Henkel AG & Co. KGaA

Microbiology

Test report

20-09373-3

on the
bactericidal efficacy

of

Biodyozon

According to DIN EN 1276:

Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas — Test method and requirements (phase 2, step 1) German version EN 1276:2019

Customer:	Mr. Florian Wüstkamp,
	Greensafer GmbH, Dreieich
Author:	Roland Breves
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H e n k e l AG & Co. KGaA
Corporate Scientific Services / Microbiology
D-40191 Düsseldorf

Tel.: +49-211-797 9630
Fax: +49-211-798 2245
e-mail: roland.breves@henkel.com

Content

1. Test laboratory

Henkel AG & Co. KGaA
Corporate Scientific Services / Microbiology
D-40191 Düsseldorf

2. Identity of the test substance

2.1.	Product name	**Biodyozon**
2.2	Batche	1000 ppm, pH 5.25
2.3	Manufacturing date	August 25, 2020
2.4	Manufacturer	GreenSafer GmbH
2.5	Date of sample entry	September 1, 2020
2.6	Active substance(s)	Hypochloric acid (HOCl)
2.7	Storage conditions in the laboratory	room temperature
2.8	Appearance	clear liquid

3. Test method and neutralization

3.1 Suspension according to DIN EN 1276:

Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas — Test method and requirements (phase 2, step 1) German version EN 1276:2019.

3.2 Neutralization by: dilution-neutralization

Neutralizer solution based on:

3% Tween 80, 0.3% Lecithin, 0.1% Histidin, 0.5% Sodium thiosulphate

4. Experimental conditions

4.1	Date of test:	November 13 – 18, 2020		
4.2	Diluent:	Water of standardized hardness		
4.3	Test concentrations:	800 – 600 - 400 ppm (final concentration in test)		
4.4	Appearance of the test dilutions:	colourless, clear liquid		
4.5	Test organisms:	*Enterococcus hirae*	*DSM 3320*	*(=ATCC 10541)*
		Pseudomonas aeruginosa	DSM 939	(=ATCC 15442*)*
		Staphylococcus aureus	DSM 799	(=ATCC 6538)
		Escherichia coli K12	DSM 11250	(=NCTC 10538)
4.6	Contact time(s):	30 sec - 60 sec - 5 min		
4.7	Test temperature:	Room temperature		
4.8	Interfering substance:	0.3 g/l BSA (low soil conditions)		
4.9	Incubation temperature:	37°C		

5. Results

An overview of the achieved reduction factors is given in the table below. Detailed test results are illustrated in the appendix. The non-interference of the chosen experimental conditions, the absence of toxicity of the selected neutralizer solution as well as the successful validation of the neutralization method were proven. Thus, the results can be regarded as sufficiently valid.

DIN EN 1276	**Biodyozon 20°C / low soil (0.3g/l BSA)**			
Test organism ▼	**Reduction factors (RF)**			
	Conc. ▼ Time ►	**30 sec**	**60 sec**	**5 min**
Enterococcus hirae DSM 3320 (=ATCC 10541)	**800ppm**	**>5.16**	**>5.16**	**>5.16**
	600 ppm	**>5.16**	**>5.16**	**>5.16**
	400 ppm	**>5.16**	**>5.16**	**>5.16**
Staphylococcus aureus DSM 799 (=ATCC 6538)	**800ppm**	**>5.22**	**>5.22**	**>5.22**
	600 ppm	**>5.22**	**>5.22**	**>5.22**
	400 ppm	**>5.22**	**>5.22**	**>5.22**
Pseudomonas aeruginosa DSM 939 (=ATCC 15442*)*	**800ppm**	**>5.33**	**>5.33**	**>5.33**
	600 ppm	**>5.33**	**>5.33**	**>5.33**
	400 ppm	**>5.33**	**>5.33**	**>5.33**
***Escherichia coli* K12** DSM 11250 (=NCTC 10538)	**800ppm**	**>5.11**	**>5.11**	**>5.11**
	600 ppm	**>5.11**	**>5.11**	**>5.11**
	400 ppm	**>5.11**	**>5.11**	**>5.11**

6. Conclusion

In the standard EN 1276 the minimum requirements for efficacy are defined as the capability to reduce the viable counts of the test organisms by a factor of ≥5lg within the defined parameters of contact time and temperature under the influence of the chosen level of interfering substances.

For the product **Biodyozon** a **sufficient bactericidal efficacy** against all test organisms prescribed in EN 1276 could be achieved at room temperature and simulated low soil conditions **30 sec at 400 ppm** of product concentration.

