Traditionally, *Nitrosomonas* and *Nitrobacter* bacteria are used for oxidation of ammonia and nitrite. These chemoautotrophs are slow growing and sensitive to fluctuations in environmental conditions.

Description of Bacteria for Aquaculture

MetaMateria regularly uses bacteria with our BIO-Lair media that contains four types of *Bacillus* bacteria, which provides a wide range of benefits for aquaculture, if used properly. The bacterial blend is provided by a third party and it will naturally digest decaying organic matter, thereby reducing organics in a tank or pond. This blend produces lipase, protease, amylase, cellulase, and urease, which are extracellular enzymes essential to the degradation of various organic compounds including proteins, starches, carbohydrates, cellulose, urine, fats, oils, greases and other organic substances. So fish waste, decaying algae, uneaten food and other organic matter are digested which naturally reduces the nutrient load. *Bacillus bacteria are* ubiquitous in sediment but are not normally found up in water in the concentrations needed for water remediation [1].

MetaMateria's highly porous Bio-Lair products are used synergistically to maintain high microbial population in the water. Bio-Lair provides an exceptionally large amount of usable surface area that is well over1,000,000 m^2/m^3. For the growth of bacteria. The hierarchical pore structure helps maintain good water flow into the product to supply nutrients and transfer bacteria into the surrounding water. This provides for the formation of biofilms on the surfaces and the production of beneficial bacteria needed for bioremediation. Because the surface area is so large, biofilms and bacterial buildup remains relatively thin, thereby maintaining excellent permeability for water to flow into the material and pore plugging minimized. A single layer of microbes covering this area would provide about 400 – 1000 kg of microbes per m^3 of system volume. Even a very conservative assumption that 90% of the pores are unavailable; this area would still provide about 40 – 100 kg of microbes per m^3 of system volume, which is considerably larger than plastic or other types of media.

Several studies have demonstrated the benefits of *Bacillus*-based products in aquaculture. For example, Bacillus strain IP5832 spores fed to turbot larvae resulted in a decrease in *Vibrionaceae* population with significant improvement in weight gain and survival of the larvae [1]. These bacteria also improved food adsorption by enhancing protease levels, decreased the number of pathogenic bacteria in the system and improved turbot larval growth. The survival of channel catfish was also found to improve in a farm trial using a mixed culture of *Bacillus* [2]. An improvement in water quality and in fish yield with lower mortality was always observed at sites using this bacteria, with or without Bio-Lair; however, performance of the bacteria is substantially enhanced by use of Bio-Lair.

Ammonia is a primary metabolic waste of fish that is also produced by bacterial ammonification of uneaten food and feces and it can also be released by mineralization of sediment waste. Ammonia is oxidized to nitrite and finally to nitrates. Traditionally, *Nitrosomonas* and *Nitrobacter* bacteria are used for oxidation of ammonia and nitrite. These chemoautotrophs are slow growing and sensitive to fluctuations in environmental conditions. Removal of TAN, nitrate and nitrite is challenge in intensive aquaculture. System fluctuations also can lead to accumulation of ammonia, nitrite, and nitrate, which can lead to fish mortality. The impact of these residues is often minimized by addition of new water and discharge of some of the existing water to improve the water quality incrementally.

Hetrotrophic bacteria are also found to remove nitrogen from water [1-6]. Some members of this group, such as *B. subtilis* and *B. cereus*, are able to grow under facultative (aerobic/anaerobic bacteria) and conditions resulting in nitrogen metabolism, which facilitate both nitrification and denitrification [3]. The pattern of nitrite metabolism by *B. subtilis* I-41 was demonstrated to show switching of nitrite and nitrate metabolism depending on the conditions.

Heterotrophs such as *Bacillus* can also survive a wider variety of environmental conditions than autotrophs; however, they decrease in numbers very rapidly without food. During periods of stress – such as limited food sources and low dissolved oxygen - autotrophs often do not survive through inactivity while heterotrophs form durable long lasting spores. Both spores and inactive autotrophic cells are activated when the right environmental conditions are established. *Bacillus* bacteria form spores that are rigid and capable of surviving harsh conditions (higher resistance to external factors such as mechanical force, desiccation, solar radiation and high temperatures). As a consequence of this resistance to environmental stress, spores are attractive for commercial applications as they endure harsh processing steps found during production and are resilient to fluctuations that can occur in systems where they used. In nature both autotrophic and heterotrophic bacteria coexist in a complimentary relationship.

Bacillus bacteria blends used by MetaMateria also thrives on decaying organic matters and digests proteins and carbohydrates using enzymes. The number of bacteria that a system can sustain will depend upon nutrients available, oxygen level, and other environmental conditions, along with the surface area needed for them to colonize. The Bio-Lair product provides so much surface area that it can maintain high microbe populations; however, growth can still be limited due to lack of nutrients and other environmental conditions. Generally, when Bio-Lair is used in already established ponds or water bodies there is sufficient organic matter to sustain the heterotrophs population. The bacteria removes many wastes, such as: (1) residual food and fecal matter (2) metabolic by-products (ammonia, nitrite etc.), (3) residues of biocides and biostats, (4) fertilizer derived wastes, (5) waste produced during moulting, and (6) collapsing algal blooms. It will also break down fats, oils and similar compounds.

The following list shows expected benefits with the application of Bio-Lair used with Bacillus and Pseudomonas bacteria strains.

- Less accumulated organic matter
- Control of nitrogen compounds (ammonia, nitrite, nitrate)
- Environmentally safe formulation, nano-pathogenic bacteria
- Faster growth of marine creatures
- Higher survival rates of fish and shrimp
- Less need to use antibiotics
- Less water exchange required
- Healthier water in ponds and control of blue green algae

Below are a number of useful bacteria references.

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