solid phase for each component of your RAS, and remember that too little is very dangerous whereas too much is inefficient.

HARDNESS AND ALKALINITY

Alkalinity and hardness (also known as ionic strength) are closely related because the major source of both alkalinity and hardness is the contact of water containing dissolved carbon dioxide with carbonate rocks (limestone), which are mostly calcium carbonate. See <u>figure 10</u>. Limestone is made up of calcium and magnesium carbonate, and when it dissolves, you end up with calcium and magnesium, which generate hardness, as well as carbonates, which generate alkalinity. If the main source of the alkalinity is calcium carbonate, the hardness will be equal to the alkalinity.

Hardness

Hardness is a measurement of water's dissolved calcium carbonate (CaCO₃). While humans' tolerance of hardness is low, the tolerance level is generally broad for fish, bacteria and aquaponic plants. However, the constituents of hardness can impart stress. Hard water resists acidification more than soft water and can form crusts when inevitable evaporation lowers water levels. It may become necessary to reconstitute water volume with distilled water. Source water should be initially

Fig. 11. Water hardness classification. <u>U.S. Geological Survey, downloaded June 2022</u>. Concentration of hardness as calcium carbonite, in milligrams per liter.



evaluated to determine its suitability for aquaculture use, especially ground water and well water that may contain iron or other metals that are suboptimal for fish health.

To give an idea of the range of hardness, Lake Superior is soft water, about 40 to 50 milligrams per liter of hardness. Lake Michigan is about 100 to 150 milligrams per liter and is considered moderately hard water. Water that is in contact with limestone or dolomite can be in the range of 200 to 400 milligrams per liter, which is very hard water. See <u>figure 11</u> for hardness of water sources across the U.S.





¹⁸¹⁻⁴⁰⁰, very hard

A desirable hardness range for aquaculture would lie between 75 and 200 milligrams of calcium carbonate per liter. Fish can tolerate these high levels of hardness. In fact, they are more tolerant of other toxic substances when the water is hard, possibly because those substances are bound up by the calcium and magnesium in the system (the most common sources of water hardness), and they are not then readily available to the fish.

Calcium and magnesium play important roles in fish health; calcium promotes bone formation and blood clotting, while magnesium is important for heart function and electrolyte balance. For these reasons, harder water is beneficial for the aquaculture system. If you have low hardness, you may have trouble growing your fish, and you may have to closely watch calcium and magnesium concentrations to ensure there is enough for healthy fish.

To learn about water hardness in your area, check the U.S. Geological Survey website (<u>usgs.gov</u>) for these two resources:

- The National Stream Quality Accounting Network (NASQAN) provides ongoing data related to sediment and chemicals in the largest rivers in the U.S.
- The National Water-Quality Assessment (NAWQA) Project has assessed multiple water-quality conditions for about 2,100 domestic wells across the United States, including water hardness.

Alkalinity

Alkalinity is a measure of the acid-neutralizing capacity (buffering capacity) of the water. Alkalinity comes from carbonates, bicarbonates and hydroxides, so it will fluctuate in your aquaculture system.



We can use the Great Lakes as a comparison again. Lake Superior, which is a soft-water lake, has low alkalinity of around 50 milligrams calcium carbonate (CaCO₃) per liter. Lake Michigan, which is a moderately hard lake, has an alkalinity around 100 milligrams per liter. Lake Mendota, near Madison, Wisconsin, is even more alkaline at 170 milligrams per liter. Lakes in northern Wisconsin are on granite rocks and not limestone, and they are very weakly alkaline, as low as less than one milligram per liter.

The most important property of alkalinity is that it acts as a buffer between the acid that is generated by the nitrification processes and the danger of increasing acid in your system. In addition to acids generated by nitrification, the fish produce CO_2 when they breathe, which dissolves in the water and slowly lowers the pH, thereby increasing the acidity. As a general rule, the higher the alkalinity is in water, the more stable is its pH. Fig. 12. Limited buffering capacity of alkaline. As acids are added to the system, the alkalinity is consumed. If not replaced, there will eventually be a rapid drop in pH, causing problems with the biofilters and the system in general. If you have a system with a high alkalinity and you add acid, the pH will stay constant for some time because the alkalinity will continue to absorb that added acid. It is a buffer against the change in pH. Over time, however, you will need to add something to increase the alkalinity, or it will drop at its breakpoint. If you continue to add more acid, the pH will drop very quickly, as illustrated in <u>figure 12</u>.

Take-Home Point: Know the chemistry, including hardness, of your source water, and make certain that dilution activities or evaporation preserve those characteristics.

