



Using PRO4000X with field results

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There are no magic bullets (only a lot of people claiming to sell them)



When shrimp are impacted by disease, the solutions often lie in several elements of the production process not in a single element. Multifactorial.

Technology requires the use of tools that are designed to do specific jobs. Aquaculturists are limited in the tools that they have available and even more so by the consistent presence of pseudoscience among their vendors and even at the production level. When tools are used correctly, they make the job easier with greater chances of a favorable outcome.



Deteriorated pond bottoms damage productivity

Enzymes

Proteins that lower the energy needed to change molecular structure.

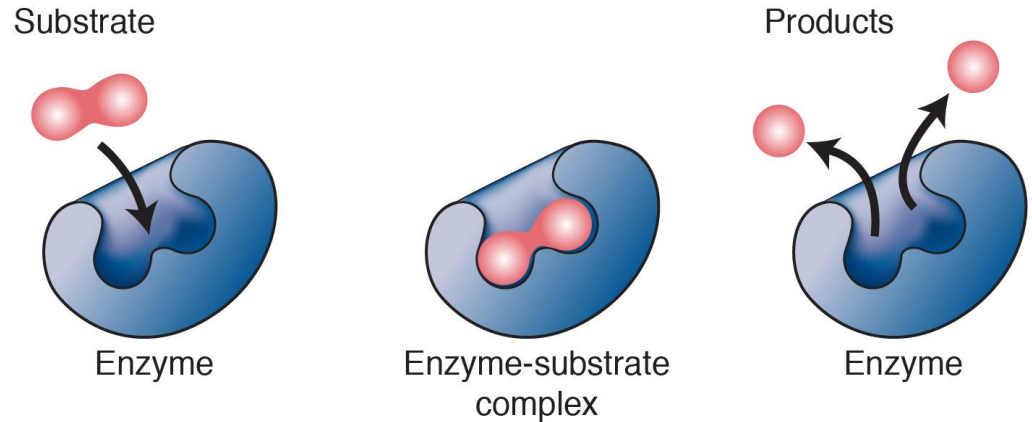
They are essential for all life. Drive all metabolic activities.

Cofactors needed for this structure.

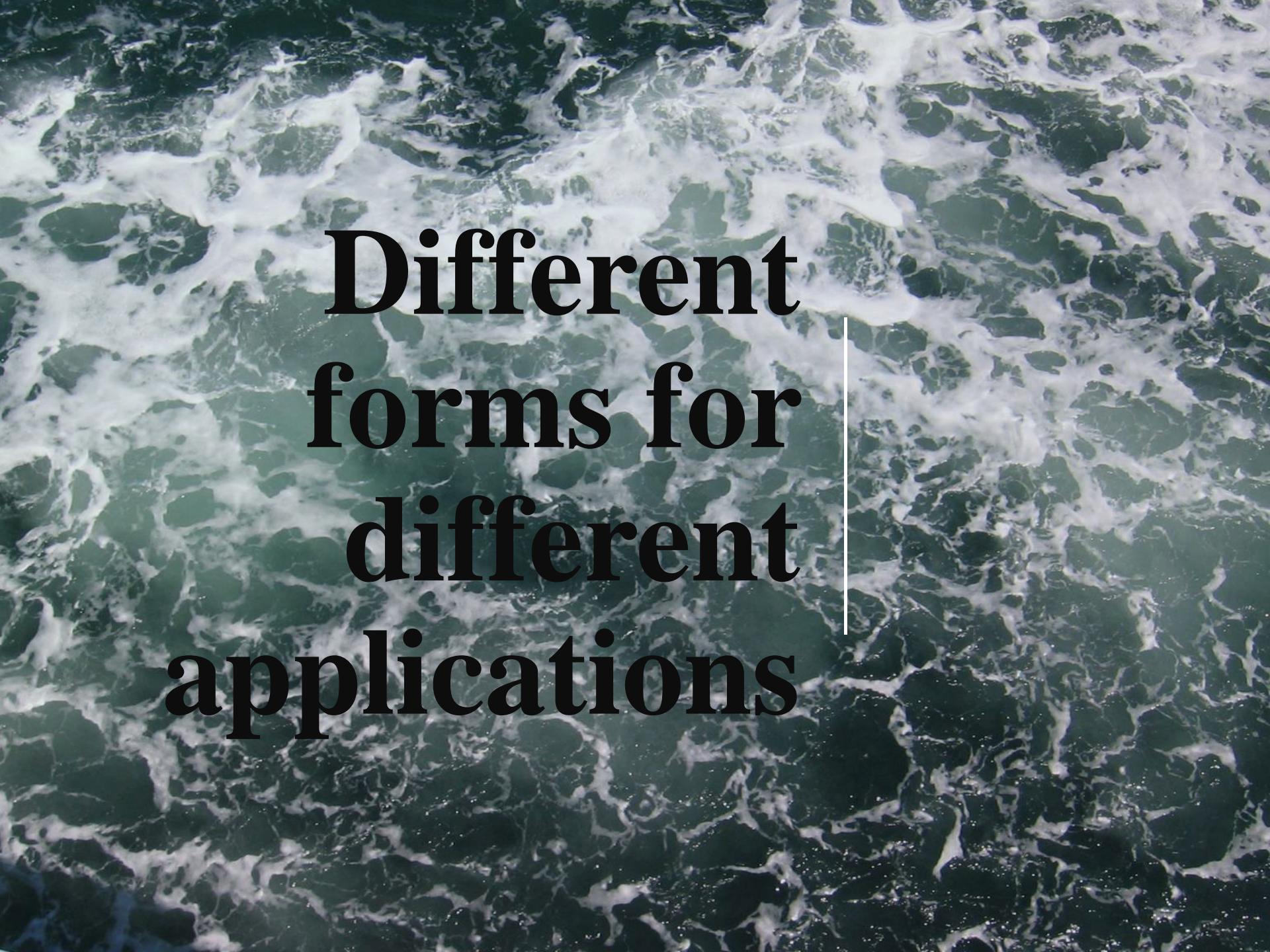
Vitamins, minerals and a variety of chemicals are needed for this catalytic shape.

