

Biocidal Effectiveness of ClO₂

Enhanced Biocidal Effectiveness for Demanding Applications

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Properties & Applications of Chlorine Dioxide

Chlorine dioxide is a broad spectrum biocide that is effective against all bacteria, viruses, mould, fungi, algae and spore formers such as Giardia and Cryptosporidium.

Chlorine dioxide acts by first destroying the cell membrane, then the nucleus of the bacteria by chemical oxidation **not** chlorination. Because the organism is totally destroyed no resistant strains can develop which enables chlorine dioxide to be used on a continuous basis without the need for alternating biocides.

General Properties Summarised

- Effective at low dosages
- Disinfection activity is very fast
- Excellent for removing biofilm
- Has 2.6 times the oxidising capacity of chlorine
- Effective over a broad pH range (up to pH 12) with no loss of activity.
- Does not chlorinate organics or react with ammonia
- Does not hydrolyse in water to form hyperchlorous or hydrochloric acids.
- Less corrosive than chlorine.
- Highly soluble in water
- No hazardous residues
- Chlorine dioxide residual does not last as long as chlorine.

When stabilised liquid chlorine dioxide is used as the source for chlorine dioxide production - as an alternative to generators there are the following advantages: -

- No expensive on site generation equipment
- Simple activation systems are available
- Flexible activation to control rate of reactivity

Typical Chlorine Dioxide Applications

Food Industry

- | | | | |
|---------------------------|-----------------|----------------|-----------------|
| * Food contact sanitation | * Flume Water | * Dairies | * Breweries |
| * Sweet Water Systems | * C-I-P systems | * Pasteurizers | * Fermenters |
| * Veg. Wash Systems | * Humidifiers | * Beverages | * Canneries |
| * Hydro-coolers | * Poultry | * Meat | * Fish |
| * Refrigeration stores | * Ice | * Mushroom | * Potato Stores |

Water Treatment , General Industry and Specialist Applications

- | | | | |
|------------------------------|------------------|------------------|----------------|
| * Potable Water | * Cooling Towers | * Process Water | * Waste Water |
| * General Sanitation | * Bleaching | * Odour Control | * Molluscicide |
| * Chemical destruction | * Cutting Fluids | * Adhesives | * Emulsions |
| * Lubricating/Hydraulic Oils | * Paper Industry | * Oil Production | * HVAC |
| * Livestock Production | * Aquaculture` | * Health Care | * Marine |
| * Pharmaceuticals | * Electronics | * Formulations | |

Biocidal Effectiveness of Chlorine Dioxide

Chlorine dioxide has been known for many years as one of the most potent biocides available. Extensive work has been carried out world-wide, we list below a range of disinfectant activity that has previously demonstrated for chlorine dioxide from various sources. Please note that this list is not exhaustive, contact Scotmas for information on strains not listed below.

Adenovirus 2	Minute virus of mice
African Swine Fever Virus	Mouse encephalomyelitis virus
Agrobacterium tumefaciens	Mouse Hepatitis Virus
Aspergillus flavus	Mucor species
Aspergillus niger	Mycobacterium bovis
Bacillus cereus	Mycobacterium kansasii
Bacillus circulans	Mycobacterium smegmatis
Bacillus megaterium	Mycoplasma pneumoniae
Bacillus subtilis	Newcastle Disease Virus
Bifidobacter liberium	Parainfluenzae
Bluetonguevirus	Parvovirus
Candida albicans	Penicillium species
Clostridium difficile	Poliovirus Type 1
Clostridium sporogenes	Proteus vulgaris
Corynebacterium nucleatum	Pseudomonas aeruginosa
Coxsackievirus Type B1	Pseudomonas species
Culex quinquefasciatus (mosquito larvae)	Rhabdovirus
Cytomegalovirus	Rubella
Echovirus Type 11	Salmonella enterica spp.
Encephalomyocarditis	Salmonella gallinarum
Enterobacter cloacae	Salmonella typhimurium
Enterobacter hafnia	Sarcinae lutea
Escherichia coli	Scopulariopsis sp.
Feline parvovirus	Sendai Virus
Flavobacterium sp.	Staphylococcus aureus
Fonsecaea pedrosoi	Staphylococcus epidermidis
Foot + Mouth Disease Virus	Streptococcus A.B.D.
Fusarium species	Streptococcus faecalis
Fusobacterium nucleatum	Streptococcus pyogenes
GD7	Swine Vesicular Disease Virus
Herpes virus 1	Trichophyton mentagrophytes
Herpes virus 11	Trichophyton rubrum
Influenzae A2 Hong Kong	Tripansoma lewisi
Influenzae A/ Bangkok	Vaccinia Virus
Influenzae A/ Brazil	Var erythrogenes
Influenzae A/ Singapore	Vesicular Stomatitis Virus
Klebsiella pneumoniae	Yersinia enterocolitica

