**Why activation is not needed using PRO4000X tablets.**

It is the spore forming ability of Bacillus species that allows for a long lasting shelf stable product. These spores form in response to nutrient limitation. Growing the bacteria up before adding them to aquatic ecosystems is neither simple or straight forward and for the average farmer there is the added risk of the addition of contaminants to their ponds that can cause problems. It is important to realize that the "nutrients" supplied with the product are heavily contaminated with lots of things besides just the spores present in the product. These can be other species of bacillus and fungi. They are not produced used sterile ingredients.

This poses a risk to the farmer in terms of possibly adding things to their systems that they do not want to. Some of the simple sugars in these nutrient packs will stimulate the growth of vibrios and are available for them to use immediately when they are added. Even if this is not a consistent problem, adding nutrient packs that contain who knows what is in and of itself a good reason not to buy a product that contains nutrients. While I can be trusted to tell you the truth, I am the exception rather than the rule. So many people think that big name companies are honest and therefore they can be trusted. This is largely very far from the reality. Many big name companies are inherently prone to taking short cuts and they only care about money and the value of the stock (in public traded companies). Furthermore, it is common practice that products that contain nutrients are usually in a form where they can be adulterated. For instance, Bayer bought our AquaproB product, cut it with something and sold our product for many years in Vietnam adulterated. Products containing included nutrients are easily adulterated and this is not necessarily benign.

Tablets cannot be contaminated, diluted, adulterated, etc. There are hundreds of products in the market in Vietnam that are adulterated by the distributor or even worse, misbranded with claims that they contain organisms that must be dead or that would cost so much to include that it would be impossible to include them and still be cost effective. This cannot be done with tablets (although of course the labels can be changed-the content cannot be).

There are two primary although potentially overlapping approaches to the use of nutrients.

**Activation**

This is addition of the spores and nutrients present in powdered formulations to water for a variable number of hours before adding to the ponds or tanks. Different strains take different amounts of time to germinate. Some are slower than others. Even within the same strains some germinate earlier and some later (this can range from 4 to 30 or more hours). Typically this is a step that is short in time and intended to get the cells closer to becoming vegetative cells. Germination is the first step in the spores becoming vegetative cells.

Since the water quality (salinity, pH, etc.), the water temperature, the source of the strains, the presence of contaminating bacteria, and above all the presence of the right type and amounts of nutrients affect the rate of germination and the amount of spores that germinate, activation typically only provides for a limited amount of vegetative cells. There are still lots of spores that are not germinated and that likely will never do so. When these suspensions are used they are poured into the ponds or tanks. The vegetative cells have to make their way to the bottom via currents created by aeration or in non-aerated ponds by random movement. Even if they are pumped onto the bottom most of the cells would not be at the pond bottom where the organic matter accumulates and the shrimp spend most of their time foraging and feeding. There is no data that supports that this is the best approach. This is entirely a historical approach from when products of this type were being used in home septic systems.

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**Grow Out**

This takes activation further by using some science. Spores are added to tanks (example 500 liter tanks) along with a specific blend of nutrients and aeration in clean sterile water for 18 to 24 hours (we can supply a formulation for this). By this time most of the spores that will germinate have and there will be large numbers of vegetative cells. For some environments, small ponds, hatchery tanks and nursery systems these can be added daily in sufficient numbers to impact the microbiome in the tanks. The potential benefits are that they limit the need to add additional sources of feed and the cells are readily consumed by the shrimp on particulates, etc. **I would not recommend this unless the company has a lab on site and the ability to run the routine QC needed to limit using heavily contaminated batches.**

The number of spores that germinate is always only a small portion of what is available to start with. Nutrients become limiting very quickly as the vegetative cells use critical micro-nutrients. When the tablets are added directly to the ponds (or tanks), they settle to the bottom, a nutrient rich environment where the tablets dissolve in a few minutes. Some spores and the earlier germinating vegetative cells enter the sediments and others the water column. Some are consumed by the shrimp that are foraging and happen upon the tablets.

Nutrients ultimately determine the amount of growth that occurs. These are available in the production environment at levels that are many, many times the level present in any nutrient additive. Since you are using the biodegradable bags you know that these contain nutrients in them. These are used in lakes and bodies of water with little to no currents and no aeration. The bags settle to the bottom where they dissolve. The nutrients that are soluble dissolve and diffuse away. In aerated ponds the nutrients are mixed thoroughly with the pond water and do nothing. All they do is add cost.

**Conclusions**

Very few farmers use the products in a manner consistent with getting the best possible results from their use. In Vietnam this is a result of the price mark ups by those in-between the first buyer and the end users. This typically results in products being priced too high to be used the way that they should be. Most companies that sell these products are not run by microbiologists and very few have competent technical backgrounds to have anything but a simple view of how these things work. They rely on hype, exaggeration, kick backs (bribery) and the statistical variability of the pond environments to market their product(s). They cling to outdated approaches towards product delivery because there is no reason for them to change. Almost everybody else is doing it, so “this must be the best approach.” This is of course nonsense and a further reflection in my opinion of the proliferation of a pseudo-scientific perspective in aquaculture that continues to damage the industry. Greed and ignorance underlie most of this.

I would not tell you that it never makes sense to grow the bacteria up. We have clients who routinely do this. They have scientists working with and for them and knowledgable microbiologists to ensure that there are not problems. They use a small number of tablets (which are not contaminated to any significant degree) and a mix of nutrients that is geared towards optimizing the process. Most farmers should never attempt this.

The data we have generated with a number of collaborators (including the largest farms in Ecuador and SIS HI) clearly shows that adding the tablets to nutrient dense environment work as well if not better than any other approach. Adding nutrients is not needed.

One more comment about spore counts. Regulatory agencies seem incapable of understanding that spores are charged and that they clump. They always undercount because of this. These ionic charges also help ensure that the spores stay on the pond bottoms and attach to like charged surfaces and use the nutrients immediately present. Many companies claim that they have super high spore counts and that

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this makes the product better, This is total nonsense and again belies a lack of understanding of what they are selling. A single spore with the right nutrients can grow to the same levels as a billion spores will. **The nutrient levels in the environment to which you add the spores is what determines how many spores germinate and grow into metabolizing cells that degrade the nutrients.** There is no relationship between spore counts and product effectiveness.

I stand by my comments. Until I see a well designed science based study that shows otherwise, from my perspective as a microbiologist and more than 40 years of working with bacteria I see no evidence that activation is needed under most circumstances.

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