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		Schulte	-441	-84	21.01.2010

" Investigation of the influence of the AQUABION® Active anode system and the principle of the DAT Aquainject on biofilm formation" and of the "Use of the AQUABIOREAKTOR for degradation of organic substances in the water phase"

- Report related to quote 10275/2009/21100 -

Summary

The DAT Dynamic Aquabion® Tower GmbH (DAT) company the AQUABION® -System for hardness stabilisation in water systems. The system is based on the cathodic corrosion protection, with the active anode being made of pure zinc. DAT postulates that the addition of zinc (zinc ions) to the water affects the covering layer formation and hence the biofilm formation as well. The calcite is converted into aragonite, which has a lesser tendency to adhere to surfaces.

To check the influence of the AQUABION® Active anode system, two rotary piston reactors were used, which were run in a closed circulation system, based on the actual conditions in practice, in selected cooling circuit systems at the IWW. The AQUABION® -system was implemented in the circulating system of one reactor; the second reactor served as a control or reference. In the water phase of the reactor system with AQUABION®, the zinc concentrations were measured.
They showed that the active anode system released zinc (zinc ions) over the entire test period. In characterising the biofilm, it was found that after four weeks, there as an accumulation of zinc from the AQUABION® system in the biofilm.

The effect of the AQUABION® mainly influenced the formation of the biofilm, less the bacteria concentration in the water phase. It could be shown that

- the hose that was installed in the system with AQUABION® was visibly less incrusted after a test period of 5 weeks than the hose from the control system. In addition, it was clear that the incrustation in the hose of the control system was coloured yellowish-brownish, whereas the biofilm in the AQUABION® was whitish in colour. This clearly showed that the biofilm in both systems is not just different in the thickness of the film, but also in composition,
- in the first two test weeks, in both test systems, short rods dominated, which were adhered homogeneously to the coupon surface. From the third test week onwards, bacterial aggregates formed, and the occurrence of protozoa was demonstrable. One important difference was the presence of filament-like bacteria only in the control system. Owing to the networking of the filament-like bacteria, biofilms that contain them generally have a higher stability,

- the cultivability of the cooling water bacteria in the AQUABION® -system was less by at least the factor 10 (after one week of testing duration, by a factor of 1000),
- the number of cultivable *Legionella* spec. in the AQUABION® system was smaller by at least a factor of 10 after 2 weeks, and after 5 weeks test duration, by a factor of 1000 than in the reference system without AQUABION®,
- there was a reduction of the cultivability of the biofilm bacteria by zinc (ions). This reduction could also be demonstrated in the water phase for the cultivability of *Legionella* spec.

When running the two reactor systems, it was noticed that more flakes sedimented in the preparation with the AQUABION® ,than in the control reactor. The chemical examination of the precipitate showed that the flakes from the two test systems had very similar compositions. The calcium concentrations were similar at 56.6 and 56.7 mg/l. There was a large difference only in the concentration of zinc in the precipitate: whereas zinc was not detectable in the precipitate from the control reactor, the flakes from the AQUABION® Active anode system contained 14 mg/l zinc.

Following the investigations with AQUABION® , the microbiocide effect in the control reactor after addition of iodine and zinc was checked according to the DAT-principle of the Aquainject on the formed biofilms. It could be demonstrated that

- in the water phase, the treatment resulted in an increase in the total cell number (acquisition of all bacteria, living and dead), but a significant reduction of the total forming units number (acquisition only of the multiplication-capable, cultivable bacteria). *Legionella* were not culturally detectable even after just 2 days of treatment.
- the treatment resulted in only a minor detachment of the biofilm.
- the cultivability in the biofilm reduced far less than in the water phase.Cooling water bacteria and *Legionella* could be detected culturally in the biofilm even after one week of treatment .

In a separate test setup, the degradation of organic substances by the AQUABIO-REAKTOR was investigated. By means of the biomass that had established in the reactor, nutrients should be locally degraded and as a result, reduce the organic load in the cooling water. It was demonstrated that

- the use of the AQUABIOREAKTOR results in a degradation of the organic substances in the water phase.
- the test results are reflected both in the determination of the CSB content as well as in the determination of the DOC-content.
- an inflow of oxygen is favourable for the fast degradation of organic substances.
- lower concentrations of Saccharose are degraded more effectively (within a shorter time) than higher concentrations.
- upon using 27 g Saccharose, the AQUABION®-system delays the degradation of the organic substance by about 2 days. This could be a pointer to the fact that the zinc ions introduced by the AQUABION® first hinder the biomass in its metabolic activity, but after 2 days, an adaptation of the bacteria to the increased zinc concentration took place.
- under these test conditions, there was no reduction in the total forming units number. The reason for this is probably that the forming units numbers in the water reflect the biofilm formation in the reactor owing to the large surface-to-volume ratio in the reactor.

Outlook

The investigations carried out clearly demonstrated that the use of the AQUABION® delayed the biofilm formation in the period of investigation as compared to the control reactor. In the selected testing period, the tests could not show whether the effect only occurs in the range of the initial weeks, and levels off thereafter, or can be sustained over a longer period of months. Therefore, further investigations of the effect of the AQUABION® Active anode system over a longer period of time would be meaningful.

Especially the cultivability of *Legionella* spec. was significantly lower in the test system with AQUABION® both in the biofilm as well as in the aqueous phase.
Information on the possible physiological state of the non-cultivable bacteria could be obtained from the FISH gene probe technique. This molecular-biological method acquires only the metabolically active bacteria, which need not, however, be capable of multiplying. To what extent the *Legionella*, which are treated with the AQUABION®, can be in a metabolically active, but non-cultivable state, will be the subject of further research work.

The results of the investigation of the AQUABIOREAKTORs showed that the test conditions selected were not optimal. For example, to check whether the use of the AQUABIOREAKTOR resulted in a reduction of the forming units numbers in the water phase, the test would have to be repeated with a greater volume of water.

We would like to express our thanks for the order and the constructive collaboration. Should there by any questions that arise, we are at your service at all times.

IWW Rheinisch-Westfälisches Institut für Wasser
Beratungs- und Entwicklungsgesellschaft mbH

i.V.

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Table of Contents

1 Introduction and Background

The microbiological and chemical quality of cooling and process water has a significant influence on the operational safety of the plant. Thus, for example, an excessive deposit of lime scale in evaporation cooling devices results in a reduction of the cooling efficiency. Thick lime scale deposits as well as the generation of corrosion products can, moreover, favour the deposition and multiplication of micro-organisms on the surfaces. The solid formation of biofilms on surfaces, e.g. of heat exchangers can result in faults during operation of the plant, and also, hygienically relevant micro-organisms can multiply in biofilms. This includes the *Legionella*, which are transferred to humans by the inhalation of aerosols entering the lungs. These bacteria find a habitat in the biofilms of many water systems, from where they contaminate the water and then get into the surroundings at places with aerosol formation. *Legionella*-containing, airborne aerosols can cause serious harm to health. In recent years, there have been reports from a few countries of spectacular outbreaks of disease through *Legionella* in aerosols from industrial cooling towers as a source of infection, causing serious illness and death in the population.

The DAT Dynamic Aquabion® Tower GmbH company uses the AQUABION® Active anode system for hardness stabilisation in water systems. The system is based on cathodic corrosion protection, with the patented active anode being made from highly pure zinc. DAT postulates that the addition of zine (zinc ions) to the circulating water has an effect on the covering layer formation and hence the bio-film formation. The calcite is converted into aragonite which has a lesser tendency for adhering to surfaces.

The DAT company plans to deploy the AQUABIOREAKTOR in some cooling water systems, to decompose organic materials locally through the biomass established in the reactor and thus reduce the organic load in the cooling water.

With this background, the DAT Dynamic Aquabion® Tower GmbH entrusted the IWW Rheinisch-Westfälisches Institut für Wasser - Beratungs- und Entwicklungsgesellschaft mbH, with their letter dated 10.09.2009, to investigate the influence of several products on biofilms in a study. In another test, in the laboratory and based on the conditions in practice, it was to be examined how effectively the AQUABIOREAKTOR can reduce the organic load in the cooling water.

