AQUA-IN-TECH Inc.

AquaInTech tools for bioremediation

Probiotics are classically defined as living microbial preparations that when administered to an animal colonize the animal's intestinal tract and impact the animal's health in a favorable manner. The use of this term is frequently misused in aquaculture and in fact, an analysis of the literature (gray and peer reviewed) suggests that the term may be largely meaningless. There are very few reproducible accounts of bacterial attachment in a consistent manner to the intestinal wall of fish or shrimp that can actually be shown to preclude the attachment of pathogenic bacteria to these same sites. Those scientists who have spent the most time in this field are of the opinion that many of the effects attributed to the application of the various bacterial preparations that have been looked at are in fact simply stimulation of the innate or non-specific immune system, an indirect effect. This would not require living cells.

Some years back an early researcher in this field coined the term probiotic for use in aquaculture in an effort to sell a product and make it appear distinctive from what it really was, <u>a microbial</u> tool for bio-remediation.

Many of the products that are commercially available contain low levels of bacteria, bacterial species that are likely dead at the time the product is being used, bacterial species that cannot possibly do what the vendors claim that they do, or finally include substances that appear to cause an effect (such as antibiotics). While it is possible to sell bacterial preparations that do not consist of bacterial spores from gram positive bacteria, most of these products would be costly, require refrigeration, have extremely short shelf lives or be acid stabilized liquids that involve selling products that are largely water.

Microbial products can be divided into two categories.

Bioremediation:Bacterial species are added to ponds to degrade organic matter.Immune Stimulation:Bacteria are fed to the fish or shrimp to enhance their ability tonon-specifically deal with a subsequent bacterial onslaught.

As a marine microbiologist with a Ph.D. in microbiology my knowledge of the current state of the art is naturally a bit greater than that of many of the salesmen that work for the various companies selling various products. Some of my conclusions, which are embodied in the approach to our products, are:

- 1. Stable bacterial preparations that can be produced inexpensively make for the best products.
- 2. Spore forming gram positive bacteria (Bacillus species) are the best candidates.

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- 3. Specific strains need to be selected that have the properties that one is interested in.
- 4. Ponds and animals already 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. Similarly in the gut.
- 5. Trying to change these through the use of generic low count products is not likely to be successful.
- 6. Powdered products that are added to pond water are all plagued by a number issue. Adding high levels of cultured bacteria is costly and has little proven efficacy. Simple mathematic analyses suggest that it is problematic to add what would likely be meaningful levels of bacteria.
- 7. Feeding living bacteria should only be done through the use of top dressing using bacterial species that are known to be shelf stable only when refrigerated, although high titer Bacillus products have some potential likely however as a result of bioremediation.
- 8. Most products do not contain the types of bacterial species at the loads that are claimed.
- 9. Many products contain bacteria that are in the product for the user and not for the fish or shrimp.
- 10. 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 need to take this into account.

The products below are our primary products. We can custom formulate products and you are free to private label products as you see fit.

<u>PRO4000X</u>



A tableted mixture of bacillus species selected for their ability to degrade organic material. Each tablet contains at least 52 billion CFU of spores. Field trail data, letters of recommendation, etc. are available. 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.

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AQUAPRO-EZ



This powdered material contains at least 4 billion CFU per gram of material. It contains a range of nutrients as well. The product is contained 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.

AQUAPRO-F

This product is for incorporation into feed at 1 to 5 kgs per MT. Although the process of milling 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 are still 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.

AQUAPRO-B



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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.

Benefits reported by clients include the following —	Less accu
The benefits your customers will see will depend on them.	Cleaner v
Every pond and every farm is different.	Less wate
We encourage addition of soluble carbon source (molasses).	Healthier hydrogen
	Lower am
	Less blue
	Higher yie
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