How do these products work?

Mechanism of enzyme activity



Competition for nutrients is an important element of the ability of some bacteria to inhibit other bacteria. When a species can tie up enough of a critical cofactor it has a competitive edge.



**Different
forms for
different
applications**



**Powder: Bulk Aqua Pro B and in
water soluble bags Aqua Pro EZ**

Product Types

Field Trial Results Aqua Pro B (1999)

\$/lb. (shrimp wholesale price)	\$ 3.50	\$ 5.00
\$ Value of crop	\$ 19253.00	\$ 27890.00
Cost of Aqua Pro B (at \$7.00/lb.)	\$ 325.50	\$ 325.50
\$ Net income	\$ 18927.50	\$ 27574.50
% ROI (\$ gain/divided by cost of Aqua Pro B)	>\$58*	> \$84
Difference in production (controls vs. treated ponds)	5578 lbs	2535 kgs

*realized \$58 for every dollar spent

Two (2) ½ acre ponds were treated with AquaPro B and two were controls. The ponds were stocked at 50-55 P. vannamei PLs/m². During the 28-week cycle 46.5 lbs. (21.1 kgs) were used.

At harvest, the farm manager (Mr. John Harvin) noted the greatly reduced organic sludge build-up in the treated ponds. He estimated the sludge layer to be ½ inch or less. He did not have to dredge the treated ponds before re-stocking. The control ponds built up organic sludge from shrimp waste and uneaten feed that had to be removed annually. Mr. Harvin noted that increased productivity, reduced stress and disease incidence were the greatest benefits.

The two treated ponds produced 2.5 MTs more than the two control ponds.

Product Properties

- Powdered products containing nutrients to allow activation in nutrient free water
- Two species of Bacillus selected for their ability to produce powerful enzymes that degrade organic matter
- No less than *4 billion spores* per gram.
- Work initially in the water column
- Soak material in clean water that is close to what you are adding the bacteria to for min 4 and max 12 hrs.
- Aeration not needed although it can help.
- Aqua Pro EZ, in a water-soluble bag, for easy application to pond bottoms.

Product Types
Tablets of
many
different sizes
and
compositions



Appearance

Small black or brown dots are known as flash rust. It is result of the tableting process and is harmless.

The tablets are a delivery vehicle for the spores.

The vehicle can change but the spores remain the same.



Properties

Approximately 16 grams per tablet (different sizes available)

Two species of Bacillus selected for their ability to produce powerful enzymes that degrade organic matter

No less than than 4 *billion spores* per gram of tablet

Work from the pond bottom up

No activation required

Targeted delivery to problem areas increases efficiency

Utilizes the nutrients present in the environment

Tablet appearance can vary --THE TABLETS ARE A CARRIER FOR THE SPORES



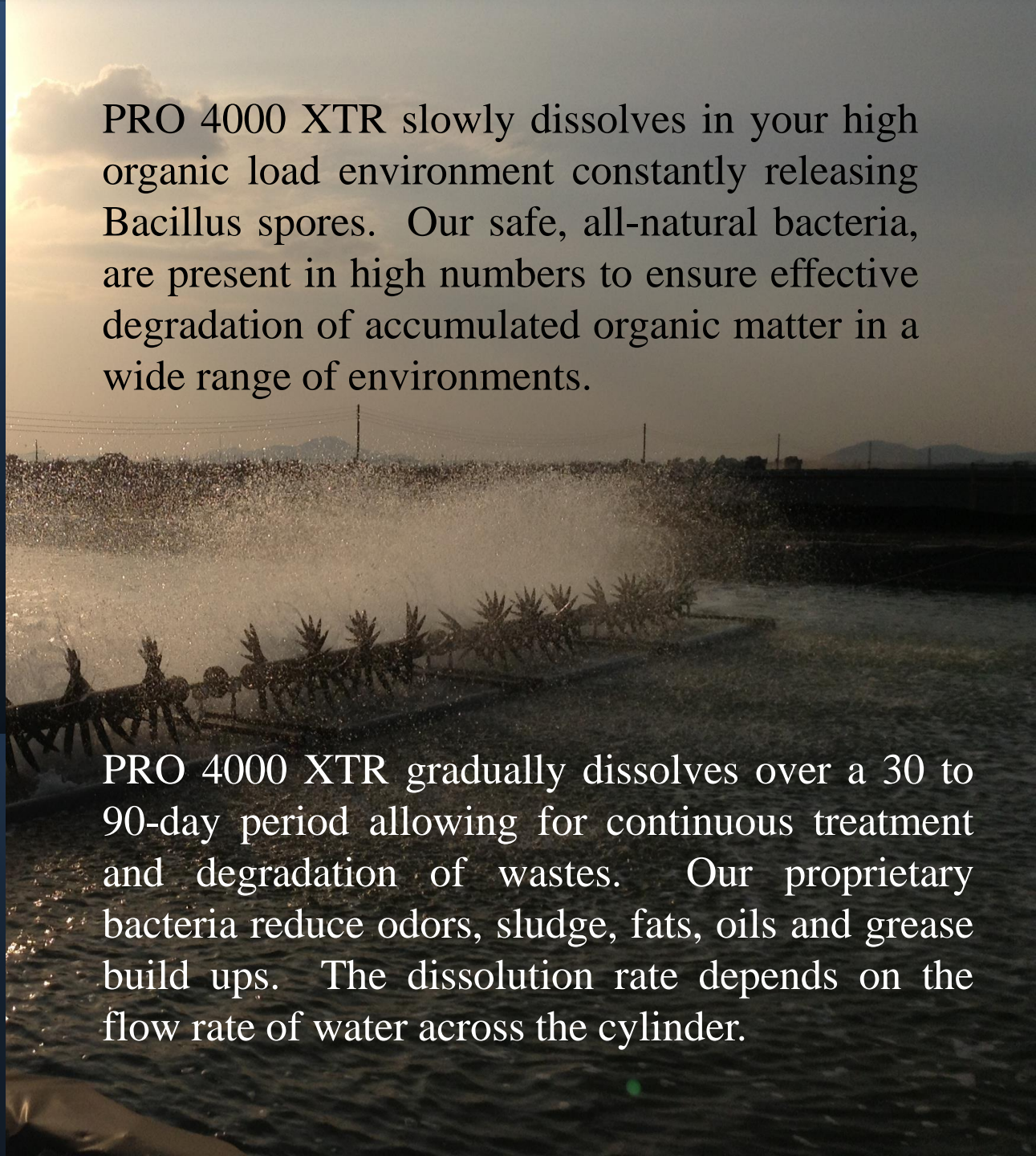
Slow constant release of spores (PRO4000XTR)

