

PUREBIOTICS AND MARINE LIFE HEALTH STUDY REPORT ON THE USE OF PUREBIOTICS CGI STABIOTIC PRODUCTS ON KOI FISH AT THE INTERKOI ARENDONK KOI FISH FARM



THE ENTITIES:

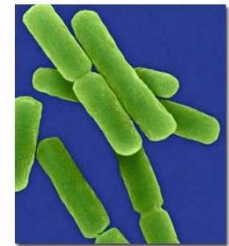
Known around the world, the interKoi Arendonk Koi Fish production facility in Germany is a major source and reference of quality in the field of Koi fish import and sales. Some of InterKoi's individual fish cost up to 50,000 Euros (\$75,000).



Therefore, to find the best solution to keeping these fish healthy, InterKoi researched the products available around the world and picked PIP (Probiotics-In-Action) specialized Stabiotic (probiotic) solutions. InterKoi spent months researching and testing the PIP microbiological products in progressively larger tests, with each stage using more fish and larger tanks.

Above is InterKoi's 2008 Grand Champion Fish

The months of testing were done on PIP's newest product release, PIP Pond PLUS. PureBiotics CGI's product line includes its Allergy Free Health Care products, innovative cleaning products and its animal care products for a complete line of animal care covering chickens, cow, pigs, fish and bees. The PIP products include breakthrough water treatment products based on Probiotic bacteria (see photograph). These bacteria have different functions, the most important ones are:



- To provide protection against harmful bacteria
- To provide deep biological cleansing, including biofilm.
- To build and sustain a healthy and stable microbiological community in both dry surfaces and water environments
- To combat odors
- To provide lasting hygiene

THE PROBLEM & NEED:



A great deal of infection causing micro-organisms (bacteria, fungi, viruses and parasites) can cause major trouble in any Koi (or other kind of fish) pond or breeding installation. On top of that, a lot of other micro organisms are responsible for the water quality. For example the nitro oxygen cycle and its benefits for the fish are controlled by the microbiological community in the water and filters.

Last but not least it is very desirable for the KOI enthusiast to actually see his fish swim in crystal clear water, which is not likely due to the fact that the micro organisms in that water have the tendency to keep developing causing the water to discolor. So it is of the utmost importance that the amount of organic material (food for the micro-organisms) in the water is kept to a minimum.

In order to get the above mentioned aspects to work and to be able to keep them this way for your pond, PureBiotics has developed Pond PLUS products in which the probiotic bacteria are responsible for:

- An optimal removal of organic material so that other, unwanted micro-organisms can not use it to manifest which leads to crystal clear water.

- Supporting the nitro oxygen cycle. The micro organisms which are responsible for this cycle will due to a lack of organic food use by preference ammonia and other sources for their energy production which leads to ideal water parameters.
- Sustaining healthy and hygienic water.

Because PureBiotics/CGI does not introduce a product into the market without thorough research and an alliance was formed with Interkoi Arendonk in order to prove the performance of these products and show the gained results are.

STUDY:

For a number of months in initial testing in a number of tanks at Interkoi in Arendonk tests were conducted on the effects of the Pond PLUS. The tests started with a shock treatment, meaning 1 liter of PIP product per cubic meter of water (1,000 liters). **Note, in later studies, PureBiotics found that there are reasons in specific cases that a shock treatment should never be used to be covered under separate cover..!** The evaluation's main focus has been:

- The bacterial composition of the water, more specifically the detection of:
 - o The total germ count as an indicator for organic stress.
 - o Entero/coliforms as an indicator for hygiene.
 - o *Aeromonas hydrophila* (cause of fin rotting) as an indicator of the level of pathogens
- The number of organic material through spectrophotometry
- The pollution levels of the filters (slime and algae buildup, visual detection)
- The clarity of the water through photometry
- pH and other relevant water parameters like hardness and ammonia
- the behavior of the fish and recovery of possibly already hurt fish.

The initial study had its start on the 6th of March for an initial 2 months. The initial test used two outside tanks of 38 m³ treated together with one inside tank of 14 m³ - plus one quarantine tank with sick fish of 2 m³.



UPDATE 10 MARCH 2008:

The results of the first water analysis, right before the addition of Pond PLUS are available. For the different sample locations the following codes are used:

BUR-W	Outside tank right – water
BUR-F	Inside tank right – filter
BUL-W	Outside tank left – water
BUL-F	Outside tank left– filter
BIV-W	Inside tank front – water
BIV-F	Inside tank front – filter
BIA-W	Inside tank rear – water
BIA-F	inside tank rear – filter
QUA-W	Quarantine tank – water
QUA-F	Quarantine tank – filter

