(R-7) The Science Behind Bio-Zyme Aquatic Applications *v3*

Bacterial Product Composition and Description

Bio-Zyme products contain completely safe, non-toxic live bacteria, which are used to purify water in lakes, ponds and in wastewater treatment. All of the bacterial strains are classified by American Type Culture Collection as Biosafety Level 1; meaning that they are not pathogenic.

Bacteria, along with other organisms, are responsible for consuming and removing impurities in water. The impurities are often classified as either inorganic or organic waste.

Inorganic wastes (generally from mineral, non-living sources) are chemicals such as ammonia and phosphate. Organic wastes (more typically from living sources) are generally more complex, and are larger chemicals such as sugars, proteins, fats, starches, etc.

The unique process of our proprietary blend optimizes production of useful enzymes by our bacteria (discussed in detail later). Enzymes are non-living biological molecules produced by bacteria that help break down organic waste into a form more easily consumed by bacteria. Enzymes are catalysts that assist in conversion of material, but aren't consumed in the process.

Bacterial Growth and Reproduction Rate

Bacterial reproduction rate and its role in population dynamics is a key element to understanding the technologies and products available from Bio-Zyme.

Different bacteria reproduce at widely varying rates (the bacterial replication time ranges from several minutes to many hours).

Most bacteria that consume organic waste reproduce every 30 to 120 minutes in a nutrient-rich environment.

In any pond, even a eutrophic pond, there is limited food, rather than an unlimited amount of food. The biological flora that is present in any pond is largely dependent on the speed of reproduction of the microorganisms that are in the water. This generally means that you will have more and more of the bacteria that reproduce quickly, and far fewer of the bacteria that reproduce slowly. The table shows what happens when one bacterial species multiplies very quickly, while a competing bacterial species reproduces very slowly:

Elapsed Time in Hours	Number of Bacteria "A" (Takes 30 min to duplicate)	Number of Bacteria "B" (Takes 60 min to duplicate)
0	1	1
0.5	2	1
1.0	4	2
1.5	8	2
2.0	16	4
2.5	32	4
3.0	64	8
3.5	128	8
4.0	256	16
4.5	512	32
After 5.0 hours	1024 count of type A	32 count of type B

Note that at the end of 5 hours, the population of Bacterial TYPE A numbers 1024. However, at the same 5 hour point, the population of Bacteria TYPE B numbers only 32 bacteria.

This is extremely important when considering what type of bacteria dominate in a given ecosystem. This disparity becomes even more pronounced when there is a true limiting nutrient or micronutrient.

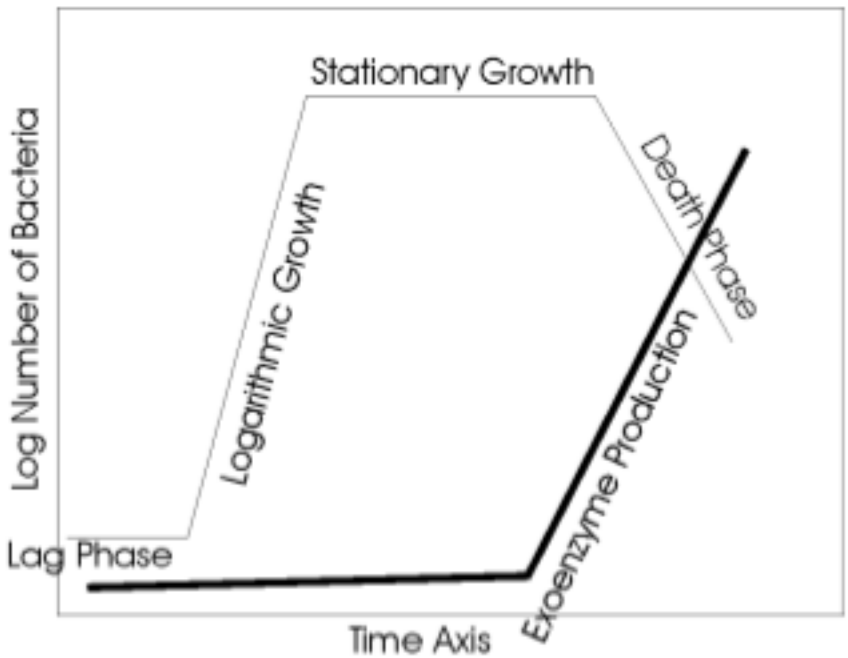
"If I already have bacteria in my lake or pond, why should I add Bio-Zyme?" This question is answered in detail below.