There are some environments where it is desirable to be able to add bacteria on a regular basis.

Safe and
effective way
to deliver
spores to the
water column

PRO 4000 XTR slowly dissolves in your high organic load environment constantly releasing Bacillus spores. Our safe, all-natural bacteria, are present in high numbers to ensure effective degradation of accumulated organic matter in a wide range of environments.

PRO 4000 XTR gradually dissolves over a 30 to 90-day period allowing for continuous treatment and degradation of wastes. Our proprietary bacteria reduce odors, sludge, fats, oils and grease build ups. The dissolution rate depends on the flow rate of water across the cylinder.



Properties

Contains 5
species/strains of
Bacillus.

Designed to add
spores constantly to a
water column.

No less than than 4
billion spores per
gram.

Requires flowing
water to dissolve the
cylinder.

No activation required

Utilizes the nutrients
present in the
environment as they
are produced.

Why Bacillus?

Long term shelf stability due to spore formation

Broad range of enzymatic and metabolic activities

Naturally occurring and safe to use

Proprietary strains with proven track record for improving water quality and increasing productivity

Compete against other bacteria such as vibrios and blue green algae (cyanobacteria) for nutrients

Excellent results reported consistently from the field with tilapia, catfish, *P. vannamei* (white shrimp), *P. monodon* (tiger shrimp), *P. stylirostris* (blue shrimp), eels, clams, *M. rosenbergii*, etc. with PRO4000X family of products

How to use Pro4000X tablets?

Direct addition. Throw tablets where you want the spores to be. The spores germinate and will move into the sediment underneath them and into the water column.

Activation. Unlike all powdered products our tablets contain pure cultures and have no non-Bacillus contaminants. They can be added to nutrient solutions for growout and addition to ponds with a smaller chance of contamination.

Dispersion. Powdered tablets can be dispersed over an area. Powder sinks or activated product can be poured over an area as well.

Where to use?

Maturation system

RAS

Hatchery tanks

Any and all ponds. Dirt, lined, concrete, etc.

Broodstock

Nursery

Production

Sedimentation ponds

Processing plants

Aquatic animals
PRO4000X has
been used with

Penaeus vannamei “white shrimp”

Penaeus monodon “tiger shrimp”

Penaeus stylirostris ”blue shrimp”

Ictalurus punctatus catfish

Macrobrachium rosenbergii

Tilapia

Clams

Barramundi

Trout

Eel



Penaeus vannamei maturation Ecuador

November 22, 2017

To whom it may concern:

As one of the leaders in selling genetically improved shrimp stocks for use by shrimp aquaculturists around the world it is important that we operate in a biosecure manner and that any products we may use in our systems add value.


We have been using a tableted product manufactured (PRO 4000X trademark) by Aquaintech Inc. for some time now. This combination of Bacillus spores and vegetative cells in this tablet settle to the bottom of our Nucleus Breeding Center recirculating floc production tanks. These bacteria degrade any accumulated organic matter and we have found that this is a convenient and easy way to keep our recirculating system healthy.

Among some of our observations:

- We use it about 1 ppm per daily (one tablet per each 16 MTs of water or so).
- We use it in the entire system from nursery through grow-out phases.
- Ammonia and nitrate levels are stabilized and remain low with infrequent spikes.
- It prevents the accumulation of rotting material in problematic settling spots in our system.
- The overall water quality is improved and our tanks are cleaner.
- It helps improve the general health of the breeders. We have observed after handling the animals during sampling and transfers that it effectively prevents exoskeleton lesions from becoming infected by bacteria.

We are quite pleased with this product and will continue to use it as an important element of our overall approach to biosecurity and improving animal health.


Regards,


David Leong
President

Testimonial for Tablet
usage in
maturation/hatchery
from a major global
broodstock provider



Hatcheries. Flow through or static.



Guidelines for maturation/hatchery usage

One tablet daily from stocking of nauplii before harvesting PLs per 5 to 10 MT of water 1 to 3 ppm.

