AN AQUACULTURE
TECHNICAL BRIEF

Microorganisms in Intensive Aquaponics

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CONTENTS

INTRODUCTION

For most people, a list of the primary components of an indoor intensive aquaponic system includes tanks, pipes, pumps, filters, fish, plants and water. A more complete list is still unlikely to include the most abundant and ubiquitous form of life in these systems: bacteria. It is often unrecognized that operating an aquaponic system means managing an entire ecosystem of microorganisms, complete with multiple habitats, each with its own unique set of microorganisms. These microorganisms are vitally important to the health and success of these systems. Although our understanding of the microbial ecosystem within aquaponic systems is expanding rapidly, there is much that is simply unknown. Fortunately, most of the principles and techniques that are used to study microorganisms in other systems, such as those associated with the human body or living in the ocean or soils, can be applied to aquaponics.

A basic knowledge of microorganisms can go a long way toward an improved understanding of how an aquaponic system operates. For instance, in a recirculating aquaculture system (RAS), microorganisms are required to remove ammonia at system start-up before fish can safely grow in the production tanks. Microorganisms also alter the pH, carbon dioxide and oxygen concentration in water, and play a large role in degrading solid waste. Although these small organisms play an outsized role in these systems, the limited knowledge of their activities means one cannot find instructions for how to maintain the microbial communities in a system. Yet if the operator controls the water chemistry and keeps it within parameters that maintain fish and plant health, it is likely the system also will maintain productive microbial communities. The goal then becomes identifying ways to alter the microbial communities to improve system performance. This approach has been very successful for municipal wastewater treatment systems. New research in the coming years will certainly advance the collective understanding of microorganisms in aquaponics and how to manipulate this unseen community to benefit production.

The goal of this two-part technical bulletin is to help you think about your aquaponic system in a different way, so that you gain an understanding of how operational choices interact with the activities of microorganisms and ultimately influence the success of your system. The first section will cover what microorganisms are and where they live in these systems, including a more in-depth look at the nitrogen cycle and biofiltration. The second section focuses on biosecurity, pathogens and new developments in pre- and pro-biotics, and how they contribute to aquaponic system health.

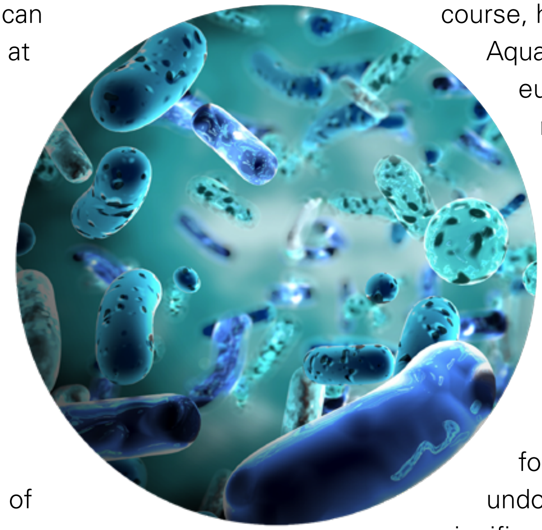
OPERATING AN AQUAPONIC SYSTEM MEANS MANAGING AN ENTIRE ECOSYSTEM OF MICROORGANISMS, COMPLETE WITH MULTIPLE HABITATS, EACH WITH ITS OWN UNIQUE SET OF MICROORGANISMS.

WHAT IS A MICROORGANISM?

Who they are, where they live and why they matter

A microorganism (or "microbe," for short) is, as the name implies, a very small organism. Typically, this means any life that is too small to be visible to the naked eye, which roughly translates to organisms less than 100 microns (*µm*) in size. This definition is based solely on the fact that an organism is small; it does not relate to a particular group of organisms.

Most places on Earth are covered in microbes, including on the surface and inside most other organisms. For instance, microbes can survive in the stratosphere and live at temperatures near boiling or deep below the Earth's surface where the pressure is immense. Also, if there is energy to be had, microbes have figured out a way to access it. Microbes can use light, consume food in a way similar to our own human cells, and even use rocks to "breathe" by shuttling electrons. We are only just beginning to understand the microbial world and the capabilities of the organisms thriving sight unseen.



Bacteria are the most prominent domain of microbial life. Bacteria are a diverse group of unicellular organisms that lack a nucleus. Similarly, *Archaea* are unicellular organisms that lack a nucleus. These two groups of microbes are visually similar in their cell size and appearance but have a vastly different genetic makeup and cellular characteristics. Microbes, especially *Bacteria* and *Archaea*, dominate the diversity of life on this planet. There are well over 100 bacterial and archaeal phyla. To compare, all vertebrate animals are part of a single *phylum* known as *Chordata*. By some estimates, there are roughly a trillion microbial species on Earth. Besides dominating the organismal diversity on this planet, microbes also have a vast repertoire of genes and capabilities for living.

Eukaryotes are organisms whose cells include a nucleus. All plants, animals, insects and, of course, humans belong to this category. Aquaponic systems contain many eukaryotes beyond those being raised for production. Small algae, micro-fungi and protozoa are all eukaryotic microbes found in these systems. As a collection, these small eukaryotes are termed *Protists*. There is not a lot of information available on the common micro-eukaryotes in aquaponic systems, especially for intensive indoor systems, but undoubtedly these organisms play a significant role in system health and the breakdown of solids.

Viruses are another inhabitor of the microbial world. Viruses are not considered to be organisms because they do not have a cellular structure, cannot reproduce on their own, and do not have their own metabolism. Although viruses by most definitions are not alive, they have a profound impact on all other organisms on Earth. Viruses are the most abundant entity on this

Most places on Earth are covered in microbes, including on the surface and inside most other organisms.

planet, being roughly 10 times more abundant than bacteria. They also have the capability to infect all other cellular organisms, including a handful of viruses that infect other viruses. This capacity to infect and ultimately destroy cells means that viruses play a formidable role in many aspects of an aquaponics facility, from causing plant and animal diseases to altering the functional capacity of a biofilter or bioreactor.