Exoenzyme Producing Bacteria

Bacteria readily consume simple, soluble food. In order to consume the larger organic food (lake detritus), bacteria require enzymatic action to convert the large compounds into small compounds that can fit through the cell wall.

Enzymes are biological macromolecules that are catalysts for biochemical reactions. Catalysts speed up reactions such as the digestion of lake detritus into small compounds. One very important type of enzyme is the exoenzyme. Exoenzymes are made inside of bacteria, and are then passed outside the bacterium, where they react with large substrate. Once in contact with large substrate, the exoenzymes catalyze the conversion of large compounds into simple, soluble substrate that bacteria can directly consume (because it is now small enough to pass through the bacterial cell wall).

The chart below illustrates the typical relation between bacterial population growth and the production of exoenzymes.



This chart shows typical, textbook progression that results from placing a nutrient solution into a flask, introducing air and bacteria, and then counting the bacteria and measuring the exoenzymes produced over time.

As can be seen, initially there is a "tooling up" phase, a lag time during which the bacteria are gearing up to grow and reproduce. After the lag phase, there is a logarithmic growth phase, which continues until the food source begins to run out. Logarithmic growth is followed by a stationary growth phase during which as many bacteria die as reproduce. Finally, there is an endogenous phase, or death phase, during which the bacteria have run out of food, and are literally consuming themselves.

Note that exoenzyme production accelerates as the soluble food source is used up, and the bacteria enter stationary and death phase.

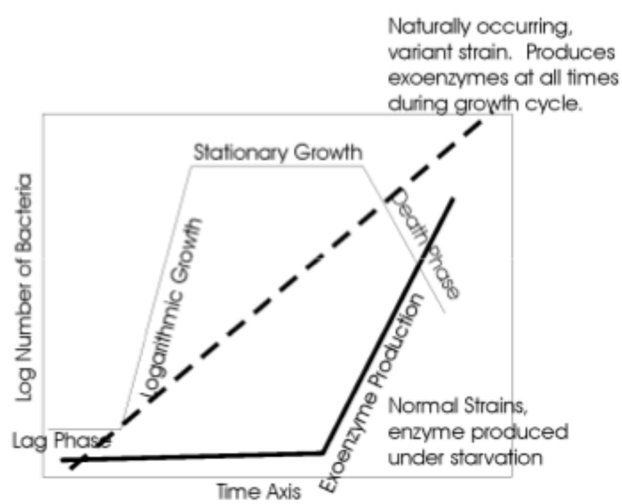
The main point is that rapidly-reproducing bacteria do not produce exoenzymes in significant quantities. *First, most bacteria do not produce these enzymes at all until they begin to starve. After all, exoenzymes are a "product" of the bacterial factory. The production of exoenzymes requires material and energy.*

When nutrients are already present, exoenzyme production would be a waste, so exoenzymes are not produced when soluble food is present.

So as long as there is at least some organic food constantly available in the pond, there are sufficient nutrients for some bacteria to grow, and exoenzymes are not produced at a high rate. The result is that sludge (organic sediment) builds up ponds. As stated before, the sludge fosters anaerobic zones, increases the likely of disease- causing bacteria to thrive, and causes foul odor problems (including sulfide).

First Method for Enhancement of Exoenzyme Production

As one option to improve exoenzyme production and reduce sludge build up, some of Bio-Zyme's bacteria include specialized (100% natural and safe, Biosafety Level 1 bacteria) that produce exoenzymes all of the time (constitutive exoenzyme production). The following chart shows how these bacteria behave with respect to exoenzyme production versus state of growth:



Bio-Zyme bacteria that constitutively produce exoenzymes are not modified or genetically engineered in any way. They are completely natural, and are in fact present at low population levels in untreated ponds.