2 Test Set-up, Material and Methods

2.1 Influence of the AQUABION® on Biofilm

To investigate the influence of the AQUABION® on biofilms and especially on bacteria of the type *Legionella* pneumophila, rotary piston reactors were employed (Figure 1).

The biofilm reactors were run based on actual practical conditions in cooling water systems

- in the circuit (volume 5 l)
- at 30°C
- with a nutrient concentration between 2 and 5 mg/l, added as a CASO solution and measured as dissolved organic carbon (DOC),
- a salt concentration of approx. 700 µS/cm measured as the conductivity and
- with a pH-value of about 8.

The two reactors required for this purpose (control reactor without AQUABION® and reactor for the continuous use of the AQUABION®) were run for 5 weeks, to be able to evaluate the influence of the system. The connections of the AQUABION® technology to the test reactor were made in collaboration with the customer.

The water of both the testing systems was injected with a mixed culture of bacteria that originated from an actual cooling water system, in a concentration of 10^6 bacteria/ml. In addition, bacteria of the type *Legionella* pneumophila were added in a concentration of 10^2 bacteria/ml.

2.2 Influence of the Principle of the DAT Aquainject on Biofilms

After the five-week trial, the biofilms in the control reactor were run with a continuous dosing of a combination of zinc and iodine according to the principle of the DAT Aquainject for a period of one week. For this purpose, two tanks connected in series with the inflow were filled with 27 g zinc and 27 g iodine respectively.
In the rotary piston reactors, there were removable test surfaces (called coupons), which formed an integral part of the outer cylinder wall. The microbial colonisation of the pipe and heat exchanger surfaces was simulated in this manner. Additional microscopic, microbiological and chemical investigations were carried out on the coupons. Thus, a separate evaluation of the action of the process used with regard to the biofilm dissolution and/or destruction could be carried out.

This differentiation was also very important for the evaluation of the efficacy of the AQUABION® against biofilm bacteria, especially *Legionella* pneumophila.
For evaluating the influence of the AQUABION® Active anode system, the biofilms on the surfaces and the aqueous phase in the reactor were examined for the following parameters at the same time:

Water phase (in the inflow of the reactor):

- Conductivity
- Temperature
- pH-value
- DOC content (dissolved organic carbon)
- Zinc concentration
- Total number of cells
  (Acquisition of all the bacteria – living and dead – by tinting the bacterial DNA with the fluorescent dye 4′,6-Diamidino-2-phenylindol (DAPI))
- Total number of colonies
  (Acquisition of the reproduction-capable bacteria, which can be cultivated on R2A-Agar at 20°C and in an incubation period of 7 days)
- *Legionella* spec.

(Acquisition of the reproduction-capable *Legionella* species, which can be cultivated after heat-treatment for 30 min at 50 °C; incubation on GVPC-Agar at 36°C and an incubation period of 10 days. Suspect colonies were created as a subculture on BCYE-Agar and NB-Agar)

Biofilm (on the test coupons):

- Zinc concentration
- Total number of cells
- Total number of colonies
- *Legionella* spec.
- Characterisation of the biofilm structures by means of fluorescence microscopy after tinting with DAPI

The test coupons have an area of 33 cm² each.

Inner cylinder with removable coupons

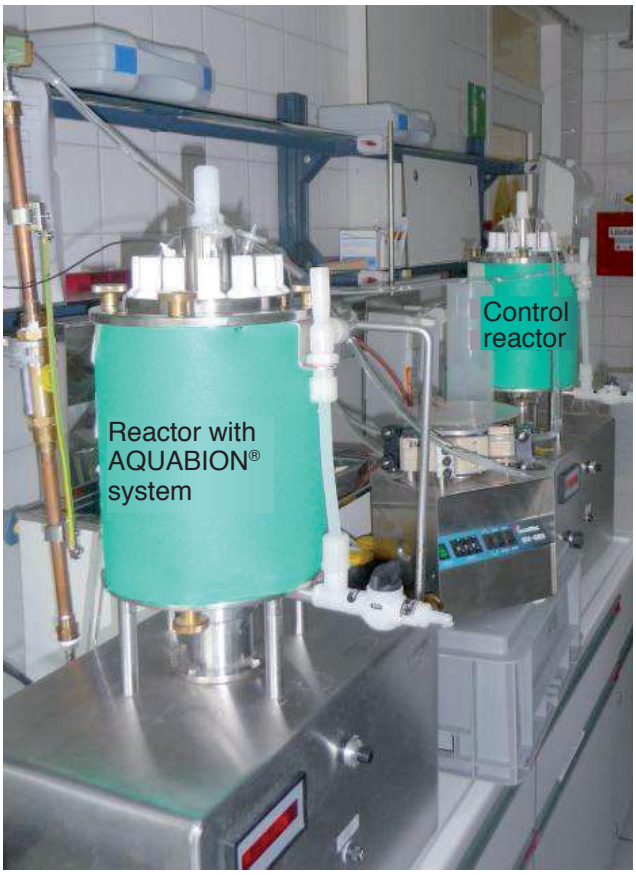


Figure 1: Test setup, heat-insulating rotary piston reactors with and without AQUABION®

2.3 Influence of the AQUABIOREAKTOR on the concentration of organic materials in the water phase

The AQUABIOREAKTOR made available by DAT was constructed of 4 PE pipes, which were each 50 cm long and had a diameter of 90 mm.
There was already a biofilm cultivated by DAT on the carrier bodies contained in it. 80 l of drinking water were introduced in the circuit at room temperature (approx. 21 °C) with a pump rating of 1 m³/hour. The pH-value was set to 7 with the help of an automatic dosing unit. To keep coarse flakes out of the system, a particle filter was integrated in the circuit with a pore size of 100 µm.

