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Hygiene- und Umweltinstitut
Cottbus GmbH

Report of results

about the analysis of the microbiological effectiveness of the UV disinfection device Purion 1.000

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1 Task definition

The device Purion 1.0 developed and built by the company PURION GmbH is supposed to be used in the field of UV disinfection from drinking water. The objective of the investigations was to test the effectiveness of the UV disinfection device Purion 1.0 in comparison to different bacteria under laboratory conditions. The analysis occurred on the basis of the offer created by the contractor on 11.02.2006 which was confirmed by the customer on 11.02.2006.

2 Testing procedure

2.1 Experimental setup and process of the different series of experiments

In the lab a specific experimental apparatus was built up (see fig. 1), that made it possible to add a defined amount of known bacteria into a drinking water stream in order to test the effectiveness of the UV disinfection device Purion 1.0 in this way.

Figure 1: General view of the experimental set up

This experimental setup consisted of following components:

- Inlet tubing (connected to an existing drinking water tap)
- electronically registering water meter
- Mixing tank (fig. 2) with device for adding of bacterium suspension and
- Sampling valve (as a sampling place for the inflow to the UV disinfection device)
- Perfusor (for the automatic addition of bacterium suspension)
- Purion UV disinfection device 1.0
- Discharge pipe (as a sampling place for the outflow of the UV disinfection device), (fig. 3):

Figure 2: Mixing tank with sampling valve

Figure 3: UV disinfection device with discharge pipe

The individual series of experiments were carried out as follows:
By opening of the drinking water valve water run continuously through the whole equipment. The mass flow of water could always be read off by the installed electronic water meter. Before the actual start of the experiment (that is before adding of bacterium suspension and initiation of the UV disinfection device) a water samples was taken at the inflow of the UV disinfection device to control the microbiological quality of the untreated drinking water. After that the dosing of bacterium suspension started and the UV disinfection device was switched on.

The rate of the dosing of the bacterium suspension was determined by the volume flow of water as well as the initial concentration of the bacterium suspension. The addition of bacteria occurred in that way that always by calculation 3×10^3 to 7×10^3 bacteria per millilitre water were contained in the inflow of the UV disinfection device. The sampling begun approx. 10 minutes after the switch-on of the UV disinfection device. A sample of the inflow and a sample of the outflow of the UV disinfection device were taken parallel in each case, in total 5 samples of the inflow and 5 sample of the outflow. The sampling occurred at intervals of 2 to 4 minutes. In addition to the microbiological tests in total 5 samples were taken during the 1st series of experiments for the analysis for trihalogenmethane (3 samples of the inflow of the UV disinfection device, 2 sample of the outflow).

2.2 Control of the microbiological water quality in the inflow of the UV disinfection device before dosing of the test bacteria

To assure that no disturbances of the test procedure occur caused by already available bacteria in the drinking water, a sample of the inflow was taken before every series of experiments, which means before the dosing of test bacteria.
This sample was checked for drinking water quality concerning the microbiological parameters. Next to the parameters to be checked routinely according to the German Drinking Water Directive TrinkwV (Colony number at 22 °C and 36 °C, Coliform bacteria and Escherichia coli) the samples were also tested for Pseudomonas aeruginosa.

2.3 Selection and cultivation of the test bacteria

As a test organism for the determination of the colony number at 36 °C Enterobacter cloacae DSM 30 054 was chosen. For Escherichia coli the strain DSM 682 was used. A wild strain Pseudomonas aeruginosa was used for the determination on Pseudomonas. The single strains were cultivated as "bemachtkultur" (ÜNK) at 36 °C on soya peptone casein-pekton-bouillon. Before the start of the experiments the number of bacteria in the ÜNK was determined

using a bacterium counting-chamber (THOMA-chamber) and then adjusted to 1 to 2 x 10^8 bacteria/ml.

2.4 Procedure of the sampling

For the microbiological analysis sterile sampling bottles were used. According to the number of analysis 100 ml to 200 ml of sample volume were taken under sterile conditions. The microbiological analysis was done immediately after the sampling. For the investigation on THMs approx. 20 ml were taken in head space bottles. The bottles were filled in a brimful way and shut immediately to the sampling air tightly with a head space forceps.

2.5 Procedure of the microbiological analysis

2.5.1 Determination of the colony number at 36°C

The determination of the colony number at 36°C occurred by means of “pour-plate methode” according to the method described in the drinking water directive 1990, enclosure 1, no. 5.
As fertile soil DEV-culture-Agar of the company SIFIN was used. The incubation time was 44 ± 4 h. The incubation temperature was 36 ± 1 °C.