Nutrient levels will affect bacterial growth

Higher nutrient levels as cycle progresses may require dosage adjustment

Can be used in **Artemia culture** (one tablet added before cysts)

Can be used in **algal tanks** (lessens vibrio loads in outdoor production tanks)

Can be used in maturation one 16-gram tablet per day per 5 to 10 MT water. (see testimonial slide 9)

Client observations from hatchery tanks

Effective control of yellow and green colonies on TCBS. (i.e. reduction in vibrio loads)

Smooth molting of larvae due to less fouling of external surfaces.

Control of the Zoeae syndrome as a result of reduction of organic loads and impact on vibrios.

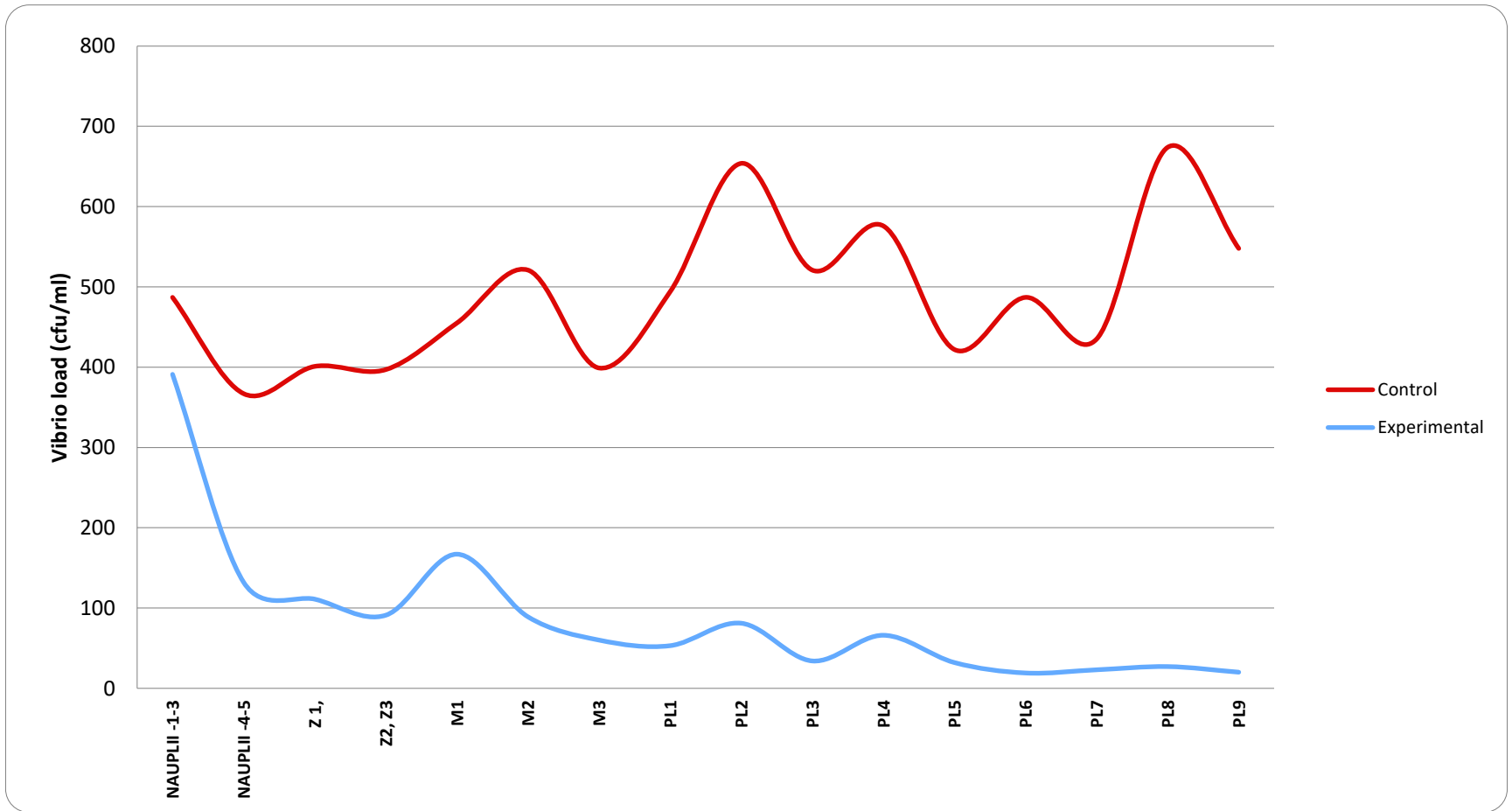
Reduction of ammonia and nitrite levels in tanks.

Better survivals when compared to the other leading probiotics (more effective reduction of organic matter).

