April
2013

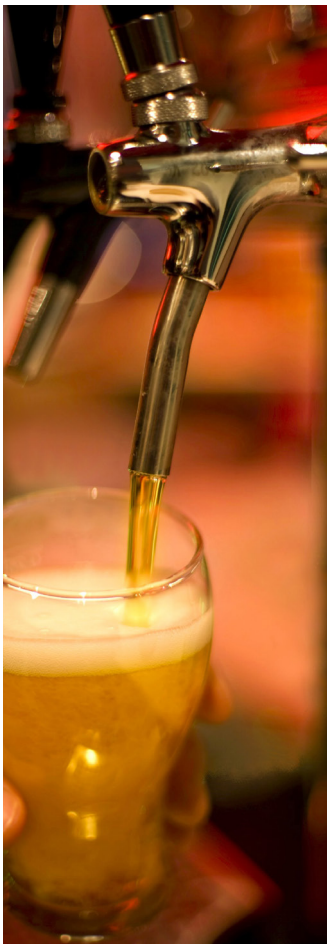
Campden BRI
food and drink innovation

Instrument Assessment Report

NovaSol DC/12

Assessment of a novel cleaning/disinfectant agent

Image source: AquaNova AG



Executive summary

The efficacy of NovaSol®DC/12, a novel disinfectant based on benzoic acid, against brewing-associated surface contaminants was evaluated. Grout, tile and stainless steel surfaces were artificially contaminated and then cleaned with either 80°C water or aqueous 10% w/w NovaSol®DC/12. A further test was carried out to investigate the potential longer term efficacy of NovaSol. For this purpose cleaned surfaces were re-incubated following the cleaning procedure.

- NovaSol®DC/12 decontaminated grout surfaces more effectively than cleaning with 80°C water did.
- For tile and stainless steel surfaces the decontamination effect of NovaSol®DC/12 was similar to that of cleaning with 80°C water. These surfaces are smoother than grout so that micro-organisms are washed off easily (>90% decrease in microbial numbers). The grout surface is rough (poor wash off) therefore NovaSol®DC/12's chemical action shows its effectiveness here.
- On the tile and stainless steel surfaces the NovaSol®DC/12 treatment showed a long-term (2 weeks) microbial inhibitory effect, i.e. less re-growth was detected following the treatment with NovaSol®DC/12 than following cleaning with 80°C water.
- NovaSol®DC/12 was predominantly active against yeast and bacteria but less so against moulds (possibly due to the presence of resistant spores). The re-growth after 2 weeks following the NovaSol®DC/12 treatment consisted primarily of mould.

Instrument Assessment Report

Background

Aquanova have developed and patented a whole range of innovative products which use the disinfectant and preservation properties of sorbic and benzoic acid and have overcome the problems associated with their use (pH dependence, solubility) by encapsulating the preservatives into micelles which form a superior transport system and are thermally, mechanically and pH stable. One of these innovative products is NovaSol®DC/12. The technology of DC/12 is based upon patents, being the property of a patent fund (3rd Patentportfolio Beteiligungsgesellschaft mbH & Co.KG) set up and managed by the Deutsche Bank AG and Clou Partners GmbH. The patent fund invested in NovaSol®DC/12 and its development in close collaboration with Aquanova having the intention to sell or license out the patented technology. NovaSol®DC/12 is specifically used for surfaces not intended to be eaten. It is water as well as fat soluble and contains 12% benzoic acid. Aquanova has carried out some trials using NovaSol®DC/12 in parts of the filler and capper area of a beer bottling line. The results appeared to be very successful. This product could be of great interest to the brewing industry. The patent fund has approached Campden BRI to independently evaluate NovaSol®DC/12 to determine its applicability in the brewing industry.

Scope of Work

- This study evaluated the capability of NovaSol®DC/12 to efficiently clean/disinfect different types of surface materials which had been artificially contaminated.
- The residual effect of limiting further microbiological growth on surfaces once they had been treated with NovaSol®DC/12 was assessed.