2.5.2 Proof and counting of Pseudomonas aeruginosa

The proof and the counting of Pseudomonas aeruginosa were carried out according to DIN EN ISO 12780. 100 ml sample (or rather 100 ml of a suitable dilution of the sample) was filtered by a membrane filter with a pore width of 0.45 µm. The filter was then put on Pseudomonas-CN-Agar. The Incubation occurred at 36 ± 2 °C for 44 ± 4 h. After the incubation all fluorescent colonies were counted as suspicious Pseudomonas aeruginosa. Random samples from the colonies were taken to examine the ammonia formation from acetamide. All colonies that form acetamide from ammonia were counted as confirmed Pseudomonas aeruginosa. In the following the number of Pseudomonas aeruginosa per 100 ml or per 1 ml sample were calculated.

2.5.3 Proof and counting of Escherichia coli

The proof and the counting of Escherichia coli (E. coli) were carried out according to DIN EN ISO 9308-1. 100 ml sample (or rather 100 ml of a suitable dilution of the sample) was filtered by a membrane filter with a pore width of 0.45 µm. The filter was then put on Laktose-TTC-Agar with Tergitol. The Incubation occurred at 36 ± 1 °C for 21 ± 3 h. After the incubation all typical colonies were counted.

For the confirmation of the oxidase-test and for the proof of the indole formation some colonies were treated as random samples. All oxidase-negative and indole-positive colonies were counted to as E.coli. Then the number of E. coli per 100 ml or 1 ml sample were calculated.

2.6 Process of the analysis on trihalogenmethane

The analysis on trihalogenmethane occurred with headspace gas chromatography with ECD-detection according to EN ISO 10 301.

3 Results

3.1 Results of the microbiological analysis

3.1.1 Results of the analysis of the microbiological water quality at the inflow of the UV disinfection device before addition of test bacteria

The results of the single analyses are shown in Table 1. The test report of the single samples are added in the enclosure 1, page 1 to 3.
The microbiological quality of the drinking water at the inflow of the UV disinfection device was analysed in each of the 3 series of experiments.

Table 1: results of the microbiological analysis in the inflow of the UV disinfection device before addition of test bacteria

Parameter	**Dimension**	**Date of sampling**		
		07.03.06	**09.03.06**	**10.03.06**
Colony number at 22 °C	1/ml	3	1	3
Colony number at 36 °C	1/ml	38	53	56
Coliforme bacteria	1/100 ml	0	0	0
Escherichia coli (E. coli)	1/100 ml	0	0	0
Pseudomonas aeruginosa	1/100 ml	0	0	0

The number of colonies at 22 °C was between 1 and 3 KBE/ml (KBE= Colony building units), the colony number at 36 °C was between 38 and 56 KBE/ml. Coliforme bacteria, E. coli and Pseudomonas aeruginosa could not be found in all 3 samples of 100 ml.

In the drinking water directive the following boundary values for microbiological parameter are fixed:

Colony number at 22 °C	**100/ml**
Colony number at 36 °C	**100/ml**
Coliforme bacteria	**0/100 ml**
Escherichia coli (E. coli)	**0/100 ml**

These limit values were kept at the 3 analysed samples, that means the water in the inflow to the UV disinfection device had drinking water quality in biological sense within the 3 series of experiments .

3.1.2 Results for the effectiveness of the UV disinfection device with respect to the colony number at 36 °C

As test the organism for definition of the colony number at 36 °C Enterobacter cloacae was used. The test organism was present with a concentration of 8540 KBE/ml in the inflow of the UV disinfection device.
In the outflow of the UV disinfection device 1 KBE/ml and 0 KBE/ml were provable.
The results of the test series are summarised in table 2.

Table 2: Results of the analysis on the colony number at 36 °C in the inflow and outflow of the UV disinfection device.

Sample No.	**Inflow colony number at 36 °C in KBE/ml**	**Outflow colony number at 36 °C in KBE/ml**
Sample 1	10000	1
Sample 2	8600	1
Sample 3	7700	0
Sample 4	1100	0
Sample 5	5400	0

3.1.3 Results for the effectiveness of the UV disinfection device with respect to the test organism Pseudomonas aeruginosa

In the inflow of the UV disinfection device Pseudomonas aeruginosa was present with an average concentration of 3880 KBE/ml. This corresponds to a concentration of 3,88 x 10^5 KBE/100 ml.

In the outflow of the device in a 1 ml sample no Pseudomonas aeruginosa was provable. In a 100 ml sample 4 to 8 KBE Pseudomonas aeruginosa were provable. The results of the test series are summarised in table 3.

Table 3: Results of the analysis on Pseudomonas aeruginosa in the inflow and outflow of the UV disinfection device

Sample No.	Inflow Pseudomonas aeruginosa in KBE/ml	Outflow Pseudomonas aeruginosa in KBE/ml	Inflow Pseudomonas aeruginosa in KBE/100 ml	Outflow Pseudomonas aeruginosa in KBE/100 ml
Sample 1	5700	0	5,7 x 10^5	8
Sample 2	2600	0	2,6 x 10^5	5
Sample 3	5800	0	5,8 x 10^5	7
Sample 4	4000	0	4,0 x 10^5	4
Sample 5	1300	0	1,3 x 10^5	4

3.1.4 Results for the effectiveness of the UV disinfection device with respect to the test organism Escherichia coli

In the inflow of the UV disinfection device Escherichia coli was present in an average concentration of 13600 KBE/ml. This corresponds to a concentration of 1,36 x 10^6 KBE/ml.
In the outflow of the device in a 1 ml sample no Escherichia coli was provable. In a 100 ml sample 2 KBE Escherichia coli maximum were provable.
The results of the test series are summarised in table 4.

