# Comparative Testing of Aquarium Cycling Additives:

**AquaLife ACTIVATE (Saltwater Formula) vs Dr. Tim's One and Only**

## I. Introduction

Establishment of a viable population of nitrifying bacteria is crucial to effective biological filtration in marine aquariums. The process of establishment, known as "cycling the tank," can be facilitated by the addition of cultured live bacteria that carry out the two-step process of nitrification. Ammonia, the primary form in which nitrogen is excreted by marine organisms, is first oxidized to nitrite by one group of bacteria, while nitrite is in turn oxidized to the relatively non-toxic nitrate by a second bacterial population. Particulate organic matter, dead organisms and uneaten food must first be broken down to simpler components by other bacterial species before the nitrogen pollution they contain can be processed by the biological filter. In this study, we investigated the ability of two commercial products for establishing the biological filter to oxidize ammonia, nitrite and organic matter.

## II. Methods

**Nitrification Potential**. The ability of each aquarium additive to promote nitrification was investigated in two laboratory experiments. In the first experiment, the total nitrification capacity of each product was measured. Identical solutions containing relatively high levels of ammonia were treated with product, aerated at 80°F, and tested daily for ammonia, nitrite, and chemical oxygen demand (COD). The experiment ran until the ammonia and nitrite were depleted in all reactors, indicating complete nitrification.

The composition of the incubation medium for the first experiment is shown in Table 1.

**Table 1: Incubation Medium for Experiment 1**

| Component | Concentration (ppm) |
|---|---|
| Ammonia ($NH_3$) | 65 |
| Magnesium sulfate | 5 |
| Ferrous sulfate | 5 |
| Casein (protein) | 50 |
| Soluble starch | 50 |
| Cellulose | 50 |
| Orthophosphate ($PO_4^{-3}$) | 1 |

The incubation medium was made with 90% distilled water and 10% tap water to add trace micronutrients. Finally, Instant Ocean™ was added to adjust the specific gravity of each test flask to 1.023. The incubation medium was placed in identical 4-liter reactors equipped with aeration. Reactor temperature was maintained by placing them in a water bath at 80°F. Sample portions of 50 ml of each product were added to each reactor. Aeration was maintained and ammonia, nitrite and chemical oxygen demand were tested daily for 12 days.

In the second experiment, the ability of each product to act as an instant aquarium cycling agent was evaluated. Identical 50 gallon reactors were set up, using the same incubation medium as in the first experiment, but without ammonia, protein, starch or cellulose (see Table 2). Beginning at time zero, product was added, and a peristaltic pump was used to deliver the equivalent of 0.1 ppm of ammonia per day to each 50 gallon reactor. All reactors were aerated at a temperature of 80°F, and tested daily for ammonia, nitrite, and COD. The experiment ran for 7 consecutive days.

**Table 2: Incubation Medium for Experiment 2**

| Component | Concentration (ppm) |
|---|---|
| Ammonia ($NH_3$) | 0 |
| Magnesium sulfate | 5 |
| Ferrous sulfate | 5 |
| Casein (protein) | 0 |
| Soluble starch | 0 |
| Cellulose | 0 |
| Orthophosphate ($PO_4^{-3}$) | 1 |

**Settleable solids test**. A well-mixed product sample was placed in a settling cone. The active ingredient (the bacteria) tend to clump together and settle out as solids in the bottom of the cone. The more solids that settle out, the higher the total bacterial population. This test measures both living bacteria and dead particulate matter in the sample.

**Bacterial plate counts**. These tests determine the actual numbers of live bacteria in a sample. Standard plate count procedures, similar to those used for municipal water analysis, were carried out on each sample.

**Sample sources**. Twelve 4 ounce packages of Dr. Tim's were obtained from a retail outlet. These were blended together to produce a 48 ounce sample. Mixing minimized variability that could theoretically exist from one package to another.

