Science Behind Treatment of Aquatic Systems with TLC Products

Introduction

TLC Products is located in Cleveland, Ohio, USA., and has been a leading manufacturer of bacterial additives since 1996. The company owner and founder, John M. Wong, is the inventor of all TLC bacterial products. He is also noted as the primary inventor on these critically important US Patents:

- U.S. Patent 4,882,059: Process Patent for Enhanced Solubilization of Colloidal and Particulate Materials in Wastewater Treatment
- U.S. Patent 5,284,587: Bacterial-Containing Polymer Gel for Wastewater Treatment (Gel-Polymer Combination)
- U.S. Patent 4,673,505: A Method for Stabilizing Bacteria Products to Provide a Long Shelf Life

The following information is a summary of our technology for nutrient reduction, sludge reduction, odor control, and general purification of aquatic systems such as ponds, aquariums, and septic tanks. Our major brands and flagship products are PondPerfect, StartSmart Complete (for aquariums), and Septic Medic.

Bacterial Product Composition and Description

TLC Products manufactures completely safe, non-toxic live bacteria, which are used to purify water in ponds, aquariums and in wastewater treatment. All of our bacterial strains are classified by American Type Culture Collection as Biosafety Level 1, meaning that they are not pathogenic.

All product bacterial strains are derived from ATCC seed cultures, and grown under sterile conditions, stabilized using patented and trade secret processes, so that the highest quality liquid and freeze-dried bacterial products with a minimum of 2 year shelf life are provided to our domestic USA and international markets.

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.

In discussions of aquariums, ponds, and other aquatic systems, the terms "bugs", "enzymes", and "bacteria" are interchangeably used by people to describe the agents involved in biological activity. We manufacture and sell bacteria. We do not specifically sell enzymes, but our unique processes optimize production of useful enzymes by our bacteria (discussed in detail later).

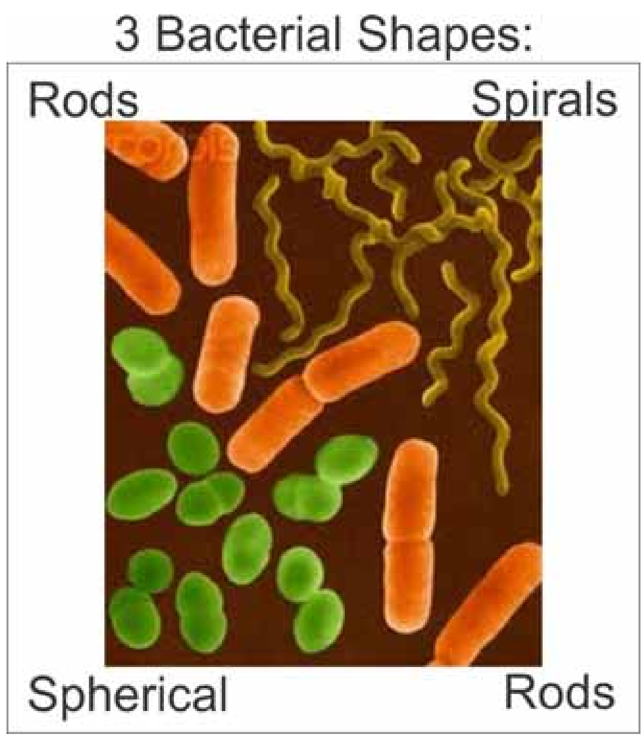
Enzymes are non-living chemicals produced by bacteria that help break down organic waste into a form more easily consumed by bacteria. Enzymes are catalysts that assist in conversion of material, but aren't consumed in the process (For more information, see our sheet, Bacteria vs Enzymes vs Chemicals).

Types of Microorganisms

We only manufacture bacteria. Bacteria are single-celled organisms between 1 to 5 microns in size (1 micron is 1 millionth of a meter). Bacteria have a cell wall, with no internal organs or body parts, and take various shapes. Bacteria are prokaryotes (no internal membranes or organelles).