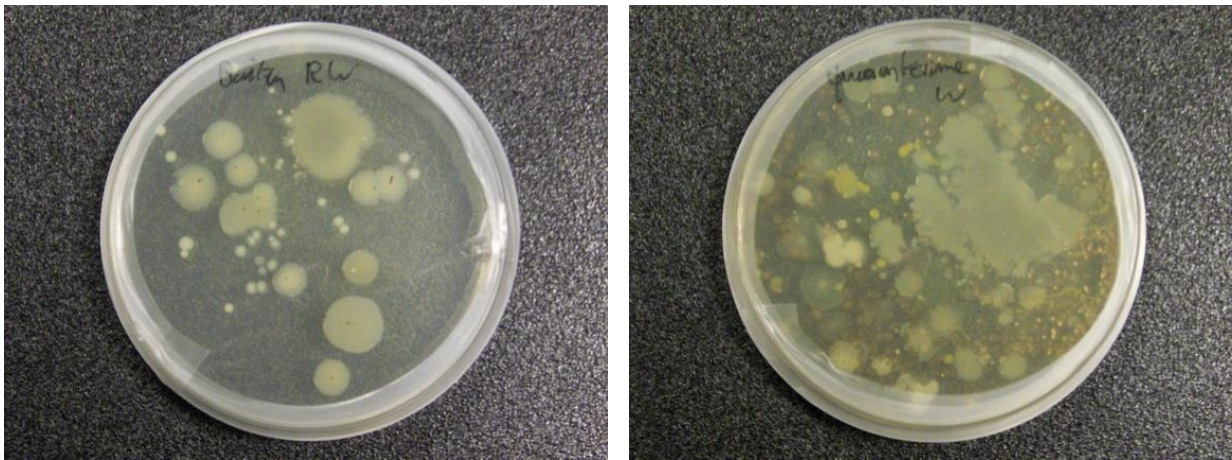
MICROBIOLOGICAL ANALYSIS:

Initially a few microbial parameters are determined, among which: total germ count stands for all the bacteria in total, coliforms are bowel bacteria and an indicator of the hygiene level of the water, Aeromonas are the cause of the rotting of the fins and an indicator of the level of infectious germs in the water. For the interpretation of the results we use a scoring system:

Score	Interpretation	Risk	Extra measure
0	Not found	None	None
1	Low amount	Very low	None
2	Moderate amount	Low	Water treatment recommended
3	Large amount	High	Water treatment necessary
4	Exceptionally large amount	Very High	Urgent intervention

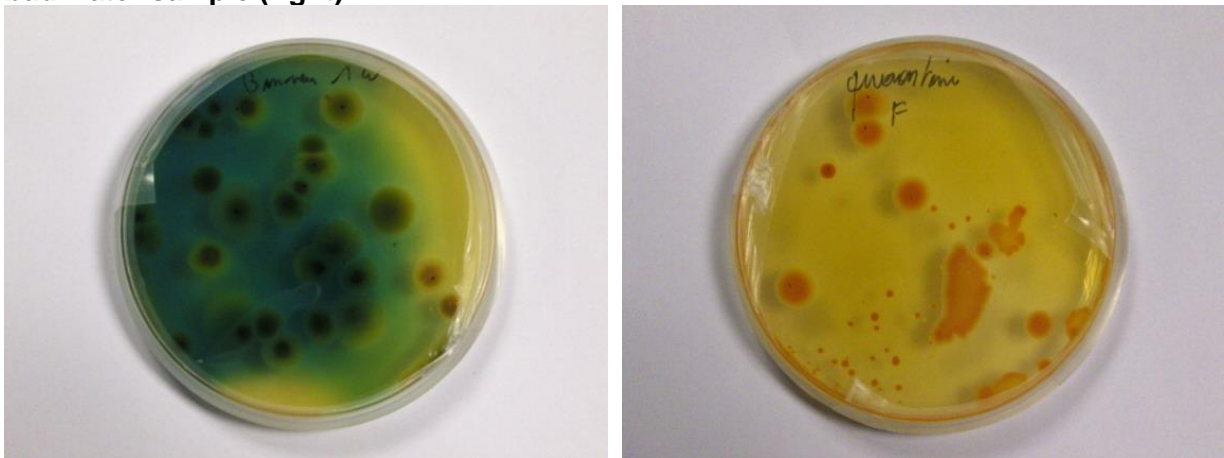
Sample	Total germ count	Coliforms	Aeromonas	TOTAL QUALITY
BUR-W	1	0	2	GOOD
BUR-F	1	3	1	AVERAGE
BUL-W	2	1	2	GOOD
BUL-F	2	1	2	GOOD
BIV-W	3	2	3	BAD
BIV-F	3	3	4	VERY BAD
BIA-W	2	2	2	AVERAGE
BIA-F	2	2	3	AVERAGE
QUA-W	4	3	2	VERY BAD
QUA-F	4	3	2	VERY BAD

In the upper chart it is obvious that a number of microbial parameters can be improved. Especially the quarantine tank has a bad microbiological water quality. Below you can find a picture of a normal bacterial growth in water (left) compared to a bad bacterial growth like in the quarantine tank (right):



Also it is noticeable that most water tanks deal with a reasonably high number of *Aeromonas* bacteria. Especially the quarantine tank has a high infection level with active *Aeromonas* bacteria.

This is clearly detectable on the specially selected growth mediums that are used for this; completely blue indicates the absence of *Aeromonas*; the more yellow you can see the more *Aeromonas*. The next pictures show a very good water sample (left) and a very bad water sample (right):



When we look for coliforms we don't see anything out of the ordinary. The outside tanks show a lower count due to their low temperature than the inside tanks. In none of the tanks we detect a concerning level of coliforms only the quarantine tanks need to be monitored closely. The acceptable values for coliforms are in alignment with the clear water and the low amount of organic material that can be found as shown through the measurements of the other water parameters.

WATER QUALITY:

In order to get an idea about the clarity and amount of organic material (BOD, COD) of the water, photometric measurements were done on the water samples. By doing this it became obvious that for all the tanks the water can be considered very clear. The gained optical density values (OD) all point out BOD and COD of less than 50 mg/ml. This matches with very clean water. The reason for these low results in regards to floating organic material is the high frequency of refreshing the water in the tanks.

The pH of the tanks is also determined and the following results are registered:

Sample	pH
BUR-W	8,01
BUL-W	7,91
BIV-W	7,90
BIA-W	7,97
QUA-W	7,80

These values are pretty high because the pH level of the spring water is 8,38.