SOLID WASTE

Removal of solid waste, or solids, is an important activity in the aquaculture system. Sources of solids include fish feces, uneaten fish food and biological growth of living and dead bacteria that sloughs off the biofilters or other places. If solids accumulate in the system, several adverse conditions can arise. Bacteria will take action to break them down, using up essential oxygen and producing excess ammonia. Solids can clog fish gills and reduce visibility in the system, making it hard for fish to see food. Solids can overwhelm the biofilter, diminishing the system's ability to work efficiently. The larger the solids are, the easier and less expensive they are to remove. The longer they stay in the system, the more they break down, making them smaller, harder to remove and more easily processed by bacteria.

Solids are defined by their size measured in microns (μ m). The four types of solids relevant to aquaculture are settleable solids (about 100 μ m in diameter), suspended solids (about 20 μ m in diameter), dissolved solids (about 0.45 μ m in diameter) and fine, floatable solids (less than 0.45 μ m in diameter).

Settleable solids are those that will settle out of water in one hour under still conditions without the aid of a filter. The traditional and easy way to measure them uses an Imhoff cone, as diagramed in <u>figure 13</u>. You put a sample of water in the cone, and after an hour the materials that settle to the bottom are settleable solids. These are easily removed from a tank of calm water. One removal technique is to pass the water through a large settling tank as the water leaves the fish tank. The solids drop to the bottom, and they are scraped out.

Suspended and dissolved solids are defined by whether they pass through a 0.45µm pore filter. See <u>figure 14</u>. Solids that do not pass through a 0.45µm filter are called suspended solids; the ones that do



< Fig. 13. Measuring the settleable solids. Settleable solids will settle out of water in one hour under still connections.

Fig. 14. Measuring suspended and dissolved solids.

Suspended and dissolved solids are defined by their size, based on how they separate after a filtration process.



are called dissolved solids. Mechanical filters with fine mesh screens are used to remove these types of solids. One type of mechanical filter passes water through a rotating drum whereby the solids are sprayed off into a trough. Another type includes a set of discs, like those of a rotating biofilter. As water passes through the screen the solids are captured, and as the screen rotates up, the collected solids spray into a trough.

Fine and smaller dissolved solids cannot be removed by settling or filtration. The most common method to remove them is foam fractionation, a process by which air is blown into the water causing bubbles of air to rise to the top. The rising bubbles collect the fine and dissolved particles and bring them to the surface where they form a foam that can be removed.

The rate of fish feces produced is proportional to the feeding rate. Plan for 25% of the feed given to the fish to go uneaten and become part of the volume of suspended solids that will be produced.

Take-Home Point: Accumulated solid waste will create adverse conditions over time. You'll use different techniques to remove different types of solids on a regular basis. (See <u>Solids Removal Filter</u> section below.)

CASE STUDY: SCIENCE-BASED DESCRIPTIONS CRITICAL TO FRAS OPERATION

The guidance provided in this case study comes from a 6,000-gallon FRAS producing nearly 12,000-15,000 market-size yellow perch per year. It has been in operation since 2000 without a so-called belly-up event (significant mortality). The system operates indoors starting with Lake Michigan domestic water supply.

The following descriptions address fundamental aspects of FRAS operation, namely, how water quality affects the biological, chemical and physical functions. This includes the nitrogen-transforming bacteria processes, starting a new system, how a balanced system operates and how to respond to common mishaps.

THE BIOFILTER AND ITS COMPONENTS

The biofilter is the heart of the FRAS system because it is essential to promoting bacterial growth and nitrification processes. In our case study system at UW-Milwaukee, there are three separate biofilter components that have solid phase materials in them, and each one is specifically chosen for the job it performs. They are the solids removal filter, the sand biofilter and the gas exchanger (aerator). Figure 15 is a diagram of the case study's system components and water sampling sites. Sampling sites 1-5 are referenced in figures <u>17</u>, <u>22</u>, and <u>24</u>. Each component uses specific media to perform particular tasks for optimal function. Let's look at each component in more detail.

Solids Removal Filter

In the solids removal filter, an upward-flowing tortuous path with low turbulence enables trapping of solids (fecal matter, uneaten food) without breaking them up. Floating, small, round media are good for this because they don't clog, as screens can, and it is easy to backwash by agitation. In this approach, raw water flows first out of the main fish tank upwards through the filter, with solid particles becoming trapped in the media matrix and low-particle water flowing out of the top to the next component.

To clean this filter, it is taken offline for a few minutes and agitated from above to dislodge trapped particles that are then drained out the bottom. Because the particles reside for hours or more in the flow matrix, they encourage the growth of heterotrophic bacteria, which eat organic material dissolved in the water or in particles, thus cleaning one of the pools of decomposing by products. The matrix itself will also support some of the nitrogen-transforming bacteria.

