

One Step Beyond Nitrification

AZ-NO₃

(Absolute Zero - Nitrates)

Thirty Day Nitrate Remover

All Natural
Oxidation/Reduction
Enzyme Catalyst



DEALER INFORMATION REPORT

AZ-NO₃ is Trademarked in the U.S.
and in International markets.

R.A.I.A.R.

To All AZ-NO₃ Dealers

RE: Dealers Information Report

Dear Sirs

A well informed sales staff is elementary in the success of any business. In the aquaria industry, understanding the functioning and usage of the products offered for resale is of prime importance to the end consumer and his livestock. An informed dealer can increase his customer base exponentially by offering quality products and knowledgeable insight into the performance and expectations of that product.

Many devices have appeared on the market that claim to reduce and/or remove nitrates. Denitrification by anoxic nitrate respirators although viable, is very cantankerous, difficult to operate, and can become life threatening to the inhabitants in the aquaria, if not adjusted and monitored properly.

Of the various chemicals and denitrifying filters that have appeared on the market (some claiming instant nitrate removal), none have performed to the expectations of the aquarist and were a very costly disappointment to the consumer, possibly resulting in lost future sales from that customer.

All dealers can benefit by knowing the contents and/or functioning of the products they offer, and should be prepared with the proper answers to all questions that can be imposed by the consumer.

Toward this end, we have included a brief synopsis of the AZ-NO₃ product, how it is administered, what effects (both short and long-term) are noticed in the customers aquarium and an in-depth study on how the product functions. For those interested in a more technical approach, the basis for how our formulation was derived is included.

Very Truly Yours



Gary V. Deutschmann, Sr.
Reef And Invert Aquarium Resources

AZ-NO₃

(Absolute Zero - Nitrates)

SYNOPSIS:

The **AZ-NO₃** (Absolute Zero - Nitrates) product works entirely by aerobic Cellular Respiration (the oxidation, within the cell, of a food molecule, to give carbon dioxide and water as end products) on the target Nitrate molecule, which is then removed by a fully functional Protein Skimmer.

The oxidoreductases (which catalyze oxidation-reduction reactions) necessary for these processes are the chemical mechanism of enzyme action, which process is thoroughly explained in the **STUDY** article near the end of this report.

In the most simplest of explanations, **AZ-NO₃** can be considered an essential food product (one of the oligosaccharides, a carbohydrate) required by the active enzyme *maltase* that functions as a cellular respirator of each Nitrate molecule. The manufacturing process renders this product as an undesirable food to certain detrimental enzymes (amylase and invertase) that could uncontrollably proliferate in the closed environment of an aquarium.

Beneficial bacterium (like nitrobacter, nitrosominas, etc.) develop naturally in the aquarium without our assistance, other bacterium must be introduced artificially. Many products on the market claim to contain such beneficial bacteria "alive" in a bottle, while the most advanced hi-tech laboratories cannot keep the same bacteria *alive* for more than only a few minutes, or, a couple of hours on those rare yet hardy specimens.

Our product utilizes a specific enzyme in the maltase enzyme group to activate the oxidoreductases necessary to reduce nitrates by cellular respiration in the aquarium. Among our test groups, it was not uncommon to hear aquarists use the term, "*Im running a maltase aquarium*" when referring to their tanks using our product, and often referred to our product, in error, as containing that enzyme.

Once initiated on the proper diet, Maltase can and will become a naturally occurring enzyme within the aquaria, as would other undesirable enzymes if not inhibited. Our product does not contain Maltose, which is usually associated with the Maltase enzyme, nor does it contain the enzyme Maltase. Our product is more like a seed, a seed contains the germ (plant) and the food for the germ (plant) to grow and get a chance at life, which is then dependent on it's surroundings, namely the soil, sunlight and proper watering.