Experimental

Testing was carried out using three different types of surfaces (tiles, grout and roughened stainless steel) which were incubated with either Campden BRI's proprietary biofilm culture (a mix of beer-specific aerobic bacteria and yeast as well as anaerobic bacteria) or the biofilm culture with an added common mould collected from a mouldy wall. The surfaces were incubated in sealed containers at 25°C for 3 weeks during which time the surfaces were inoculated/sprayed daily with the two different types of microbial culture. Additionally, uncontaminated growth media was sprayed onto the surfaces regularly to provide sufficient nutrients for the microorganisms and to optimise the growth conditions. This process allowed cells to attach and a biofilm to start developing. 12 samples of each surface type were prepared (3 samples in duplicate to be incubated with biofilm and 3 samples in duplicate to be treated with biofilm containing mould).

After the 3-week incubation period, duplicate samples of each type of surface were analysed directly (controls) and the remaining surface samples were sprayed with approximately 10mls of either NovaSol®DC/12 (at a 10% w/w concentration, contact time 30 minutes), or water at 80°C (contact time 30 minutes) as a reference cleaning method. The controls and a set of duplicates cleaned using the 2 different methods were swabbed to collect microorganisms attached to the surfaces; the swabs were re-suspended in sterile diluent. Serial dilutions were then performed from each re-suspended sample and an aliquot of each plated onto growth media chosen to determine the presence and respective numbers of aerobic bacteria, yeast and mould or anaerobic lactic acid bacteria. The plates were incubated either for 3 days at 25°C for aerobic bacteria, yeast and mould or in a CO_2-enriched environment for 5 days at 25°C for the detection of anaerobic bacteria.

The sets of duplicates of different surfaces treated either with NovaSol®DC/12 or water at 80°C which remained un-swabbed were incubated for a further 2 weeks at 25°C to test the residual effect of both methods to limit further microbial growth. During the period of incubation, nutrients were sprayed onto the surfaces every 3 days to allow any possible viable microorganisms to grow. After the two weeks, the surfaces were swabbed and analysed following the same method described previously.

Results

After the 3-week inoculation and incubation of the surface samples mould was visible on all the surfaces whether or not they had been contaminated with mould. It is likely that the surfaces themselves had contained some spores that developed during the incubation period. Table 1 shows the percentage reduction in viable microorganisms present on the different surfaces, which had been contaminated with biofilm, treated with either water at 80°C or NovaSol (10% w/w). The numbers of cells recovered from the surfaces pre and post treatment are tabulated in the Appendix. In the case of tile and stainless steel surfaces cleaning with NovaSol resulted in a similar reduction of viable surface

microorganisms compared to the hot water treatment. Both these materials have relatively smooth surface textures allowing cells to be washed off quite easily. For the rough grout surface cells do not wash off as easily. Thus, following the hot water treatment 48% of micro-organisms remain on the surface, whereas only 1% and 12% remained on the tile and stainless steel surfaces. NovaSol exhibits its antimicrobial activity on the grout surface with only 21% of cells surviving on the surface following the chemical treatment. Table 2 shows the percentage reduction in viable microorganisms present on the different surfaces which had been contaminated with biofilm culture containing a common mould and treated with either water at 80˚C or NovaSol at 10% w/w. As before, compared to the hot water treatment NovaSol showed the greatest surface microbial reduction effect for the grout.

Both sets of results are also presented graphically in Figure 1. In both situations NovaSol performed better than the hot water clean for the grout surface. However, the tiles were cleaned more efficiently with the hot water. This difference may be due to the difference in roughness of the surfaces. The tiles are relatively smooth and if cells are not well attached they may be washed away just with water, whereas cells will not wash off the rough grout surface easily. NovaSol's chemical antimicrobial action becomes apparent in this situation performing better than the water treatment.

Surface	Cleaning method	Average reduction in surface micro-organisms (%)
Grout	Water at 80°C	52
	NovaSol 10% (w/w)	79
Tiles	Water at 80°C	99
	NovaSol 10% (w/w)	93
Stainless Steel	Water at 80°C	88
	NovaSol 10% (w/w)	98

Table 1: Percentage reduction in viable micro-organisms present on the different surfaces contaminated with biofilm culture and treated with either water at 80°C or NovaSol at 10% w/w.

