PRO4000X Tablet usage

Why there is no one best way to use PRO4000X in any given production system?
Science based aquaculture must contend with the inherent variability of aquaculture production environments. Rarely will one approach work for all.

The definition of the term probiotic, *as defined by the FAO*, is:

Living bacteria –not spores-and not dormant. ***Must be metabolically active.***

Applied orally. ***Must be ingested via the mouth to conform to definition.***

Changes the microbiome. ***Must impact this in a consistent fashion.***

Must have a positive impact on the animal health

Aerial view of shrimp farm in Guatemala.

Why Bacillus species are not probiotics?

More than 30 years ago, one of the first companies selling bacteria for bioremediation of farmed aquatic environments (ponds) used the term probiotic to apply to a microbial suspension added to the water to affect water quality. They used a product for use in lakes and other bodies of water.

They found that when they marketed the product using the term probiotic it had much wider market appeal. This started an avalanche of companies selling products containing many different bacteria in them. Many of these products contain dead bacteria or species that, if present, are at very low levels of what is purported to be present. Some contain bacterial species that cannot possibly be included at meaningful levels (because of costs or being very exacting in their growth requirements, etc.).

While *scientists are still debating* the concept of probiotics in aquaculture, the reality is that adding living bacteria to the animal via the water and seeing a direct benefit on animal health that is statistically meaningful is problematic and far from certain. In fact rare. Even if it were the case this would not be a probiotic.

At Aquaintech Inc., we understand that no two ponds are the same, even if they are right next to each other. Not only may ponds be different in surface area and depth, they also differ in the composition of soils that contact the water (or there may be no interaction in 100% plastic lined ponds), the water that is added to the ponds (ranging from fresh water to full strength seawater) with different compositions (rainwater versus river water versus well water versus seawater, etc.), and so forth.

Just as no two human beings will have identical microbial flora, neither will two ponds be the same. Although we are still in the very early stages of understanding what shapes the microbiome, we do know that it depends on nutrient loads and types, both macro and micro, what types of bacteria, bacterial viruses and predators are present, the ability of these indigenous strains to compete against each other, what metabolites are there to be degraded, etc. This has been borne out by countless studies.

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Why there is no one right way to use the tablets.

Facts that shape this are:

Living bacteria are not in any way akin to chemicals where the activity is consistent at a given level of application. Bacteria grow and as they do, so does their range of activity.

Water and sediments have large numbers of bacteria, viruses (including bacteriophages that infect and kill bacteria), algae, fungi, protozoa and metazoans in them. This can range well into the tens of millions per ml of water or sediment. The composition will vary.

Ponds are dynamic with nutrients and water being input at a variable rate. Outputs are also dynamic.

The microorganisms in PRO4000X are strains of **naturally occurring bacteria** that have been selected for the ability to digest organic matter. As with all bacteria, They have preferred sources of nutrients and environmental conditions that allow for optimal growth.

They **must be added repeatedly** as they do not colonize the pond ecosystem long term (no products sold today do). After the spores germinate (hatch) due to the presence of water in an environment conducive to optimum growth and where needed levels of nutrients are present, they experience a burst of activity digesting the nutrients and eventually return to a steady state level. At some time thereafter they must be re-added as they are intruders, and the microbiome pushes hard against them.

Day	0	7	14	28	42	56	70	84	94	105	112	121	128	135	142	Total
Week			2	4	6	8	10	12	13	15	16	17	18	19	20	
Amount	15	15	15	20	25	30	35	40	45	50	55	60	75	100	100	680

The **above table** is a starting point for a low to medium intensity density farms over the course of a production cycle. These are not fixed application rates. Only guidelines to start from.

There are many nutrients present in ponds and we want to make sure that the levels of nutrients in the ponds never become the immediate limiting factor affecting the surge of bacterial growth. We suggest that before this surge occurs (typically within 24 hours after the product is added to the ponds) a source of organic carbon should be added as this tends to be the limiting factor. Molasses used to be considered a good source of this, although due to the ability of vibrio strains to grow on simple sugars, it is now widely held that one should use more complex carbohydrates such as fermented rice, wheat bran, soybean meal, etc.

AquaInTech Inc.
16825 48th Ave W. Suite 322
Lynnwood, WA 98037 USA

Tel: 425-787-5218 C: 425-239-7682
Email: sgnewm@aqua-in-tech.com
URL: www.aqua-in-tech.com,
www.bioremediationaquaculture.com

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Following the outlined logic an ethical company can only recommend an approach to start from. Most clients find that they may need to adjust the suggested levels of usage and use less (or more) tablets based on the observed impact. Adjusting the dosage can be done either by changing the number of tablets one uses and/or the frequency of application. Some additional suggestions:

1. **In a pond where you are exchanging water periodically, do a flush from the bottom prior to the addition of the tablets if there are large amounts of anaerobic sludge present.**
2. **Use the tablets as soon as you add water to the ponds.**
3. **Add the tablets to those areas of the pond where you see problems, know that the organic matter accumulates or expect that there will be a problem.**
4. **The Bacillus move via the currents. They start at the bottom and move through the water column. The tablets are a convenient way to get them into the pond bottoms.**
5. **Supplemental aeration never hurts.**
6. **Add a carbon source (molasses or soybean meal are good sources of carbon and yeast that will help with the process) at 24 hours or so post stocking. The more complex carbohydrates seem to be better overall than the simpler sugars.**

Let the buyer beware regarding claims of an impact on animal health. "Caveat emptor"

Perhaps the most egregious aspect of the way many companies are marketing their products are the claims about impacting animal health. These are not legal in the US without a large investment to prove to the FDA that there is an impact. *The Bacillus alter the environment for a short period of time. This impacts the microbiome and the water quality. Any impact on health is indirect and* ***NOT*** *a result of the bacteria acting directly on the animal-a prerequisite for a probiotic.*

Without paying attention to the role of biosecurity in disease management and ensuring that appropriate strategies are consistently in place to keep pathogens out of production systems preventable disease will always be a challenge to shrimp farmers. Exclusion through PLs by ensuring that all adults are from nucleus breeding centers is an essential first step. Reducing the stressors acting on a crop is essential as well and PRO 4000X is a field proven tool for reducing the impact of many potential stressors on the population. Exclusion, stress reduction and optimizing production parameters are the keys to lessening the impact of disease.

I would not hold my breath for this to change. The diseases of tomorrow are already present in todays stocks.

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