Microbes are a critical component of life on Earth for many reasons. They can cause disease in humans, animals, plants and insects. They are the basis of many of our foods such as yogurt, wine, beer, cheese, coffee and chocolate. They have the ability to degrade and detoxify organic matter and pollutants, as well as mediate biogeochemical processes that impact climate, including producing about one-half of the oxygen that we breathe. But how are they specifically important to aquaponics?

IMPORTANCE OF MICROBES IN AQUAPONICS

The sheer fact that microbes are everywhere means nearly all operational areas in an intensive aquaponic system are influenced by microbial activities. For perspective, it is estimated that every hour, each person sheds about 30 million microbes into the air and that each person contains 10 to 100 trillion microbes — about five pounds' worth. Besides being ubiquitous, in aquaponics, microbes also carry out many of the chemical transformations that affect water quality. These systems benefit from microbes in two critical ways: first, microbes serve as a *biofilter* removing *ammonia* and *nitrite*, as shown in figure 2; and second, beneficial microbes prevent pathogen growth. Microbes also are commonly used in the removal of solid waste from these systems.

A microbial community is the collection of all microbes living and interacting in a particular habitat. An aquaponic facility harbors many distinct microbial communities. For instance, there are microbial communities that that do the following:

- are associated with the other organisms (termed *hosts*) in the system, such as the fish or plants, where the microbes living on the outside surfaces of the hosts are different from those that live in their guts or that are associated with plant roots,
- thrive in the water and generally transform major nutrients in the water such as carbon, nitrogen, and phosphorus,
- inhabit *anoxic* (low oxygen) environments such as in waste clarifiers, which are used to remove complex solid waste products,
- contain specific microbial partnerships that live in biofilters and act to remove ammonia and nitrite from the water and
- live outside the production system, such as on building surfaces or the people operating the system.

Microbes are divided into groups by a few basic lifestyle characteristics that can provide clues as to where those microbes will be found. One characteristic in aquatic systems, such as an aquaponic facility, is whether microbes prefer a *free-floating* (non-surface associated) lifestyle or one where they are attached to particles and/or surfaces. The free-floating microorganisms will be found in the production water and will travel continuously with that water flow, thereby connecting all system components. Those that prefer an attached lifestyle live in biofilms (collections of many microbes encased in extracellular polymers, akin to a microbial slime) and can be found coating the side of tanks, covering uneaten feed

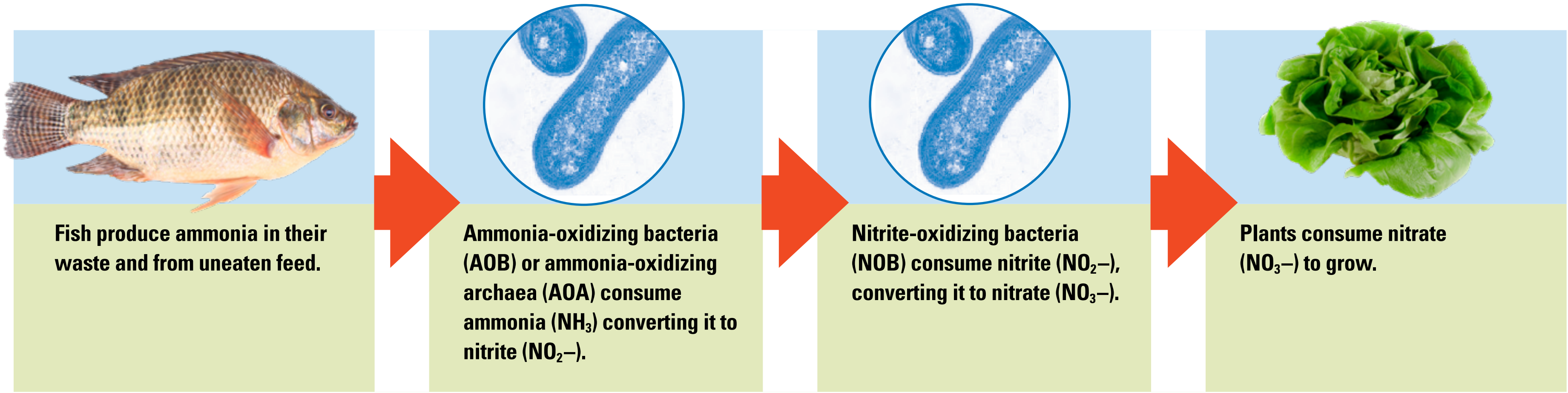


Fig. 2. Nitrification process. (R. Newton, 2020.)

