

## **Autotrophic vs. Heterotrophic Bacteria**

There's a lot of confusion among aqua culturists about nitrifying bacteria. This is due in large part to the recent emergence of a wide variety of bacterial products claiming to be nitrifiers or nitrifying aids. The confusion results from the plethora of misinformation presented in advertisements and the aqua culturists general lack of knowledge about bacteria. Most of these products actually contain species of heterotrophic bacteria from the genera *Bacillus* and others.

True nitrifying bacteria are considered to be those belonging to the family NITROBACTERACEAE. These bacteria are strictly aerobic, gram-negative, chemolithic autotrophs. They require oxygen, utilize mostly inorganic (without carbon) compounds as their energy source, and require carbon dioxide (CO<sub>2</sub>) for their source of carbon. In the case of the Nitrobacteraceae these energy sources are derived from the chemical conversion of ammonia to nitrite, or, nitrite to nitrate.

Five genera are generally accepted as ammonia-oxidizers and four genera as nitrite-oxidizers. Of these, *Nitrosomonas* (ammonia-oxidizers) and *Nitrobacter* (nitrite-oxidizers) are the most important. Species of marine nitrifiers are different from those that prefer fresh water, and yet, are very closely related. Each species has a limited optimum range for survival. They are very slow growing because of the manner in which they must obtain energy. Under optimal growth conditions, they will double in population every 15-24 hours. Heterotrophic bacteria, on the other hand, can reproduce in as little as 15 minutes to 1 hour.

Where does ammonia in the pond come from? Some of it is released directly by the shrimp/prawn/fish; by diffusion from the blood across the gill membranes. Excreted urea or uric acid is also converted to ammonia through a process called mineralization. Solid organic, nitrogenous, waste material (a.k.a.- sludge) is also converted to ammonia through mineralization. Sources of this waste material are from fecal material, the decay of plant and animal tissues, and from the decay of excess food. Mineralization is accomplished by any of a number of species of heterotrophic bacteria. Species from the genus *Bacillus* are the most common.

Ammonia is the primary compound produced by this process. Some species of heterotrophic bacteria can oxidize or reduce nitrogenous compounds directly to nitrites (NO<sub>2</sub>), nitrate (NO<sub>3</sub>), or other forms of nitrogen (as NO or N<sub>2</sub>). In the absence of an organic nitrogen source, many heterotrophs can utilize ammonia instead. This is much more likely to happen in the laboratory, under ideal conditions, than in actual practice. In the pond, aquarium, as in nature, an organic, nitrogen rich, food source is constantly being produced and is readily available for these bacteria to utilize. Heterotrophic bacteria have little or no need to resort to utilizing ammonia as their source of nitrogen.

This ability of heterotrophic bacteria to utilize ammonia has led to the erroneous belief that they are as effective as true nitrifying bacteria in establishing the nitrogen cycle. These bacteria, however, generally cannot utilize nitrites.

Experimental data has shown that up to one million times more ( $10^3$  -  $10^6$ ) of these heterotrophic 'nitrifiers' are required to perform a comparable level of ammonia conversion that is attained by true autotrophic nitrifiers. When using heterotrophic 'nitrifiers', the nitrogen cycle in the aquarium basically follows the same course as when no bacteria are added and the system cycles naturally.

Another negative aspect to heterotrophs is that under certain environmental conditions they can operate in the reverse direction. In other words, they can convert nitrate back to nitrites and ammonia through a process called dissimilation. This is generally an anaerobic process, but, can occur during periods when dissolved oxygen levels are very low (DO  $\leq$  2.0 ppm). Dissimilation is a part of the denitrification process. Denitrification is the conversion (reduction) of nitrites and nitrate to gaseous nitrogen ( $N_2$ , NO,  $N_2O$ ).

Heterotrophic bacteria can be either gram-positive (ex: Bacillus) or gram-negative (ex: Pseudomonas). Some are strictly aerobic, but many are facultative anaerobes (they can survive in either the presence or absence of oxygen). Many species tolerate a wide range of environmental conditions: temperature, pH, salinity, etc.

They can also survive adverse conditions by forming spores, increasing the shelf life of the products. Because of this ability, they can be dried, packaged, and sold as viable cultures. Nitrifying bacteria do not form spores and cannot survive drying. This process kills the bacteria leaving no viable bacteria which might inoculate another culture. It has been debated whether or not nitrifying bacteria could survive freeze drying, but, even this is doubtful.

Dry product formulations are by far the most common. These products consist of sludge removing and other nonpathogenic, heterotrophic species of bacteria and their culture media. Formulations may vary to some degree, but, they all perform the same functions. They all contain bacteria species that digest proteins, fats, oils, cellulose, and starch.

These are similar to liquid sludge removers with broader applications. They often contain species of bacteria that function well in fresh, brackish, or salt water. Most liquid type bacterial products available in the industry contain little or no true nitrifying bacteria. Instead, they rely on their ability to maintain tolerable levels of ammonia until naturally occurring Nitrosomonas and Nitrobacter bacteria can become established. Both of these liquid and dry products should be considered as biological aids to nitrification.

Some manufacturers recommend the addition of gravel or water from an established aquarium. This is their only source of nitrifying bacteria. An element of risk is involved with this method because the potential exists for introducing disease causing pathogens into the new tank.

**BioRemid-Aqua** contains pure cultures of lyophilized Nitrosomonas and Nitrobacter bacteria along with heterotrophs. **BioRemid-Aqua** gives nitrification a tremendous boost by introducing over two billion nitrifying bacteria per gram of product to rapidly accelerate the nitrification process. Ammonia and nitrite levels are quickly and significantly reduced to safe levels.

## Key Points

- True nitrifying bacteria are strictly aerobic autotrophs. They can only use nitrogen from inorganic sources such as ammonia and nitrite. Nitrosomonas (ammonia-oxidizers) and Nitrobacter (nitrite-oxidizers) are the most common.
- Heterotrophic bacteria are generally considered to be organic sludge degraders. They are mostly from the genera Bacillus and Pseudomonas. Most of these are facultative anaerobes; meaning they can function with or without oxygen. They will do completely different functions depending on the level of dissolved oxygen present.
- Heterotrophic "nitrifiers" prefer to obtain their nitrogen from organic sources such as decomposing organic debris. Those that can convert ammonia do so only when an organic nitrogen source is not available. This is unlikely to happen in an aquarium or pond where fish are present. The explosion of "nitrifying" bacteria products in the industry is due to research that some heterotrophs can use ammonia-nitrogen. However, this is under ideal laboratory conditions.
- Heterotrophic "nitrifiers" generally cannot utilize nitrites. Only a few species are capable of reducing nitrite to free nitrogen, but, under strictly anaerobic conditions.
- Scientific studies indicate that, depending on species, between one thousand to one million heterotrophic bacteria cells are required to perform the same ammonia conversion rate as one Nitrosomonas bacteria cell.
- **BioRemid-Aqua** has a cell count of 2 billion bacteria per gram, majority of which is Nitrosomonas and Nitrobacter. To obtain the same ammonia conversion rate, a competitive product composed of heterotrophic "nitrifiers" would require the addition of 15 trillion bacteria. This would probably require several kilograms of another product. No quantity of heterotrophic "nitrifiers" would reduce the generated nitrites.
- Heterotrophic "nitrifiers" can also operate in the reverse direction; that is they can convert nitrate to nitrite or ammonia, especially during times of low dissolved oxygen levels. In a pond, this could potentially happen during the hours before sunrise when DO levels are at their lowest.