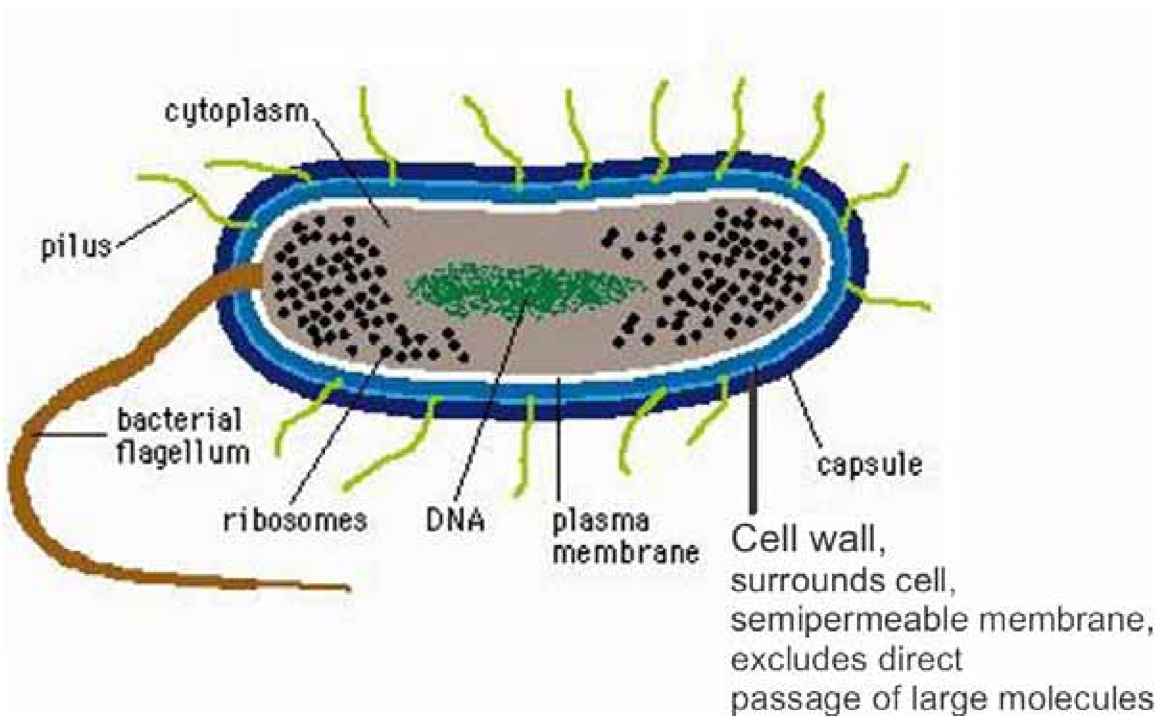
Classification of Bacteria Based on Shape

Bacteria have three common morphologies (shapes). Remember that the size ranges from about 1 to 5 microns (1 to 5 millionths of a meter).



Larger, more complex life forms involved in biological activity in water / wastewater include algae, protozoans, worms, and rotifers, which can be over 1000 microns in size (many times larger than bacteria).

Bacterial cell walls have pores that allow passage of small, low molecular weight, soluble material to the inside of the bacterium. Bacteria can't "eat" particles. The only way food can get inside of bacteria is by passing through the cell wall. This means that only very small, very simple foods can get inside of bacteria, and larger, polymers and particulates (such as sludge particles) are excluded.



Only simple, soluble material like sugars and amino acids can pass through the cell wall. More complex potential food sources, such as milk proteins, starches, and fats can't pass directly through pores. Sludge particles are many thousands of times larger than bacteria, and certainly cannot directly pass into the interior of the bacterium.

In order for the bacteria to consume these complex foods, the large, complex food must first be broken down into smaller pieces. This is done by special enzymes known as exoenzymes.

Classification of Bacteria Based on Functional Temperature

Bacteria that are active within a moderate temperature range are the type of bacteria manufactured by TLC Products. There are other bacterial types of course. The chart below gives a quick summary:

Cryophilic bacteria. Like cold temperatures (near freezing)
Mesophilic. Common in gardens, dirt, etc. Thrive between 50 to 100 F
Thermophilic. Survive hot temperatures in excess of 100 F.