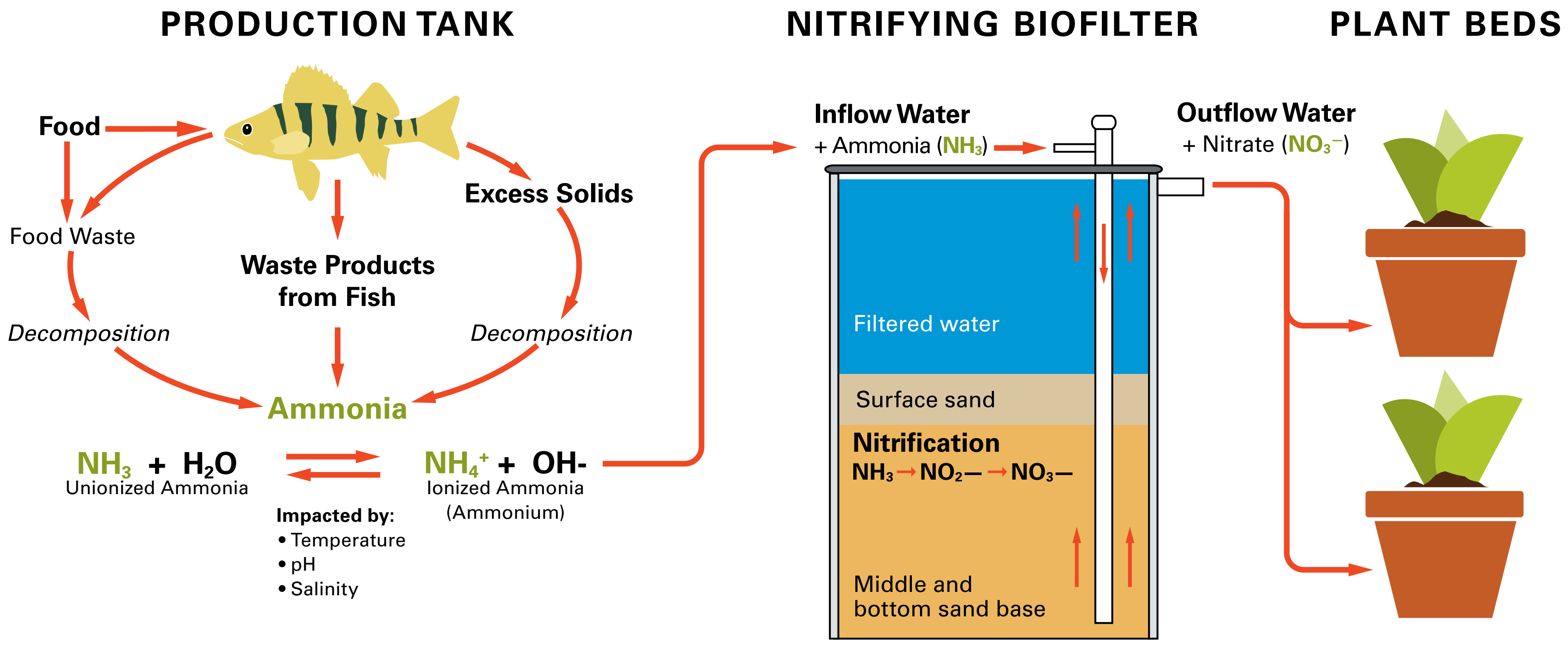


Fig. 3. Biofilter concept. (R. Newton, 2020.)

or living on any solid surfaces in the system. These microbes tend to be more habitat-specific and thus the communities vary more across locations in the facility.

Microbes are also distinguished by the ways they obtain carbon (i.e., how they eat) and how they generate energy to maintain life and thrive in their environment. For carbon acquisition there are two basic types of microorganisms: *autotrophs* and *heterotrophs*. Autotrophs obtain carbon for cell production by converting carbon dioxide into more complex organic carbon molecules, such as sugars. Plants are also autotrophs. They use sunlight to drive this process. In intensive aquaponic systems, microbial autotrophs are common, but they do not use sunlight. Instead, these microbes use the oxidation of ammonia or nitrite to get energy for carbon dioxide uptake and transformation. These microorganisms are found primarily in the biofilter (nitrifying biofilter). In contrast to autotrophs are heterotrophs, which are microbes that take up complex carbon molecules (e.g., sugars) to build biomass (i.e., basically eat parts of other organisms). Humans are heterotrophs. Heterotrophic microbes are widespread throughout aquaponic facilities and live in all components. In many ways, because the removal of ammonia and nitrite is so important, managing microbes in an aquaponic facility is about balancing conditions that allow for both autotroph and heterotroph activity.

For energy generation, microbes are divided into two primary categories, *chemotrophs* and *phototrophs.* Chemotrophs obtain energy by oxidizing molecules from their environment. Humans are chemotrophs. We eat food and oxidize components in that food to generate energy. In indoor aquaponic facilities, nearly all organisms are chemotrophs, so they are found in all facility components. Phototrophs use sunlight to generate energy. Plants are phototrophs. Some microbes, for example algae and cyanobacteria, are phototrophs. These organisms are not common in indoor aquaculture facilities but are common in aquaponic facilities because light is used to produce plants. Phototrophic microbes will be found wherever sunlight or artificial light that mimics sunlight is present.

Although there is information about the lifestyle characteristics of microbes in aquaponic systems, researchers are still learning about the actual microbes that live in these systems, i.e., who they are and exactly what they are doing that impacts production. The following few sections outline some of what is known.

Nitrifying Biofilters

A biofilter is a container designed to grow microbes. A *nitrifying biofilter* is designed specifically to grow two types of microbes defined by the way they generate energy: 1) *ammonia oxidizers* and 2) *nitrite oxidizers*. Nitrifying biofilters are designed to remove ammonia from the system production waters because even at fairly low levels, ammonia is toxic to fish. Nitrite is a byproduct of ammonia removal through oxidation. Ultimately this two-step reaction produces *nitrate*, which is also harmful to fish, but only at much higher concentrations. In aquaponics this nitrate-rich water can be circulated to plants. Nitrate is a primary nutrient, or fertilizer, that fuels plant growth. The nitrifying process is illustrated in figure 3. Figure 4 shows an operating nitrifying biofilter.

A next logical question about biofilter operation is: What is needed to grow ammonia and nitrite oxidizers? Collectively, ammonia and nitrite oxidizers are known as *nitrifiers*. For all microbes, growth is a function of available carbon and energy. The microbes need carbon to build more biomass and need energy to maintain cellular integrity and carry out the function of dividing to reproduce (i.e., create more cells). Nitrifiers are defined by this combination of carbon acquisition and energy generation, and are part of the group known as

Fig. 4. Biofilter in operation. (P. Wilborn, 2020.) **PortFish Ltd., a Wisconsin-based non-profit organization, operates each of its aquaponic systems using a biofilter of two 55-gallon barrels. The biofilter incorporates layers of media, from bottom to top: large aggregate, medium aggregate, small aggregate, pea gravel and sand. More information is available at portfish.org.**

chemolithoautotrophs. This means they use preformed molecules (chemo) that are of an inorganic nature (litho) and carbon dioxide (auto) to live and grow. Nitrifiers need either ammonia or nitrite, which are inorganic molecules, and oxygen to generate energy. They use the oxygen to oxidize the ammonia or nitrite.

Nitrifiers are also *autotrophs*. That is, they use carbon dioxide to obtain carbon for biomass. At a very basic level these microbes need to be supplied with a steady supply of ammonia or nitrite, oxygen and carbon dioxide. Ammonia is produced by fish in their waste and in uneaten fish feed, so it is in ready supply – and it is also the primary reason a nitrifying biofilter is needed. Nitrite is produced by the ammonia-oxidizing microbes and is taken up rapidly by a second group of microbes that oxidize the nitrite. All cells also need a variety of other cell components like phosphorus and trace metals (e.g., iron and manganese), but these components are present in ample supply in fish waste/uneaten feed, etc., in the system. Finally, it benefits all cells to have their waste products removed from their surroundings; therefore, a steady water flow is beneficial for nitrifier growth. Because nitrifiers require ammonia or nitrite to thrive, they ultimately remove them from the production waters, thereby returning concentrations to safe levels to maintain fish health.