Duesseldorf, November 30, 2020

Dr. Roland Breves
Head of Microbiology

Julia deBache-Boy
Head of lab Product Efficacy

Appendix: Detailed test results

Appendix: Test results, validation and controls - Bactericidal efficacy EN 1276

DIN EN 1276:2019		Sample 20-09373/7	T = 20°	Soil: low (0.03% BSA)	Biodyozon				
	Validation and controls				Product tests (5.5.2.2): test conditions : concentration [ppm] and contact times [sec/min]				
Test organism	Validation suspension Nv (Nv_0) (5.4.1.5)	Validation of the selected experimental conditions A (see 5.5.2.3)	Verification of the absence of toxicity of the neutralizer B (5.5.2.4)	Validation of the dilution-neutralization method C (5.5.2.5)	Test suspension N (5.4.1.4)		800 ppm	600 ppm	400 ppm
Enterococcus hirae DSM 3320 (=ATCC 10541)	V_c: 115/123 Nv_0: $1.19x10^2$ [x]yes [ ]no	**30 sec** V_c: 115/ 88 A: $1.02x10^2$ [x]yes [ ]no	V_c: 94/120 B: $1.07x10^2$ [x]yes [ ]no	**30 sec / 800 ppm** V_c: 105/ 94 C: $9.95x10^1$ [x]yes [ ]no	10^{-6} : 185/228 10^{-7} : 16/24 N: $2.06x10^8$ **lg N: 8.31** **lg N_0: 7.31** [x]yes [ ]no		**30 sec**	**30 sec**	**30 sec**
						Vc_0:	0 / 0	0 / 0	0 / 0
						Vc_{-1}:	0 / 0	0 / 0	0 / 0
						Vc_{-2}:	0 / 0	0 / 0	0 / 0
						Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
						lg Na	<2.15	<2.15	<2.15
						R:	**>5.16**	**>5.16**	**>5.16**
		60 sec V_c: 121/121 A: $1.21x10^2$ [x]yes [ ]no		**60 sec / 800 ppm** V_c: 122/124 C: $1.23x10^2$ [X]yes [ ]no			**60 sec**	**60 sec**	**60 sec**
						Vc_0:	0 / 0	0 / 0	0 / 0
						Vc_{-1}:	0 / 0	0 / 0	0 / 0
						Vc_{-2}:	0 / 0	0 / 0	0 / 0
						Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
						lg Na	<2.15	<2.15	<2.15
						R:	**>5.16**	**>5.16**	**>5.16**
		5 min V_c:123/116 A: $1.19x10^2$ [x]yes [ ]no		**5 min / 800 ppm** Vc: 82/100 C: $9.1x10^1$ [X]yes [ ]no			**5 min**	**5 min**	**5 min**
						Vc_0:	0 / 0	0 / 0	0 / 0
						Vc_{-1}:	0 / 0	0 / 0	0 / 0
						Vc_{-2}:	0 / 0	0 / 0	0 / 0
						Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
						lg Na	<2.15	<2.15	<2.15
						R:	**>5.16**	**>5.16**	**>5.16**
Staphylococcus aureus DSM 799 (=ATCC 6538)	V_c: 113/116 Nv_0: $1.15x10^2$ [x]yes [ ]no	**30 sec** V_c: 78/112 A: $9.50x10^1$ [x]yes [ ]no	V_c: 123/137 B: $1.30x10^2$ [x]yes [ ]no	**30 sec / 800 ppm** V_c: 107/121 C: $1.14x10^2$ [x]yes [ ]no	10^{-6} : 240/225 10^{-7} : 24/22 N: $2.32x10^8$ **lg N: 8.37** **lg N_0: 7.37** [x]yes [ ]no		**30 sec**	**30 sec**	**30 sec**
						Vc_0:	0 / 0	0 / 0	0 / 0
						Vc_{-1}:	0 / 0	0 / 0	0 / 0
						Vc_{-2}:	0 / 0	0 / 0	0 / 0
						Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
						lg Na	<2.15	<2.15	<2.15
						R:	**>5.22**	**>5.22**	**>5.22**
		60 sec V_c: 103/148 A: $1.25x10^2$ [x]yes [ ]no		**60 sec / 800 ppm** V_c: 115/117 C: $1.16x10^2$ [X]yes [ ]no			**60 sec**	**60 sec**	**60 sec**
						Vc_0:	0 / 0	0 / 0	0 / 0
						Vc_{-1}:	0 / 0	0 / 0	0 / 0
						Vc_{-2}:	0 / 0	0 / 0	0 / 0
						Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
						lg Na	<2.15	<2.15	<2.15
						R:	**>5.22**	**>5.22**	**>5.22**
		5 min V_c:110/ 88 A: $9.90x10^1$ [x]yes [ ]no		**5 min / 800 ppm** Vc: 115/110 C: $1.13x10^2$ [X]yes [ ]no			**5 min**	**5 min**	**5 min**
						Vc_0:	0 / 0	0 / 0	0 / 0
						Vc_{-1}:	0 / 0	0 / 0	0 / 0
						Vc_{-2}:	0 / 0	0 / 0	0 / 0
						Na:	$<1.4x10^2$	$<1.4x10^2$	$<1.4x10^2$
						lg Na	<2.15	<2.15	<2.15
						R:	**>5.22**	**>5.22**	**>5.22**