Surface	Cleaning method	Average reduction in surface micro-organisms (%)
Grout	Water at 80°C	79
	NovaSol 10% (w/w)	90
Tiles	Water at 80°C	94
	NovaSol 10% (w/w)	78
Stainless Steel	Water at 80°C	95
	NovaSol 10% (w/w)	98

Table 2: Percentage reduction in viable micro-organisms present on the different surfaces contaminated with biofilm culture containing a common mould and treated with either water at 80°C or NovaSol at 10% w/w.

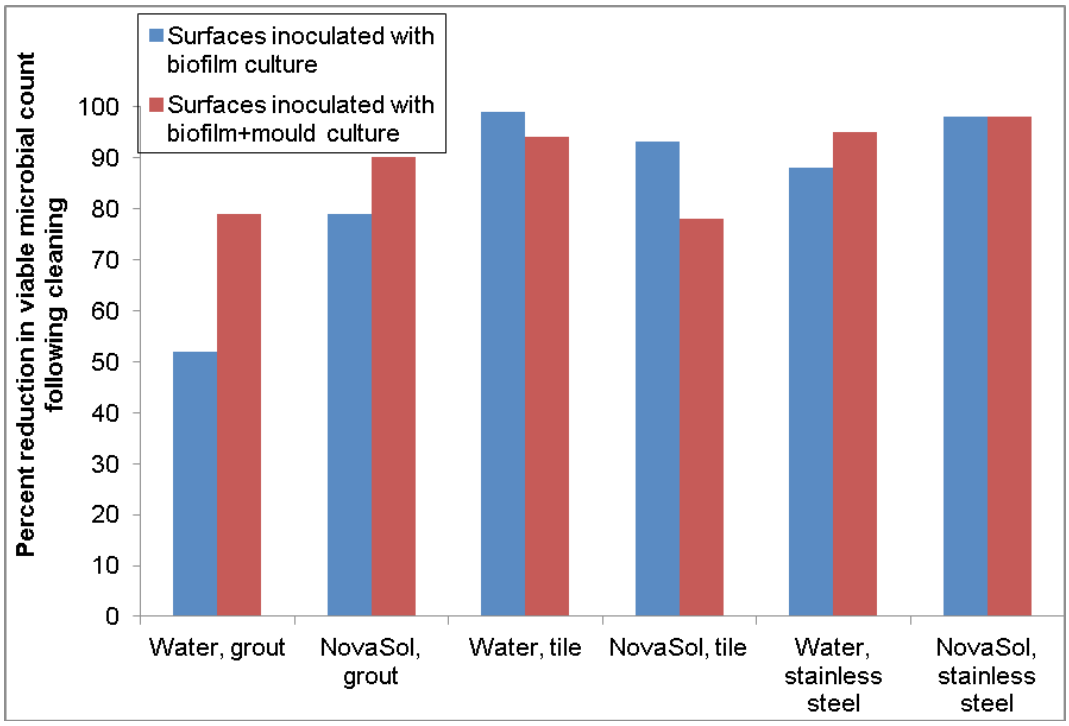


Figure 1: Percentage reduction in viable micro-organisms present on the different surfaces contaminated with biofilm culture with or without a common mould and treated with either water at 80°C or NovaSol at 10% w/w

Following the two cleaning procedures a set of sample surfaces were re-incubated for 2 weeks to investigate whether the treatments could inhibit further microbial growth. Results are tabulated in the Appendix. Figures 2 and 3 show the results for the tile surfaces following cleaning and re-incubation. All surfaces exhibited an increase in micro-organisms independent of the cleaning method. Due to the initial better clean with NovaSol the cell numbers attached to these surfaces was lower than for the water-cleaned tiles. It was noted that re-growth was considerably lower following the NovaSol treatment than the hot water treatment. This would indicate that NovaSol had a 'long-term' microbial inhibitory effect.