Nitrifying microbes like to grow in close and dense specialized microbial communities known as *biofilms*; therefore, any nitrifying biofilter needs to promote biofilm production. Biofilms are produced in stepwise fashion, as shown in figure 5. Some microbes are adept at sticking to surfaces, and they initially attach to a surface and colonize (or grow on) it. They serve as the seed for biofilm development. After this initial attachment other microbes begin attaching to the colonizers and a stack of microbes begins to build up. As this stack is building, some microbes secrete extracellular *polysaccharides*, a slime-like substance, that adheres all the microbes together into a matrix. This matrix of slime gives the biofilm a three-dimensional shape and helps create unique internal habitats that different microbes can exploit for growth. The microbes in a biofilm interact and cross-feed, creating an enormously complex system with high microbial diversity. Nitrifiers are slow-growing microbes. They take advantage of the protection of the biofilm, which keeps them from washing out of the biofilter, and the available nutrients in the biofilm to grow to fairly high concentrations.

To promote and maximize biofilm growth, biofilter design is focused on maximizing the solid surface area that contacts the production water. This water brings in the needed ammonia, oxygen and carbon dioxide for nitrifier growth, and solid surfaces provide an opportunity for a biofilm to develop. There are many types of solid surface configurations, known as biofilter media, including sand, gravel and plastic-based media, such as *biowheels* or *biospheres*. See figure 6 for examples of biofilter media. All media are used in a variety of biofilter designs, but ultimately the goal of all nitrifying biofilters is to grow as many nitrifiers as possible (maximum biofilm formation), in as small of a space as possible, and to do this in a consistent manner that produces stable ammonia and nitrite removal.

When operating a nitrifying biofilter, close attention must be paid to the flow of solid waste from the upstream production tanks. A high flow of solid waste can be detrimental in two ways. First, larger suspended solid waste can clog the biofilter, reducing flow and potentially preventing biofilm growth on the biofilter media, which quickly decreases the

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removal of ammonia by nitrifiers. Second, even if there is suspended solid waste removal, a high load of dissolved solids can fuel the rapid growth of heterotrophic microbes in the biofilter biofilm. Recall heterotrophs consume the remnants of other organisms, such as that found in solid wastes. Using the oxygen in the water to oxidize this waste generates more energy than that generated from ammonia or nitrite oxidization (the food for nitrifiers). This energy differential allows these microbes to grow more rapidly than the nitrifiers, and thus outcompete them for space and additional resources needed for growth, ultimately reducing the concentration of nitrifiers, or altogether preventing their growth.

The Nitrifying Microorganisms

Nitrification is the microbial process of converting ammonia to nitrate. This process is carried out in a two-step reaction by two different groups of microbes: *ammonia oxidizers* and *nitrite oxidizers*. The first step in this reaction series is the conversion of ammonia to nitrite. Both bacteria (*ammonia-oxidizing bacteria*) and archaea (*ammonia-oxidizing archaea*) are capable of carrying out ammonia-oxidation, but only bacteria are known to carry out nitrite-oxidation (*nitrite-oxidizing bacteria, NOB*). The microbes carrying out these processes are common in nature, occurring in environments from oceans and lakes to many different soil environments.

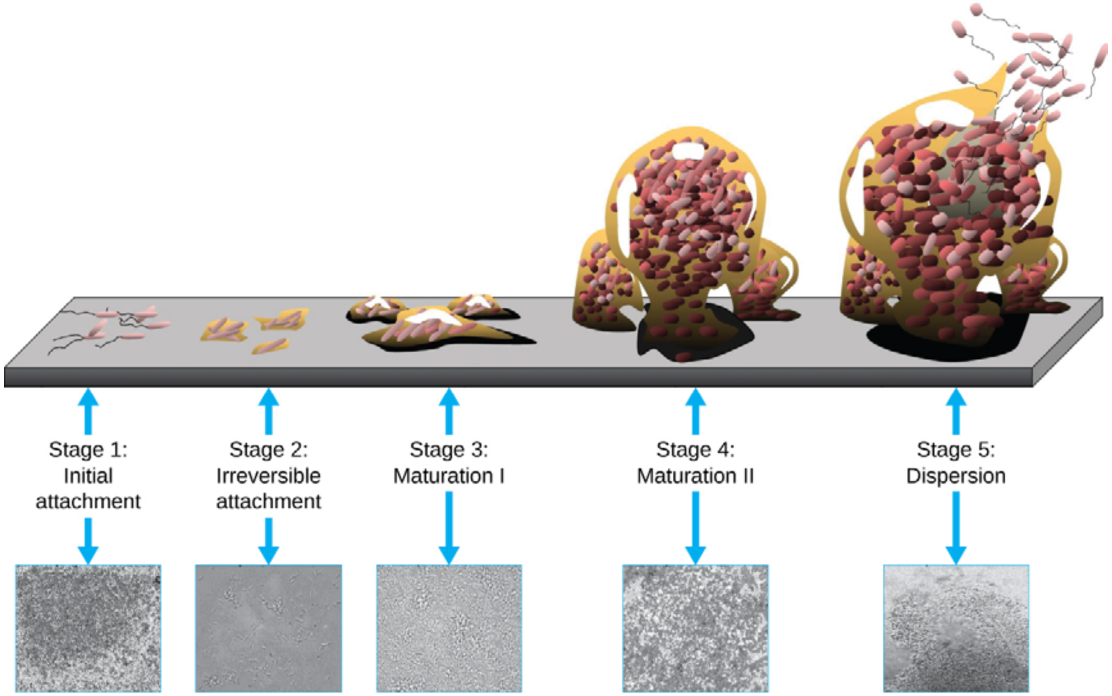

Fig. 5. Creating a biofilm. (Wikimedia Commons, 2016.)