DIN EN 1276:2019		Sample 20-09373/7	T = 20°	Soil: low (0.03% BSA)	Biodyozon				
Test organism	Validation and controls				Product tests (5.5.2.2): test conditions : concentration [ppm] and contact times [sec/min]				
	Validation suspension Nv (Nv_0) (5.4.1.5)	Validation of the selected experimental conditions A (see 5.5.2.3)	Verification of the absence of toxicity of the neutralizer B (5.5.2.4)	Validation of the dilution-neutralization method C (5.5.2.5)	Test suspension N (5.4.1.4)		80%	50%	30%
Pseudomonas aeruginosa DSM 939 (=ATCC 15442)	V_c: 236/236 Nv_0: $2.36x10^2$ [x]yes [ ]no	**30 sec** V_c: 140/134 A: $1.37x10^2$ [x]yes [ ]no	V_c: 170/230 B: $2.00x10^2$ [x]yes [ ]no	**30 sec / 800 ppm** V_c: 124/136 C: $1.30x10^2$ [x]yes [ ]no	10^{-6} : 295/300 10^{-7} : 28/42 N: $3.02x10^8$ **lg N: 8.48** **lg N_0: 7.48** [x]yes [ ]no	Vc_0: Vc_{-1}: Vc_{-2}: Na: lg Na **R:**	**30 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**	**30 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**	**30 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**
		60 sec V_c: 111/148 A: $1.30x10^2$ [x]yes [ ]no		**60 sec / 800 ppm** V_c: 154/112 C: $1.33x10^2$ [X]yes [ ]no		Vc_0: Vc_{-1}: Vc_{-2}: Na: lg Na **R:**	**60 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**	**60 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**	**60 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**
		5 min V_c:112/126 A: $1.19x10^2$ [x]yes [ ]no		**5 min / 800 ppm** Vc: 182/160 C: $1.71x10^2$ [X]yes [ ]no		Vc_0: Vc_{-1}: Vc_{-2}: Na: lg Na **R:**	**5 min** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**	**5 min** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**	**5 min** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.33**
Escherichia coli K12 DSM 11250 (=NCTC 10538)	V_c: 91/ 97 Nv_0: $9.40x10^1$ [x]yes [ ]no	**30 sec** V_c: 75/ 83 A: $7.90x10^1$ [x]yes [ ]no	V_c: 89/ 94 B: $9.15x10^1$ [x]yes [ ]no	**30 sec / 800 ppm** V_c: 70/ 70 C: $7.00x10^1$ [x]yes [ ]no	10^{-6} : 175/190 10^{-7} : 18/19 N: $1.82x10^8$ **lg N: 8.26** **lg N_0: 7.26** [x]yes [ ]no	Vc_0: Vc_{-1}: Vc_{-2}: Na: lg Na **R:**	**30 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**	**30 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**	**30 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**
		60 sec V_c: 102/ 92 A: $9.70x10^1$ [x]yes [ ]no		**60 sec / 800 ppm** V_c: 96/117 C: $1.07x10^2$ [X]yes [ ]no		Vc_0: Vc_{-1}: Vc_{-2}: Na: lg Na **R:**	**60 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**	**60 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**	**60 sec** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**
		5 min V_c: 81/ 95 A: $8.80x10^1$ [x]yes [ ]no		**5 min / 800 ppm** Vc: 86/ 76 C: $8.10x10^1$ [X]yes [ ]no		Vc_0: Vc_{-1}: Vc_{-2}: Na: lg Na **R:**	**5 min** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**	**5 min** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**	**5 min** 0 / 0 0 / 0 0 / 0 <$1.4x10^2$ <2.15 **>5.11**

Legend:

N:	Cell counts in the test suspension
Nv:	Cell counts in the validation suspension
A:	Cell counts in the experimental conditions control
B:	Cell counts in the neutralizer control (absence of toxicity)
C:	Cell counts in the validation of the neutralization method

Criteria of validity:

Lg N:	8.17 – 8.70
lg N_0:	7.17 – 7.70
N_v :	300 – 1600
N_{v0} :	30 -160
A, B, C:	each ≥ 0.5 N_{v0}