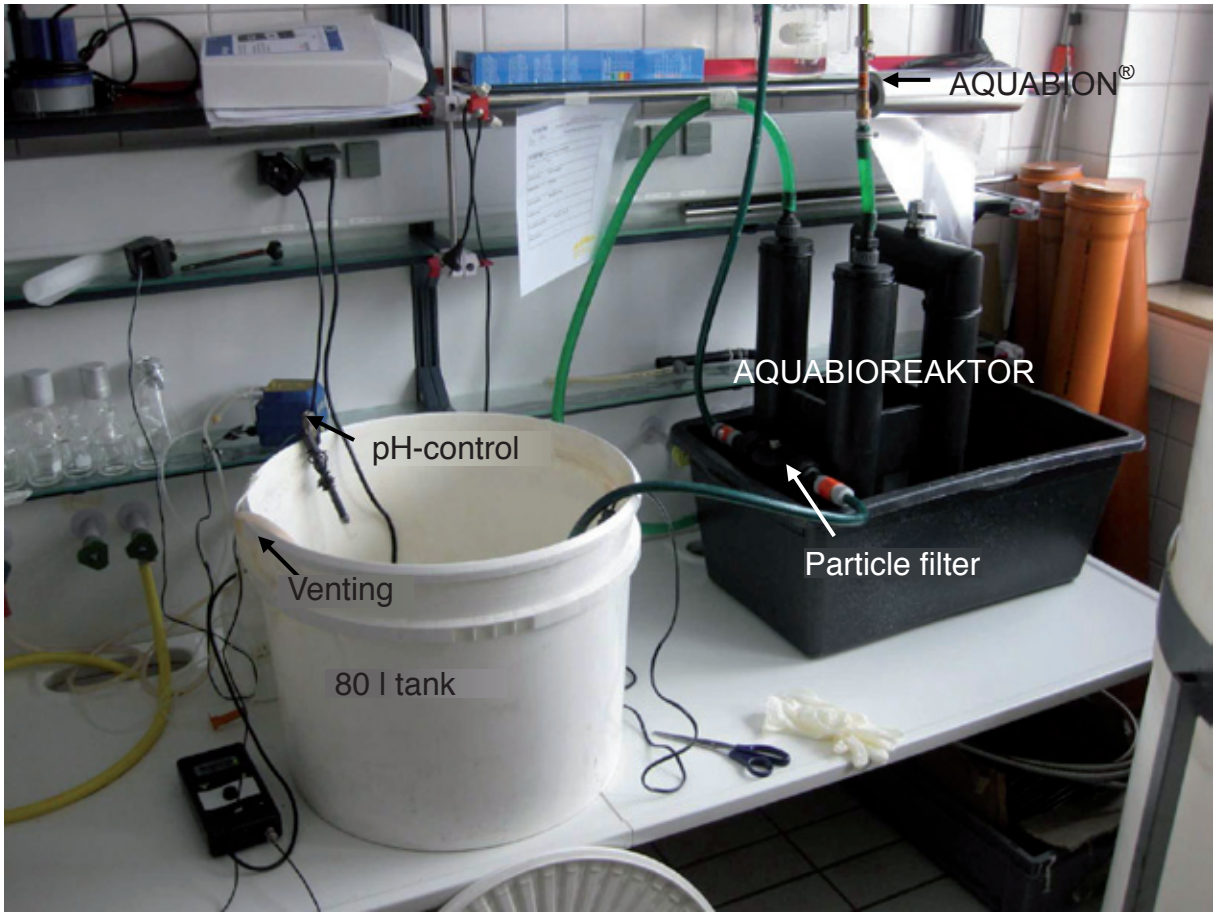


Figure 2: Test setup AQUABIOREAKTOR with AQUABION®

In various different test preparations, either CASO-Bouillon was used as a full medium with carbon (C), nitrogen (N) and phosphorus (P) or Saccharose as the sole carbon source in different concentrations. In some test versions, the water was vented and in some others, it was additionally treated with the AQUABION®. The different test versions are shown in Table 1. Before every restart, the particle filter was cleaned and the water removed completely from the reactor. Thereafter, the reactor was rinsed with drinking water and subsequently, filled with the medium with the desired nutrient content.

Table 1: List of the test preparations with the AQUABIOREAKTOR

Test no.	Carbon source	Concentration	Oxygen supply	AQUABION®
1	Caso-Medium	1.25 l	no	no
2	Saccharose	19 g	no	no
3	Saccharose	19 g	yes	no
4	Saccharose	27 g	yes	no
5	Saccharose	27 g	yes	yes
6	Saccharose	27 g	yes	yes
7	Caso-Medium	1.25 l	yes	yes

To check the reduction of the carbon content in the water, the chemical oxygen requirement (CSB) and the dissolved organic carbon (DOC) were analysed. In addition, the total forming units numbers in the water samples were determined to check whether the bacteria on the filling material of the biofilm reactor get immobilised and hence removed from the water phase.

3 Results and Discussion
3.1 Influence of the AQUABION® on Biofilms
3.1.1 Chemical and physical water parameters

During the entire testing period, the parameters pH-value, DOC content, zinc concentration, temperature and conductivity were measured, to firstly set concentrations typical for cooling water and secondly to check the functioning of the AQUABION® system by determining the zinc concentration.

The pH value was relatively constant with values between 7.45 and 8.62 (Table 2).

The DOC-content was low at the beginning of the test at 1.6 or 1.7 mg/l, but was then set to values above 3 mg/l. Upon sampling after 5 weeks, the DOC concentration, at 2.5 and 2.6 mg/l respectively, was once again below 3 mg/l (Table 2).

The temperature reliably reflects the warm water conditions with values between 26°C and 32°C (Table 2).

The conductivity was also constant, with values around 700 µS/cm (Table 2).

The exception here is a value after 3 weeks testing duration in the reactor with AQUABION®.

Table 2: Summary of the physical and chemical water parameters over the test period (K = control, AB = AQUABION® System)

Test duration	3 hours		1 week		2 weeks		3 weeks		4 weeks		5 weeks	
Reactor	K	with AB	K	with AB	K	with AB	K	with AB	K	with AB	K	with AB
pH	8.08	8.14	8.14	7.45	8.36	8.18	7.74	8.03	8.08	8.47	8.62	8.28
DOC (mg/l)	1.6	1.7	3.5	4.5	3.8	3.4	3.4	3.4	2.9	3.2	2.5	2.6
Zn (mg/l)	0.037	0.725	<0.01	11	0.024	26	0.019	4.6	0.014	0.56	<0.01	0,93
T (°C)	26.4	27.1	30.8	30.6	31	30.1	31.5	30.5	27.2	26	31	31
LF (µs/cm)	674	655	707	732	762	696	679	900	631	621	702	686

In the case of the analysed chemical and physical water parameters, no differences could be determined between the two investigated systems with and without AQUABION®. One exception here is the parameter Zinc, which, in the control reactor, was always close to the detectable limit of 0.01 mg/l and in the reactor system with AQUABION®, reached values up to 26 mg/l. This value was reached after 2 weeks of running the test.

water to which nutrients had been added. Next, after every sampling, 2 litres of the test volume were replaced.
The increased zinc concentrations in the water phase of the reactor system with AQUABION® indicate that the active anode system worked over the entire testing duration and the zinc concentration is the only difference in the two test systems.

3.1.2 Zinc in the biofilm

The concentration of zinc in all the examined biofilms showed that up to a period of 3 weeks of testing, the zinc concentrations were close to the detectable limit. When the biofilm was sampled after four weeks, it was found that there had been an accumulation of zinc in the biofilm from the AQUABION® System. The reason for this could be the high zinc concentration in the water at the time of sampling, i.e. after two weeks. The zinc concentration in the biofilm (per 10^6 biofilm bacteria), which was treated with the AQUABION® System, was 1-2 orders of magnitude higher than in the control system after 4 to 5 weeks of testing period (Table 3).

Table 3: Concentration of zinc in the biofilm over the period of the test

	Zinc (mg/10^6 biofilm bacteria)	
Duration of the test	Control	with AQUABION® System
3 hours	3.57×10^{-8}	3.17×10^{-8}
1 week	$< 6.95 \times 10^{-9}$	1.93×10^{-7}
2 weeks	$< 8.72 \times 10^{-9}$	$< 3.11 \times 10^{-8}$
3 weeks	$< 2.40 \times 10^{-8}$	$< 2.30 \times 10^{-8}$
4 weeks	7.08×10^{-6}	2.89×10^{-4}
5 weeks	2.88×10^{-5}	5.12×10^{-4}

3.1.3 Microbiological water analyses

At the start of the test, total cell numbers of 2×10^6 bacteria/ml were measured in the water, which is in the typical range for cooling water. Up to three weeks of testing, there is no clear trend recognisable whether the cell numbers in the AQUABION® System or in the control system were higher.

This shows that zinc does not have a toxic action on the cooling water bacteria. However, from the fifth week onwards, the bacteria concentration in the AQUABION® system increased to values of 5 x 10^6 bacteria/ml, whereas the bacteria concentration in the water of the control system was just approximately 4 x 10^5 bacteria/ml. This could be an indication of the fact that in the AQUABION®, there was less biomass adhered to the surfaces, but there was more in the water phase.

This trend was reflected preparation-wise in the results of the total forming units number determination (Figure 3).

The *Legionella* concentration did not show this flow. At all the five sampling times, the concentrations in the AQUABION® system were always clearly below those in the control system.

⇨ This gives a pointer to the fact that the AQUABION® degrades the cultivability of *Legionella* in the water, which must originally be owing to the concentration of zinc ions in the water phase.