## III. Results

**Standard plate counts**. For the Dr. Tim's product, the standard plate count (SPC), performed in triplicate, was 500 million live aerobic bacteria per ml of product. For AquaLife ACTIVATE saltwater formula, the SPC was 2.5 billion live aerobic bacteria per ml of product.

**Settle-able solids test**. The Dr. Tim's product contained 100 ml/L settle-able solids, while AquaLife ACTIVATE saltwater formula contained 80 ml/L settle-able solids. These results are shown in Figures 1 and 2.

Figure 1: Dr. Tim's

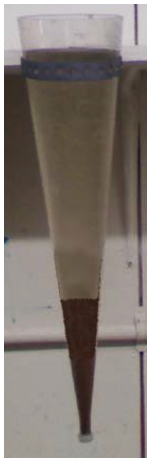
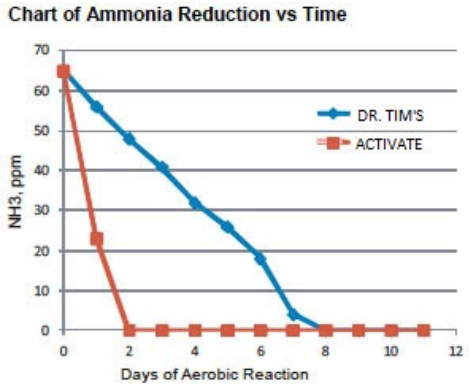


Figure 2: AquaLife ACTIVATE

**Nitrification Potential, Experiment 1**. Table 3 presents the test results from this experiment. These results are also presented graphically in three charts.

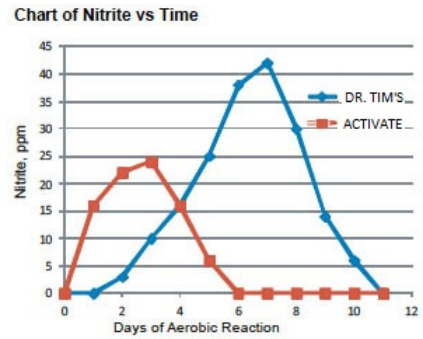
*Table 3: Absolute Nitrification Potential of Two Saltwater Cycling Products*

| Day | Ammonia (ppm) | | Nitrite (ppm) | | COD (ppm) | |
|---|---|---|---|---|---|---|
| | Dr. Tim's | ACTIVATE | Dr. Tim's | ACTIVATE | Dr. Tim's | ACTIVATE |
| 0 | 65 | 65 | 0 | 0 | 440 | 440 |
| 1 | 56 | 23 | 0 | 16 | 440 | 302 |
| 2 | 48 | 0 | 3 | 22 | 425 | 185 |
| 3 | 41 | 0 | 10 | 24 | 435 | 110 |
| 4 | 32 | 0 | 16 | 16 | 430 | 50 |
| 5 | 26 | 0 | 25 | 6 | 400 | 26 |
| 6 | 18 | 0 | 38 | 0 | 350 | 30 |
| 7 | 4 | 0 | 42 | 0 | 310 | 32 |
| 8 | 0 | 0 | 30 | 0 | 285 | 30 |
| 9 | 0 | 0 | 14 | 0 | 260 | 25 |
| 10 | 0 | 0 | 6 | 0 | 221 | 31 |
| 11 | 0 | 0 | 0 | 0 | 195 | 22 |



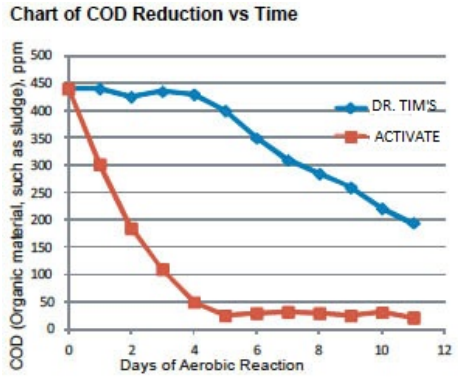

As is clear from the above chart, AquaLife ACTIVATE removed all of the ammonia within two days of reaction time. Since the starting ammonia concentration was 65 ppm, and it took two days to complete ammonia removal, the rate of removal was 32.5 ppm/day. Dr. Tim's took 8 days to remove all of the ammonia, or a rate of 8.1 ppm/day. AquaLife ACTIVATE was three times faster at removing ammonia Dr. Tim's.

