REMOVAL OF *KLEBSIELLA TERRIGENA* FROM MAINS WATER - ASSESSMENT OF THREE STERASYL FILTER ELEMENTS

Report No.: CO 5014

October 2001

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SUMMARY

The performance of three Sterasyl filters was assessed for their ability to remove *Klebsiella terrigena* cells from mains water.

The test procedure employed was the United States Environmental Protection Agency (USEPA) Guide Standard and Protocol for Testing Microbiological Water Purifiers. The test was of 14 days duration during which the filters were challenged with two types of water, both of which were amended with the test organism at a concentration of 1×10^7 cells per 100 ml. The initial phase of the test, which was of duration 8 days, used dechlorinated mains water and the final stage of the test a challenge water, in which mains water was amended to elevate the TOC (total organic carbon), turbidity, TDS (total dissolved solids) and pH in accordance with the USEPA protocol.

Each of the filters tested did not show any breakthrough of the test organism during the entire duration of the test. The removal efficiency of the filters is therefore at least 7 logs under the prescribed test conditions. The filter effluents were also analysed for silver but no residues were detected throughout the duration of the trial.

1. INTRODUCTION

Fairey Industrial Ceramics commissioned WRc-NSF to assess the removal efficiency of three Sterasyl filter candles using the bacterium *Klebsiella terrigena* as the challenge organism.

The filter elements tested were 2" diameter x 10" long Sterasyl Ceramic Filter elements.and were taken directly from Fairey Industrial Ceramics Ltd. (FICL) production and are a typical example of FICL products.

The test was performed according to the USEPA Guide Standard and Protocol for Testing Microbiological Water Purifiers. The test is designed to substantiate the microbiological removal capabilities over the effective use life of the purifier. The procedure is designed to replicate the field use conditions (worst case) and to this end employs clean mains water for the initial phase of the test and a challenge water for the final stressed challenge phase of testing.

2. METHODS

2.1 Preparation of challenge culture

The challenge suspension was prepared using a freeze-dried culture of *Klebsiella terrigena* (NCIMB 12053) obtained from NCIMB Ltd., Aberdeen, AB24 3RY.

The culture was rehydrated aseptically with 0.5 ml of nutrient broth (Oxoid). The contents of the tube were transferred to a culture tube containing 5 ml of nutrient broth and incubated at 35°C for 24 hours. A loopful of culture was streaked onto a nutrient agar plate which was incubated at 35°C for 24 hours. Individual colonies, aseptically removed from the plate, were used to inoculate 200 ml of nurtrient broth which was incubated at 35°C for 24 hours. The cells were centrifuged (8000 *g* × 10 min) and the pellet resuspended in 100 ml sterile phosphate-buffered saline (Oxoid). The resuspended cells were washed twice using the same centrifugation conditions. The *Klebsiella* suspension was serially diluted in ¼ strength Ringers solution (Oxoid) and dilutions of the suspension assayed using a standard membrane filtration procedure (Clesceri *et al.* 1998). The membranes (Gelman) were transferred to petri dishes containing modified M-FC agar and were incubated for 24 hours at 35°C. The density of the washed cell suspension of *Klebsiella terrigena* was determined as 2×10^9 ml^{-1}.

2.2 Test waters

Two types of water were employed during the evaluation. The general water was mains water which was supplied to the holding tank through a dechlorinating filter.

The challenge water was prepared by amending dechlorinated mains water. Analysis established that the addition of 20 mg humic acid sodium salt (Sigma Aldrich) and 1.15 g of sea salts (Sigma) to 1 litre of water gave a TOC of 6.4 mg l^{-1} and TDS of 1.15 g respectively. Appropriate quantities of the materials were dissolved in the mains water to form the challenge water as shown in Table 1. The pH of the challenge water was adjusted with 0.2 M sodium hydroxide and the temperature maintained at 4°C using a Techne Dip Cooler Model RU200. The turbidity of the water was adjusted with ISO 12103-1 Standardised Arizona Test Dust Contaminant (Powder Technology Inc. Minnesota, USA, obtained through Ellis Components, Alfreton, Derbyshire). The addition of 50 mg l^{-1} of the test dust produced the specified turbidity.

Table 2.1 Water quality parameters

Test water	General	Challenge
pH	6.5	9.0 ± 0.2
Total organic carbon	0.70 mg l^{-1}	10 mg l^{-1}
Total dissolved solids	350 mg l^{-1}	1500 mg l^{-1}
Turbidity	0.1 NTU	30 NTU
Temperature	20 ± 5°C	4 ± 1°C

2.3 Silver analysis

Inductively coupled plasma mass spectrometry was used to determine the concentration of silver in the common feed and filter effluent samples. All samples were collected into nitric acid. The sample was spiked with an internal standard and introduced in aerosol form into an argon plasma which is maintained at a temperature of ca. 7000 K. The plasma is produced and sustained by electromagnetic coupling through a coil in an RF circuit. Determinands in the sample are ionised in the plasma, and a small portion of these ions are sampled and introduced into the mass spectrometer. The ions are separated (and identified) by their mass:charge ratio and are detected using a dynode array detector (SCA 1996). The detection limit of the assay is 0.1 µg $litre^{-1}$.