For the stainless steel surfaces the results were very similar with rising numbers of surface microbial contaminants in all cases (see Figures 4 and 5). For the stainless steel surfaces contaminated with the biofilm culture NovaSol was more effective than hot water at inhibiting re-growth. However, for the biofilm+mould inoculated stainless steel surfaces more re-growth was detected for the hot water treated sample surfaces. This may be due mould (spores) being resistant to the chemical treatment.

In general, it was noticed that the surfaces cleaned with NovaSol showed high levels of mould following the 2-week re-incubation. The samples inoculated with biofilm culture containing mould were also seen to have a higher re-growth. This seems to indicate that NovaSol may be quite efficient against the bacteria and yeast used in this study but not as efficient against mould (spores).

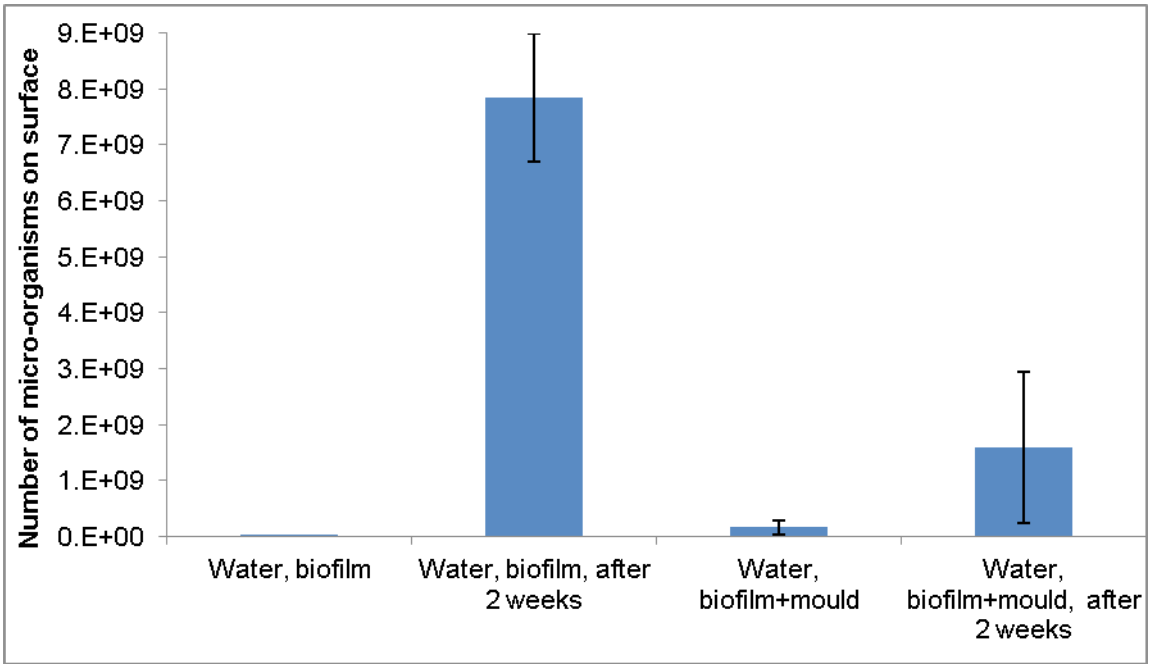


Figure 2: Level of contamination on tile surfaces contaminated either with biofilm or biofilm containing mould and cleaned with 80°C water. One set of surface samples was re-incubated for 2 weeks following the cleaning procedure.