The activating ingredient in our product is Cozymase (a group of dormant, very hardy enzymes termed dehydrogenases) which function in the oxidation of proteins and of many other compounds important in the intermediary metabolism and the resulting active enzyme *Maltase* in the aquarium. The bulk of the product (Massequite), like the bulk portion of a seed, is merely food for both the Cozymase to develop and the Maltase to thrive. A descriptive of the Massequite is in the manufacturing process that renders the product distasteful to detrimental enzymes thus inhibiting their development and growth.

AZ-NO₃

(Absolute Zero - Nitrates)

DOSAGE:

The **AZ-NO₃** product is now packaged in a convenient flip top dropper cap dispenser bottle, which more easily facilitates the suggested **Safe-Start** method of product introduction to the aquarium. The **AZ-NO₃** product is completely Non-Toxic, thus the empty rinsed bottle can be reused around the aquaria for dispensing other aquatic use liquid products as well.

The proper denitrifying dosage of the **AZ-NO₃** product is 1/4 ounce per each 50 to 60 gallons of water in the aquarium system daily, until nitrates are undetectable, a period usually not exceeding 30 days, or the contents of the bottle. Larger aquariums will naturally require more of the product, but nitrates (even if as high as 100ppm) are normally reduced to zero within the same time period of 30 days. If an aquarium is over 100ppm, it may take slightly longer to reduce the nitrates to absolute zero.

Many aquarists are inadvertently introducing nitrates on a continuing basis, either through their water supply, food, additives, etc. therefore requiring that they maintain the enzyme activity at a low maintenance dosage holding the nitrates in check. Initially the maintenance dosage should be 1/2 of the denitrifying dosage, decreasing the amount of **AZ-NO₃** used until nitrates can be maintained at an undetectable level. The intent is to slowly wean the aquarium off of the product entirely, allowing the natural denitrification properties of the aquariums live rock or plenum to come into full functioning. The maintenance dosage can be cut to bi-weekly or even weekly additions as the live rock and/or substrate develops to its full potential.

An alternative method is to use 1/2 the recommended amount or less (see **Safe-Start** method on bottle label) for the first 7 days, then increasing to the proper dosage during the denitrifying period of usage and then slowly decreasing the maintenance dosage until the aquarium is weaned from the product. This alternative method not only extends the usefulness of the product, it also reduces the undesirable bloom effect mentioned under **SHORT TERM EFFECTS** on the next page.

Over the past several years, many aquarists have been utilizing the nitrate removing capabilities of the **AZ-NO₃** product to also remove undesirable algae growths from their aquaria. We did not formulate the product for such a purpose and although the product is quite safe for long term usage and does remove undesirable algae growths, it does also remove a slight amount of beneficial foodstuffs for many of the more delicate invertebrates.

Aquarists have experimented with varying and/or different higher maintenance dosages than suggested to keep these undesirable algae growths in check. I would not condone such usage of the **AZ-NO₃** product, however, there has never been any adverse effects reported from using the product for this purpose.

AZ-NO₃

(Absolute Zero - Nitrates)

EFFECTS:

AZ-NO₃ provides many other benefits to the aquarium besides nitrate reduction to Absolute Zero. Upon addition of the product to the aquarium, you will notice that the Protein Skimmer becomes more efficient and remains that way for approximately 30 days after the final use of the **AZ-NO₃** product. In addition, as the enzymes become active, all sediment and pre-filters in the aquarium remove larger amounts of organics than ever before, thus requiring cleaning more often.

SHORT TERM EFFECTS:

If the aquarist has never used an enzyme activating product in their aquarium, it is most likely that they will experience a short bacterial bloom, lasting normally from day number 3 through day number 5 of the program, indicated as a whitish haze in the water. The extent of clouding is dependent on many factors relative to each specific aquaria. The alternative start-up methods of using the product is recommended, to reduce the amount of cloudiness in the aquarium, as the beneficial bacteria proliferate and become active. If the customer's aquarium currently has an ionic imbalance, or undesirable enzymes already exist, a very prominent white haze will result. This clouding is harmless and will dissipate as the enzymes become active or the imbalance corrected.