The Sand Biofilter

The sand biofilter is the main biofilter where the majority of nitrogen detoxification occurs. The two

Fig. 15. Case study system biofilter components with sample sites. The solids removal filter (15A) is a closed tank that contains floating round plastic beads. As the water flows through the beads from the bottom, solid particles get trapped on the path full of twists and turns up through the beads, using gravity as one of the operators. The sand biofilter (15B) is a large cylindrical fiberglass tank that contains several tons of quartz sand. Water pressure keeps the sand fluidized (suspended) so that each grain, and the bacteria growing on it, is circulating through the water, performing nitrification as it makes passing contact. The gas exchanger (15C) is the last step for water on its path back to the fish tank. It is a tower of bioballs or other very low-resistance media that mixes air back into the flowing water. This last step, known as aeration, serves to replenish oxygen used by both fish and bacteria, and to vent carbon dioxide into the atmosphere.



Fig.16. Comparison of amount of biofilter sand to amount of nitrite removal. Nitrite oxidation rate was directly proportional to the amount of sand in short-term incubations. Zero is measured as undetectable.

Fig. 17. Nitrification capacity of water from various FRAS sites with no solid phase material. All sites stable for five days. Site 3 water (downstream of the bead filter) started most quickly, suggesting dislodgement of nitrite oxidizers preferentially from smooth plastic bead material. All samples developed ammonia oxidation capacity after seven days (low initial population with slow growth rate), probably due to bacteria growing on bottle walls.



most important requirements of the biofilter are first, enough substrate surface area (in this case sand) for bacterial adhesion, and second, a capacity for water flow-through, as moving water improves efficiency of all the components.

The surface area of the substrate in the biofilter is important because it serves as home for attached bacteria. These surfaces are where most of the nitrification happens, rather than in the water itself.

As a point of comparison, <u>figure 16</u> shows that more surface area for bacteria habitation results in increased nitrite removal; whereas in <u>figure 17</u> we see that a lack of solid phase material slows nitrification significantly.

In the experiment diagrammed by figure 17, water from five different sites in the FRAS was spiked with 550 units of ammonium and incubated at room temperature in shaker bottles. It took more than six days for any discernable ammonia removal. Where the water passes through biofilters that have media surfaces with attached bacteria (figure 16), nitrification starts right away. It is likely that in the figure 17 experiment, the bacteria accomplishing the nitrification after six days were those growing on the surface provided by the walls of the shaker bottle.

There are four basic biofilter designs to consider depending on your system. The simplest design is a submerged biofilter (figure 18A), which is simply a tank with filters. The water goes up and down through these filters, and bacteria live on the filter material in contact with the water. The rotating biofilter (figure 18B) consists of a group of disks in a tank that rotate slowly in and out of the water. As they come out of the water, they pick up oxygen and return it to the water as added oxygenation. Nitrifying bacteria live on the discs



and perform their nitrification function when submerged in the water.

A fluidized bed and bead biofilter (figure 18C) is the design used in our case study. Generally, the design consists of a filter medium, such as little beads or other small particles, suspended in water in a container. The water enters from the bottom and is filtered by bacteria growing on the small particles. With a trickling biofilter (figure 18D), water flows down from the top through a filter medium where bacteria live. Used often in aquaponics, the raw water is pumped out of the fish tanks into a header tank and then distributed to the plant beds, which serve as the media that filters the water. The filter



Fig. 19. Various biofilter media include (left to right) quartz sand, plastic beads, nylon woven pads, "bioballs" and oyster shells.

both removes ammonia and solids and serves as the growing media for plants.

Figure 19 shows a variety of materials with varying amounts of surface area that can be used in a biofilter. All materials have pros and cons to consider — some are best for solids removal, some for bacterial communities and others for aeration. In a fluidized bed for example, sand is the best choice because it provides the greatest amount of surface area. Porous materials like oyster shells have protected space for bacterial growth and they also neutralize acid from nitrosofication. The goal is to maximize surface area, which controls the maximum potential nitrosofication, but not every system requires the same potential. Bioballs and fluidized sand provide good conditions for nitrosofication but have vastly different surface-to-volume ratios. Plastic fiber pads have a high surface area, however they clog easily and thus reduce efficiency of the system. The best media will depend on the water chemistry in each FRAS.

Gas Exchanger (Aerator) and Turbulence

We've seen why it is necessary to have a large amount of biofilter surface area on which the bacteria can grow. **Fig. 20. The effect of turbulence on ammonia removal.** It is important that the water flow through the turbulence, and not over it.