All TLC Products bacteria are **mesophilic bacteria**. That means that they are functional in the range of about 7 to 43 C (or about 45 to 110 F). Outside of this temperature range, the TLC Products bacteria work either very slowly (slow below 45 F), or they are destroyed (above 110 F). We believe this to be true of all bacteria sold in other bacterial additives for aquariums, ponds, and septic tanks. We are highly skeptical of claims to the contrary.

Classification of Bacteria Based on Oxygen Requirement

Oxygen Environment

1. Anaerobic – live without dissolved oxygen. True anaerobic bacteria do not survive in the presence of dissolved oxygen. Disease-causing bacteria (pathogens) are often anaerobic bacteria.
2. Facultative – can function with or without dissolved oxygen. Bacteria in this class use SO4, NO3, etc. as alternative oxygen sources when dissolved oxygen is unavailable.
3. Aerobic – require dissolved oxygen to live and reproduce.

Anaerobic bacteria die in the presence of dissolved oxygen. Anaerobic bacteria are found in pond bottoms, in aquarium gravel and sand, and throughout septic tank systems.

Anaerobic bacteria are often pathogenic (cause disease), and some species produce hydrogen sulfide (H2S is an odorous, toxic compound, with a rotten-egg odor). Basically, in any healthy pond or aquarium, the anaerobic zones must be minimized. TLC bacteria work to digest the accumulated sludge in these systems. By getting rid of sludge, the anaerobic habitat is minimized. That means that the preferred habitat and potential for pathogenic bacteria, foul odors, and sulfide production is minimized.

Sulfide is the rotten-egg odor that one associates with stagnant ponds. Sulfide is not only unpleasant, but is harmful to fish. Digestion of excess organic sludge means less habitat for anaerobic bacteria in which to thrive, which means less sulfide, which is an important benefit we offer our customers.

Facultative bacteria can function either in oxygenated systems (with dissolved oxygen, or D.O.), or they can function when the oxygen is very low. When the oxygen is very low, facultative bacteria use another oxygen source such as NO3 (nitrate). Important biochemistry occurs in facultative conditions, and TLC bacteria include facultative bacteria as part of the formulation.

Aerobic bacteria require DO (dissolved oxygen) to function. Most of the TLC bacteria are aerobic. This means that they function well in aquatic systems that have at least some dissolved oxygen.

Classification of Bacteria Based on What They Eat

Bacteria can be broadly characterized as consumers of either organic or inorganic compounds.

In either case (organic or inorganic food), the food must be small, simple, and soluble before the bacteria are able to consume the food. Remember, the bacteria are very small, and are enclosed in a semi-permeable cell wall. Bacteria cannot "bite or chew" large substrate (or food). They depend on passage of food through the cell wall in order to acquire nutrients. Food that is too large must first be hydrolyzed (or solubilized into smaller subunits). After the larger food particle is converted into a smaller subunit, the bacteria can consume the food.

Examples of ORGANIC BACTERIAL FOOD

Some examples of organic bacterial food would be simple sugars, starches, cellulose, fats, or proteins. Typical pond pollutants such as dead algal mass, excess food, solid animal excretory waste, leaves, etc. are organic, and are too large to be consumed by bacteria directly. Due to their large size, these substrates cannot pass through the bacterial cell wall. Instead, they must first be solubilized into small, low molecular weight subunits before bacteria can consume them. This is an extremely important function provided by certain TLC bacteria (and produced only under certain conditions), and accounts for the sludge reduction and water purity in aquatic systems treated by TLC Products.

Examples of INORGANIC BACTERIAL FOOD

Ammonia is an inorganic nutrient that is excreted by aquatic animals, and often enters lakes and ponds through fertilizer run off, sewage infiltration, etc. Very specialized bacteria are required to consume NH_3 in large quantities. These bacteria are known as nitrifying bacteria. TLC Products's greatest strength is the special process by which we manufacture bacteria nitrifying bacteria and preserve them in a highly concentrated, liquid form.

Bacteria 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 TLC.

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 Minutes to Duplicate	**Number of Bacteria "B"** Takes 60 Minutes 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 hrs	**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 aquarium, pond or septic tank. This disparity becomes even more pronounced when there is a true limiting nutrient or micronutrient.

A very common question is: *"I already have bacteria in my aquatic system. Why should I add more TLC bacteria?"* This question is answered in detail below.