Since these bacteria produce exoenzymes all of the time, they reproduce very slowly.

As was demonstrated in the reproduction vs time chart, when a species reproduces more slowly, its' population count remains very low, while those that reproduce rapidly quickly dominate. The useful, slowly-reproducing bacteria that constitutively produce the valuable exoenzymes get lost from the population due to their relatively slow growth.

In summary, even though these constitutive exoenzyme- producing bacteria are present in untreated ponds, since they produce exoenzymes all of the time, they will have a naturally low reproduction rate. With a slow reproduction rate, by definition, these bacteria will always be lost in population dynamics. Dosing Bio-Zyme periodically helps boost the numbers of these slow glowing but very important bacteria in the lake's microbe population.

Second Method for Enhancement of Exoenzyme Production

The first method we use (dosing Bio-Zyme directly to the lake) to enhance exoenzyme production is based on inclusion of constitutive exoenzyme-producing bacteria in our formulations (previously described). A second method we use is a special process that induces high-rate exoenzyme production using our Bio-Generator technology.

On-Site Bio-Generator:

In larger applications, we include use of an on-site Bio-Generator. The Bio-Generator is equipped with vigorous aeration. To the Bio-Generator, we add the following:

- Specialized nutrients to act as "recognition compounds" for production of specific exoenzymes by bacteria.
- Our products that contain bacteria capable of producing the required quantity and diversity of exoenzymes to convert sludge into simple, low molecular weight, soluble substrate that bacteria can consume.

By adding the right recognition nutrients, the right bacteria, and growing them for the right length of time, we starve the bacteria and force them into stationary and death phase conditions. This causes these bacteria to produce exoenzymes at the maximum possible rate. The majority of these bacteria do not produce the exoenzymes at all when they are in logarithmic growth.

Without the Bio-Generator, the correct recognition nutrients, the right bacteria, and the proper growth conditions (time and temperature), these bacteria would not produce the needed exoenzymes. With these elements in place, our bio-generators produce the required amount of exoenzymes needed to maximally digest sludge (lake detritus).

What Happens to the Solubilized Sludge?

Once sludge solubilization occurs, the preferred habitat for sulfide generating, anaerobic bacteria (such as desulfovibrio desulfuricans) is eliminated. The majority of the sulfate reducing /sulfide producing bacteria live at the sludge-water interface. Sulfide reduction occurs with sludge reduction because the sludge-water interface is quickly interrupted by the solubilization technology, depriving the sulfide producers of a place to live. Generally, odor control can be achieved within the first 4 to 8 weeks of product application, even in rather severe situations.

Another consideration is the result of solubilized particulate organic matter. The newly available (small and soluble) food is now equally available to ***all*** of the microorganisms in the aquatic environment.

Even more important, the conversion of particulate sludge to soluble food is the *rate-determining step* (rather slow). In contrast, the consumption of low molecular weight, soluble food is very rapid.

When solubilization occurs, all of the bacteria in the pond are recipients and beneficiaries of the newly available soluble food. The simple, soluble substrate is rapidly consumed, and largely converted to CO2 and water, plus new bacterial cells.

As the lake's existing bacterial population benefits from consumption of newly available soluble food, the lake's bacterial population is more robust and diverse. The lake's more robust, more diverse bacterial population is better able to compete with various aquatic species for micro-nutrients. In many situations, however, sludge digestion also releases N and P into the system (N and P that was previously bound in organic sludge deposits).

The powerful nitrifying ability of Bio-Zyme products is used to consume any N and P released through sludge digestion. That is the subject of the next section.

Bio-Zyme Nitrifying Bacteria

The greatest competitive advantage is our technology for manufacturing and stabilizing nitrifying bacteria.