The greater the amount of biofilter material surface area there is — whether it is beads, sand, Wiffle Balls, oyster shells or any other medium — the faster and more complete is the nitrogen processing.

Additionally, the bacterial community must also get the nutrients it needs to grow and thrive, including oxygen. This is best done by adding turbulence with a gas exchanger. The goal of this aeration is to maximize the exchange of atmospheric oxygen to saturation, while also venting carbon dioxide to desaturate.

Figure 20 illustrates this effect. Using two identical bottles with biofilter sand, we added 14 mg/L of ammonium to the overlying fluid in each. One bottle was kept in motion and the other was stationary. The rate of ammonia removal was normal at about 3% per hour for the bottle in motion. However, there was little change over two days for the stationary bottle (flat line at 1000 = 14 mg/L).

Cascading water through a tower of Wiffle Balls is an inherently stable, long-term, zero-energy method for this last-step water conditioning. Bacteria will grow on these also, but if the biofilter is effective, these bacteria will be primarily organic-consuming bacteria that help to prevent disease outbreaks. The aeration component should be free of places that accumulate debris and cause flow restrictions.

Aerating keeps oxygen in the water. There are three common aeration techniques: mechanical, bubble contact and packed column. Large mechanical aeration devices (21A) stir air into the water and are not often used in aquaculture systems. They are more commonly found in ponds or lakes.

Bubble contact aerators (<u>21B</u>) use pumps to push oxygen-loaded air into the water. They are commonly

Fig. 21. Types of water aerators.

A. mechanical aerator

drawn-in air



B. bubble contact aerator

9" diffuser disc



C. packed column aerator



used in aquaculture because they can create a large quantity of very tiny bubbles. The sphere of each little bubble is a surface area permeable to oxygen. Tiny bubbles are the most efficient because they have the highest surface area per volume of gas.

Another common aquaculture technique is the packed column aerator (21C). This is the aerator used in our case study FRAS system. Water drops down over material packed in a column, where it bounces around getting into contact with air. As the water touches air, the surface area is increased so that when it leaves the column, the water is saturated in oxygen.

Take-Home Point: Create water flow to go through the aeration media, rather than over it, to provide sufficient turbulence for gas exchange.

TESTING AND RECORDING YOUR COMPONENTS' FUNCTIONS

So how do you assess which solid medium is best — for each component or for the main biofilter? First, you need to know what each component does, how it is performing and what it can do under strenuous circumstances. This requires regular testing and accurate record keeping, and this is where monitoring and experimentation come into play. You will be keeping these records as long as your system is running, using them to diagnose and respond to potential problems. Knowledge combined with experience is the key to success!

Note that there are several companies that sell basic test equipment that is easy to use and relatively inexpensive. They will identify an issue, but their level of detail is limited. (See the section <u>A Note About</u> <u>Chemical Measurements.</u>) Alternatively, you can connect with a local water scientist to arm yourself with more technical proficiency. For example, every town has a wastewater treatment plant, and every plant has a chemistry section because nitrogen removal is one of their main functions. They, or experts at a university, can help you develop your own chemistry lab that will greatly improve your understanding. You will have an initial investment, but the value of the output is great, and if you are serious about aquaculture, you need to outfit yourself for the long run.

Start by collecting water samples from your source water, as well as upstream and downstream of each major unit (fish tank, particle removal, biofilter, aeration, others, see figure 15). This is known as a black box approach where you subtract values downstream from upstream, and that difference gives evidence for processes occurring in the spaces between. Use the tests to determine the amount of each nitrogen compound (TAN and nitrite) and pH. Look for detectable differences that indicate activity. For example, you might find high amounts of ammonia in the fish tank but lower amounts in the water flowing out of the particle clarification unit that follows. The outflow might have slightly more nitrite than the fish tank. This would indicate that the particle removal unit was converting ammonia at a faster rate than it was converting nitrite. The same concept applies to each upstream and downstream sample pair in your system.

These assays are recommended to be done on a weekly basis. On a daily basis, you should be measuring the water flowing into the fish tank. But if you don't see anything in your analysis, how do you know that the system is even working? This is where experimentation, as described in the <u>System in Balance</u> section, provides answers.

SYSTEM IN BALANCE

As you run a RAS, you'll continually ask yourself certain questions. Is the system in balance with respect to fish nutrition and water purification? How can you tell if there is a risk of failure, or when everything is operating smoothly? When things are not in balance, the risk is a morbidity event.

There are two ways to determine your system status. The first is through regular water quality analysis and good recordkeeping. The second is an occasional foray into system testing or experimentation, which is fun and instructive. Intense experimentation should only be done after a complete harvest to avoid risking the health of the fish. Some experimentation can be done at any time with material removed from the system and tested in bottles.