The chart below shows the development of nitrite as the ammonia is oxidized:




When ammonia is consumed by nitrification, it is converted into nitrite. Nitrite is then converted into nitrate. Theory predicts that there will be an increase in nitrite from zero at the beginning, with a maximum reached at some point, followed by a decline towards zero as the system completely cycles. As seen in the chart above, this is precisely what occurred. ACTIVATE peaked at day 3 (about 25 ppm of nitrite), while Dr. Tim's peaked at day 7 (41 ppm nitrite). ACTIVATE reached zero nitrite at day 6, while Dr. Tim's reached zero nitrite at day 11. AquaLife ACTIVATE thus out-performed Dr. Tim's by about 2 to 1 in oxidizing nitrite.

The next chart presents our results for COD reduction:



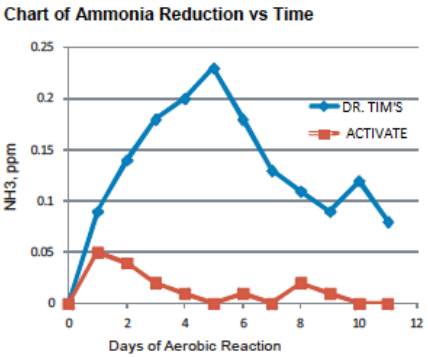

In this chart, ACTIVATE showed superior organic pollutant removal capability, reaching low levels by day 5. Dr. Tim's never achieved COD reduction to the same extent during the duration of the test.

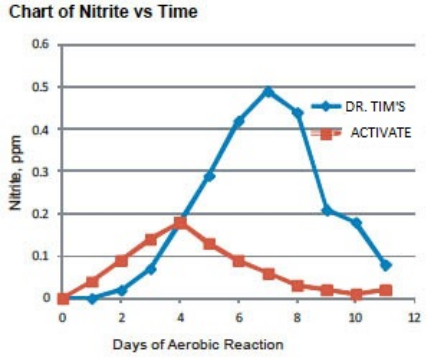
**Nitrification Potential, Experiment 2**. The data from this experiment are presented in Table 4.

*Table 4: Ammonia and Nitrite Oxidation Under Simulated Aquarium Conditions*

| Day | Ammonia ppm | | Nitrite ppm | |
|---|---|---|---|---|
| | Dr. Tim's | ACTIVATE | Dr. Tim's | ACTIVATE |
| **0** | 0 | 0 | 0 | 0 |
| **1** | .09 | .05 | 0 | .04 |
| **2** | .14 | .04 | .02 | .09 |
| **3** | .18 | .02 | .07 | .14 |
| **4** | .20 | .01 | .18 | .18 |
| **5** | .23 | 0 | .29 | .13 |
| **6** | .18 | .01 | .42 | .09 |
| **7** | .13 | 0 | .49 | .06 |
| **8** | .11 | .02 | .44 | .03 |
| **9** | .09 | .01 | .21 | .02 |
| **10** | .12 | 0 | .18 | .01 |
| **11** | .08 | 0 | .08 | .02 |

From the data, it can be shown that both products were effective in preventing accumulation of ammonia in the reactor vessel. These data are presented graphically in the charts below:




When ammonia is consumed by nitrification, it is converted into nitrite. Nitrite is then converted into nitrate. Theory predicts that there will be an increase in nitrite from zero at the beginning, with a maximum reached at some point, followed by a decline towards zero as the system completely cycles. As seen in the chart above, this is precisely what occurred with both products. The reactor treated with AquaLife ACTIVATE

reached maximum nitrite concentration on day 4 (less than 0.2 ppm nitrite), while the one treated with Dr. Tim's did not reach maximum nitrite concentration until day 7 (about 0.5 ppm nitrite).