Many aquarists will not notice any clouding or haziness in the water column because their aquarium is in perfect ionic balance and/or they have previously (even if unknowingly) used an enzyme activating product. Some frozen foods also provide certain enzyme activating compounds that keep the aquarium from hazing when using the **AZ-NO₃** product.

LONG TERM EFFECTS:

The **AZ-NO₃** product has been extensively tested in rigorous laboratory testing and scrutinized by home marine and reef aquarists for over 7 years now. No two aquariums are exactly alike, as a result the reports from each aquarist, members of the test panel, varied slightly in their observations of the same animals, time period to undetectable nitrates, start and length of cloudiness if present at all.

The only reports that could be considered detrimental consisted of certain animals showing a temporary negative reaction to the product's usage during, or immediately following the start-up phase. These include: Sea Mat, Green Star and Mushroom Polyps; Leather Corals (all species); Bubble and Elegance Corals; and a few Feather Duster Worms. However, in all cases, after the initial start-up period had passed, and the aquariums containing high levels of Nitrates were reduced to less than half of their initial Nitrate reading, the animals began to reopen and became more vibrant and luxurious than before using the product.

As expected, when conducting open tests consisting initially of over 100 amateur aquarists, some loss did occur during this 6 month period. However, the total loss was well below that of a smaller control group who did not use our product.

AZ-NO₃

(Absolute Zero - Nitrates)

STUDY:

In an effort to explain how the **AZ-NO₃** product functions, one must understand the enzyme, the catalyst, how they function and what reactions are produced.

Enzymes:

Enzymes are substances that cause and direct the numerous chemical reactions that occur in living organisms. Many chemical reactions will not proceed at an appreciable rate except in the presence of minute amounts of certain substances called catalysts. Enzymes are the catalysts of living cells.

In order to understand the full significance of this definition, it is necessary to grasp the fact that the living cell or the living organism may be regarded as a system of chemical reactions, beautifully integrated and co-ordinated, of extraordinary complexity, but nevertheless amenable to experimental investigation and to some measure of comprehension or understanding. Most of these chemical changes that occur in living cells are caused and controlled by enzymes.

Enzymes are proteins, they exhibit the physical and chemical properties of protein molecules. Enzyme molecules range in molecular weight from about 10,000 to Several Million, and they cannot pass through semipermeable membranes that readily allow the passage of water and other substances of small molecular size.

Enzymes show a remarkable degree of specificity for the substances on which they act (substrates). A particular enzyme catalyzes only one kind of reaction and acts only on one kind of substance or group of closely related substances. This high degree of specificity is one of the characteristics that sharply differentiates enzymes from other catalysts and allows the **AZ-NO₃** product to single out and remove nitrates from the aquarium.

The first step in the action of an enzyme is a combination of the substrate molecule(s) with the enzyme at the so-called active site. A chemical change then occurs, and the final step is the dissociation of the product(s) of the reaction from the enzyme. The high specificity of the enzyme is related to the fact that the structurally complex protein can combine in a particular way necessary for the catalytic action only with molecules that fit very particularized structural requirements.

Enzymes do not appear to undergo any net change during the reactions which they cause. This apparent absence of change is of course due to the fact that the reaction sequence involves a cyclic regeneration of the original enzyme, so that one molecule of enzyme reacts over and over again within a brief space of time, each time with a different molecule of substrate.

One of the Enzymes activated by the **AZ-NO₃** product is flavoproteins, which function in respiratory metabolism to transfer hydrogen and/or electrons from food molecules to oxidants such as molecular oxygen.

AZ-NO₃

(Absolute Zero - Nitrates)

STUDY Cont.:

When flavoprotein oxidase acts, two hydrogen atoms are removed from the substrate molecule and added to the flavin prosthetic group, thereby reducing it. The reduced flavin then passes the hydrogen (in the form of hydrogen ions and electrons) on to oxygen, after which the regenerated oxidized prosthetic group can act on another molecule.