First let's consider monitoring basic system function. As we will see, each component of almost any setup has a specific role and its own requirements, whether the setup is designed for purely fish aquaculture or a combination of aquaculture and hydroponics (i.e., aquaponics). From the fish perspective, the rule is: dirty water leaves the tank, clean water returns to it. In this case, dirty and clean have to do with the status of nitrogen content, with additional consideration of pH, oxygen, total carbon dioxide and particle waste.

For this example, let's say that 15 mg/L of TAN can become dangerous. So, pH of 6.8 to 8.5 may be tolerable, but lower (7-7.5) is best. Why? Because it uses that ammonia equilibrium to drive the toxic ammonia (alkaline pH) to benign ammonium (neutral pH) even though TAN remains constant. For nitrite-N, 2 mg/L is dangerous and must be removed. The goal is a consistent state where nitrite isn't seen and ammonia cycles through with bacteria feeding in barely detectable levels.



Fig. 22. A 26-hour cycle of nitrification, showing net nitrogen decrease after feeding cessation. In the TAN graph (top), ammonia was always lower after passing the solids removal component (Site 3), the first location of active nitrification. In the Nitrite graph (bottom), there is a spike in the first two hours immediately after backwash at Site 3. Compared to the other sites, Site 3 is always higher showing imbalance of nitrifying process in solids removal component.

A Day in the Life of a Mature FRAS

Figure 22 illustrates a day in the nitrogen life of our case study system, a mature FRAS with 15,000 near-market yellow perch. This system is on a 12:12 light-dark cycle, meaning that the day and night are each 12 hours. The feeding is stopped a couple of hours before sunset.

This system is in balance because the test results are ideal: in the top graph, the ammonia in the tank with the fish (site 1), in the solids removal bead filter (site 2 minus site 3) and the flow into the biofilter (site 3) increases with feeding during the day. Maximum TAN levels reach only 30 μ M (0.42 mg/L; upper left axis). More importantly, the water coming out of the biofilter itself (sites 4 and 5) contains virtually no ammonia. Likewise, the nitrite shown in bottom panel is always barely detectable everywhere (<2 μ M or 0.028 mg/L) except just downstream of the particle removal unit (site 3), where it spikes after a backwash. It is highly unlikely that consistent deviations from the observed trends occurred in the absence of feeding at night.

There are systematic differences in chemistry among sampling sites (refer to figure 15) and each differs for ammonia and nitrite. This is how you first assess component function. In the example illustrated in figure 22, the fact that ammonia is lower at site 3, after the particle removal unit, indicates that this is a site of nitrosofication (upper graph). But, the same site is the only one in which nitrite is found in any quantity (lower graph). On the other hand, neither compound is evident in samples downstream of the main biofilter. Everything is now in balance. Note that the bead-based solids filter (2a, between sites 2 and 3) is out of balance with net nitrite production, as emphasized in the spike of nitrite at the beginning of the bottom panel. That is the backwash water from morning cleaning of the particle trap when that component was taken off-line. We use this as a positive control for our analyses because we know we will get a signal that validates the chemistry assay.

However, there is a hint of the potential for problems of imbalance even in this example. It is first observed in the ammonia, top graph, where the amount at site 3 is distinctly lower than any location upstream of the biofilter itself. The difference indicates that the plastic balls in the bead filter are taking out ammonia, which should be good. But at the same time, analysis of nitrite (lower graph), shows that there is a stark elevation of nitrite in the same samples. This means that the beads are indeed nitrosofying ammonia to nitrite, but the nitrifiers that remove the nitrite are not keeping up completely. The levels are truly small and inconspicuous, but the protocol of monitoring each component on a regular basis is justified. It demonstrates that all sites in a system are not equal.

Testing and Experimentation to Learn More

Our case study data are from a fully mature FRAS, but what about the early developmental stages of a new system operation? This is where we find experimentation to be informative.

First, let's look at the function of the plastic beads. Figure 23 shows the results of removing some beads from the particle removal filter, putting them in two bottles of clean system water and adding ammonia to one bottle and nitrite to the other. The experiment exhibited three salient points.

First, with ammonia addition at 15 mgN/L (a large dose), the beads removed ammonia right away and almost eliminated ammonia by 48 hours (top graph).

Fig. 23. Effects of added ammonia and nitrite on water from mature FRAS. Top: Rate of CO_2 loss (-34.0/hr) is twice the rate of ammonia loss (16.9/hr). Nitrite grows because its removal is slower than production from ammonia.