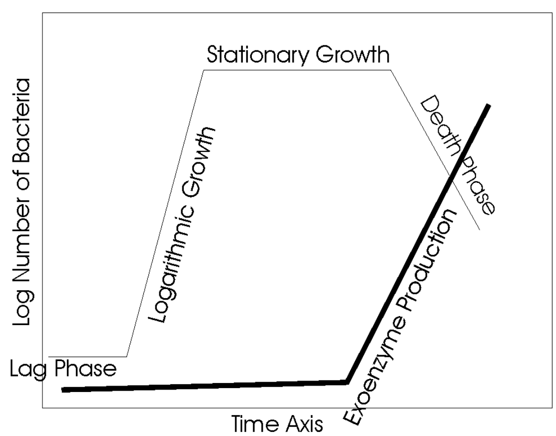
Exoenzyme Producing Bacteria

Simple, soluble food is readily consumed by bacteria. But in order to consume the larger organic food, bacterial 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. 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 (speed up) 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).

It is helpful to think of bacteria as the factory, and of enzymes as the end product. Certain bacteria are capable of producing the types of enzymes that digest large, complex particles and convert them into small, soluble compounds.

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



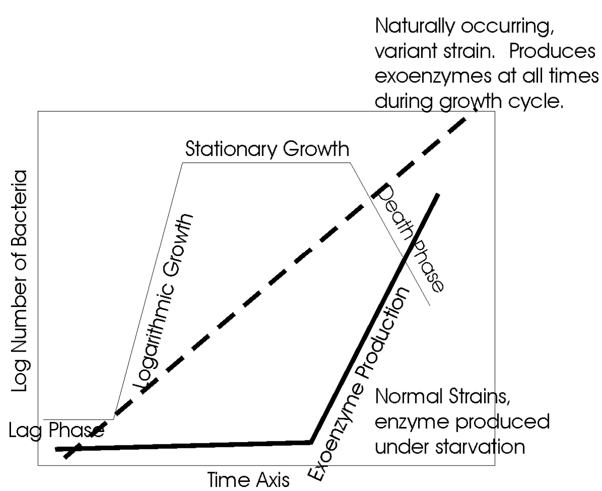
This chart shows typical, textbook progression that results from placing a nutrient solution into a flask, introducing air and bacteria, and then counting the bacteria and measuring the exoenzymes produced over time.

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).

As one option to improve exoenzyme production and reduce sludge build up, some TLC bacteria include specialized (but 100% natural and safe, Biosafety Level 1 bacteria) that literally 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:



TLC 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.

Second Method for Enhancing Exoenzyme Production

The first method we use to enhance exoenzyme production is 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 activation technology.

On-Site Bioreactor

In larger applications, we include use of an on-site bioreactor. The bioreactor is equipped with temperature control and vigorous aeration. To the bioreactor, 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 bioreactor, 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 bioreactors produce the required amount of exoenzymes needed to digest sludge.

What Happens to the Solubilized Sludge?

First, 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 is 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 aquarium, pond, or septic tank environment.

Even more important, the conversion of particulate sludge to soluble food is a 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 CO_2 and water, plus new bacterial cells.

As the entire bacterial population benefits from consumption of newly available soluble food, the bacterial population is better able to compete with various algal species for micro-nutrients. In many situations, treating solely for sludge reduction and odor control assists with algae reduction. 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 TLC Products products is used to consume any N and P released through sludge digestion. That is the subject of the next section.

TLC Products Nitrifying Bacteria

Our 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 excessive algae growth. Nitrifying bacteria consume NH_3, NO_2, and also consume large amount of Phosphate (PO_4). These nutrients are also items that algae consume very rapidly.

Key Point: Algae and nitrifying bacteria consume similar nutrients

Because nitrifying bacteria closely resemble algae in their nutritional requirements, the addition of large numbers of active nitrifying bacteria into ponds constitutes an effective algae reduction program.

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 ponds and aquariums is that NH3 is highly toxic to fish and other aquatic organisms. Aquarists are trained to carefully note the NH3 levels in their aquariums and ponds. 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).