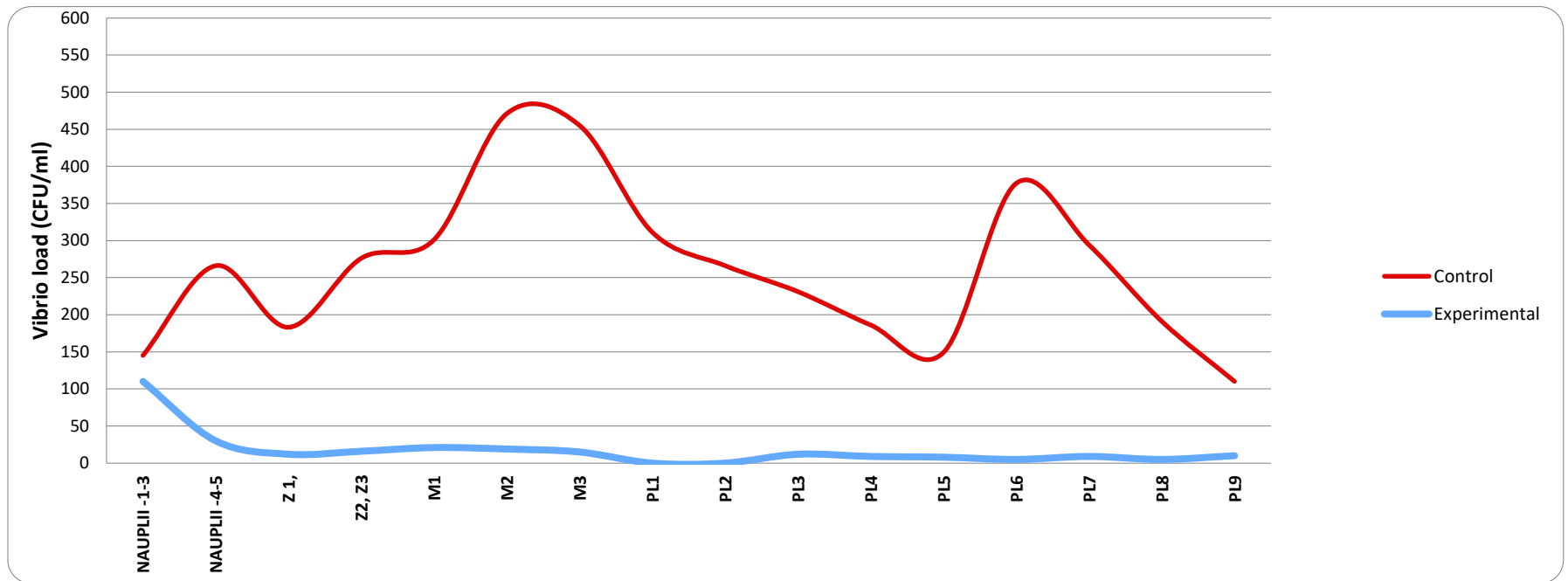
Impact on vibrio loads

Test in India enumerating vibrio loads in production tanks treated with Pro4000X compared with non-treated controls

TCBS yellow vibrios in hatchery reduction by Pro4000X



Pro400X reduces green vibrios in hatchery tanks.



- *TCBS Green loads reduced to almost zero in hatchery tanks*



Penaeus monodon PLs

What happens when I use these products?

Spores in the tablets germinate when exposed to moisture.

They grow into metabolically active Bacillus cells. Healthy cells are fat gram positive non-motile rods.

Through the production of enzymes these bacteria rapidly utilize the nutrients that are readily available.

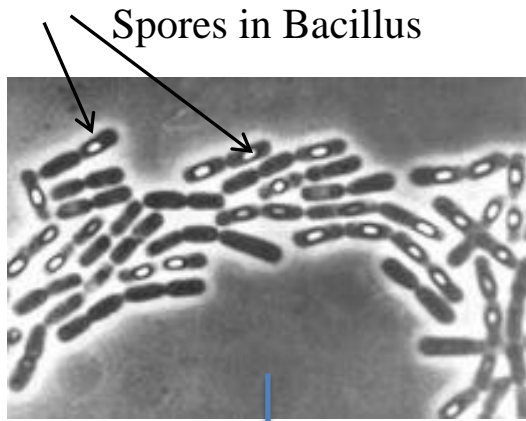
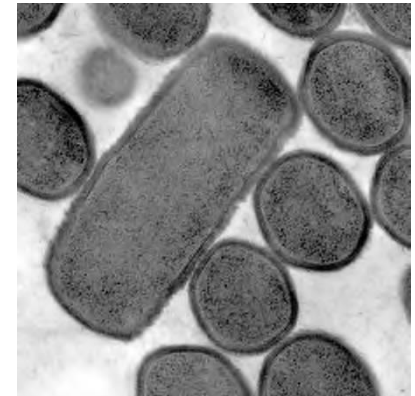
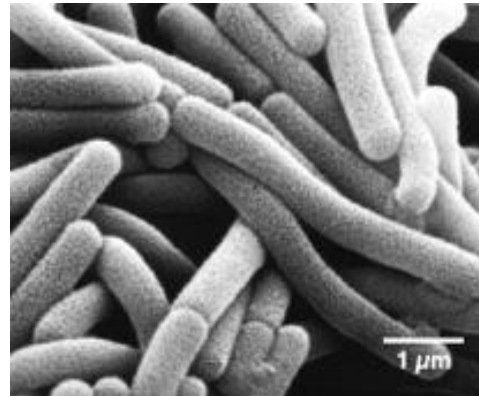
They “shape” the microbiome over a short period of time until they die off and return to background levels.

Gram positive fat rods



Targeted
Delivery
Germination

Non stained cell appearance (EM)



Fermentation and spore concentration
blending and tableting



Growth on agar



Degradation of organic matter resulting in improvement of water quality.
Degradation of ammonia and nitrites.
Degradation of hydrogen sulfide.
Used properly will compete against other bacteria (vibrios) and blue green algae.
Action on the environment benefits the animal.
Cleaner animals. Less fouling. Less stress. Better health.

Farm in Indonesia (before use)





BENEFITS OF USING PRO4000X

Benefits Reported by our clients*

- Less organic matter
- Cleaner animals at harvest
- Healthier looking animals
- Better growth
- Higher survivals
- No need to use antibiotics
- Lower vibrio loads
- Lower ammonia levels
- Less water exchange required
- Healthier ponds with less blue green algae
- Lower hydrogen sulfide levels

*Benefits vary. When used correctly all clients experience a reduction in organic matter. Secondary benefits depend on your environment and culture conditions.