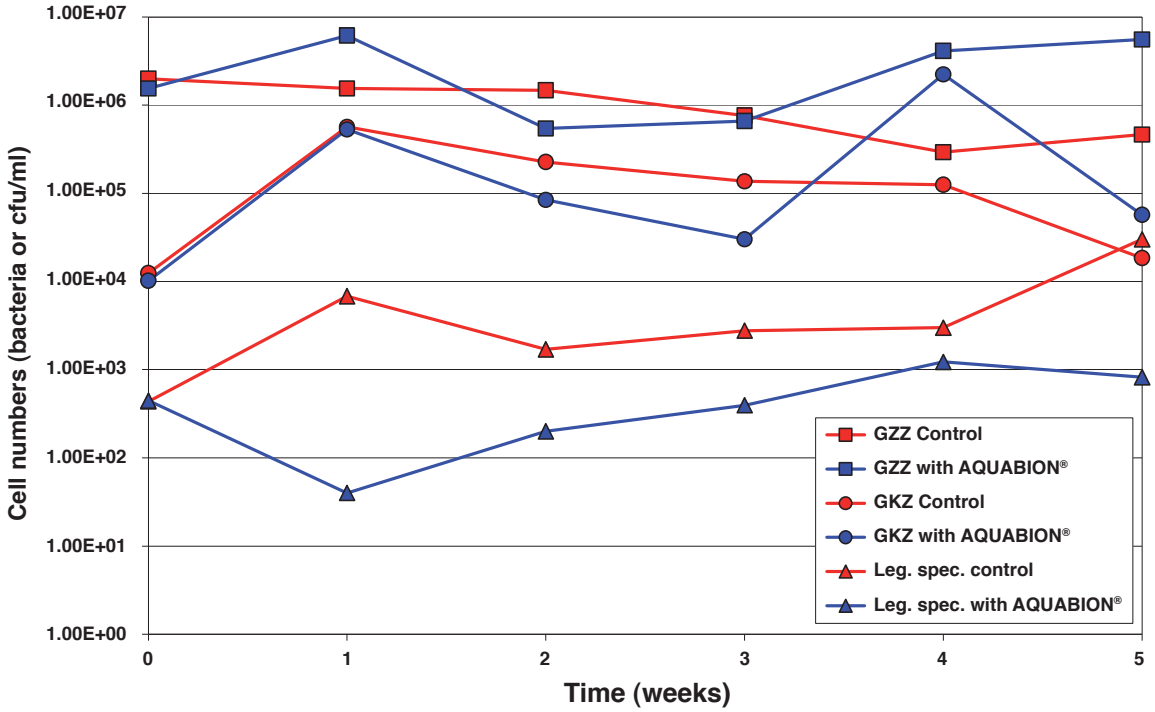


Figure 3: Summary of all the microbiological parameters determined in the water (GZZ = total number of cells, GKZ = total forming units number, Leg. spec. = *Legionella* species)

3.1.4 Microbiological biofilm analyses

In the biofilms of both the test systems, at the start of the test, total cell numbers of about 3 x 10^6 bacteria/cm² were measured (Figure 4). Up to three weeks of testing time, it was clearly seen that the cell numbers in the biofilm of the AQUABION® Active anode system were less than in the control system. From the fourth week, however, the concentrations of the total cell numbers came closer to each other.

However, the results of the total forming units number determination in the biofilm clearly showed that the cultivability in the AQUABION® system was less by a factor of at least 10 (after one week of testing duration, even by a factor of 1000) (Figure 4).

Even in the determination of the *Legionella* concentrations in the biofilm, it could be shown that the number of cultivable bacteria in the AQUABION® system was less by a factor of at least 10, and after 2 weeks or 5 weeks testing, even by a factor of 1000. One exception here are the *Legionella* concentrations, which were determined after 4 weeks of testing.
However, it must be taken into account here that the *Legionella* concentrations in both the systems were so high that the selected dilutions were not suitable and it has to be assumed that the *Legionella* growth on the Agar plates was hindered, which gave rise to a lower result.

⇨ In the critical examination of the results of the cultivability of the biofilm bacteria, it is noticeable right in the water phase, that in the AQUABION® system, the cultivability of the native cooling water bacteria and the *Legionella* is significantly reduced. It can also be causatively explained only by the action of the zinc ions that were released by the AQUABION® system.

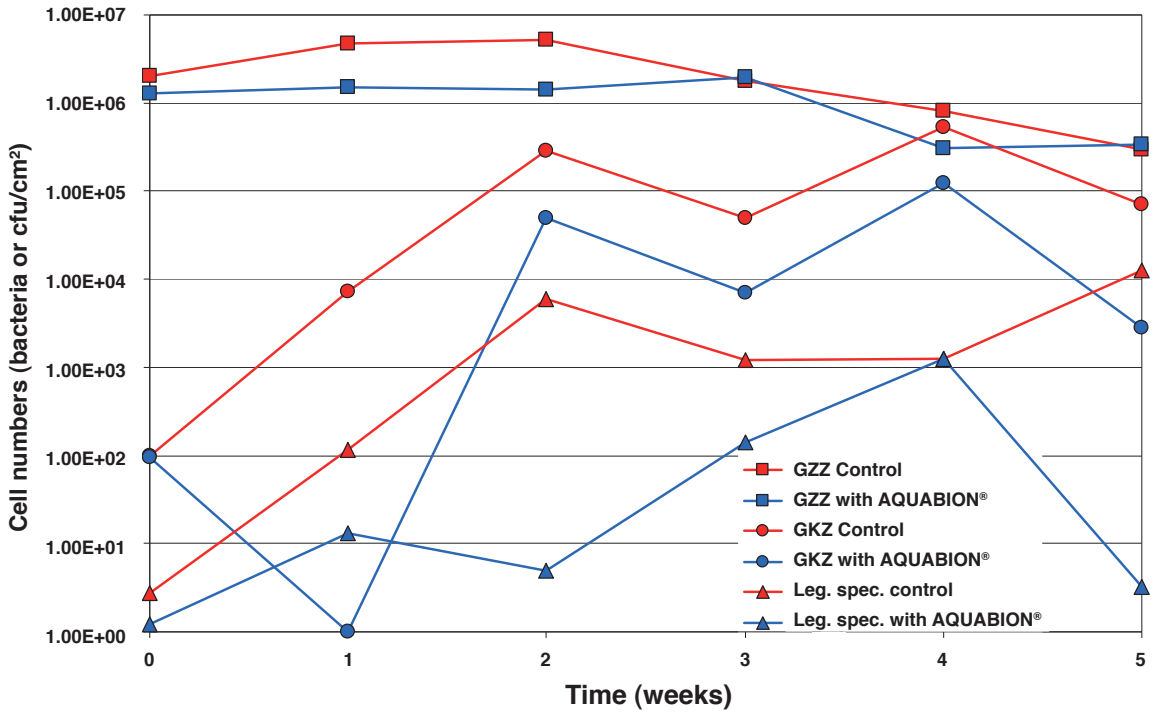


Figure 4: Summary of all the microbiological parameters determined in the biofilm (GZZ = total number of cells, GKZ = total forming units number, Leg. spec. = *Legionella* species)

3.1.5 Surfaces

3.1.5.1 Macroscopic examination of the hose surfaces

After five weeks testing time, the Tygon hoses through which the water flowed during the entire testing period were macroscopically compared. It can be quite clearly seen that the hose that was installed in the system with AQUABION®, was less incrusted than the hose from the control system (Figure 5). In addition, it was clear that the incrustation in the hose of the control system was coloured yellowish-brownish, whereas the biofilm in the AQUABION® Active anode system is coloured whitish. This leads to the conclusion that the biofilm in both systems is not only different in the biofilm thickness, but also in the composition.