Bottom: When nitrite was added to a separate water sample, the bead filter media removed it at a rate of 10.3/hr.

Starting at one thousand, ammonia declined with time at a constant rate of 16.9 per hour.

Second, note that total CO_2 decreased nearly in parallel, but twice as fast in molecules (34 units per hour, right axis label). The CO_2 loss is a result of acid production by bacteria during nitrosofication and shows that two acids are released for each ammonia transformed. This is consistent with the nitrogen transformation series already presented.

The nitrite story for these same beads is crucial to understanding the system in use. When we added about 2 mg/L of nitrite-N alone (bottom panel), nitrite began to disappear immediately, and at a constant rate (10 per hour). That is nice, but the same beads could release nitrite from ammonia faster (17 per hour). The difference means that nitrite could build up, as it did in this example. In fact, more than one-quarter of the added ammonia built up as nitrite with the beads, then slowly declined as the ammonia became very low.

The third salient point is that this demonstrates one recurring feature of the plastic solid phase materials: the ammonia oxidizers like them more than the nitrite oxidizers. In this example, as long as nitrite production by ammonia is less than 10 per hour, the chemistry will remain good for fish, but the potential for toxic buildup is very real.

You should test this in your system regularly by placing some of the material into a bottle of clean system water, adding some ammonia or some nitrite, and measuring the chemistry twice a day until it is gone. Add enough to get a good signal with your kit (10 mgN/L ammonia, 1-2 mgN/L nitrite should work) and analyze after 12 hours. Sample frequently if it is down a significant amount, and measure time as shown in this figure.





Hours After FRAS System Ammonia Spike

Fig. 24. Ammonia and nitrite removal over two-hour period. The TAN graph (left) shows that the addition of ammonia in the biofilter beads resulted in maximum concentration of 450uM of TAN downstream at Site 3. Maximum concentration downstream of the biofilter at Site 4 was 180uM (not shown on graph). TAN in the entire system, measured at Site 5, then rose to only 50uM and residual ammonia was rapidly removed. The Nitrite graph (right) illustrates that nitrite was consumed nearly as fast as it was produced. Excess nitrite production at Site 3 is seen in elevation over Site 5 after one hour, however the scale is 0.1% of the added ammonia.

Fortunately, the particle removal system with beads is upstream of the main fluidized sand biofilter, which can take care of both the remaining ammonia and nitrite. Under balanced "healthy" conditions, the biofilter should be able to withstand a substantial dose of nitrogen without risk to the fish.

An example is shown in <u>figure 24</u> for a whole system spike between loads of yellow perch. Above we saw that daily ammonia rose to about



Hours After FRAS System Ammonia Spike

25 of these units during a normal day of feeding. Here we added enough ammonia to make a thousand (40 times; 14 mgN/L) in the bead filter and we only saw half that downstream. By two cycles of water through the whole system (1 hour) the ammonia was down to daily levels (about 20 or 0.28 mgN/L). In addition, nitrite was never seen at more than trace levels at any time during the spike (scale of nitrite is 300x less than for ammonia). This is a resilient system with a very well-functioning biofilter.

It is worth considering that a FRAS is like a good sports team: the team plays together with a uniform goal, but each of the members has some individual skill in addition to the overlapping team skills. It is not unusual for different components to maximize part of the detoxification process. It is the cumulative function that determines the efficacy of the system. For this reason, it is necessary to both test the function of each component on occasion and to test the combined function frequently.

STARTING A NEW SYSTEM

There are several ways to successfully initiate a FRAS, and each system will have a different microbiological outcome. That is because the microbial strains will vary with the water source (i.e., well water, city groundwater, city lake or river water). For all FRAS startups, the safest method is to add water, feed the system with ammonium chloride and sodium nitrite, analyze the chemical response and repeat feeding until a stable, robust consortium of activities is seen. There are many other required nutrients, such as phosphate and iron, that are part of nitrification system activity. Most of these are abundant in the fish food and present in excess, which then feed the bacteria.

There are several steps involved in starting a new system; then patience and constant testing are required to get your system in balance.

First, it is necessary to remove construction byproducts such as PVC cement fumes and fiberglass resins, among others. This can be accomplished by effective ventilation and by filling and emptying the system with water. Every pipe and void space must be thoroughly flushed.

Second, feed the system for both the nitrosofication and nitrification processes. The ammonia- and the nitrite-oxidizing bacteria are different organisms. They work well together, but we have shown earlier that they can also work well independently. Because they may thrive in different components of your system, it is necessary to initially feed separately for both.