Nitrifying bacteria play a critical role in reducing ammonia and nitrite levels in ponds, and in the control of water quality. Nitrifying bacteria consume NH3, NO2, and also consume a large amount of Phosphate (PO4). These nutrients are largely food source molecules for many aspects of the aquatic ecosystem.

Key Point: Our nitrifying bacteria consume these nutrients.

What is Nitrification?

Nitrification is the SEQUENTIAL aerobic, bacterial conversion of ammonia (NH3) to nitrite (NO2) and then to nitrate (NO3). One broad class of nitrifying bacteria converts NH3 into NO2. Another broad class of nitrifiers converts NO2 into NO3.

Of particular importance in lakes and ponds is that NH3 is highly toxic to fish and other aquatic organisms. Aquatic Specialists are trained to carefully note the NH3 levels in their lakes and ponds on a regular basis. Ammonia spikes (when NH3 concentration rises) can be lethal to fish! Meanwhile, NO2 (nitrite) is less toxic than ammonia but can still harm fish, while NO3 (NO3) is relatively non-toxic.

The two groups of nitrifiers as they relate to our nitrification technology are:

Nitrosomonas, which converts NH3 to NO2 (ammonia to nitrite), and Nitrobacter, which converts NO2 to NO3 (nitrite to nitrate).

With our exceptional nitrification technology, which allows us to grow nitrifying bacteria to the highest concentration, and keep them stable for use for 2 years, we are able to add huge numbers of completely non-toxic, live, actively nitrifying bacteria to ponds and lakes.

Nitrification to Denitrification

Denitrification is the facultative reduction of NO3 to nitrogen gas. Many naturally occurring bacteria perform this function; however, denitrification has special requirements. The table below gives a good contrast between the two processes:

Nitrification	**Denitrification**
NH3 to NO2 to NO3	NO3 to N2
Requires nitrifying bacteria	Requires denitrifying bacteria (very common)
Requires oxygen (aeration)	Requires low dissolved oxygen (less than 0.5 ppm)
Releases Hydrogen ions , Consumes alkalinity, lowers ph	Increases alkalinity, increases pH
Requires relatively clean environment (low soluble BOD)	Soluble organic food is required

Note that NO3 is the byproduct of nitrification (NH3 to NO2 to NO3). By enhancing nitrification, we convert ammonia to nitrite to nitrate. However, that is not enough. We need to remove nitrate (enhance denitrification) as well.

Fortunately, all lakes and ponds have zones where there is less oxygen, which means that there is low dissolved oxygen in at least part of the system. Low dissolved oxygen zones would include the gravel, sand, or other treatment zones of the bottom of any lake or pond.

Note that the presence of soluble organic food is a requirement for denitrification. Since Bio-Zyme Products include solubilizing bacteria, there will always be some low level of soluble organic food available to support denitrification.

Through denitrification, NO3 (the end product of nitrification) reacts with soluble organic food and denitrifying bacteria. The end product is nitrogen gas (which makes up 79% of our atmosphere!).

Through denitrification, some NO3 is eliminated from the pond as N2 gas.

Why Continue to Dose Bio-Zyme Products After Initial Application?

It is true that the bacteria we supply are common bacteria found in natural, untreated aquatic ecosystems. However, the natural biochemical environment of a lake or pond precludes the long-term establishment of bacteria that produce exoenzymes at a high rate (this function is required to minimize sludge build up).

Real world experience proves that sludge builds up, odors, and decreased water qualities occur. These problems are typical, and prevalent throughout the aquatic industry. Through use of Bio-Zyme products, our customers solve these problems safely, naturally, and quickly.

Nitrifying bacteria are by nature very slow growing. Regular additions of large numbers of actively nitrifying bacteria are needed to help eliminate ammonia, nitrite, and nitrate.

Even after dosing with our Bio-Zyme products, after some time expires, the other bacteria (the faster growing ones existing before we added our products) tend to re-establish their dominance over the slow growing bacteria. That is why it is necessary to continue to add Bio-Zyme.

In general, dosing between once per week to once per month is recommended depending on your specific lake or pond's need. This is true in both small and large applications.