Comparative Biocidal Properties of Disinfectants

Chlorine dioxide has been compared with numerous other disinfectants, and the results published. The results here are taken from various papers, commercially published. Details of further specific tests are available on request

Peracetic Acid

Condition	ppm	Viable Cells / ml (60 second exposure)	
		Pediococcus	Lactobacillus
Control		2.5×10^6	1.6×10^6
Chlorine Dioxide	20	$<2 \times 10^0$	$<2 \times 10^0$
	50	$<2 \times 10^0$	$<2 \times 10^0$
Peracetic Acid	100	$<2 \times 10^0$	$<2 \times 10^0$
	100	$>10^5$	$>10^5$
	200	8.5×10^2	$>10^5$
	500	$<2 \times 10^0$	7.3×10^5

The results indicate that 20 ppm Chlorine Dioxide could reduce viable counts of lactic acid bacteria at least 99.999% in a 60 second speed-of-kill assay. The results suggest that about 1000ppm of Peracetic Acid Would be required to achieve a similar reduction in viability, using this test. These results are for comparative purposes only. - Dr R.S. Tanner, Sept 14 1991

Level Of Disinfectants Required to Achieve A 99.999% Kill

Disinfectant	Microorganism		
	Pseudomonas aeruginosa	Staphylococcus aureus	Saccharomyces cerevisiae
Chlorox™	1000	1000	1000
Sodium Chlorite	820	820	1000
Chlorine Dioxide	48	93	95
Iodophor	440	440	450
Peroxide	36000	68000	270000
Gluteraldehyde-Phenol	2300	1200	620
Acid gluteraldehyde	6600	2200	18000
Quaternary	580	140	740
Acidified quaternary	150	1200	300
Phenolic	1500	380	190

Published by ALLTECH Biotechnology Center, USA

Biocidal Test Results

The table below represents some laboratory test results published in Germany. The Chlorine Dioxide was obtained from a 3% solution of stabilised Chlorine Dioxide. These results support the claims made for the powerful biocidal effectiveness of Chlorine Dioxide.

BACTERIA	DENSITY OF ClO ₂ (ppm)	TIME (MINS)
Staphylococcus aureus	0.304	0.5
Streptococcus faecalis	0.19	2
Bacillus anthracis	0.95	120
Clostridium botulinum	0.95	120
Pseudomonas Florescens	2.09	0.25
E. Coli	0.02	1
Salmonella typhi	0.04	1
Mycobacterium Tuberculosis	19	1-3
Aspergillus niger	38	60
Adeno virus	0.08	61
Polio virus	0.114	16
Hepatitis type B	0.66	2
Pseudo Rabies Virus	0.10	1
Corona virus	0.09	2 - 3
Hog Cholera virus	0.08	1 - 2
Parvo virus	1.1	2 - 3

EFFECT OF ACIDIFICATION AND TIME ON CIDL EFFECTIVENESS OF ANTHIUM DIOXCIDE

Standardised test material was exposed to solutions of Anthium Dioxide which had been "activated" by various levels of pH. The results are expressed over both pH level and contact time.

Test Method: Mueller's Macro method
 Test Organisms: Micrococcus pyrogenes var. aureus
 Standard ATCC No 6538 or FDA No 209
 Germicidal Tests: At 250C
 Inoculum: Within the limits of 75 to 125 million organisms per ml.
 100% Kill = 99.9999% or more

Hydrogen concentration adjusted with HCl

Anthium Dioxide solution adjusted to 100ppm available ClO₂ with distilled water.

Percentage kill after various contact times

pH	5 min	10 min	15 min	30 min	1 hr	2 hr
6.05	0	14.0	19.3	22.8	33.3	100
5.00	35.3	45.1	75.5	95.1	99.9	100
4.00	44.7	55.3	78.9	99.7	100	100
3.50	100	100	100	100	100	100

EFFECT OF TEMPERATURE ON ANTHIUM DIOXCIDE

Anthium Dioxide will begin to release free ClO₂ at temperatures above 60°C.