CONCLUSION:

The analysis of the different tanks before the start of our study shows that the water in general is very clear with a high pH. Because of the clarity and the lack of microbial competition especially the *Aeromonas* bacteria have an open playing field. Especially in the warmer fish tanks the number of *Aeromonas hydrophila* is much too high and definitely pose a high infection risk for the fish. The main focus in this study will consequently be on these infectious germs.



Update 17 march 2008:

After the application of the product on Thursday the 6th of March with a ratio of 1/5000 the following was registered:

- foaming (too much to be comfortable)
- the fish react calmly to the application
- after 24h changes are noticeable with the sick fish in the quarantine tank.

The first 24h the water refreshing was disabled, after 24h this was enabled again and set at 60% per day. The foaming disappeared after 48 hours.

The initial plan was to add product weekly in a 1/10.000 ratio but because of the water refreshing system we decided on Tuesday the 11th of march to add daily 500 ml of product to each of the tanks. This matches an increasing concentration according to the microbial pollution of the tanks (less in the outside tanks, more in the quarantine tanks).

On Wednesday March 12th new samples were gathered. In regards to microbiology, water parameters and pH no significant changes were detected compared to the initial situation. In general the number of disease causing germs has lowered with 10% which is not enough in order to talk about an improvement.

The reason why the desired results were not reached is to blame to the much too high frequency of water refreshing. On top of that we only started with adding a daily dose the day before this measurement and thus no effect can be measurable. Because of these results it was decided to lower the water refreshing frequency to a max of 20% per week.

Analysis of the spring water show that this water does not contain *Aeromonas* nor coliforms and only a very low total count of bacteria. The real question is if the water contains enough nitro oxygen processors.

INJURED FISH PUT IN STABIOTIC PIP TREATED WATER TO TEST HEALING



Fish that normally take up to six months to heal from major injuries (see above) when put in PureBiotics Pond PLUS treated water develop a coating over the wound in the first few days and have been found to be fully healed in approximately six weeks.

UPDATE MARCH 21ST 2008:

Last week the decision was made to severely decrease the amount of water refreshing flushes and to add a daily dose in stead of a larger amount on a weekly basis. On Wednesday March 19th new samples were extracted from the stones in the upper filters of the water tanks.

GENERAL OBSERVATIONS:

In almost all tanks the water is turbid. This is most likely due to the combination of a lowered water refreshing percentage and the workings of the StaBiotic bacteria. These bacteria will release in the whole water system as well as the filters excess dirt which floats in the water. This causes the turbid water and has to disappear in the next faze.

The fish themselves still display an overall calm and healthy behavior. In tank 1 two fish were detected with the first symptoms of an Aeromonas infection, this has to be focused on and this also shows us that the problems regarding the Aeromonas definitely are not in the past yet.

MICROBIOLOGY OF THE SAMPLES:

For almost all of the samples a clear improvement is detectable on a microbiological level. With the StaBiotics bacteria becoming dominant and the number of disease causing germs lowering. Especially the coliforms show a spectacular decrease which shows us that the pollution of the water with bad bacteria is lowering considerably because the beneficial StaBiotics bacteria are becoming dominant.

Sample	Total germ count	Coliforms	Aeromonas	TOTAL QUALITY
BUR-W	1	0	2	GOOD
BUR-F	1	0	1	VERY GOOD
BUL-W	2	1	2	GOOD
BUL-F	2	0	2	GOOD
BIV-W	2	2	3	BAD
BIV-F	2	3	3	BAD
BIA-W	2*	1	2	GOOD
BIA-F	2	0	1	GOOD
QUA-W	2	0	2	BAD
QUA-F	2	0	2	BAD

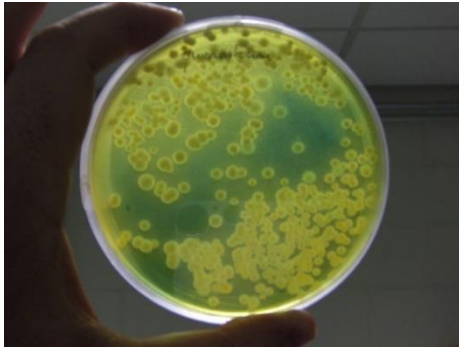
* With the total germ count of the rear inner tank it is noticeable that the StaBiotic bacteria is becoming dominant, but an unknown red bacteria shows its face, this can be a harmless bacteria but needs to be monitored closely.

PHOTO'S TO ILLUSTRATE THE MICROBIOLOGY:

Quarantine tank : Bacillus takes over (especially white colonies in stead of a multi colored mix)



Quarantine tank : Less yellow = less Aeromonas



Inside tank front : less yellow = less aeromonas



Inside tank front: Bacillus takes over



WATERQUALITY:

The other parameter stay pretty much the same, except for the turbid water (COD/BOD) which now balances around 100 mg/ml for most water samples. This by itself is not a problem and even protects the fish better but its not a desired effect for the fish breeder because it reduces the visibility. Like mentioned before this is released dirt from the filters which will be removed in the next faze of the study by the StaBiotic bacteria.

The pH balance of the water has lowered in general with 0,2 units:

Sample	pH
BUR-W	7,98
BUL-W	8,01
BIV-W	7,84
BIA-W	7,78
QUA-W	7,68

This decrease is the consequence of the lower in stream of spring water which had a high pH of 8,38.

CONCLUSION:

We notice in all water tanks and filters on microbiological level an improvement; there is no more sample that gets the score 'very bad'. In all samples its noticeable that the StaBiotic bacteria are becoming dominant and that they because of this are able to control a lot of other, potential bad bacteria. We do have the issue of the turbid water as mentioned before due to the releasing of the dirt which will cease to happen in the next week. The floating dirt will disappear and clear water will be gained.