As mentioned previously, the nitrifying power of TLC bacteria is independently recognized as world class. In the aquarium hobby, and in commercial aquaculture, ammonia concentrations are dangerous for the fish, and great measures are taken to prevent increases in ammonia levels.

NITRIFYING BACTERIA ARE NOTORIOUS FOR GROWING SLOWLY. In aquaculture systems, particularly intensive aquaculture, where high fish density prevails, water changes are performed when ammonia levels become high (because the nitrifying bacteria in that system do not grow fast enough to consume all of the ammonia produced). In the aquarium hobby, fish are not added to tanks until the system has had nearly a month to develop its' own population of nitrifying bacteria.

A unique solution is offered by our aquarium and aquaculture product, StartSmart Complete. Other companies offer nitrifying products that claim to assist in lowering ammonia levels in days rather than weeks. In contrast, StartSmart Complete, which contains the same nitrifying bacteria as PondPerfect, completely removes ammonia and nitrite from aquaculture systems in just one day. This result is unprecedented in the industry. And this result has been independently verified by the testing staff of the largest aquarium magazine in the world, Aquarium Fish International.

Cover image, Aquarium Fish International Magazine, September 2010 (pg 110)

The professional testing personnel of this prestigious magazine set up an evaluation with identical, high fish concentration loads, in test and control tanks. The test tank received the standard dose of StartSmart (the aquaculture version of PondPerfect), while the control tank did not. The control tank immediately showed ammonia concentration that reached lethal levels in 48 hours, with 100% fish mortality. The test tank showed zero ammonia increase, zero nitrite increase, and had zero fish mortality.

The study author, David Lass, commented as follows:
"StartSmart performed exactly as claimed by the manufacturer…it provided sufficient nitrification activity to instantly remove all toxic ammonia and nitrite. We have never seen anything close to this level of nitrifying activity in any other product, and we applaud TLC Products for this excellent tool for the aquarist."

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.

Nitrification and Denitrification

Denitrification is the facultative reduction of NO3 to nitrogen gas. This function is performed by many naturally occurring bacteria, and it has special requirements.

Denitrification is nearly the opposite of nitrification in many respects. The table below gives a good contrast between the two processes:

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

Algae thrive on the entire N series: ammonia, nitrite, and nitrate (NO3). 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 aquariums 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 aquarium, and the bottom of any pond.

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

Through denitrification, NO_3 (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 NO_3 is eliminated from the pond. This further reduces the food source for algae, which reduces the algae bloom.

Summary of TLC Action in Aquariums, Ponds, and Septic Tanks

- First, TLC nitrifying bacteria rapidly convert toxic ammonia (NH_3), into less toxic nitrite (NO_2), and finally into relatively non-toxic nitrate (NO_3).
- Second, TLC nitrifying bacteria are excellent at binding phosphate (PO_4). Phosphate is a well known algal nutrient.
- Third, TLC bacteria digest organic sludge and sediment. This reduces the anaerobic zones where pathogenic bacteria live. It thus reduces the potential for disease and foul odors.
- Fourth, by combining all of the actions above, TLC bacteria enhance denitrification as well. This means that much of the NO_3 is actually converted to nitrogen gas. Therefore, much of the N completely leaves the system.

TLC Products and Shelf Life

Some competitors will claim that nitrifiers in the "bottle" just die. That is simply untrue. One of our main patents involves the use of metabolic inhibitors to keep the nitrifiers dormant in the bottle.

As long as the product is in a capped bottle, TLC bacterial products have a two-year shelf life after departing our facility.

When TLC bacteria are added an aquarium, pond, or septic tank, the TLC bacteria are exposed to nutrients and become active within one hour of product addition.

Tender Living Care

Why Continue to Dose TLC Products After Initial Application?

It is true that the bacteria we supply are in fact common bacteria found in natural, untreated aquatic systems. However, the natural biochemical environment of an aquarium, pond, or septic tank 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 are common, and excessive algae blooms occur. These problems are typical, and prevalent. Through use of our 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 products, after some time expires, the other bacteria (the fast growing ones that were there 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 TLC bacteria such as StartSmart Complete, PondPerfect, or Septic Medic.

Generally, we recommend dosing between once a week to once a month. This is true in both small and large applications.

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