Ammonia and nitrite oxidation are fairly unusual ways to generate energy — there are relatively few microbes that have this capability. *Nitrosomonas* is a bacterial genus containing several species, all of which are capable of ammonia oxidation. It is the best-known genus of ammonia oxidizers and traditionally has been cited as carrying out ammonia oxidation in nitrifying biofilters. Recent studies have shown that *Nitrosomonas*, although regularly found in aquaponic nitrifying biofilters, may not be the dominant organism performing ammonia oxidation. Instead, it has been shown that ammonia-oxidizing archaea, potentially of the genus *Nitrososphaera*, are more common and abundant in these biofilters. All known ammonia oxidizers are given the prefix "*nitroso*" in their genus name. Additional ammonia-oxidizing genera include *Nitrosospira, Nitrososcoccus, Nitrosolobus* and *Nitrosopumilis*.

The second step of nitrification is the oxidation of nitrite to nitrate. Traditionally, *Nitrobacter* was thought to be the dominant bacterial genus carrying out nitrite oxidation in nitrifying biofilters, including those used in

Fig. 6. Examples of biofilter media. (P. Wilborn, 2020.) **Aggregates, pea gravel and sand (left); polyethylene Hydroballs and clay Hydroton (right).**

aquaponics. In fact, most biofilter engineering designs are based on a combination of ammonia-oxidation rates, growth rates and environmental controls for *Nitrosomonas*, paired with the equivalent measures for *Nitrobacter* nitrite-oxidation capabilities. Recently studies of aquaponic nitrifying biofilters have shown that *Nitrobacter* is not common to these systems. Instead, the bacterial genus *Nitrospira* appears to be the dominant nitrite-oxidizing group, especially in freshwater systems.

This understanding of nitrification became more complicated in 2016, when it was discovered that some *Nitrospira* are capable of complete ammonia oxidation (known as *comammox*); that is, the conversion of ammonia to nitrate within a single organism. This broke the longstanding paradigm — lasting more than a century — that biological nitrification required a partnership between two microorganisms. Since this discovery, it has been shown that the *comammox*-capable *Nitrospira* are present and sometimes abundant in aquaponic nitrifying biofilters. Additionally, the genus *Nitrotoga* is abundant in some aquaponic nitrifying biofilters. *Nitrotoga* appear to dominate in systems run at a slightly acidic pH. Other nitrite-oxidizing genera include *Nitrospina, Nitrococcus* and *Nitrolancetus*. All nitrite-oxidizing genera have the *"nitro"* prefix in their genus names.

There is no one-size-fits-all approach to the nitrifiers in aquaponic nitrifying biofilters. Much work remains to identify the optimal mixes of nitrifiers under different operational scenarios, but as more of the players in these cycles are revealed, there is great potential for breakthroughs in biofilter performance optimization or alternate modes of system design.

Starting a New Nitrifying Biofilter

There are many ways to start a new nitrifying biofilter. Once the physical biofilter and full system are operational, the establishment of the nitrifying function of the biofilter can begin. Often nitrifying biofilters are initiated by flowing water through a new system, and then dosing the water with ammonium chloride (or equivalent ammonium salt) or by adding fish to generate ammonia waste. After adding ammonia, the operator then waits for the microbes to arrive and remove the ammonia. This process typically takes several weeks. As the ammonia is removed, nitrite accumulates and then is removed subsequently. When ammonia and nitrite levels are maintained at a stable and safe concentration, production may begin. Ultimately this process works on the premise of the adage, if you build it, they will come. Nitrifiers are common in many habitats, and some of them will randomly end up in any new system where they will initiate nitrification if given the right conditions for growth (oxygen, carbon dioxide, ammonia, growth medium). Although this process produces desired results, it requires a long start-up phase, typically a month or more, and relies on chance as a key component to success.

Alternatively, there are options to enhance the success rate and decrease the start-up phase of the biofilter. One option is to purchase a commercial nitrifying microbe seed/starter system. There are many commercial options available. All contain a concentrated stock of live nitrifying microbes. These starter systems may produce results, but remember there are many nitrifying microbes, each having a particular environmental condition (temperature, pH, ammonia/nitrite load, substrate for growth) where it will thrive, and many conditions where it will not. Identifying the conditions in one's system in relation to the needed conditions for growth of the starter culture is not easy and may be impossible. Ultimately, adding a nitrifying seed system will not harm biofilter initiation, but it also may not help.

Another nitrifying biofilter start-up option, and the one with the most proven success, is to seed a new biofilter with substrate (sand, bio-plastic media, etc.) from a nitrifying biofilter that is in operation with water chemistry conditions similar to those anticipated for the new system. This process provides the new biofilter with a dose of the nitrifying microbes that are likely suited for it, so there is less reliance on chance matches between microbe and a suitable environment. If enough material is available to seed the system with a 1% by volume seed material, some reports indicate full nitrifying biofilter function within a few days. Adding less seed material will add time to the start-up as the nitrifiers need to time to grow, but any addition should aid start-up. With any seeding procedure, those nitrifiers need food to grow, therefore a steady dose of ammonia and possibly phosphorus (as phosphate) is also needed, whether via chemical or natural (via fish) addition.

Microbial Processes Affecting Water Quality

The nitrifying biofilter is usually the component most carefully considered when relating microorganisms to water quality in aquaponics. This makes sense given the toxicity of ammonia and nitrite, and the need for the fairly large physical presence of the nitrifying biofilter in the facility design. There are, however, a number of other ways in which microbes can affect water quality in these systems. Microbes are intimately connected to their environment; therefore, changes in pH, water temperature, the concentrations of organic carbon, carbon dioxide, oxygen, ammonia, nitrite, nitrate, phosphorus, etc., all can impact microbial activities.

In turn, the activities of microbes can affect pH, alkalinity, percent saturation of oxygen or carbon dioxide, nitrogen speciation and concentration, etc. Aquaponic systems are ecosystems in the same way that lakes, oceans or grasslands are, albeit with a few control points. Because of this, altering one parameter can have rippling effects on several other parameters in the system. Following are a few examples of common connections between microbes and water quality in aquaponic systems. It is important to note that there are many more connections than highlighted here, and many interactions that are not yet fully understood.

(Example 1) Nitrification is just one component of the larger biological nitrogen cycle, as illustrated in figure 7. The microbes that carry out nitrification use carbon dioxide as a carbon source when they reproduce. In doing so, they remove it from the water, which over time reduces the alkalinity of that water. If the alkalinity falls too far, then the water has little buffering capacity and is subject to rapid pH changes, which can be harmful to animals, plants and microbes alike.