UPDATE MARCH 27TH 2008:

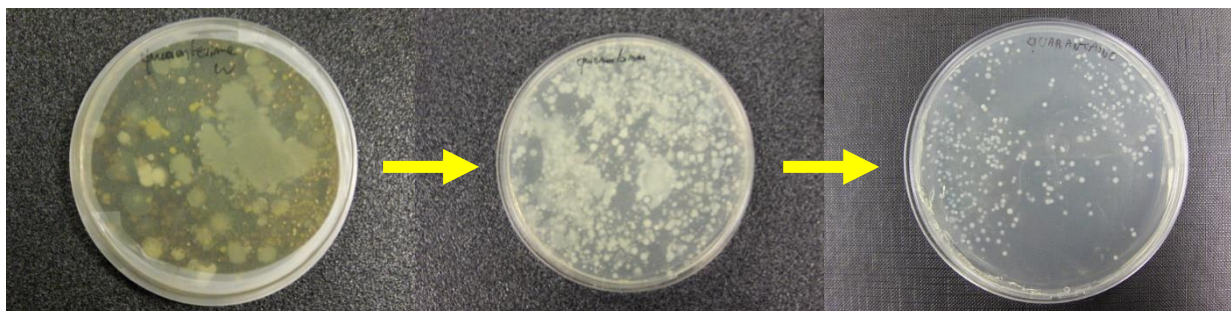
One more week into the treatment and the trends in the results keep persisting. We are still adding a daily dosage. Gradually we can see the StaBiotic bacteria gain the upper hand and a stable system is developing. Especialy the quarantine tank is improving considerably.

MICROBIOLOGY OF THE SAMPLES:

The trends of the last weeks keeps continuing; the good bacillus takes over and the coliforms and Aeromonas continue to lower. Because of this we see an improvement in the tanks and filters on a microbiological level. In most tanks the number of Aeromonas decreased even more (up to 30%) and the coliforms are almost nowhere to be detected. This points out a very good water hygiene. Only in the rear inside tank the number of the Aeromonas has stayed the same, most likely because the circulation pump malfunctioned for 2 days and everything kept floating around in the water.

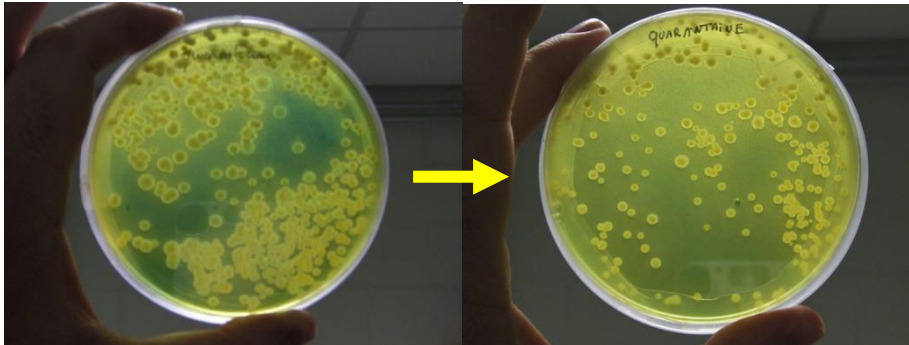
Sample	Total germ count	Coliforms	Aeromonas	TOTAL QUALITY
BUR-W	1	0	2	GOOD
BUR-F	1	0	1	VERY GOOD
BUL-W	2	1	2	GOOD
BUL-F	2	0	2	GOOD
BIV-W	1	1	1	VERY GOOD
BIV-F	2	1	2	GOOD
BIA-W	2*	1	2	GOOD
BIA-F	2	0	1	GOOD
QUA-W	1	0	2	GOED
QUA-F	2	0	2	GOED

As shown in the upper chart at this time there are no more BAD samples. All the water and filters have a good to very good microbiological quality! Especially the quality of the quarantine tank has improved tremendously. See the evolution below:



A very obvious evolution from a big mess of bacteria and fungus to a much lower number of good bacteria (from the products) is clearly noticeable.

Also the number of Aeromonas keep lowering, despite of the strong contamination coming from the sick fish themselves:



There are much less bacteria colonies (spots) seen on the growth media and also less orange colored.

GENERAL WATER PARAMETERS:

The most water parameters are unchanged, the pH stays the same for all samples. The clarity of the water has improved in the quarantine tank. The removal of the dirt in the water has clearly started and is expected to be totally gone by next week. Because the pumps broke down in the rear tanks the water has not cleared up.

FISH INJURIES:

According to Luc, who is a specialist, there is a noticeable improvement in the healing process in regards to Aeromonas wounds in the quarantine tanks. The usual healing process takes 9 months while now we detect a difference after only three weeks! It is too soon to draw any conclusions but we will follow up closely.

CONCLUSION:

On a microbiological level and the overall water quality the condition of the water samples is at this time good to very good. Only the turbid water in certain tanks still has to clear up which should happen within the next week. If so, we can speak of a very stable and secure water system which from that point on can be maintained with a lower dosage of product.

UPDATE APRIL 3RD 2008:

The samples of Wednesday April the second show another improvement of the water quality in microbiology, pH and clarity.

MICROBIOLOGY OF THE SAMPLES:

For all the water samples again an improvement has been detected, especially in regards to the Aeromonas. The filters stay pretty much the same.