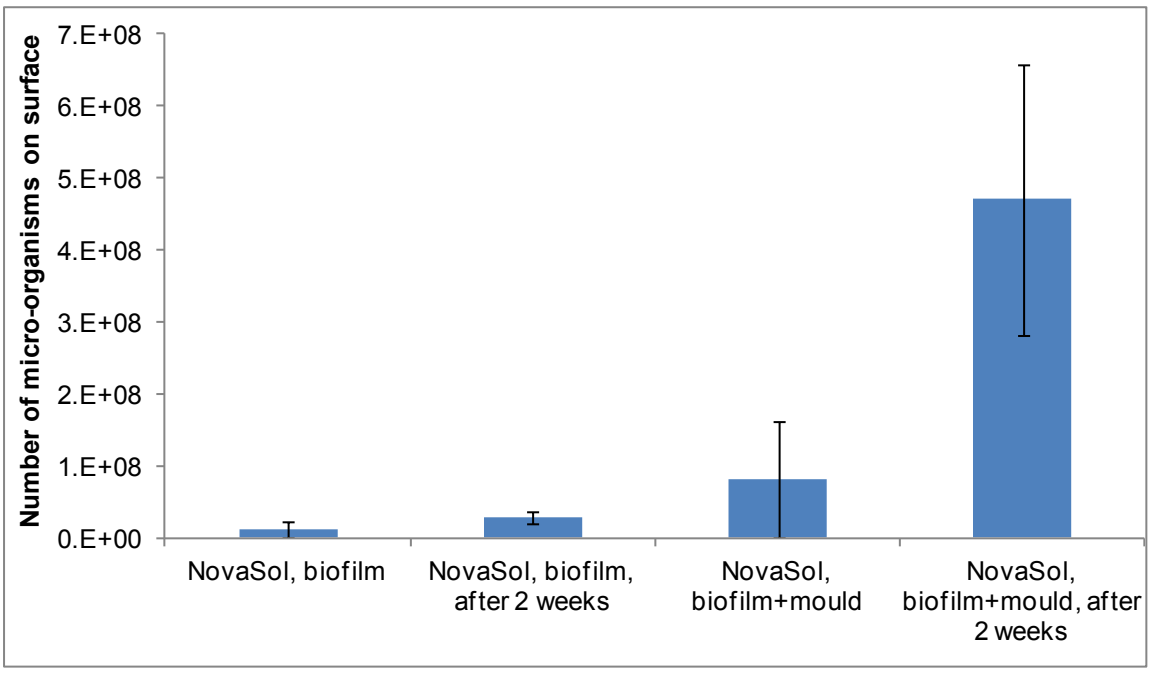


Figure 3: Level of contamination on tile surfaces contaminated either with biofilm or biofilm containing mould and cleaned with 10% w/w NovaSol. One set of surface samples was re-incubated for 2 weeks following the cleaning procedure.