(Example 2) Under low oxygen conditions, which typically result from high levels of organic matter (i.e., solids), two alternate microbial nitrogen transformations may occur. Both ultimately remove nitrogen from the water through production of nitrogen gas. In an aquaponics setup, this is generally undesirable, because that nitrogen could be used to feed the plants.

The first reaction, anaerobic ammonia oxidation (*anammox*) converts ammonia to nitrogen gas (figure 7), thereby removing ammonia from the production waters. This process is frequently found in aquaponic systems in solids clarifiers or sump systems. The second reaction, called *denitrification* is a multistep process to convert nitrate to nitrogen gas (figure 7). Again, this process is common in solids clarifiers or sump systems. Anammox is a highly specialized reaction, so it is carried out by only a few microbes (members of the bacterial phylum *Planctomycetes*). Denitrification, on the other hand, can be carried out by many different bacteria and is much more common.

In either case, if these reactions are occurring in an aquaponic system, it is a good sign that oxygen has been depleted. Engineers and scientists have harnessed microbial processes to reduce solid wastes and convert solids into reusable energy sources such as methane gas. These systems, known as *anaerobic digesters*, are very common in land-based agriculture and wastewater treatment but have not been widely adapted into the aquaponics industry, except in the largest facilities. Certainly, widespread adoption of these waste reuse systems across the aquaponics industry would facilitate decreased waste and impact on natural environments.

(Example 3) Oxygen is a critical component of any aquaponic system, as animals need it to maintain life. Microbes can have a big impact on oxygen concentrations, particularly when organic matter (solids) is high. This abundant source of nutrients fuels rapid heterotrophic growth, which under the right circumstances can rapidly deplete the oxygen in the water. Temperature has an impact on this process, as warmer temperatures fuel faster metabolic activity and growth of microorganisms. Likewise, low oxygen leads to non-oxygenic metabolisms by microbes, such as denitrification, mentioned above, or sulfate reduction to hydrogen sulfide, which produces a rotten egg odor.

Overall, the connections between microorganisms, their environments and the impact of various available operational controls make it difficult to predict water quality outcomes. However, a basic understanding of these connections can lead to both quick responses when imbalances arise and better long-term planning for creating a stable system.

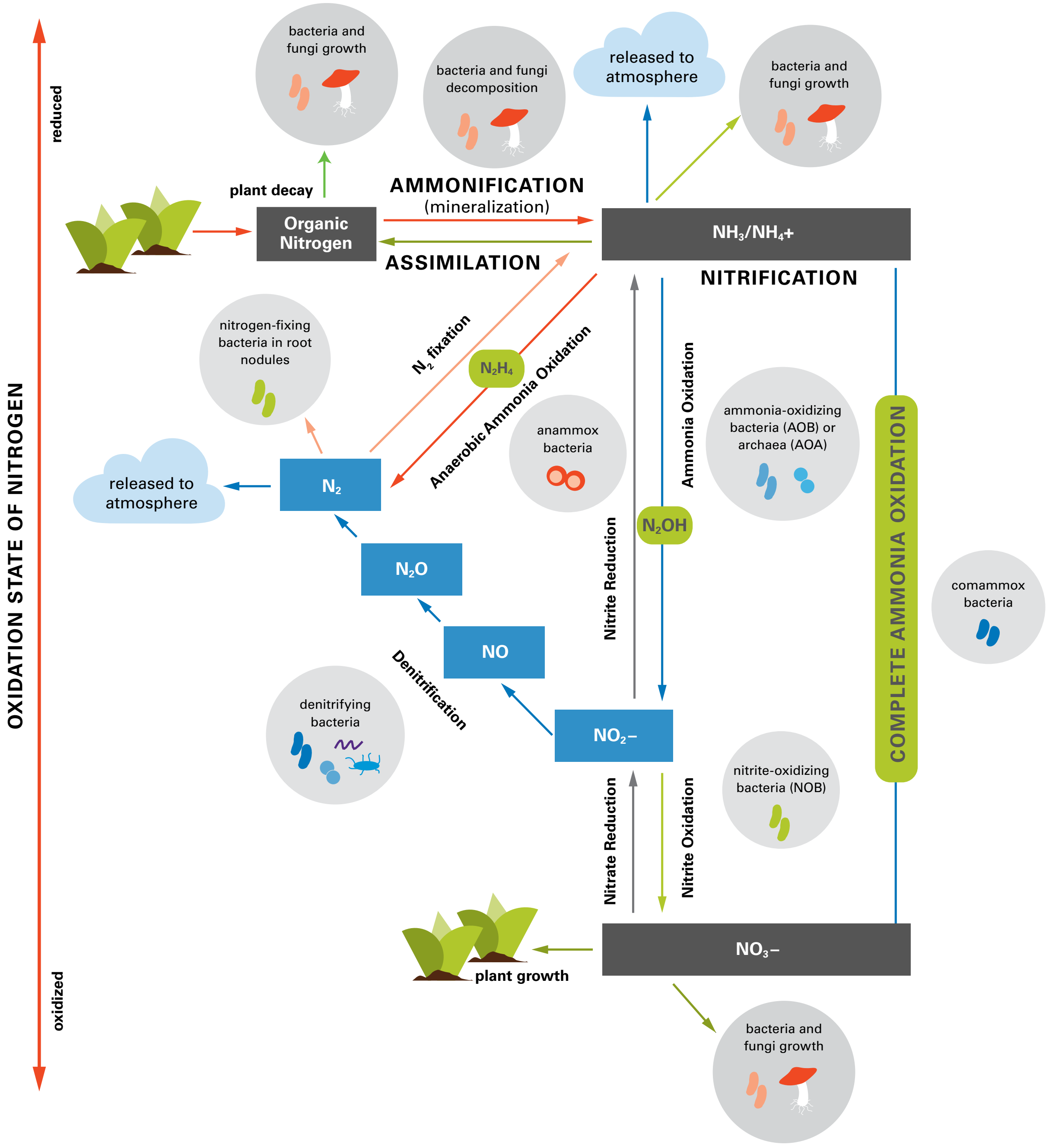

Fig. 7. Microbial processes impacting nitrogen transformation in aquaponics. (R. Newton, 2020.)