Isla de Escalante Ecuador



Ecuador 2020

Before application



After application



Proof that PRO 4000X tablets work.



Fish farm in Bangladesh

Ecuador 2020



Ecuador 2020



Ecuador 2020



Farm usage



How best to use Pro4000X?

There is no one right way.

Each pond is distinct with unique inputs and outputs.

Adjust your usage to fit your environment.

Experiment with dosage levels and observe impacts. Adjust usage rates and frequency as feedback from the environment dictates.

This means you adjust the dosage when it appears that it is not doing the desired job and that you increase the dosage as the cycle progresses.

Concept of titration



Titration

Clients are urged to start from our framework and modify their usage pattern to optimize the products functionality.

This can involve starting out at high levels and backing off to those levels that work the best.

The bacteria in the product do what we claim they do. This is not in doubt.

How well they work in a production environment is controlled by the user.

Critical point to consider: Organic matter accumulation is a function of water exchange, the density of the biomass in the ponds and how much feed you add. As the cycle progresses these all increase. More tablets should be added as the cycle progresses. This can be done by increasing frequency of application and using more tablets.

Suggested application rates to start from

Day	Tablets per ha		Day	Tablets per ha	
Density/m ²	High (> 75)	Low (< 75)	Density/m ²	High (> 75)	Low (< 75)
0	10	0	77	60	27
7	15	5	84	60	30
14	20	7	91	75	40
21	20	10	98	90	40
28	25	12	105	90	45
35	30	13	112	90	45
42	35	15	119	90	45
49	40	17			
56	50	20	Tablet #	925	431
63	50	25	kg	15	7
70	60	30			

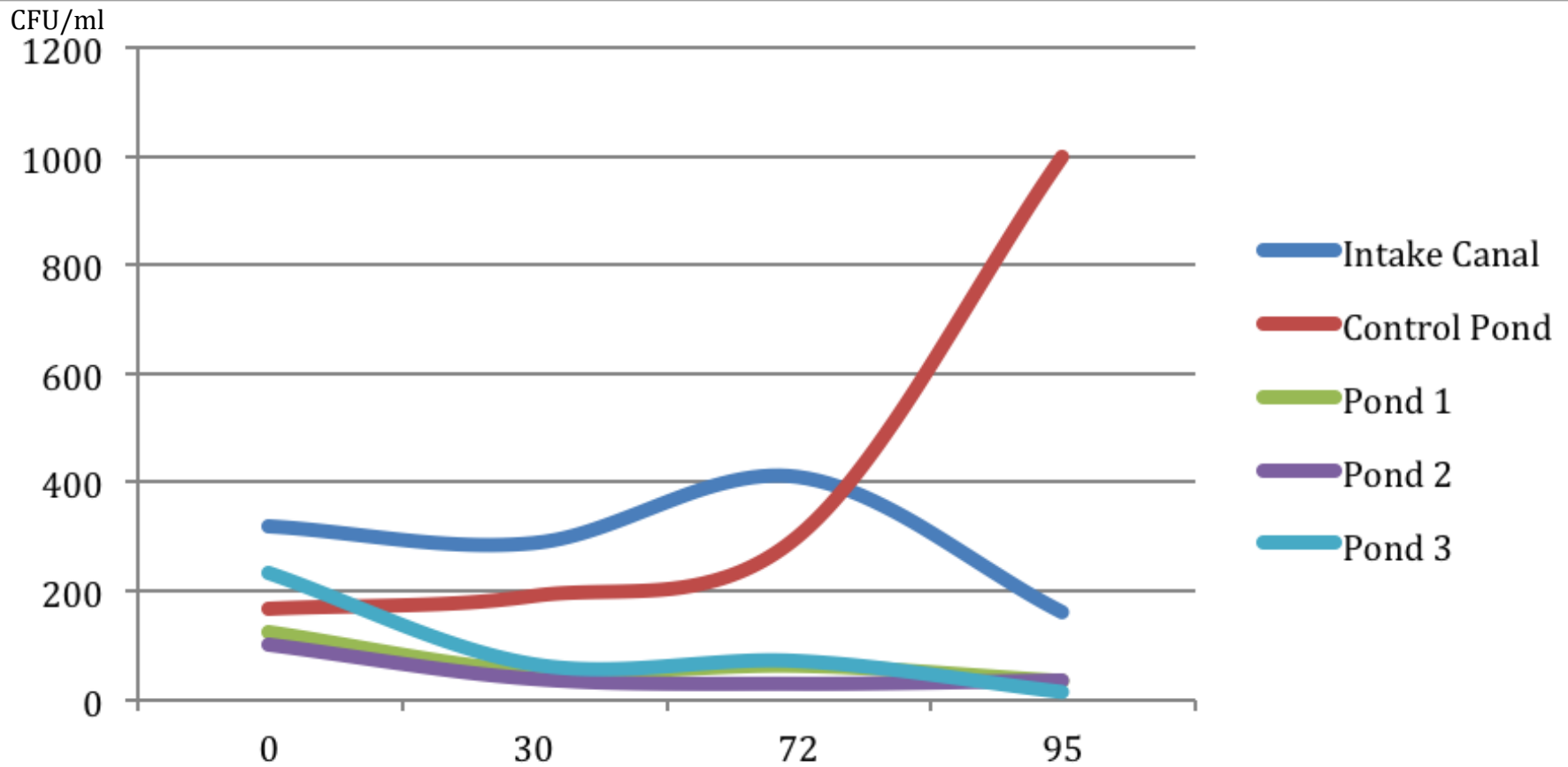
This is for guidance only. Your rates may vary.