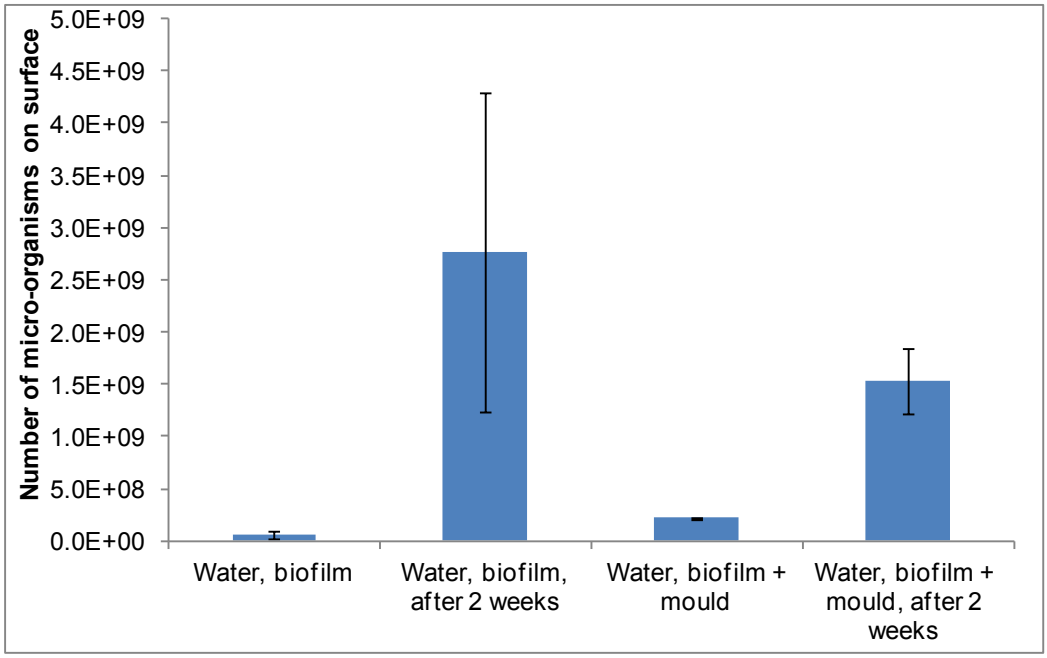


Figure 4: Level of contamination on stainless steel surfaces contaminated either with biofilm or biofilm containing mould and cleaned with 80°C water. One set of surface samples was re-incubated for 2 weeks following the cleaning procedure.

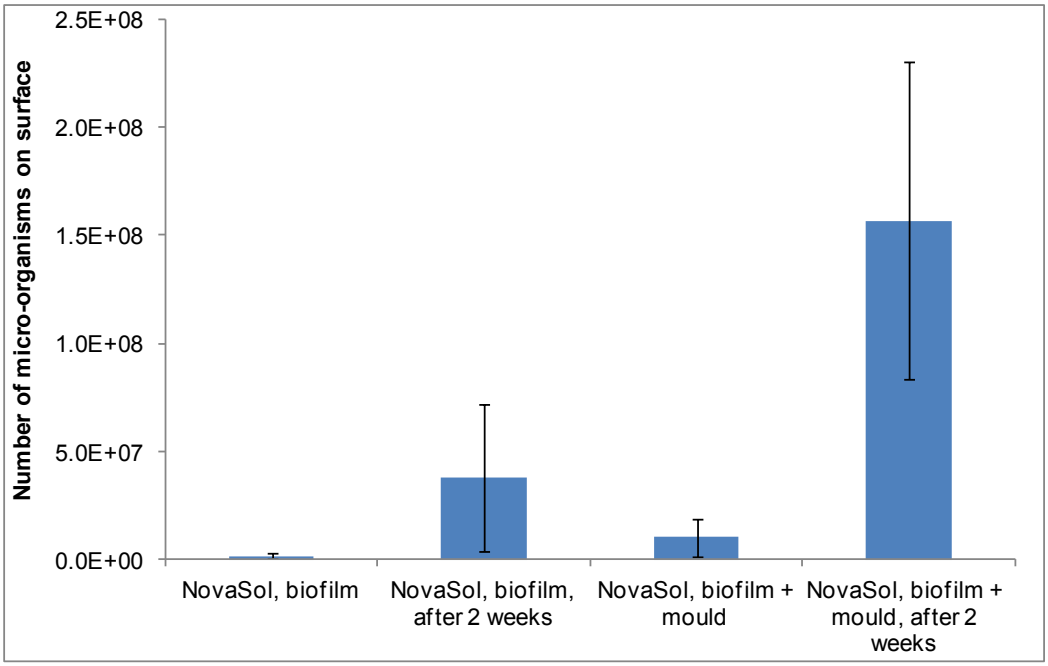


Figure 5: Level of contamination on stainless steel surfaces contaminated either with biofilm or biofilm containing mould and cleaned with 10% w/w NovaSol. One set of surface samples was re-incubated for 2 weeks following the cleaning procedure.

Summary and conclusions

In this study the efficacy of NovaSol®DC/12 against brewing associated microbial surface contaminants was compared to that of an 80°C water treatment. Grout, tile and stainless steel sample surfaces were inoculated and incubated with a biofilm culture, a mix of aerobic and anaerobic bacteria as well as yeast, or biofilm with a common mould species added. Following the 3-week artificial contamination a set of surfaces was cleaned with either 80°C water or an aqueous 10% w/w NovaSol®DC/12 solution. The surfaces were then swabbed to retrieve any surviving microbial cells together with a set of non-cleaned surfaces as controls. A set of cleaned surface samples was re-incubated for a further 2 weeks to see whether the treatments would be able to inhibit microbiological growth.

