AquaInTech Tools for Bioremediation

<u>AQUA</u>culture <u>IN</u>formation <u>TECH</u>nology

Biotechnology Benefiting Aquaculture ™

Probiotics are defined as <u>live</u> microbial preparations that when fed to an animal colonize the animal's intestinal tract and positively impact the animal's health. This term is widely misused in aquaculture and an analysis of the literature (gray and peer reviewed) suggests that the term may be largely inaccurate. There are very few accounts of bacterial attachment to the intestinal wall of fish or shrimp demonstrating the inhibition of pathogenic bacteria.

Some years ago an early researcher in this field coined the term probiotic for use in aquaculture in an effort to sell their product and make it appear distinctive from what it really was, a microbial tool for bioremediation.

These products manipulate the microbial ecology in the pond. Any action in the gut is irrelevant to the effect. Some lab trials suggest otherwise but the reality is that these lab trials mean little in the field.

Many of the commercially available products contain low levels of bacteria, bacterial species that are likely dead at the time-if present at all (such as Nitrosomonas and Nitrobacter) and/or bacterial species that cannot possibly do what the vendors claim that they do. In some instances they may include substances that appear to cause an effect (such as antibiotics) and are not on the label. While it is possible to sell bacterial preparations that do not consist of bacterial spores from gram positive bacteria, most of these products are costly, require refrigeration, have extremely short shelf lives or are acid stabilized liquids that involve selling products that are largely water.

- ⇒ Stable bacterial preparations that can be produced inexpensively make for the best products. Spore forming gram positive bacteria (Bacillus species) are the best stable candidates.
- ⇒ Ponds and animals contain very high levels of naturally occurring bacterial populations composed of hundreds, if not thousands of species. Typically in pond water and pond bottoms natural bacterial counts are in the millions per ml or gram much as in the intestinal tracts.
- ⇒ Trying to change these through the use of generic low count products is not likely to be successful.
- ⇒ Powdered products that are added to pond water all have a viable cell count issue. Adding high levels of bacteria is costly and has little proven efficacy. Mathematical modeling suggests that it is problematic to add what would likely be meaningful levels of bacteria.
- ⇒ Feeding live bacteria can best be done via the use of top dressing using bacterial species that are known to be shelf stable only when refrigerated. High titer Bacillus products can also be fed this way although it is likely that the observed benefits are a result of bioremediation.
- ⇒ Most products do not contain the types of bacterial species or the levels that are claimed. Many products contain bacteria that are in the product for the farmer and not for the fish or shrimp.
- ⇒ The first clue that things are not what they appear to be is when vendors make claims based on a standardized addition of product (i.e. add one kg per ha, 5 liters, etc.). Each pond is different and effective programs must take this into account. These are not chemicals and the use of recipe type approaches to the application of living bacteria to production systems will not work.
- ⇒ The proper use of products requires range finding tests to determine optimal dosages and timing of application. As the cycle progresses higher levels must be added and usually at higher frequencies.
- ⇒ Nitrosomonas and Nitrobacter cannot be sold in dried products. They are expensive to culture and are dead in dry products.
- ⇒ Lactobacillus do not form spores and die rapidly when held at high temperatures and humidity.

 Products that contain them are best held refrigerated prior to use. If not the contents are not viable.

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Our main products are below. We do custom formulate products and clients are free to private label as they see fit. We can add many different organisms, enzymes, prebiotics, immune stimulants, etc. to custom blends.

PRO4000X

A tableted mixture of bacillus species selected for their ability to degrade organic material. Each 16 gram tablet contains approximately 64 billion CFU of spores of special strains of *Bacillus subtilis and B. licheniformis*. These bacteria have been selected for their ability to degrade organic matter, degrade ammonia and compete against other bacterial species. Field trial data, letters of recommendation, etc. are available on our product web sites. Tablets can be added to ponds, lakes, golf course lakes, koi ponds, shrimp ponds, fish ponds, shrimp and fish hatchery tanks where they dissolve and the bacteria germinate and grow.

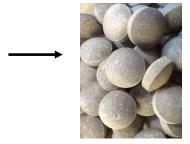
<u>AQUAPRO-EZ</u>

This powdered material contains at least 4 billion CFU per gram of *B. subtilis* and *B. licheniformis*. It contains a range of nutrients as well. The product is in biodegradable bags. These are thrown into the water where, similarly to the tablets, they sink to the pond bottom and dissolve. Nutrients are then immediately available for bacterial growth. Best for use in low density minimally aerated environments. The bag is a convenient delivery tool and many clients like the convenience of having a prepackaged amount of product.

<u>AQUAPRO-F</u>

This product is for incorporation into or onto feed (top dressing) at 1 to 5 kgs per MT. It is only made fresh. Although the process of milling in the feed does result in high heat and shear for a short period of time, spores are heat resistant and while some will be killed, there will still be very high spore counts in the final feed product, approximately 4 million CFU per gram of feed per kg of product per MT of feed. These germinate after the shrimp defecate and the bacteria begin to digest the feed in-situ. It contains 5 species of bacteria, added enzymes to aid in feed digestion and a potent prebiotic (MOS) to condition the digestive tract. It is very similar in appearance to Aquapro-B.

The tableted product was designed for ease of use and has some excellent data to support its use in farms and hatcheries. It is an economical and useful tool.





AQUAPRO-B

This is a high titer product that is used in the same manner as traditional products are. The powder is soaked in warm clean water prior to use and added to the ponds after 12 or so hours. Product is the same as EZ except it is sold in bulk, not in bags.



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AquaInTech tools for Bioremediation (3)

Our client base is growing. Currently we work with farmers in many different countries including Ecuador, Peru, Venezuela, Mexico, India, Indonesia, Singapore, Vietnam, Egypt and elsewhere.



- Less accumulated sludge
- ♦ Cleaner water
- ♦ Less water exchange = less cost
- Healthier pond bottoms
- Lower hydrogen sulfide levels
- ♦ Lower ammonia levels
- ♦ Less blue green algae (by competitive exclusion)
- Higher yields
- Higher survivals
- ♦ No antibiotic usage
- ♦ Lower vibrio loads (by competitive exclusion)

Every pond is different. Application rates will vary depending on pond type, size, stocking density, water exchange rates, pond location, amount of biomass in the ponds, amount of protein in the feed, the presence of stress related disease problems, etc. We work closely with distributors and individual clients to optimize usage rates. Please ask.

Our products work. They do what we say they do. Our many satisfied clients include those who understand that the use of science is important for optimal results and have the hard data to support the observations above.

It is important though to understand that these are tools. They are not solutions. Like any tool they have to be used in the right way.

It is always better to be **PROACTIVE** rather than **REACTIVE** when it comes to managing aquatic animal health. When farmers report that our products do not work, it is because they do not understand how to properly use them. Used correctly, they are valuable tools for improving profitability and ensuring sustainable productivity.

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