3. FILTER EVALUATION

A test rig supplied by Fairey Industrial Ceramics was set up in the microbiology laboratory at WRc-NSF.

The water tank of the test rig was filled with 105 litres of the general water and adjusted to 20 ± 2°C. The filters were flushed in the rig with the general (dechlorinated mains) water for 12 minutes at a flow rate of 3 litres minute^{-1}. The tank was then spiked with the suspension of *Klebsiella terrigena* calculated to give a cell concentration per 100 ml of approximately 1×10^7 in the test water.

The operating cycle of the test system used a 30 minute cycle time with an 20% on and 80% off period for the filters under evaluation. This cycle was maintained for 8 hours per day. The influent pressure to the filters under test was maintained at 60 pounds per square inch throughout the course of the trial.

Samples for bacterial analysis were taken from the filter influent and effluent according to the schedule in the USEPA protocol. The sampling plan is summarised in Table 3.1 below.

All samples were taken in duplicate and assayed using membrane filtration on modified MFC agar using the method described for the challenge culture in Section 1.1 above. The influent samples were serially diluted to yield counts between 20 and 60 colonies per membrane. The sample size of the effluents which were assayed using the membrane filtration procedure were 1 ml, 10 ml and 100ml.

Table 3.1 Filter test schedule

Test Point[1]	Test Water	Tests			
		Filter cleaning	Microbiological analysis of the mains water supply (background)	Microbiological analysis of influent and filter effluents	Silver residue analysis of influent and filter effluents
Start	General		×	×	×
Day 3 (middle)				×	×
Day 6 (middle)				×	×
After 48 hours stagnation		×		×	×
Day 7 (middle)	Challenge			×	×
Day 8 (near end)				×	×
After 48 hours stagnation		×		×	×
Day 10½				×	×

[1] All days are running days and exclude stagnation periods

4. RESULTS

The concentration of *Klebsiella terrigena,* assayed using membrane filtration according to the filter test schedule (Table 4.1), in the common water feed and from the individual effluents of each Sterasyl filter element are shown in Table 4.1

Table 4.1 Assay of the test organism, *Klebsiella terrigena*, and silver residues in the common influent and effluent samples of each filter element during the trial period

Test Point	Test Water	*Klebsiella terrigena* (colony forming units per 100 ml) and, in parenthesis, silver concentration[1]				
		Influent background	Influent	Filter effluent		
				Filter 1	Filter 2	Filter 3
Start	General	0 (nd)	3.5×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
Day 3 (middle)			1.7×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
Day 6 (middle)			1.5×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
After 48 hours stagnation			3.7×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
Day 7 (middle)	Challenge		1.2×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
Day 8 (near end)			2.9×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
After 48 hours stagnation			2.7×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)
Day 10½			1.8×10^7 (nd)	0 (nd)	0 (nd)	0 (nd)

[1]nd – not detected

Table 4.2 shows the removal efficiency of the test bacterium during the course of the trial for each Sterasyl filter element. The removal efficiency is better than 7 log throughout the course of the trial. Samples of the influent to the filters and the filter effluents were also assayed for silver at the test points shown in Table 4.2. Silver was not, however, detected in any of the samples. The concentration of the metal is therefore below the level of detection of the assay (0.1 µg litre^{-1}).

Table 4.2 Removal efficiency of the test organism during the trial period

	log reduction of *Klebsiella terrigena* for each filter element		
Test Point	**Filter 1**	**Filter 2**	**Filter 3**
Start	7.5	7.5	7.5
Day 3 (middle)	7.2	7.2	7.2
Day 6 (middle)	7.2	7.2	7.2
After 48 hours stagnation	7.6	7.6	7.6
Day 7 (middle)	7.1	7.1	7.1
Day 9 (near end)	7.5	7.5	7.5
After 48 hours stagnation	7.4	7.4	7.4
Day 10½	7.3	7.3	7.3
Mean removal efficiency (± 1 standard deviation)	7.35 ± 0.17	7.35 ± 0.17	7.35 ± 0.17

5. CONCLUSIONS

Three Fairey Sterasyl Ceramic filter elements underwent the USEPA protocol for the microbiological testing of water purifiers. The test programme revealed that each filter element excluded the challenge organism, *Klebsiella terrigena*, to an extent that exceeded the USEPA requirements to qualify as a microbiological water purifier with respect to pathogenic bacteria. The minimum required reduction is at least 6 log throughout the entire duration of the test programme. The actual removal efficiencies were better than 7 log for each filter element.

Assay of the filter effluents for silver showed that the metal did not leach from any of the three filter elements under test during the course of the trial.

6. REFERENCE

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