As already mentioned, the enzyme molecule is large - usually much larger than the substrate molecule on which it acts. One might therefore suppose that one enzyme molecule could combine with and act on many substrate molecules simultaneously, but this is not the case. Current data show that there are only one or a few sites on the large enzyme molecule where the reaction with the substrate takes place.

In very simple terms, once the now large enzyme molecule oxidized the nitrate molecule, taking on part of the nitrate molecule, it has done its job and regenerated another to take its place. It is then filtered out of the aquarium via the Protein Skimmer, taking whatever contaminants it can along with it. The oxidation process that occurred, left behind only a small amount of carbon dioxide and water, the former of which escapes as gas from the aquarium. I might note that the amount of CO₂ released is too small to detect on a home test kit.

AZ-NO₃ (Absolute Zero - Nitrates) is the only product of its kind on the market, it is not instant, but does reduce 100ppm of nitrates (when used as directed) to Absolute Zero in 30 days or less. Higher nitrate levels will take a little longer, however, do not increase the dosage relative to the nitrate level in your tank, the product does not work that way. Whether your nitrates are 10ppm or 110ppm the product works by enzymatic action based on the volume of water in your total aquarium system and the performance of your Protein Skimmer to remove the molecule before it breaks back down through the normal nitrification cycle into nitrates again. Thus, you must have a properly adjusted Protein Skimmer for the product to perform at its best, successfully and rapidly.

Thank you for offering the **AZ-NO₃** product to your customers!

AZ-NO₃

(Absolute Zero - Nitrates)

TECHNICAL DATA:

Although we cannot disclose our exact formula, nor the processes involved in the manufacture of the **AZ-NO₃** product, we can give you the basis on which the **AZ-NO₃** formula was derived.

For comparison, we will show first, why the acid based products, tried by so many aquarists, seemed to begin to work and then failed miserably, then we will explain the unique base enzyme action formulation of the **AZ-NO₃** product.

It is well known that by combining certain reactions, it is possible to pass from any monosaccharide to one containing an additional carbon atom. As an example only; the addition of hydrogen cyanide to an aldose gives two products (nitriles) which on subsequent hydrolysis to the aldonic acid and appropriate reduction of the lactone (inner ester) of the acid yields the two higher aldoses.

In the case of aquarists who have used yeast or cane sugar in their aquarium, We'll use the cane sugar example for reference; Sucrose is hydrolyzed by the action of dilute acid (not found in the marine or reef aquaria), thus it's breakdown must occur slowly by the enzyme invertase. The end result being that the remaining chemical combination of sucrose with D-glucose and D-galactose provides a group of oligosaccharides (found to be detrimental in a closed seawater system), including melzitose, raffinose and gentianose, which are left behind in your aquaria to proliferate.

An entirely opposite procedure, or degradation of the nitrate molecule, can be accomplished by several methods (more suitable in the marine and reef aquaria environment). In one of these methods, the calcium salt of, for example, D-gluconic acid is oxidized with hydrogen peroxide in the presence of ferric acetate, whereupon the D-arabinose is formed.

In the marine or reef aquaria, the action of mild bases on the aldonic acids results in the inversion of configuration (2-epimerization) on the carbon (second) adjacent to the carboxyl function. Thus, a means is provided for the conversion (after subsequent reduction of "carbon one" to the aldehyde stage) of D-glucose into its 2-epimer D-mannose. This action of mild bases (as found in marine and reef aquaria) on the aldoses or ketoses leads to a mixture in which the ketose predominates, plus ketoses may also be synthesized from the next lower aldoses on the suitably substituted derivatives used in the **AZ-NO₃** base formula. This formulation resulted in a product that allowed a specific enzyme in the maltase group to proliferate in the aquaria, while discouraging the other undesirable acetic enzymes.