To do this you must know the total volume of your system. To begin the third step, dissolve enough ammonium chloride (NH_4Cl) or sodium nitrite ($NaNO_2$) in water to make 15 mg/L or 1.5 mg/L of nitrogen (200

or 20 grams per 1,000 gallons respectively). Pour them both into the pumping system and start testing. Take measurements at the same location (the flow back into the fish pond is most relevant), and test the consumption of each addition.

Step three takes patience, time and testing. When all the ammonia and nitrite are gone, feed again. Test to see how long it takes for them to disappear completely. Feed again. When both are decreasing immediately after the addition, the system is nearly ready to add fish.

Commercial companies make solutions that promise successful development of nitrosofying biofilter populations. Often these contain dried strains of the bacteria, which may accelerate the initial phases of colonization. Others provide the initiating nutrients as described above. As a point of comparison, in a mature FRAS, there are kilograms of nitrosofying bacteria, while the packet you buy from a company might be half a kilogram. Your system will develop its own population perfectly tuned to the starting conditions you provide. It just takes time.

How long does the third step take? This is the most important question new growers need to answer, and it is different for every system. One must test and observe repeatedly, and once the system has become resilient, there will be a little sigh of relief. If initiation is difficult, there may be another limiting nutrient, or a bacterial poison, that would need to be identified and resolved.

In our model system, a semi-commercial-scale FRAS, it took about forty days to become ready for the first load of fingerling yellow perch. Baby fish are more sensitive than adults to suboptimal conditions of



Elapsed Time (hrs)

water chemistry. In the construction there was a lot of fiberglass resin and PVC cement, along with five tons of virgin quartz sand and its dust. The system was filled with dechlorinated tap water, which in Milwaukee is direct from Lake Michigan. After two days of wetting everything, we began the initiation process.

Remembering that chlorination of drinking water involves chloramine formation, we were confident that the bacteria we needed were already in the water and would thrive. Chloramine breaks down spontaneously to chloride ion (CI-) and ammonia, which stimulates nitrosofiers. You can test this by sampling your tap water after a day or two unopened, then sample again after flowing the water for two minutes. For us, the first sample was loaded with nitrite and had little ammonia, while the second had ammonia but very little nitrite. Nitrification was occurring in the faucet nozzle. So, we started with addition of both ammonia (1000 units) and nitrite (100 units) together.

At the early stage shown in figure 25 (0-300 hrs), two important outcomes are apparent. First, total ammonia began to decrease right away, but the rate was very slow. It wasn't until almost ten days that ammonia removal began to approach practical rates. Second, nitrite elimination did not begin right away, and nitrite started to accumulate further as rapid ammonia use was initiated. By 15 days the entire nitrogen addition was converted. That meant that about 1100 units (15 mgN/L) of nitrite, the deadliest poison, had been detoxified in total.

To test that observation, we added a very large dose of nitrite (center action at 425 hrs) and saw that removal began right away, with linear removal right to the zero point in only four days. By this time quite a bit of turbidity was apparent from the washing of sand, and most of the resins had dissolved into the water, so a few days were spent flushing and filling to exchange out the organics and nitrate residue that had built up. Because the bacteria live on biofilter surfaces, we could do this without losing much of the population.

At this point we expected that all the nitrosofying bacteria were in a growth mode, and now was the time to begin feeding the bacteria to develop a robust population. Addition of 750 units of ammonia alone (10 mgN/L) resulted in immediate and fairly rapid removal at 10 units per hour (0.15 mgN/L/hr), which is about one-third of the sustained maximum observed later in the mature system. Nitrite began to accumulate immediately and did not start to disappear until the ammonia

Fig. 25. Initiation of nitrification activity in Case Study FRAS

start-up. Nitrification activity is initiated by sequential additions of ammonia and nitrite over a 40 day period, using virgin sand in the biofilter. The nitrite spike at hour 430 is caused by a backwash in the solids filter. Gray area at hour 925 indicates the chemistry has stabilized enough to add fish. was exhausted, but then decreased at about the same rate as ammonia did before it. This was about half the sustainable rate of the mature system. Knowing that these very lethal levels could be readily handled, the first batch of fish was added after return to zero toxin conditions at 40 days.

CRISES AND HOW TO RESPOND

Throughout the operation of a FRAS, there will be different crises that need antidotes. Some are visually obvious, and some require analysis to recognize.

Mineral Precipitates on Pipes and Walls

Most water contains calcium ions (Ca++, hardness), and some, such as well water, may contain a high concentration of calcium. Calcium combines spontaneously with sulfate, another common component of water, to make insoluble calcium sulfate, otherwise known as gypsum (drywall), plaster of Paris and other household materials. It also combines with carbonate system components to form calcium carbonate, the material of oyster, clam and snail shells; marble; etc. In addition, calcium is crucial for the formation of bones in fish. But when you see white crusts on the mouth of outflow pipes or waterlines, the chemicals are too concentrated. Often this arises from evaporation, which concentrates all dissolved solids, followed by making the volume up with the same water as originally used. Over time the fluid becomes very concentrated.