Table 4: Results of the analysis on Escherichia coli in the inflow and outflow of the UV disinfection device

Sample No.	Inflow E. coli in KBE/ml	Outflow E. coli in KBE/ml	Inflow E. coli in KBE/100ml	Outflow E. coli in KBE/100ml
Sample 1	13000	0	$1,3 \times 10^6$	2
Sample 2	10000	0	$1,0 \times 10^6$	1
Sample 3	15000	0	$1,5 \times 10^6$	1
Sample 4	17000	0	$1,7 \times 10^6$	0
Sample 5	13000	0	$1,3 \times 10^6$	0

3.2 Results of the analysis on trihalogenmethane

At the 3 samples from the inflow to the UV disinfection device as well as in the two samples from the outflow of the UV disinfection device no trihalogenmethane were provable. The concentration of Trihalogenmethane was smaller than 0.0001 mg/l in all samples and in this way below the detection limit of the investigation method. The individual results of the analysis on trihalogenmethane can be understood from the test reports (see enclosure 2, pages 1,3,5,8 and 10).

4 Evaluation of the microbiological tests and discussion of the results.

For all 3 analysed test bacteria a clear reduction could be proved by the use of the UV disinfection device Purion 1.0 (see Table 5).

Table 5: Reduction of the test bacteria

Parameter	**Colony at 36°C**	**Pseudomonas aeruginosa**	**Escherichia coli**
Concentration at inflow (Average)	8.540/ml	388000/100 ml	1.360.000/100 ml
Concentration at outflow (Average)	0,4/ml	5,6/100 ml	0,8/100 ml
Reduction factor*	4,33	4,84	6,23
Reduction in %	99,9953	99,985	99,9999

***Reduction factor:** $\log_{10}$ bacteria concentration at inflow - $\log_{10}$ bacteria concentration at outflow

At Enterobacter cloacae, as a test organism for the determination of the colony number at 36 °C, a reduction of the test organism through the UV disinfection device occurred around approx. 4 $\log_{10}$ -stages, that means the test organism was reduced to 99,99 %. In the outflow the limit value of the drinking water directive for the colony number at 36 °C of 100 KBE/ml was kept in all of the 5 samples without problems. The maximum value in the outflow was near 1 KBE/ml (at 2 samples). In 3 samples the value was near 0/ml, which means the bacterium concentration lay below the detection limit of the procedure.

At Pseudomonas aeruginosa a reduction of the bacteria occurred around almost 5 $\log_{10}$ -stages. On the average Pseudomonas aeruginosa was reduced around 99,9985 %. In the outflow of the UV disinfection device small amounts of Pseudomonas aeruginosa were still provable, 5,6 ml KBE/100ml on the average.

At E. coli a reduction of the bacteria occurred around approx. 6 $\log_{10}$ stages. Through the UV disinfection device E. coli was reduced around 99.9999%. In the

outflow minimum amounts of E. coli were still proved in 3 samples (1 and 2 ml KBE/100). Two samples of 100 ml were free of E. coli. In total with the test operation carried out a very good effectiveness can be certified to the UV disinfection device Purion 1.0.

With the described test conditions the used test organisms were reduced around 4 to 6 $\log_{10}$-stages. If one takes the minimum reduction of bacteria of around 4 $\log_{10}$-stages as a basis, waters with colony numbers up to almost 106 KBE/ml or with E. coli concentrations smaller than 10000/100 ml can be disinfected under the described test conditions with the UV disinfection device Purion 1.0 in a way that the microbiological limit values of the drinking water directive are kept.

With the use of the UV disinfection device for the disinfection of already purified drinking water and/or the disinfection of water from own water supply installations that was not subjected to any previous preparation it is to be assumed that in the water to be cleaned the above-mentioned bacteriological values are normally not exceeded and accordingly the water does have the above-mentioned bacteriological values after the UV disinfection and it is drinking water quality in a bacteriological sense. The case of the simultaneous appearance of different bacteria was not examined within the framework of the test operation. There are not, however, any plausible reasons that fundamentally other results have to be expected in this case. The analysis on Trihalogenmethane showed, that during the test operation using the UV disinfection device no IHM was generated. No statement can be made about the effectiveness of the UV disinfection device in a continuous operation mode because the device was operated only for approx. 20 to 30 minutes for each individual test series. For the UV disinfection device a maximum volume flow of 40 l/min is indicated, at which the UV disinfection is possible. Within the tests carried out the volume flow was 26 to 29 l/min. Higher volume flows were not reached in the available test installation in the building of the HUC GmbH at any place.