Both, the hot water and the NovaSol cleaning procedures resulted in a drop in contaminants on all surfaces but more so for the tile and stainless steel surfaces. These materials are relatively smooth so that any cells that are not strongly attached can be washed away easily. NovaSol appeared to perform similarly to hot water on the tiles and stainless steel surface. However, for the grout NovaSol worked more efficiently. This may be due to this surface being rougher and cells not being washed off so easily – the chemical action is more important in this case.

Following re-incubation of the cleaned surfaces an increase in surface cell numbers was seen in most cases indicating that cell growth is not completely inhibited for either of the cleaning procedures. For the tile and stainless steel surfaces NovaSol appeared to limit microbial re-growth compared to the hot water treatment, thus indicating a microbial inhibitory effect. It was noticed that after re-incubation moulds were pre-dominant after the NovaSol treatment. This would indicate that NovaSol is more effective against bacteria and yeast than against mould (spores). The product is based on benzoic acid which is known to be most effective against yeasts.

Appendix 1

Surface	Cleaning method	Pre-treatment (average cells/ surface)	Post treatment (average cells/ surface)	Average reduction in surface micro-organisms (%)
Grout	Water at 80°C	9.97×10^9	4.74×10^9	52
	NovaSol 10% (w/w)	1.13×10^7	2.35×10^6	79
Tiles	Water at 80°C	1.45×10^8	1.62×10^6	99
	NovaSol 10% (w/w)	1.61×10^8	1.18×10^7	93
Stainless Steel	Water at 80°C	4.95×10^8	5.98×10^7	88
	NovaSol 10% (w/w)	1.19×10^8	1.90×10^6	98

Reduction in viable micro-organisms present on the different surfaces contaminated with biofilm culture and treated with either water at 80°C or NovaSol at 10% w/w

Surface	Cleaning method	Pre-treatment (average cells/ surface)	Post treatment (average cells/ surface)	Average reduction in surface micro-organisms (%)
Grout	Water at 80°C	9.67×10^9	2.03×10^9	79
	NovaSol 10% (w/w)	1.02×10^7	1.00×10^6	90
Tiles	Water at 80°C	2.97×10^9	1.66×10^8	94
	NovaSol 10% (w/w)	3.78×10^8	8.25×10^7	78
Stainless Steel	Water at 80°C	4.61×10^9	2.19×10^8	95
	NovaSol 10% (w/w)	6.97×10^8	1.05×10^7	98

Reduction in viable micro-organisms present on the different surfaces contaminated with biofilm culture containing a common mould and treated with either water at 80°C or NovaSol at 10% w/w

Surface	Cleaning method	Surface contaminants	Post treatment (average cells/ surface)	2 weeks later (average cells/ surface)	Average multiple increase in surface micro-organisms
Tiles	Water at 80°C	Biofilm	1.62×10^6	7.85×10^9	4,846 times
		Biofilm + mould	1.66×10^8	1.60×10^9	10 times
	NovaSol 10% (w/w)	Biofilm	1.18×10^7	2.76×10^7	2 times
		Biofilm + mould	8.25×10^7	4.70×10^8	6 times
Stainless Steel	Water at 80°C	Biofilm	5.98×10^7	2.77×10^9	46 times
		Biofilm + mould	2.19×10^8	1.53×10^9	7 times
	NovaSol 10% (w/w)	Biofilm	1.90×10^6	3.79×10^7	20 times
		Biofilm + mould	1.05×10^7	1.57×10^8	15 times

Increase in viable micro-organisms present on the different contaminated surfaces and treated with either water at 80°C or NovaSol at 10% w/w, following 2 weeks re-incubation

Contacts

Kristina Dietsch, T: 49 (0) 89-3090484-61
DIETSCH@CLOUPARTNERS.DE

Marcel Tilmann, T: 49 (0) 174-6747773
MT@MARCELTILMANN.COM

Prof. Dr. Dr. Markus J. Seewald, T: 49 (0) 177-8345652
M.SEEWALD@LOEL.HS-ANHALT.DE