To alleviate this, make-up water should be as close to distilled water as possible. Because this is usually a small amount, a small mixed-bed deionizing filter can work, such as a commercial scale Brita filter. Water softeners are less ideal because they replace the calcium with another ion.

Lethargy from Low Oxygen Concentrations

Both fish and biofilter bacteria become lethargic from oxygen depletion. For fish, concentrations below 5 mg O_2 per liter begin to approach dangerous levels. A rarely discussed outcome of low oxygen is toxic products of bacterial anaerobic (no oxygen) processes such as sulfate reduction, which produces dangerous hydrogen sulfide (recognized as rotten egg smell). Oxygen solubility is less in warmer water, but warm-water fish are also better adapted to withstand occasional low oxygen. The nitrifying community bacteria are less sensitive, but still require dissolved oxygen.

An obvious sign of low-oxygen problems is fish breaking the water surface and gulping air. First, check the pumping system and be sure that water is flowing throughout. In many cases water flow and movement through a simple aerating tank will provide adequate dissolved oxygen. However, stagnant areas can cause locally depleted oxygen levels. Remove accumulations of uneaten food from the bottom of the fish tank and backwash the particle removal chamber frequently. Smell them. If they are putrid, you need greater water flow. Also, be sure that the room housing your unit is not so tightly insulated that oxygen depletion in the atmosphere is possible.

Lethargy from High Ammonia Concentrations

When the system becomes unbalanced and the fish waste and food decomposition exceed the rate of ammonia oxidation, ammonia can build up to dangerous levels. This requires analytical capability, most often a commercial kit for ammonia analysis using a color comparator and vials or ampoules for chemical testing.

Ammonia toxicity can be controlled by pH, such that a spike of ammonia can be temporarily neutralized by decreasing the pH to about seven, which changes the form to safe, ionized ammonium but does not remove it.

It is important to keep the system in balance; increasing ammonia concentration can cause stress in fish. Ammonia poisoning can increment with time and increase suddenly in the system. Fish start gasping for air at the surface of the water, their gills look red, they avoid eating, become increasingly lethargic and soon die.

To correct the conditions, lower pH to a value that produces a safe ammonia concentration and/or change 50% of the water. Remember that changing the form of ammonia to ammonium does not remove it from the system, and a sudden increase in pH can still be deadly. Ammonia must be kept at a low concentration consistently.

Lethargy and Death from Nitrite Toxicity

Nitrite toxicity causes methemoglobinemia, known as brown blood disease, and occurs when there is an imbalance between ammonia oxidation and nitrite oxidation. Nitrite chemically and irreversibly oxidizes hemoglobin, the oxygen-carrying molecule in red blood cells, preventing oxygen from being moved through the fish tissues. In essence, because the blood doesn't carry enough oxygen to maintain the fish's metabolic processes, the fish suffocates even if there is abundant dissolved oxygen.

We have shown that some surfaces favor ammonia-oxidizing bacteria, but the large biofilter usually has enough capability to maintain balance. When the imbalance is pronounced, nitrite can build up to dangerous levels, about 100 μ M (1.4 mgN/L). It is valuable to use a test kit regularly to follow nitrite and detect increases before they become dangerous.

There are two primary methods for responding to nitrite toxicity. On an immediate level, addition of chloride ion, (Cl⁻), will compete with nitrite ion (NO₂⁻) for uptake at the gills and instantly prevent further competition for binding sites, but reversibly. Clean salt, sodium chloride, is an adequate source of chloride, and may be used at about five times the concentration of the toxic nitrite (500 µM or 30 mg NaCl per liter in this example). This is an emergency response that needs to be followed up with one or both of the more involved treatments: restoring nitrite oxidation capacity and exchanging the water in the system. Chloride is not toxic at this low concentration but should not be allowed to build up. Assessment of cause of nitrite accumulation is crucial if it is to be prevented in the future.

CONCLUSION

Keeping your FRAS operating at optimal level with all chemistry parameters in order is critical to a successful operation. You are in control of the environment where your fish live, and healthy water chemistry is the primary factor in preventing stress on any of the biological components, especially bacteria. Although bacteria are invisible, they are essential to your success because they transform naturally occurring toxins into non-toxic energy for their bacteria communities. Water chemistry is the guide to understanding the health of your fish and the bacterial communities on which they depend.