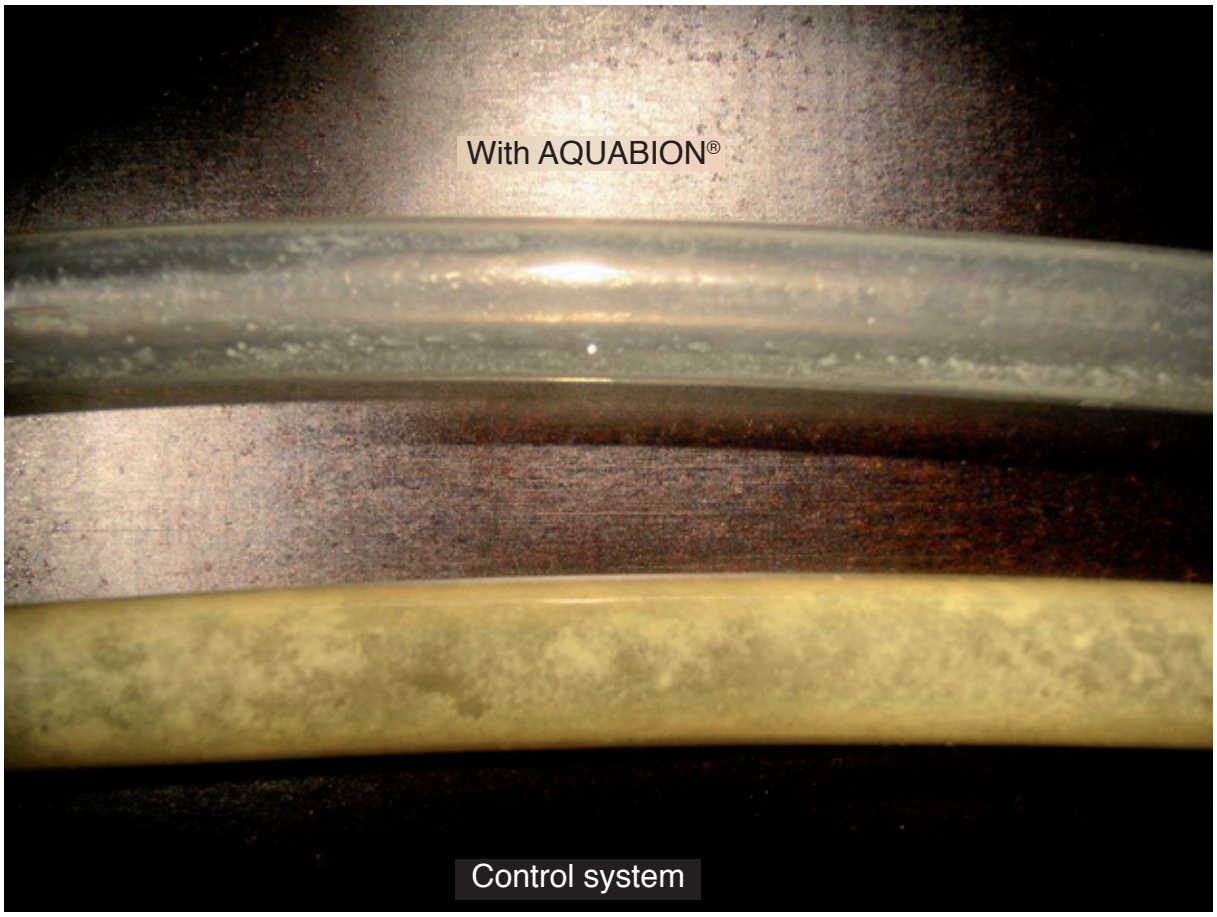
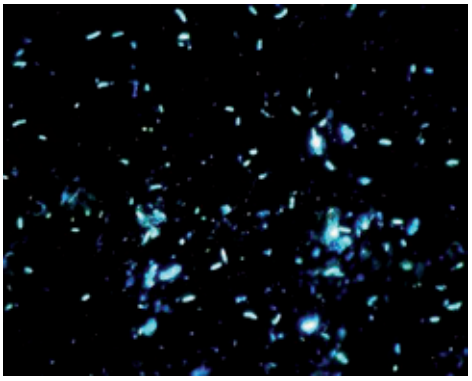
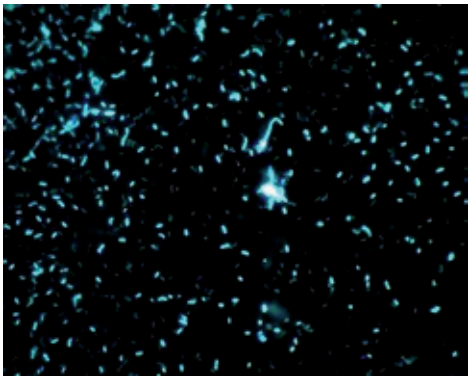
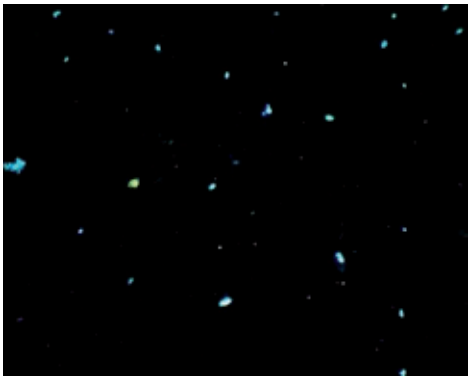
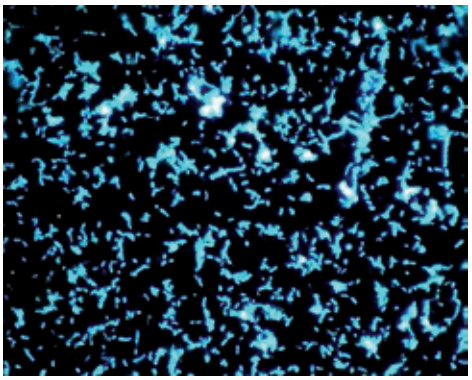
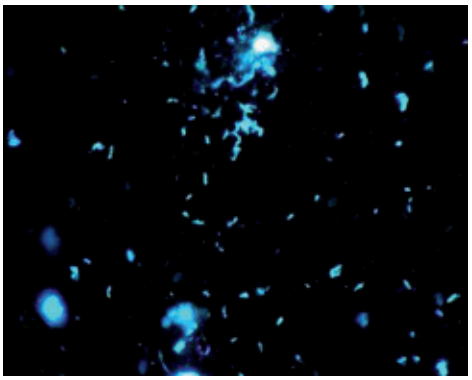
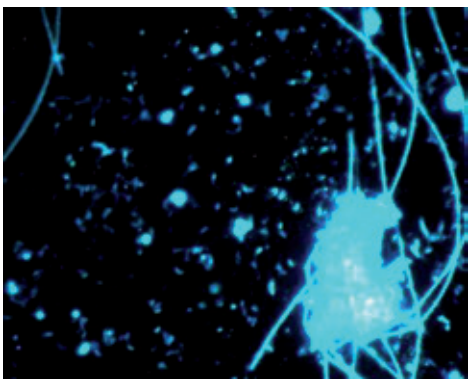
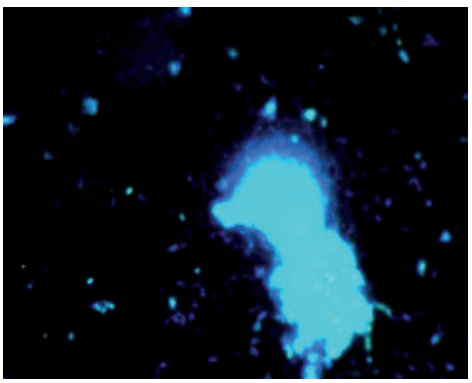


Figure 5: Macroscopic examination of the hoses from the system with AQUABION® (top) and the control system (below).

3.1.5.2 Direct examination of the coupon surfaces

In the fluorescence microscopic examination of the tinted coupon surfaces, it became clear that it was populated more densely with biofilm bacteria in the control reactor, than the coupon surfaces that were taken from the AQUABION® active anode system. It was also quite clear to see that the biofilms of the second week were distinctly different from those of the third week. Whereas in the first two testing weeks, shorter rods that were adhering homogeneously to the coupon surface dominated, from the third testing week onwards, aggregate formation and the occurrence of protozoa were clearly recognisable. One important difference in the structure of the biofilms on the surfaces was the presence of filament-like bacteria in the control system. Through the networking of filament-like bacteria, biofilms that contain them generally have a higher stability. The reduced stability of the biofilm owing to the presence of zinc ions from the AQUABION® could be an additional reason for the fact that it did not develop to the same extent as in the control system because of the shearing forces prevailing in the hose (see 3.1.5.1).