Field Trials India 2013 PRO4000X

Results of three ponds treated with PRO 4000X compared with a pond that was not treated.

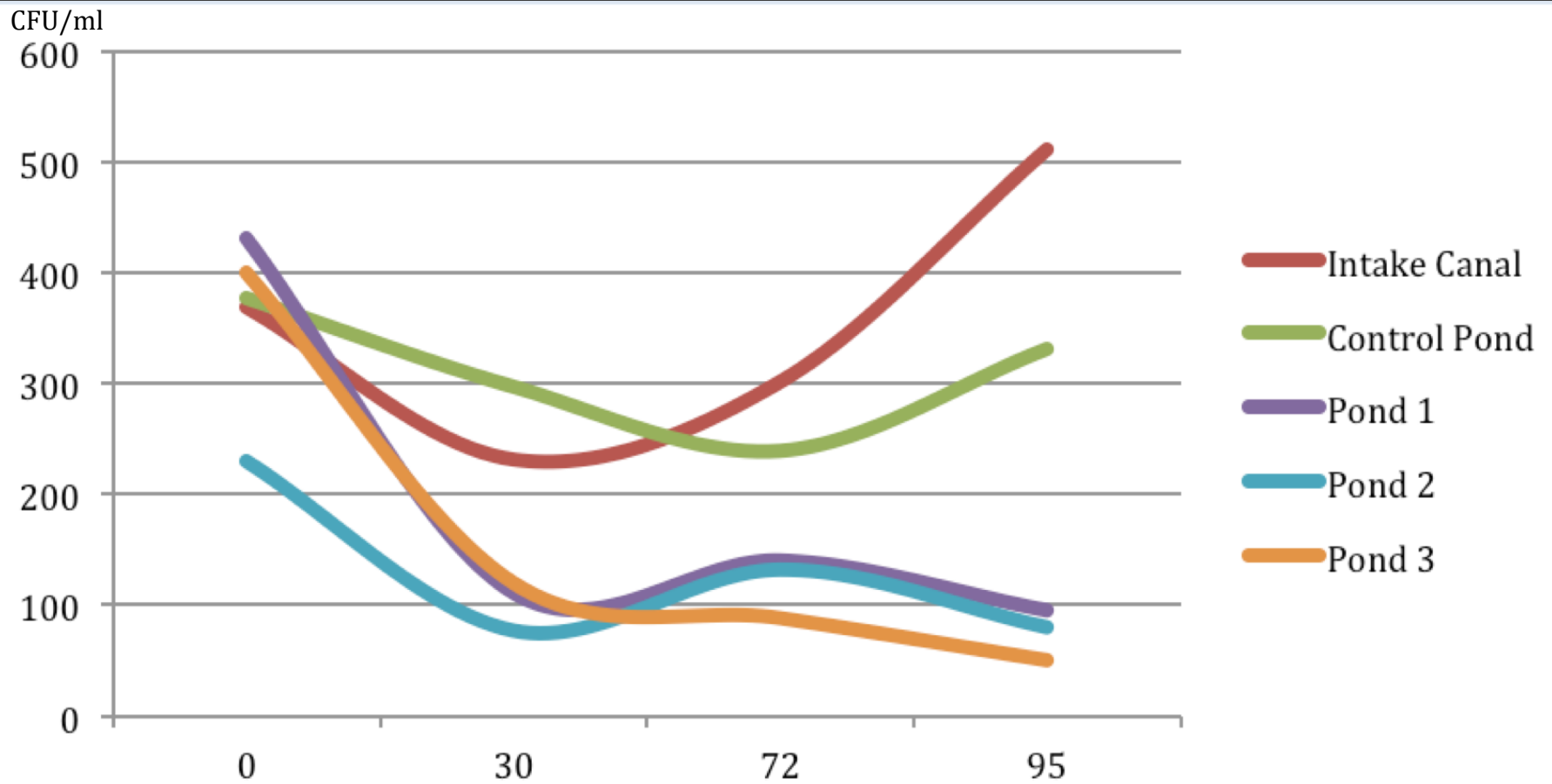
Clearly demonstrated there a dramatic impact on productivity. Subsequent pages show why.

Pond #	Animals per sq m	Area (ha)	Depth (m)	Water Exchange (%)	Cycle (days)	weight grams	MT harvest	% surv	FCRs
1	35	1	1.5	0	115	24.5	>7.7	>90	1.1
2	35	1	1.5	0	115	23.3	>7.3	>90	1.1
3	28	0.9	1.4	0	115	25.9	>6.5	>90	1.2
Control	35	1	1.5	10 to 15	122	15.1	5.3	82	1.7



**Reduction of TCBS
green vibrio loads
over the course of
the production cycle.**

TCBS green vibrio loads were significantly reduced in ponds that used PRO4000X tablets.