Sample	Total germ count	Coliforms	Aeromonas	TOTAL QUALITY
BUR-W	1	0	1	VERY GOOD
BUR-F	1	0	1	VERY GOOD
BUL-W	2	1	1	VERY GOOD
BUL-F	2	0	2	GOOD
BIV-W	1	0	1	VERY GOOD
BIV-F	2	1	2	GOOD
BIA-W	1	0	1	VERY GOOD

BIA-F	2	0	1	GOOD
QUA-W	1	0	2	GOOD
QUA-F	2	0	2	GOOD

Visually there are not many more improvements detectable on the growth media because the good bacteria of the products now totally dominate. The pictures of last weeks results are still representative for what was seen this week. Only the number of colonies on the growth media has lowered once again in regards to the coliforms and Aeromonas. Also in the ponds where the BIO-UV lamps were used we continued to find our good bacteria which proves that both systems can be combined.

HEALING OF THE FISH:

Both Luc and Rini closely monitored the healing of the fish in the quarantine tank where they observed an accelerated healing process of the wounds. Also Dr. Maarten Lammens, 'Koi Doctor' was brought in to help monitor this aspect closely.

CONCLUSION:

We can definitely claim that all waters and filters are of good to very good quality with a low infection risk in regards to Aeromonas and other infectious germs. Despite the heavy contamination of the quarantine water by the sick fish (= constant source of Aeromonas) also this water stays stable at the level of good (coming from very bad 4 weeks ago). An additional improvement will be hard to reach in the upcoming weeks because the water quality is optimal right now.

UPDATE APRIL 14TH 2008:

The trend we are measuring the last weeks continues, the number of disease causing germs keeps lowering even though the decreases are less strong than previously measured due to the fact that we are almost at an optimal effect already. There will always be a minimal amount of infectious germs in the bowels of the fish and in certain parts of the filters. This rest fraction is desirable in order for the fish to keep building resistance.

MICROBIOLOGY OF THE SAMPLES:

The scores of the samples stay the same as last week:

Sample	Total germ count	Coliforms	Aeromonas	TOTAL QUALITY
BUR-W	1	0	1	VERY GOOD
BUR-F	1	0	1	VERY GOOD
BUL-W	2	1	1	VERY GOOD
BUL-F	2	0	2	GOOD
BIV-W	1	0	1	VERY GOOD
BIV-F	2	1	2	GOOD
BIA-W	1	0	1	VERY GOOD
BIA-F	2	0	1	GOOD
QUA-W	1	0	2	GOOD
QUA-F	2	0	2	GOOD

Here are a group of graphs from the first 6 weeks of testing of the different water tanks.

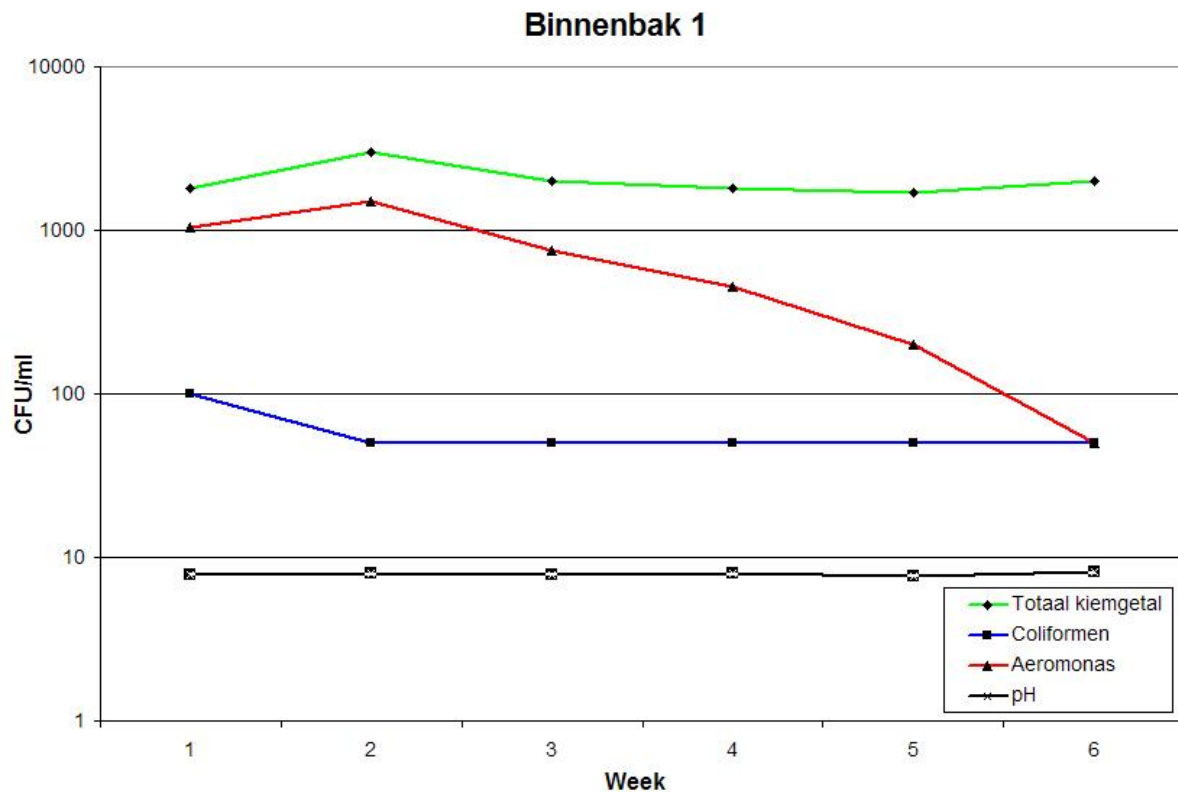
Here also you can see constantly the same trend: the total germ count maintains a stable level because the StaBiotic bacteria become dominant.