BIOSECURITY AND PROTECTING YOUR SYSTEM

Microorganisms are everywhere, and controlling their movement is not possible. Everyone and everything that moves around in a facility brings with them their microbes. While this cannot be prevented, there are precautions and techniques that can be employed to reduce the risk of disease in the facility. *Biosecurity* is the term used to describe good practices that prevent movement of pathogens into a facility or their proliferation. There are two main tactics that aid in the prevention of pathogen spread in a facility.

First is reducing the transfer of microorganisms into the facility from the outside environment by creating and executing standard operating procedures, including wearing protective clothing, washing hands, wearing gloves, disinfecting shoes and sanitizing equipment and production systems before use. Second, stress must be kept as low as possible in the production animals and plants. As with most animals and plants, a low-stress individual has a healthy immune system, which can protect that animal or plant from most pathogens. Increased stress dramatically increases the chances of an animal or plant succumbing to illness or disease.

Keeping accurate records of the procedures in the facility and the water chemistry in the system is one of the best ways to understand what may have happened should a pathogen outbreak occur — and to protect the system from future disease outbreaks. Changes in system water properties (pH, solids content, etc.) can trigger disease outbreaks, often through stress inducement. Specific changes in system operations around the time of the outbreak may indicate the potential pathogen involved. For example, some bacteria in the genus of *Flavobacterium* are of serious concern in aquaponic systems because they are opportunistic pathogens (i.e., pathogens that do not need a host animal or plant to survive). These bacteria are excellent feeders on complex solids, which under the right circumstances become the fish in production. A recent change in solids management may explain the outbreak of a *Flavobacterium* species.

Pathogens

Microbes carry out a large number of beneficial activities in aquaponics systems, but some microbes can cause disease in plants or animals. Microbial eukaryotes like some fungi, bacteria and viruses can cause disease. Notably, Archaea are not known to infect and cause disease in other organisms.

There are two types of pathogens, *obligate* and *opportunistic*. An obligate pathogen requires a host to grow and therefore is transmitted directly between hosts (i.e., fish to fish). Viruses are considered obligate pathogens because they are not alive and need a host to replicate. However, since viruses are not cellular entities, they can persist in the absence of a host for some time. In contrast, opportunistic pathogens have life cycles outside of a host. Generally, these pathogens are able to infect and cause disease in a host under the right circumstances. Most opportunistic pathogen infections occur during periods of host stress that reduce immune activities or with the loss of a physical barrier, such as an open wound through the skin of a fish or epidermis of a plant.

Many opportunistic pathogens can be found in biofilms; therefore, reducing biofilm growth on solid surfaces can help to minimize outbreaks. On the other

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hand, fish and plants are covered in microbes, most of which are not capable of causing disease. This external microbial barrier plays a critical role in preventing infections, including as competitors with pathogens for space and nutrients. Disruption to this animal- or plant-associated microbial community, which often is the result of host stress, can lead to pathogen growth. There is still much to be discovered about the microbes associated with fish or hydroponically grown plants and how these microbes play a role in host health and growth, as well as disease suppression.

There is virtually no way to eliminate all pathogens from a facility, especially opportunistic pathogens. These microbes always will be present as they have adapted to life outside of hosts. For example, members of the bacterial genus *Flavobacterium* are one of the most common fish pathogens in freshwater aquaponics. Two species, *Flavobacterium* columnare and *Flavobacterium psychrophilum* are especially problematic because they are found commonly in production system biofilms but cause disease when given the opportunity. The key to disease prevention is to reduce fish and plant stress, which reduces opportunistic pathogen outbreaks, and to use biosecurity protocols to minimize introductions of microbes from outside the facility to prevent obligate pathogen outbreaks.

The intensification of agriculture and aquaculture has been associated with dramatic increases in severe disease outbreaks. There are hundreds of microbes that can be pathogens of fish or plants. In Table 1, we will cover in more detail a few common freshwater fish pathogens. You can learn more about fish pathogens using reputable online resources such as your local department of natural resources or via fishpathogens.net. (In Wisconsin, see dnr.wisconsin.gov/topic/Fishing/fishhealth) If you encounter symptoms of pathogen infection that are difficult to identify, consult people who are reputable experts at raising fish or plants, such as the American Association of Fish Veterinarians.

TABLE 1. COMMON FRESHWATER FISH PATHOGENS

Aeromonas salmonicida is a non-motile psychrophilic (cold-loving) bacterium that causes the disease furunculosis. The acute form of the disease commonly affects younger salmonids and can lead to rapid septicemia and death with a few days. The disease is named for the characteristic boils (furuncles) that develop on the skin or musculature of diseased fish. *A. salmonicida* is distributed worldwide.

Flavobacterium columnare is the causative agent of columnaris disease. *F. columnare* is a gram-negative aerobic bacterium that typically appears in the form of long slender motile rods. *F. columnare* is distributed worldwide and infects many freshwater fish species, including carp, channel catfish, goldfish, eel, perch, salmonids and tilapia. *F. columnare* causes both acute and chronic infections that affect the gills, skin and fins of host fish. Skin lesions and fin rot often are present.

Flavobacterium psychrophilum is a gram-negative, motile, aerobic psychrophilic bacterium that is the causative agent of rainbow trout fry syndrome (RTFS) and bacterial cold water disease (BCWD) in salmonids. *F. psychrophilum* can be found on both external and internal fish surfaces including the skin, gills, brain, kidney and spleen. Affected fish usually display a whitish discoloration along the adipose fin.

Lactococcus garvieae is a gram-positive bacterium that causes disease in both freshwater and marine fish. Freshwater trout, salmonids, eels, catfish and tilapia are all susceptible. Most disease occurs in warmer water (18-25°C). The primary symptom is bulging eyes. Skin darkening or petechiae of the eyes or gills may also be observed.

Streptococcus iniae is a gram-positive bacterium, and the etiological agent of septicemia and meningitis (streptococcosis) in many different finfish, including trout, tilapia, bass and various marine species. *S. iniae* is found in warm water, particularly in North America, Asia and the Middle East. *S. iniae* can cause invasive disease in humans who handle infected fish.