Testing duration	Control reactor	Reactor with AQUABION®
3 hours		
1 week		
2 weeks		
3 weeks		

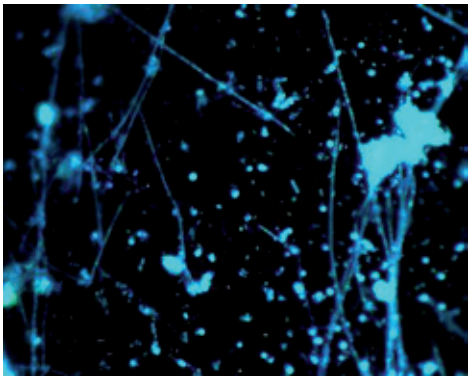
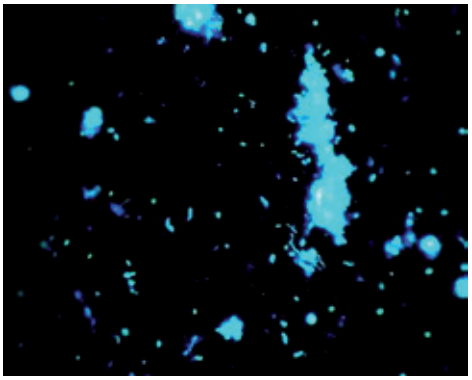
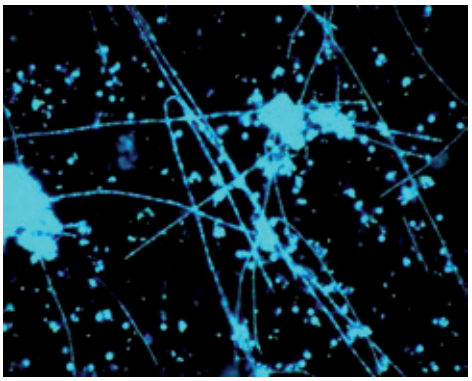
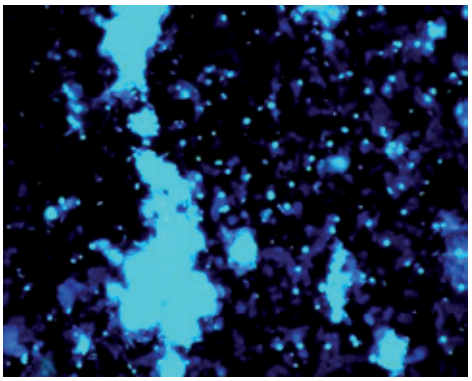
Testing duration	Control reactor	Reactor with AQUABION®
4 weeks		
5 weeks		

Figure 6: Direct examination of the coupon surfaces at the sampling times after tinting with the fluorescent dye DAPI

3.1.6 Chemical investigation of the precipitates of the circulation systems

During the operation of the two reactor systems, it was noticed that in the preparation with the AQUABION® Active anode system, far more precipitates were deposited to the floor of the 5 litre barrel than in the control reactor (Figure 7). Since it is postulated by DAT that by the addition of zinc (zinc ions) to the water, calcite ($CaCO_3$) is converted into aragonite ($CaCO_3$), which has a lesser tendency to adhere to surfaces, it is necessary to check whether the precipitates contained different concentrations of different elements. Therefore, the chemical composition of the flakes was determined by means of ICP-OES (Table 4).

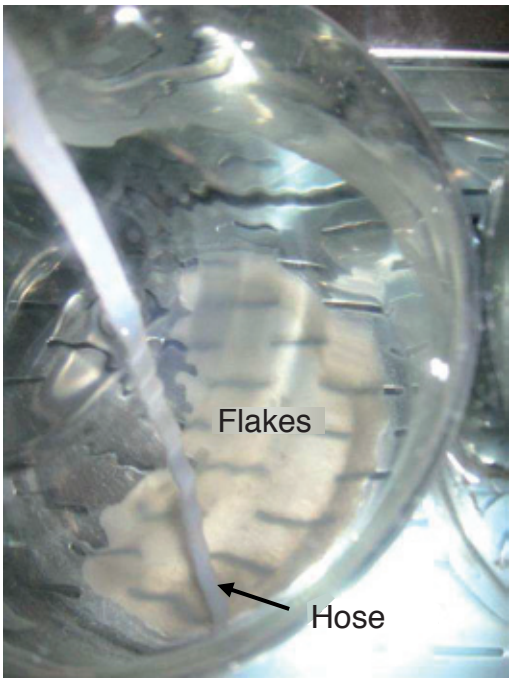


Figure 7: Flakes on the floor of the 5 litre barrel in the preparation with AQUABION® -System

The investigation showed that the flakes from both the test systems had a very similar chemical composition. The calcium concentrations were similar at 56.6 and 56.7 mg/l. There was a large difference only in the concentration of zinc in the flakes: whereas zinc was not detectable in the flakes from the control reactor, the flakes from the AQUABION® Aktive anode system contained 14 mg/l zinc. From the literature, it is known that zinc causes a change in the crystalline structure during the formation of carbonates, especially calcium carbonate (Coetzee, P.P. et al., 1998, “Incrustation reduction and incrustation modification effects through Zn and other metal types in the physical water treatment”, Water SA, Bd. 24, Nr. 1, S. 77-84). During the crystallisation, large crystals, so-called aragonites, are formed. According to the literature, the adhesion forces of the aragonite by and on surfaces are much smaller than in the case of simple calcite. The measured presence of zinc in the precipitates and the reduced incrustation formation on the hoses of the system with AQUABION® (see 3.1.5.1) provide indications of the anti-scaling properties of the AQUABION®.

In addition, there were minor differences in the copper and sodium concentrations. They were both higher in the AQUABION® system than in the control system. Copper probably entered during the installation of the AQUABION® -System.

Table 4: **Summary of the chemical investigations of the flakes in the two reactor systems; the elements that were present in different concentrations are shown shaded.**

Parameter	Flakes composition, Control reactor		Flakes composition AQUABION®	
Aluminium	< 0.05	mg/l	< 0.05	mg/l
Calcium	56.6	mg/l	56.7	mg/l
Magnesium	9	mg/l	9.7	mg/l
Sodium	65.4	mg/l	71.8	mg/l
Potassium	5.2	mg/l	5.8	mg/l
Boron	0.089	mg/l	0.099	mg/l
Cadmium	< 0.001	mg/l	< 0,001	mg/l
Cobalt	< 0.005	mg/l	< 0,005	mg/l
Chromium	0.0085	mg/l	< 0.0051	mg/l
Copper	0.056	mg/l	0.18	mg/l
Nickel	0.011	mg/l	0,016	mg/l
Lead	< 0.01	mg/l	0.041	mg/l
Iron	0.099	mg/l	< 0.05	mg/l
Manganese	< 0.01	mg/l	< 0.01	mg/l
Silicon	2.89	mg/l	2.73	mg/l
Zinc	< 0.052	mg/l	14	mg/l
Total phosphorus	0.14	mg/l	0.16	mg/l

3.2 Examination of the microbiocide effect after the addition of iodine and zinc according to the DAT Principle of Aquainject on formed biofilms

Following the investigations with the AQUABION® Active anode system, two tanks were connected in series in the inflow of the control reactor, which contained 27 g zinc or iodine respectively. Once again 5 litres were pumped over a period of one week in the circuit.

After two days, a water sample was examined, and after one week, a water sample and a biofilm sample, with regard to the total number of cells, total number of colonies, concentration of *Legionella* spec., DOC and Zink investigated.

Water phase:
The results show that the treatment resulted in an increase in the total number of cells (living and dead bacteria), but a significant reduction of the total number of colonies (bacteria capable of multiplying). After 2 days, the total number of colonies reduced by 2 logarithmic levels and after 7 days, by 3 logarithmic levels. *Legionella* were not detectable any more after 2 days of treatment.

Biofilm:
The treatment of the biofilm with the combination of zinc and iodine according to the DAT principle of Aquainject resulted in only a small dissolution of the biofilm. The cultivability of the biofilm bacteria, however, reduced significantly less than in the water phase.