Both coliforms and Aeromonas lower strongly while the pH level stays pretty much the same. With the coliforms we often see a short raise after the application of the product, because these are being detached from the filters and end up in the water.

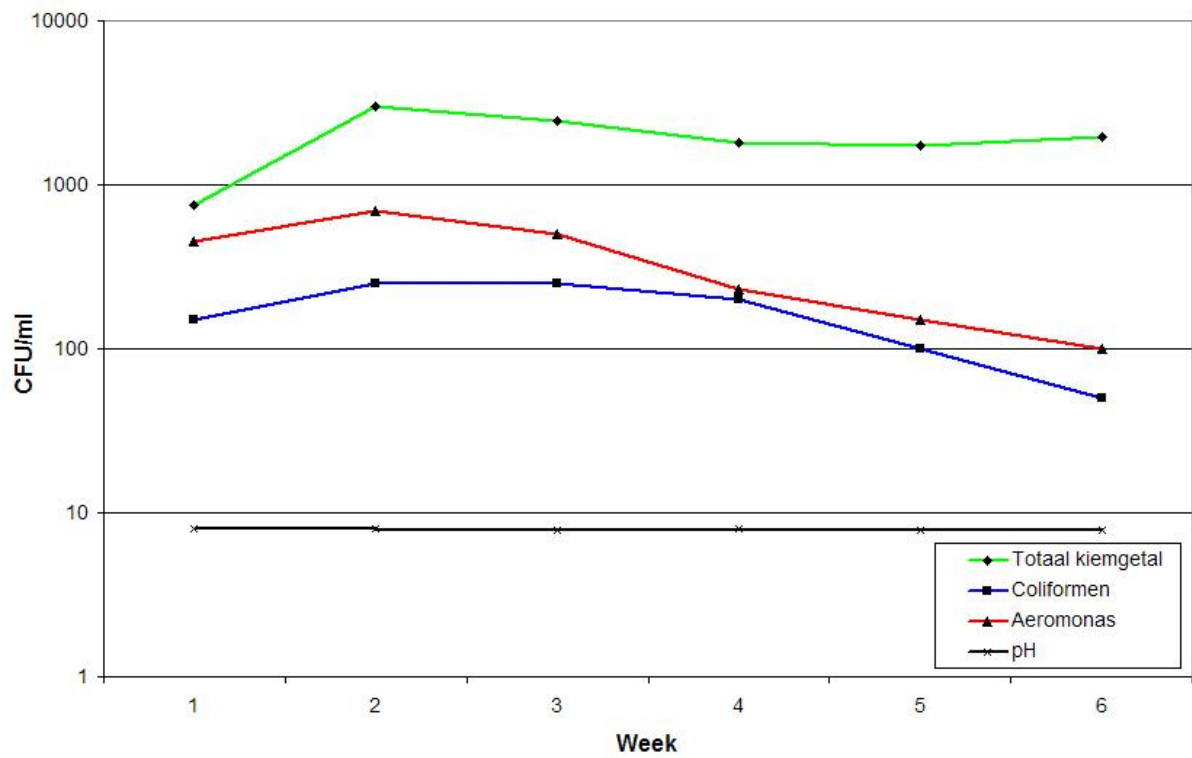
Two weeks after the start of the treatment with our products the water becomes stable on a microbiological level.

The following graphs are logarithmic, the scale in the y-axis leaps in ten fold, because of this a lowering looks less strong, but the numbers indicate that with out a doubt strong decreases are detected in regards to infectious germs.

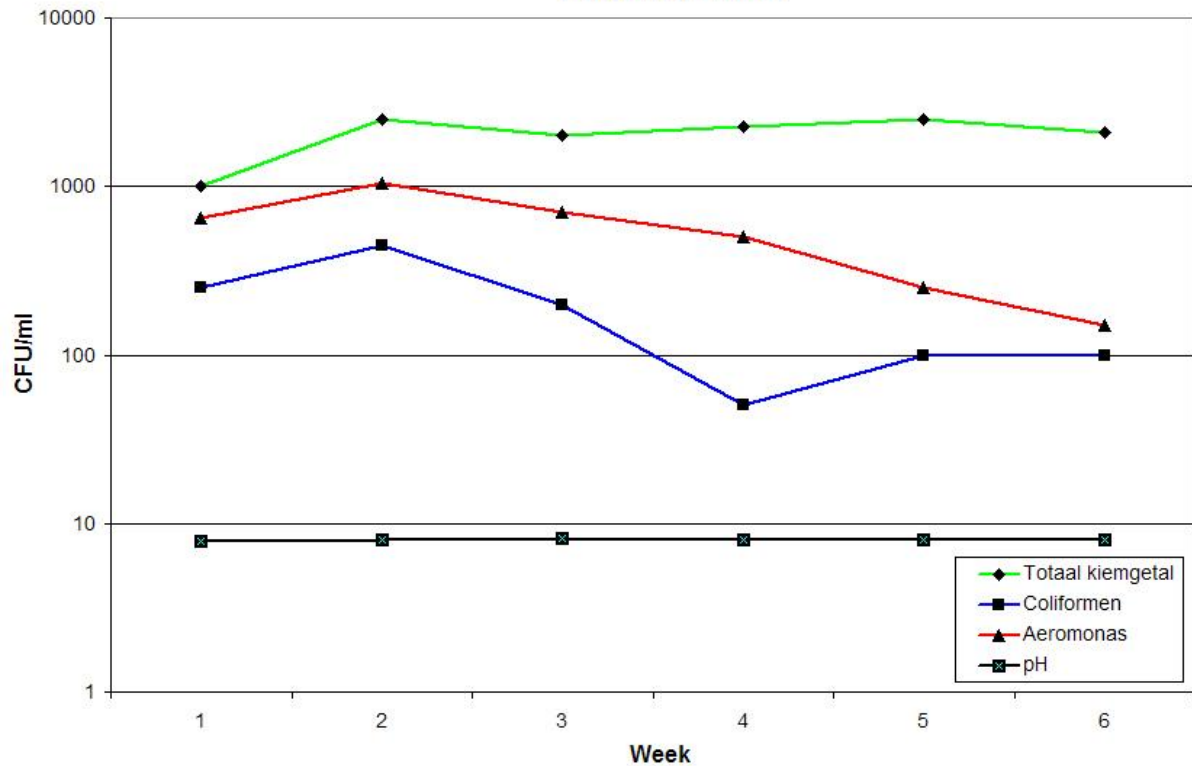
Very often we do not measure anymore coliforms, but since the detection limit of the used measurement system is 50 CFU/ml we conclude that this is a minimum amount that can always be present.