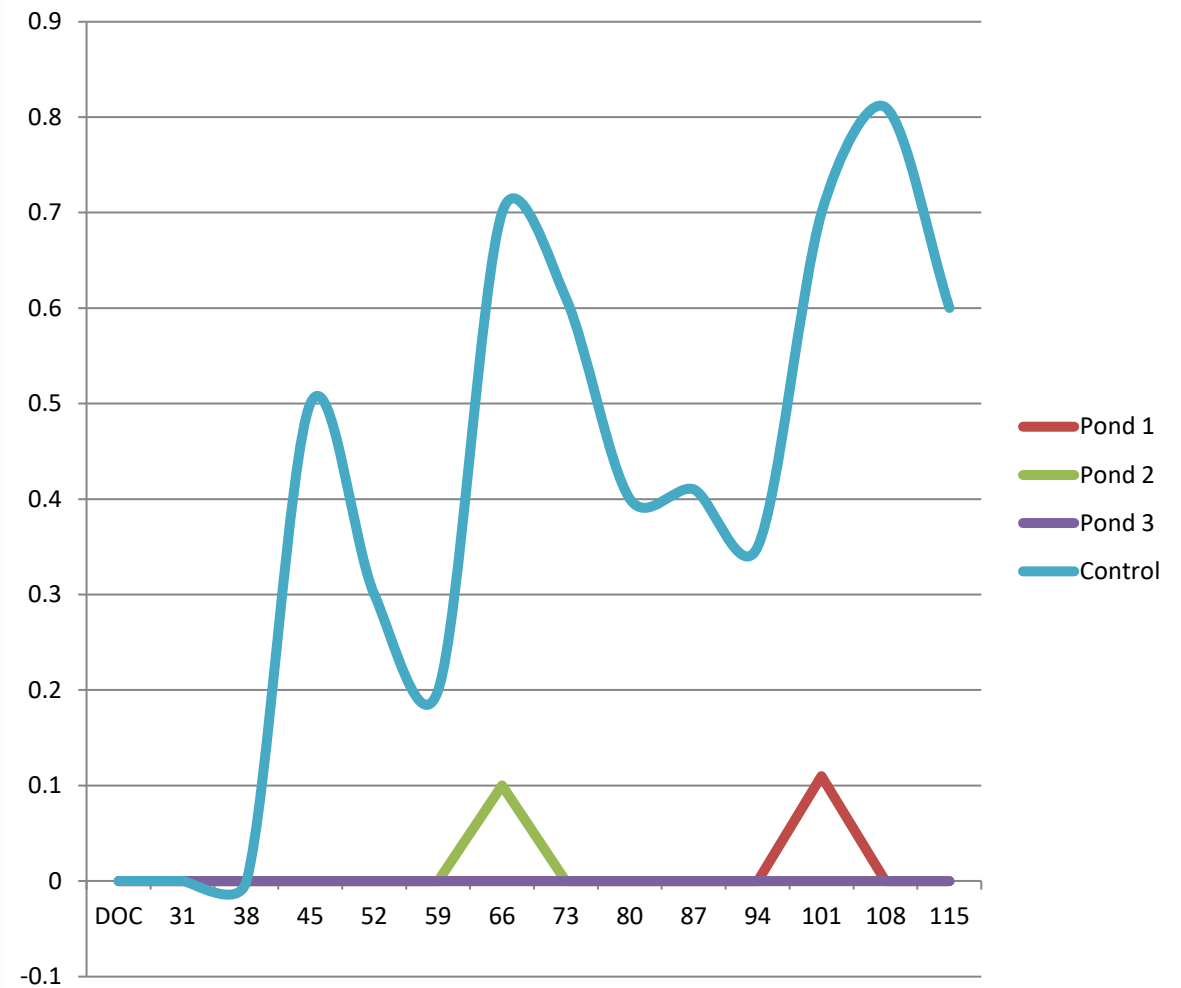


**Reduction of TCBS
yellow vibrio loads
over the course of
the production cycle**

TCBS yellow vibrio loads were significantly lower in ponds treated with Clean Pond tablets

Weekly ammonia levels in control and experimental ponds

PRO 4000X controlled ammonia levels in the three experimental ponds

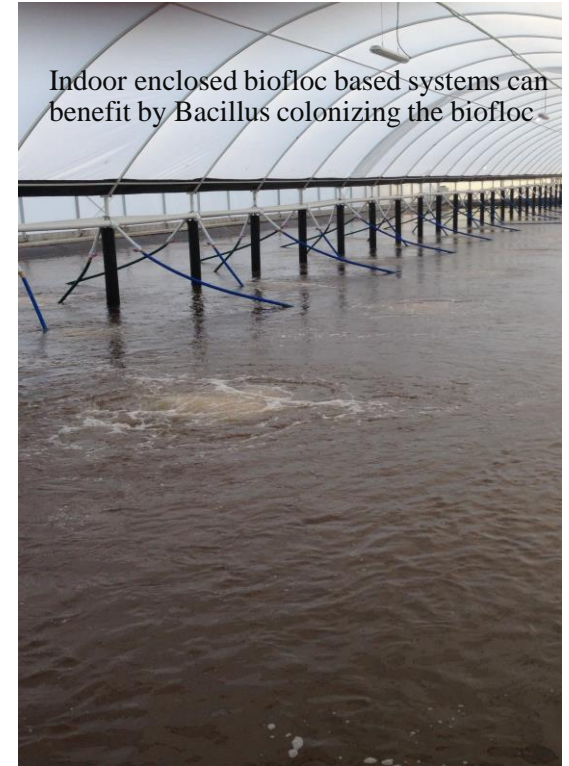


Larger lined ponds



Smaller lined ponds with high levels of aeration

Indoor enclosed biofloc based systems can benefit by Bacillus colonizing the biofloc



Where else can tablets be used?

and using well water for makeup and exchange can benefit by using tablets in drainage areas



Final thoughts.....

Shrimp and fish production environments are complex. What we see is only a part of what is happening. Much of what is going on we cannot see. The iceberg photograph to the left models this well although I am sure that there are some who think that this model is upside down.

Those things that contribute to the problems we see are not always obvious or readily understood. There are some that are never figured out and others where no matter what we try, our efforts end up in vain. Many of the challenges that aquaculturists face are multifactorial. A combination of several things are causing the problem.

Taking reasonable cost-effective measures to limit the potential impact of any bacterial disease problem is smart.

PROACTIVE disease management is a critical element of biosecurity that should not be ignored