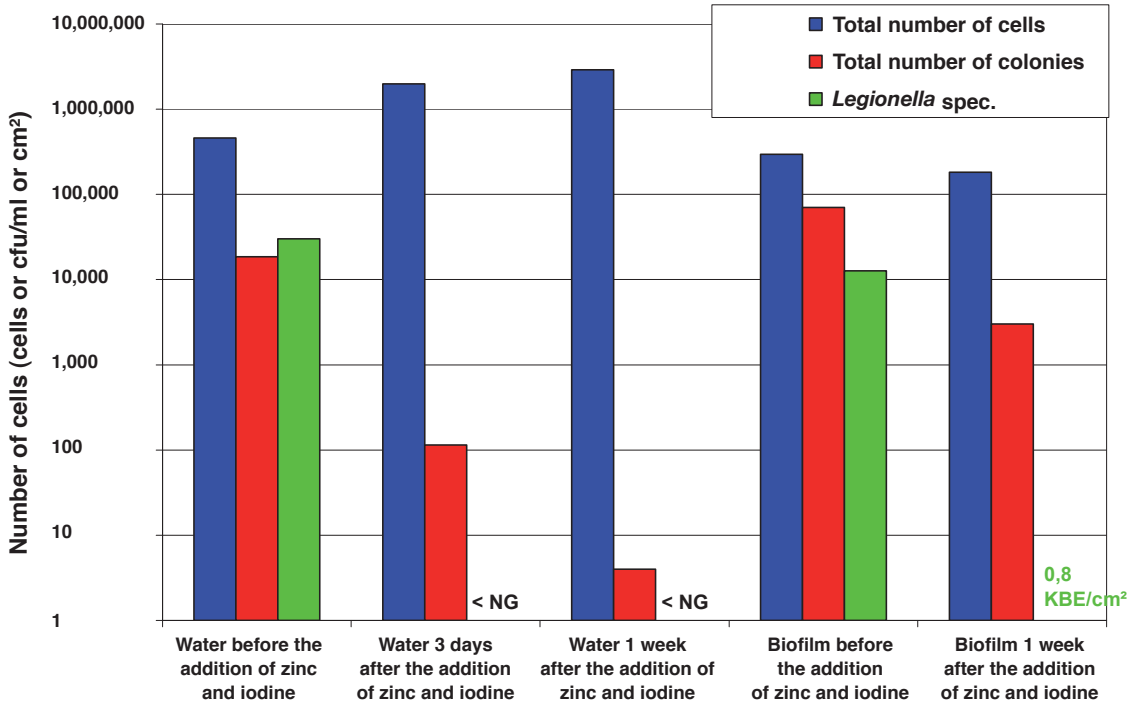


Figure 8: **Influence of the treatment with zinc and iodine on the biofilm (< NG = below detectable limits)**

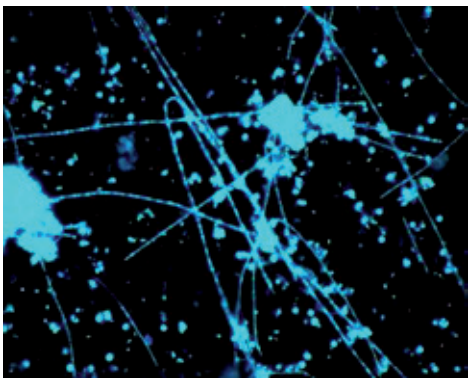
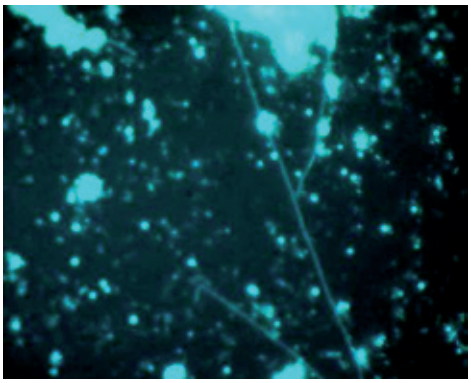
Duration of test	Before the addition of zinc and iodine	After the addition of zinc and iodine
1 week		

Figure 9: **Documentation of the influence of the treatment of the biofilm with zinc and iodine directly on the coupon surface**

3.3 Influence of the AQUABIOREAKTOR on the degradation of organic substances in the water phase

The results show that the use of the AQUABIOREAKTOR gives rise to a reduction in the organic substances in the water phase (Figure 10, Figure 11). For the degradation, in the selected test periods, (3-4 days) it is immaterial whether what is involved is a full medium with C,N and P-source (CASOMedium) or a sole C-source (Saccharose). The test results are reflected in both the determination of the CSB-content and in the determination of the DOC-content, because the curve flows of the CSB- and the DOC-concentrations are comparable (Figure 10, Figure 11). The following findings could be obtained from the different trial preparations:

- The addition of oxygen is favourable for the fast degradation of organic substances.

- Lower concentrations of Saccharose are degraded more effectively (within a shorter time) than higher concentrations, but in percentage terms, after 100 hours (approx. 4 days) approximately the same target value of 5.9 or 3.7 % is reached.

- Upon using 27 g of Saccharose, the AQUABION® system delays the decomposition of the organic substance, but after about 2 days, the same DOC or CSB concentrations are reached. This could be an indication of the fact that the zinc ions introduced through the AQUABION® first hinder the biomass in its metabolic activity, but after 2 days, the bacteria have adapted to the increased zinc concentration.

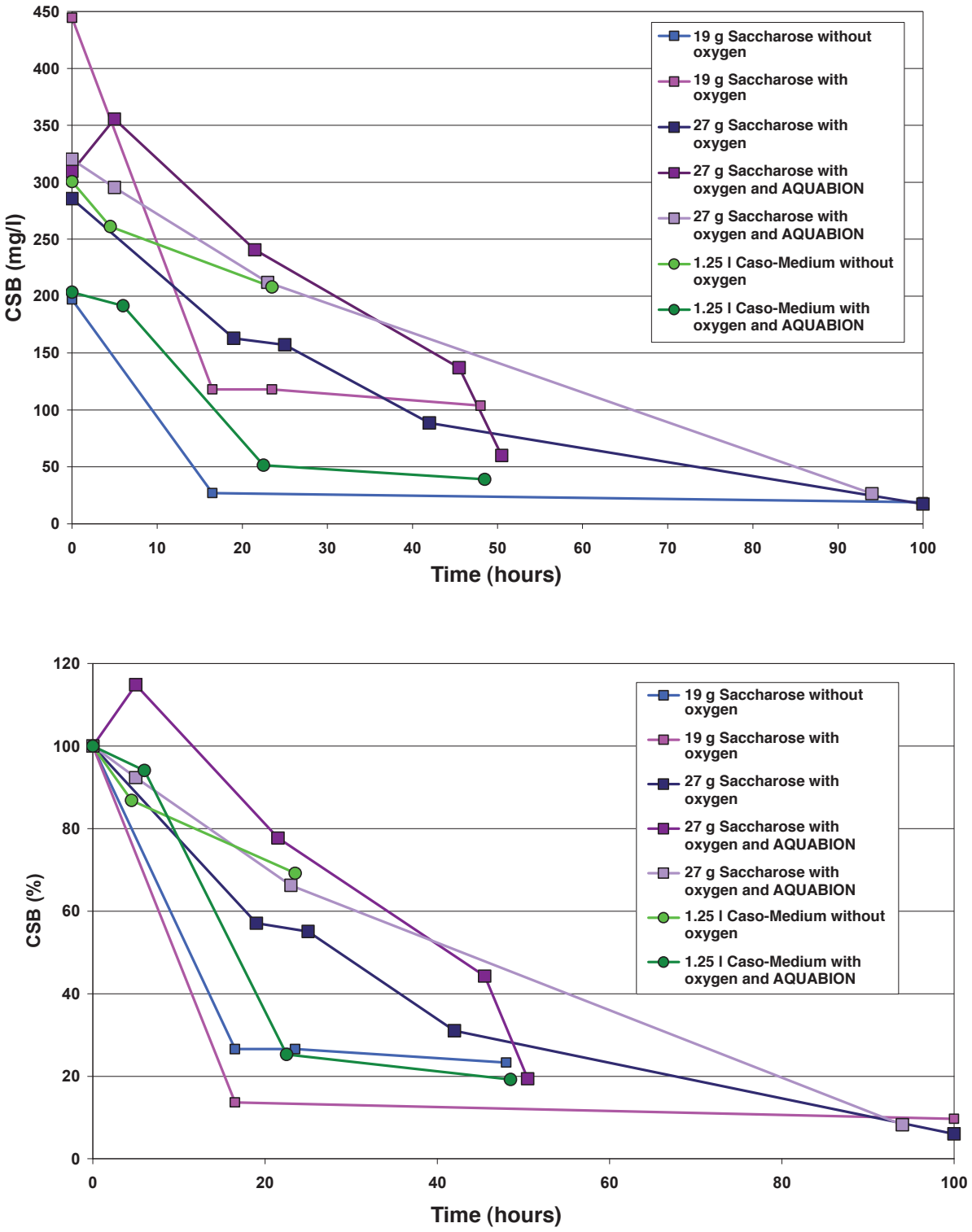


Figure 10: Graphical depiction of the reduction of the concentration of organic substances measured according to the CSB content by the AQUABIOREAKTOR; top: absolute concentrations (mg/l) bottom: relative reduction (%)