*Technical Note: Calculation of Total Daily Feed of $NH_3$ Using Peristaltic Pump*

The stock solution contained 19 ppm (or 19 mg/l) of ammonia, and 1 liter of solution was pumped on a continuous basis to each reactor per day, the ammonia addition rate was as follows:

One liter of 19 mg/l solution added to 50 gallons, at 3.785 liters/gallon: 19 mg / 50 gal / 3.785 L/gal = 0.1 mg/l $NH_3$/day.

If no nitrification occurred, the ammonia level in each reactor would increase by 0.1 ppm each day, reaching 0.7 ppm by the end of a week.

## V. Conclusions

AquaLife ACTIVATE out-performed Dr. Tim's in all tests and experiments we carried out.

**Nitrification potential at high ammonia concentration**. AquaLife ACTIVATE oxidized 32.5 ppm $NH_3$ per day, requiring 2 days for complete oxidation. Dr. Tim's oxidized 8.1 ppm $NH_3$ per day, and required 8 days for complete oxidation. ACTIVATE is therefore 3 to 4 times more effective at $NH_3$ oxidation.

The reactor treated with ACTIVATE showed a nitrite peak at day 3 (25 ppm), and reached zero nitrite at day 6. The reactor treated with Dr. Tim's peaked at day 7 (41 ppm nitrite), and reached zero nitrite at day 11. ACTIVATE completely cycled 65 ppm of ammonia to nitrite then to nitrate in a little more than half the time, 6 days, compared 11 days for Dr. Tim's.

**Nitrification potential at simulated aquarium ammonia concentration**. The reactor treated with AquaLife ACTIVATE reached maximum nitrite concentration on day 4 (less than 0.2 ppm nitrite), while the one treated with Dr. Tim's did not reach maximum nitrite concentration until day 7 (about 0.5 ppm nitrite). Ammonia concentration in the reactor treated with ACTIVATE never rose above 0.05 ppm, while in the reactor treated with Dr. Tim's, the ammonia concentration reached a maximum ammonia concentration of 0.23 ppm before showing a decline. Thus, not only was ACTIVATE effective in about half the time, the reactor treated with ACTIVATE never reached an ammonia concentration that would be considered toxic to marine organisms.

**Organic pollution reduction**. From the chart, it is clear that AquaLife ACTIVATE is vastly superior to Dr. Tim's in removing organic pollution. It may be that Dr. Tim's does not contain bacteria designed to remove organic pollution, and in fact the product literature does not mention this function. However, for rapidly establishing a healthy biomass, the ability to degrade organic pollution is an obvious advantage for ACTIVATE.

**Product concentration**. AquaLife ACTIVATE was found to contain 5 times the concentration of live aerobic bacteria compared to Dr. Tim's product. This test counts all aerobic bacteria in the product samples. ACTIVATE includes sludge reducing, organic carbon consuming, and nitrifying bacteria. Dr. Tim's product claims to contain only nitrifying bacteria, and this could account for the observed difference.

**Settle-able solids**. Dr. Tim's contained 100 ml solids per liter, while AquaLife ACTIVATE contained 80 ml of solids per liter. This result is only indicative of the amount of material that settles to the bottom of the settling cone, and is not necessarily proportional to the amount of live bacteria in the product. However, the following observations can be made. The ACTIVATE product solids are amber to brown in color. The Dr.

Tim's product has a high solids content, and is composed of fluffy white material. This suggests that the bacteria in Dr. Tim's may be different from those in ACTIVATE. The volume of solids does not represent viable bacterial count nor the rate of nitrification of which the product is capable. Solids could consist of inert particulate matter.