Description of Bacteria Used with Bio-Lair

Bio-Lair products allow large colonies of multiple bacteria to co-exist and thrive on its high surface area. These can be naturally forming or specialized bacteria designed for treatment of certain types of contaminants. Bacteria are absolutely essential for natural recycling of nutrients throughout the environment and these biologically maintain healthy water by breaking down nitrogen, carbon, and other contaminants; they can even biologically incorporate phosphorus. This is nature's way of controlling contaminants, and for a normal environment, already existing bacteria can often do the job; however, when excess contaminants exist, such as proteins, oils or pathogens, or when oxygen is too low, water quality suffers and supplemental bacteria can provide the extra help needed. Often, special blends of bacteria can be used to handle a broader array of organic contaminants, which cleans water faster.

MetaMateria uses formulated brands of bacteria with Bio-Lair. These contain 4 types of enzymatic *Bacillus* and sometimes pseudomonas bacteria that provide a wide range of benefits for use in aquaculture and waste water treatment where control of organics and nitrogen compounds are important. These bacteria will also out-compete many types of algae for nutrients and blooms do not form. Pseudomonas bacteria compliments Bacillus and works at lower temperatures to provide effective treatment of sludge and other contaminants, even in low dissolved oxygen.

Bacillus bacteria are ubiquitous in sediment but not normally found in water at levels needed for remediation [1]. These bacterial blends naturally digest decaying organic matter, thereby reducing organics in water. Blends produce lipase, protease, amylase, cellulase, and urease enzymes essential to degradation of organic compounds including proteins, starches, carbohydrates, cellulose, urine, fats, oils, greases and other organic substances. These will digest fish waste, decaying algae, uneaten food and other organic matter to reduce nutrient load in a water body.

Bacteria reproduce on solid surfaces; therefore, the more surface area available for beneficial bacteria to multiply, the larger their colonies can be. 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 often above 2,000,000 m^2/m^3 for growth of bacteria. A hierarchical pore structure helps maintain good water flow into the product to supply nutrients and transfer bacteria into the surrounding water. Formation of biofilms on surfaces and production of beneficial bacteria needed for bioremediation is enhanced. Because surface area is so large, biofilms and bacterial buildup remain 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; about 40 – 100 kg of microbes per m^3 of system volume occurs, considerably larger than available with plastic or other types of media.

Bacillus-based bacteria have been shown to be effective in aquaculture and waste water systems. 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]. It 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 found to improve in a farm trial using a mixed culture of *Bacillus* [2]. An improvement in water quality and fish yield with lower mortality was always observed at sites using these bacteria with or without use of Bio-Lair; however, performance of the bacteria is enhanced by use of Bio-Lair and less bacteria are needed.

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 nitrate and nitrite is a challenge in intensive aquaculture. System fluctuations can lead to accumulation spikes 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 existing water to improve the water quality incrementally.

Heterotrophic 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 to 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 thrive on decaying organic matter and digest proteins and carbohydrates using enzymes. The number of bacteria will depend upon nutrients available, oxygen level, and other environmental conditions, along with the surface area needed for them to colonize. Bio-Lair provides so much surface area that it can maintain high microbe populations; however, growth can still be limited due to lack of nutrients or other environmental conditions. 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 are expected benefits using Bio-Lair with Bacillus and Pseudomonas bacteria strains.

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

Useful Bacteria References

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