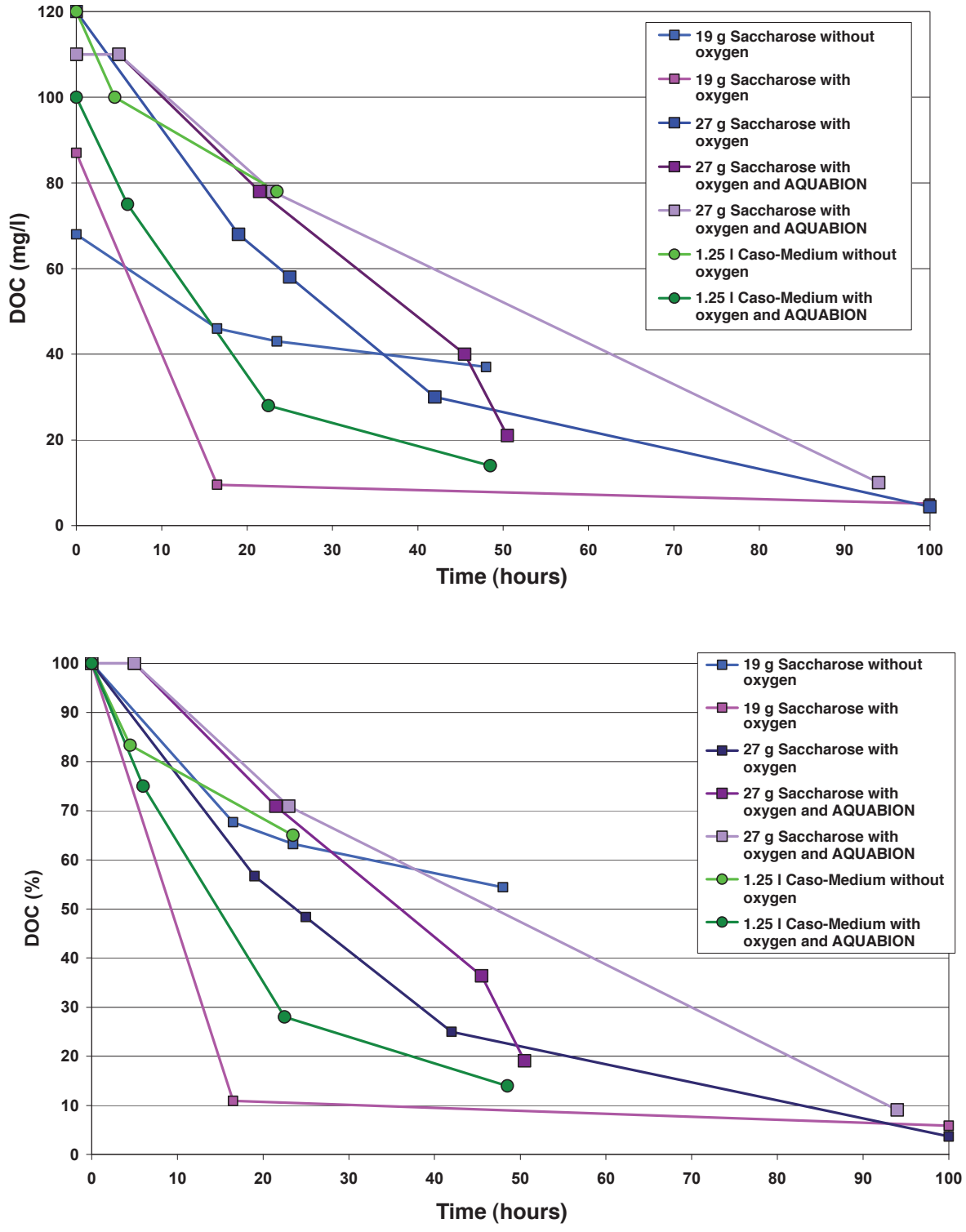


Figure 11: Graphical depiction of the reduction of the concentration of organic substances measured according to the DOC-content by the AQUABIOREAKTOR; top: absolute concentrations (mg/l) bottom: relative reduction (%)

Moreover, the results show that in the selected test preparation, there was no reduction of the bacteria concentrations, measured with the help of the total forming units number (cfu/ml) (Figure 12). The reason for this is probably that the number of colonies in the water reflect the biofilm formation in the reactor owing to the large surface to volume ratio. To check whether the deployment of the AQUABIOREAKTOR results in a reduction of the number of colonies in the water phase, the test would have to be repeated with a larger water volume.

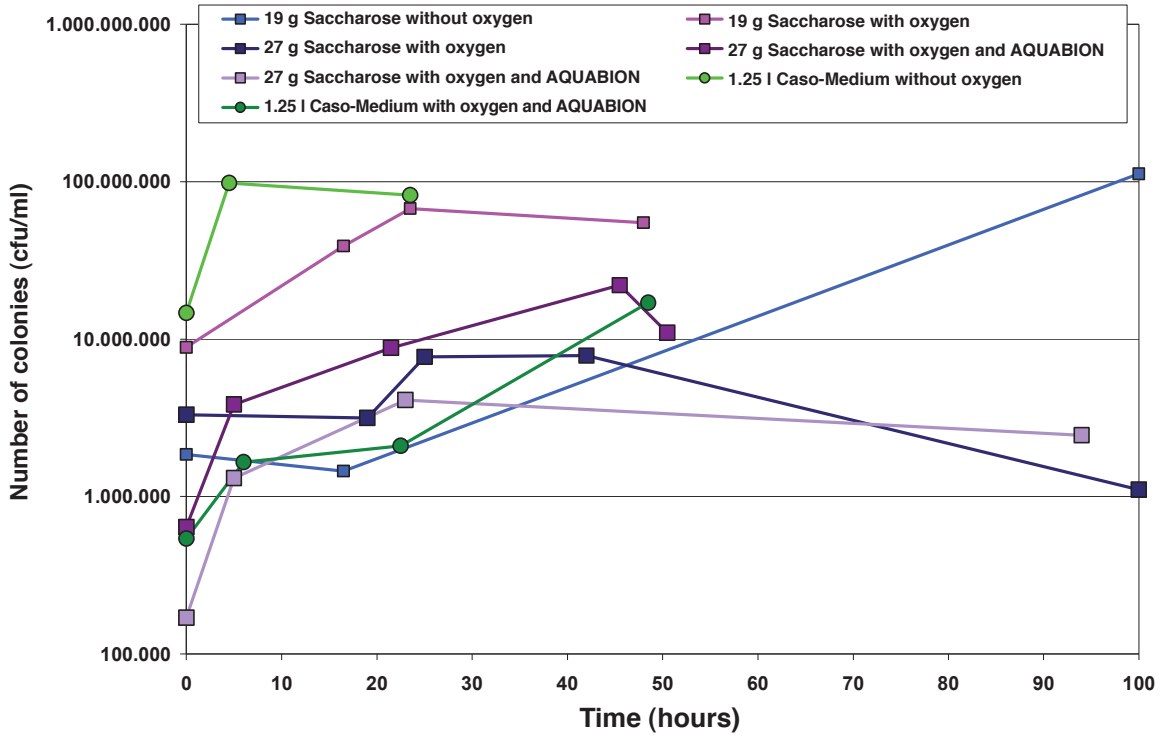


Figure 12: Graphical depiction of the development of the concentration of the total cfu number of colonies in the water phase