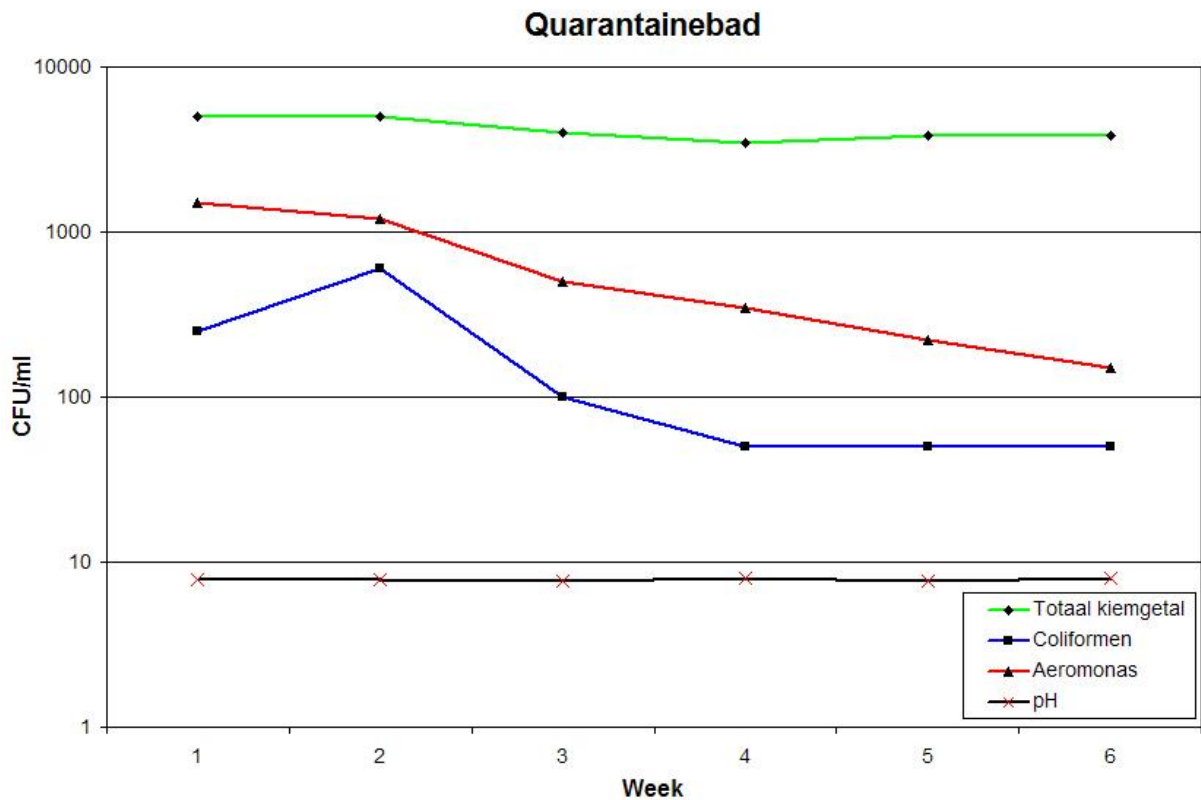
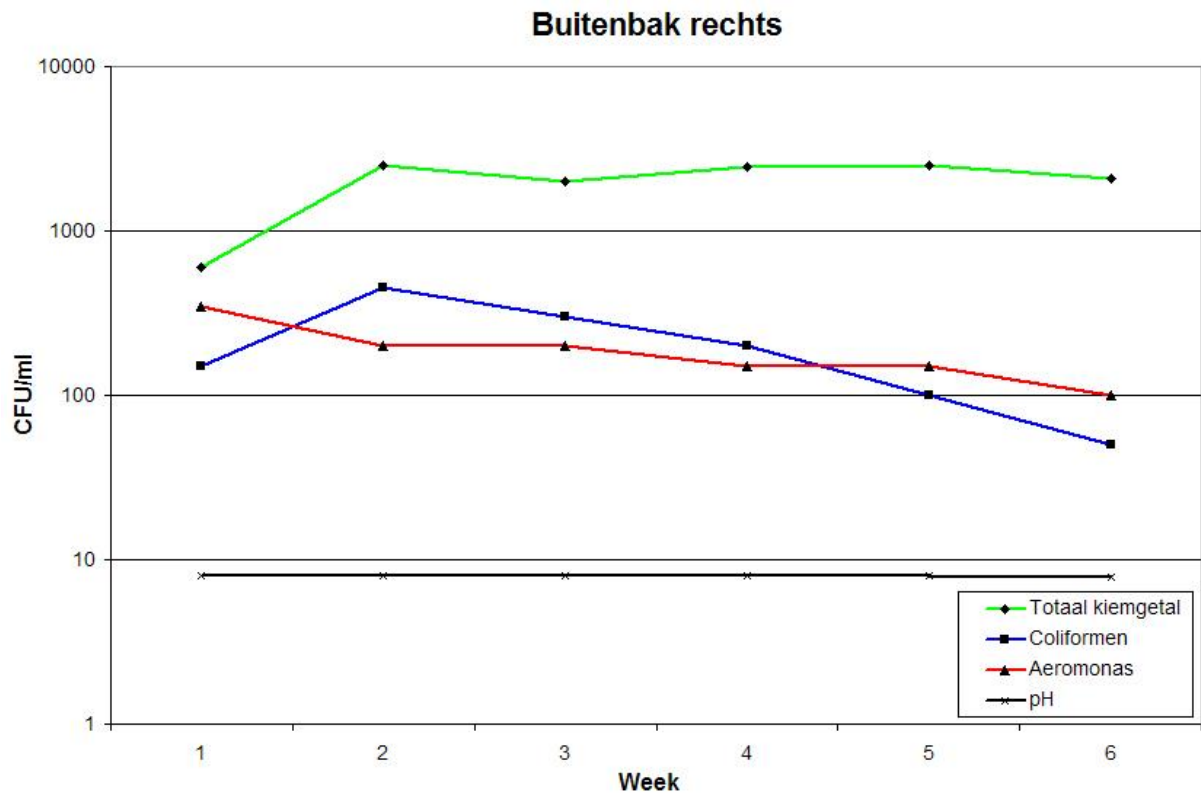