Yersinia ruckeri is the etiological agent of enteric redmouth disease (ERM). *Y. ruckeri* is a gram-negative rod-shaped bacterium that infects salmonids worldwide. ERM can impact fish of all age classes, but it is typically acute in young fish and chronic in older/larger fish. Changes in behavior are often the first sign of disease and include lethargic movements, loss of appetite, darkening of the skin or subcutaneous hemorrhages in and around the mouth and throat.

TABLE 1. COMMON FRESHWATER FISH PATHOGENS *(continued)*

Cyprinid herpesvirus 3 (CyHV-3) is a virus and the causative agent of a highly contagious and lethal disease in carp and koi. It was previously known as koi herpesvirus. The CyHV-3 virus is a member of Herpesvirales group, which are linear double-standed DNA viruses surrounded by a capsid. The CyHV-3 virus has been identified in most parts of the world. To date it has not been found in South America, Australia and northern Africa. Younger fish appear to be more susceptible to infection, and the infection spreads rapidly throughout the fish's body in as little as 24 hours. Horizontal transmission (between fish) is known to occur. Disease progression occurs only at temperatures >16ºC.
Viral Hemorrhagic Septicemia (VHS) is a highly infectious virus for both freshwater and marine fish. VHS causes disease in cool-water fish, typically those reared at temperatures <15ºC. VHS has caused significant die-offs in the Laurentian Great Lakes. Over 50 fish are known to be susceptible, with significant mortality for muskies, walleye, lake whitefish, freshwater drum, yellow perch, shad and round gobies. VHS is not known to be harmful to humans. VHS typically causes hemorrhaging, bulging eyes and bloated abdomens, and it leads to rapid death. Horizontal transmission (between fish) occurs readily as the virus can survive in water outside a host for up to 14 days.
Infectious pancreatic necrosis virus (IPNV) is a highly infectious member of the *Aquabirnavirus* genus of viruses. It mainly affects young salmonids (<6 months old) and is more infectious in cold water. IPNV disease is characterized by a swollen abdomen or eyes, darkening of the skin and spiral swimming. Transmission can occur in either fresh water or salt water.
Ichthyophthirius multifiliis is a parasitic ciliate often called ICH. It is an endoparasite (lives inside its host) that can infect most freshwater fish. *I. multifiliis* causes a disease commonly called white spot disease because of the visible trophonts (the motile stage of ciliates) and aggregation of host cells that produce an elevation of the skin, which appears as white spots. *I. multifiliis* damages the skin and gills, eventually leading to severe ulceration, respiratory problems and death. Besides the characteristic white spots, fish with the disease often exhibit anorexia, increased breathing rates and bottom resting behavior.

Pre- and Probiotics

Fish and plants need their microbial communities. There is a large amount of current microbiology research on host-associated microbes. This research field has been spurred on by a large endeavor to understand the human microbiome or the ways microbes influence human health. Much of the knowledge and general principles determined by the human microbiome project may be applicable directly to understanding how microbes influence fish health. This research area is relatively new, and we are only beginning to learn about the complexity of these host-microbe systems. In the future, one can anticipate that very specific methods will be developed to promote the growth of microbes that improve fish or hydroponic plant production. These techniques are already widely applied in soil-based crop production.

Another interesting aspect of the human microbiome project is that there is now wide availability of human *probiotics* and *prebiotics* that claim to improve human health. Similarly, there has been a big increase in these products for aquaponic production. To clarify, a probiotic is a live microbe that when given in an adequate amount benefits host animal or plant health. Yogurt is a probiotic because it contains live *Lactobacillus* species or other common gut bacteria that have positive impacts on human gut health. A prebiotic does not contain live organisms. It is a non-digestible ingredient that stimulates the metabolism of desirable microbes.

Remember that as a human being, you are full of microbes. You contain an established community, and each person's microbial community is unique. The same is true for fish and plants whether or not they are raised in an aquaponic system. Although there are many pro- and prebiotics available for humans, it is not yet clear

how effective they are, and it is likely the effectiveness varies greatly between individuals. This is the same for fish, and there has been much less research in this area. To understand why developing a probiotic is difficult, consider the following. In order to be effective, probiotics need to be able to

- survive in a package,
- survive delivery to its intended site (like the gut),
- out-compete some existing microbes in order to grow and establish in its intended location,
- grow to densities that confer some benefit to the host and
- perform the intended benefit once reaching its targeted destination.

This is a daunting list for success. In fact, many probiotics that are known to have health benefits in some people do so for only a short period of time (24 hours) so repeated dosing is needed. The concept of using probiotics to improve fish or plant health is sound; however, more research is necessary to make the practice effective and reliable. It is possible that any probiotic product available currently will improve the health of fish or plants in an aquaponic system, but there is no good way to predict its effectiveness. Using the trial and error method is the only way to find out whether a product will work.

Therefore, before introducing pre- and probiotics — or any new element — into a system, read available research and consult experts. Think critically about what products you might purchase. If you decide to use a new product, note that it is doubly important to keep accurate records of the procedures in the facility, the water chemistry in the system and the growth/health of the fish and plants. (See the "Biosecurity and Protecting your System" section on page 14.)

CONCLUSION

An aquaponic system contains an ecosystem of microorganisms, so to run one successfully you must also manage your microbial ecosystem. This technical brief aims to illustrate how an aquaponic system is a complex, interdependent ecosystem. When you manage an aquaponic system, you are not only managing fish and plants, you are also managing microbes.

Water chemistry is the ultimate indicator that the microbes are being managed properly, and the best pathogen management is prevention. Your goal is to promote beneficial microbial growth and prevent pathogen outbreaks through careful water chemistry management and implementing biosecurity protocols.

Diligent practices and documentation are critical because they will provide the best clues on how to correct issues that occur in the system

In many ways, aquaponic systems are replicas of natural ecosystems, but with a few engineered constraints. Studying these systems is somewhat equivalent to studying what happens in nature, and because there is more control (e.g., choosing system size and materials, regulating indoor temperatures, controlling light regimes), it is possible to tease apart microbial interactions and activities that are not easily identified in nature and then both improve system design and further our understanding of the microbial world.

WHEN YOU MANAGE AN AQUAPONIC SYSTEM, YOU ARE MANAGING MICROBES.

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