Binnenbak 3



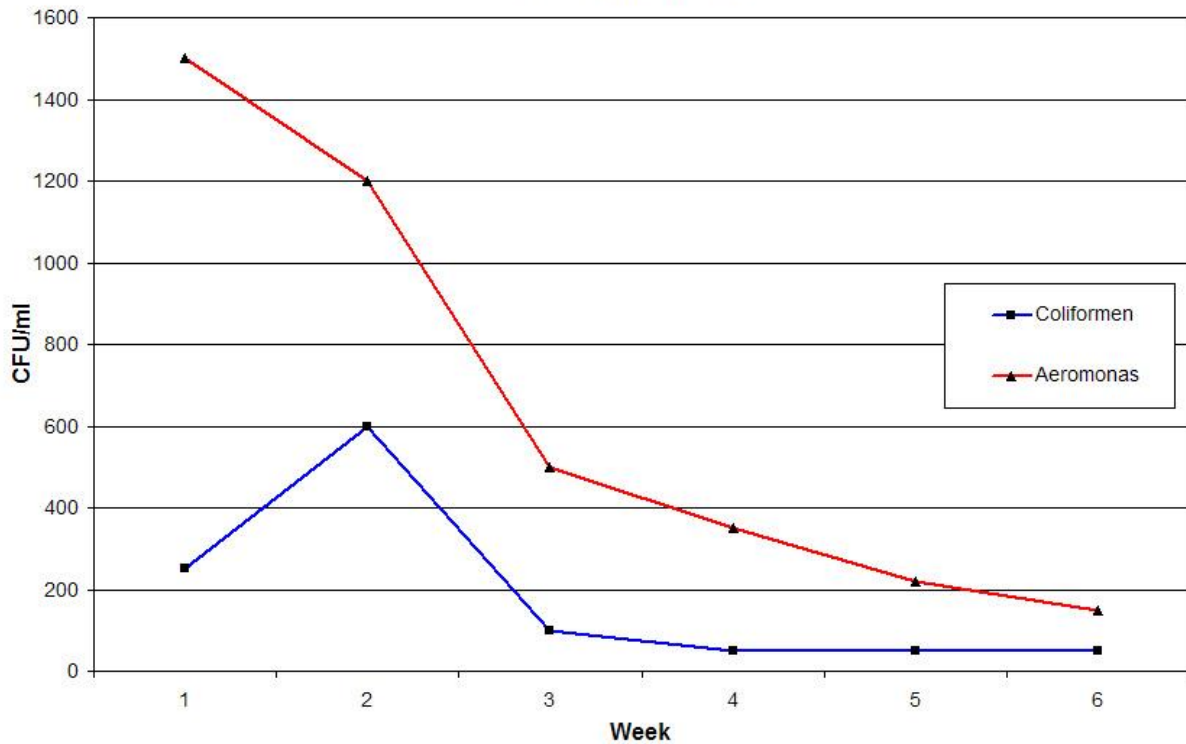
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When we put the evolution of the water in the quarantine tank into a normal graph we can see how strong the decrease of the infectious germs really is:

Quarantainebad



CONCLUSION:

WITH THE USE OF STABIOTIC PIP IN THE INERKOI FISH FACILITY TANKS, THE WATER BECOMES A STABLE AND HEALTHY MICROBIOLOGICAL COMMUNITY.

BECAUSE OF THIS, THE FISH ARE FAR LESS STRESSED AND ARE FAR MORE ABLE TO CONCENTRATE ON THEIR DEVELOPMENT (GROWTH) INSTEAD OF HAVING TO FIGHT OFF INFECTIOUS AND HAVE THEIR ENERGY DRAINED BY THE TANK'S OLD ENVIRONMENTS.