Residuals of Anthium Dioxide in hot water systems can therefore be most useful in keeping systems free of contamination particularly when fluctuating temperatures occur.

EFFECT OF TEMPERATURE ON THE EFFICIENCY OF SOLUTIONS OF CHLORINE DIOXIDE

Contact time required to achieve a 99% kill of E. Coli. used to contaminate a water sample. Treatment rate was 0.25mg/l of ClO₂.

Temperature	5°C	10°C	20°C	30°C	40°C
Time (seconds)	110	74	41	16	7

Bacterial Test Reports

GLASGOW CALEDONIAN UNIVERSITY COMPANY LIMITED
MICROBIOLOGY CONSULTANCY SERVICE

Test Material BI-OX, converted to a solution of Free Chlorine Dioxide by the addition of a proprietary activator.

Test Organism	Concentration	Contact Time	Free ClO ₂ Concentration	Reduction Achieved
Bacillus Cereus	10 ⁵	5 mins	100 ppm 50 ppm	100% 2 log

To pass this part of the test it is required that the disinfectant achieves a 1 log reduction in 5 min.

Test Organism	Concentration	Contact Time	Free ClO ₂ Concentration	Reduction Achieved
Pseudomonas Aeruginosa	10 ⁶	5 mins	50 ppm 25 ppm	100% 100%
Salmonella Typhimurium	10 ⁶	5 mins	50 ppm 25 ppm	100% 100%
Staphylococcus Aureus	10 ⁶	5 mins	50 ppm 25 ppm	100% 100%
Saccharomyces Cerevisiae	10 ⁶	5 mins	50 ppm 25 ppm	100% 100%

To pass this part of the test it is required that the disinfectant achieves a 5 log reduction in 5 mins. Both parts of the test were passed when free ClO₂ solutions of 50ppm were used.

Test Reports by: N.A. Logan BSc PhD L.P. Macham BSc PhD I.M. Packer BSc MSc PhD

Effect Of Temperature on the Efficiency Of Solutions of Chlorine Dioxide

Contact time required to achieve a 99% kill of E. Coli. used to contaminate a water sample. Treatment rate was 0.25mg/l of ClO₂.

Temperature	5°C	10°C	20°C	30°C	40°C
Time (seconds)	110	74	41	16	7

Bacterial Test Reports

Short Contact Times for Activated Anthium Dioxide According to AOAC Tests

Micro-organism	Level of ClO ₂ (ppm)	Contact Time (sec)
Salmonella Chloreraesius	50	60
ATCC 10708		
Staphylococcus Aureus	50	60
ATCC 6538		
Pseudonomas Aeruginosa	50	60
Saccharomyces cerevisiae	50	60

Method: AOAC section 4001/4011, 48 hour culture, Munneecue (1981)

Cidal Effectiveness of Anthium Dioxide

Organism	Initial Conc.	Conditions Time	Contact Count/ml.	Final Conc.
Listeria monocytogenes ATCC 7644	2.54 x 10 ⁷	A	10 sec	0

Initial concentration was 2.54 x 10⁷.

2 ml of 0.9% saline harvested culture (Adjusted to a density of N x 10⁸ cell/ml) was added to 18 ml of solution containing 134 ppm of free ClO₂ and 191 ppm of total available ClO₂

TEST ORGANISMS SUSCEPTIBLE TO ACTIVATED ANTHIUM DIOXIDE

Bacillis cereus	Salmonella enteritidis
Escherichia coli	Salmonella gallinarium
Proteus vulgaris	Salmonella typhimurium
Pseudomonas aeruginosa	Staphylococcus aureus
var erythrogenes	Streptococcus faecalis
Yersinia enterocolitica	
Campylobacter jejuni	(5 strains supplied by Glasgow Dept. of Microbiology)

Reference: Deans & Stephenson, May 1986.
West of Scotland College of Agriculture, Auchincruive, Scotland

Comparison of the Effectiveness of Chlorine Dioxide and Chlorine in differing pHs and temperatures

Microorganism (OT Ortholidine) (OTA Ortholidine arsenite)	Chlorine Dioxide mg/l			Chlorine mg/l		
	Proportion	Residual (OT)	Residual (OTA)	Proportion	Residual (OT)	Residual (OTA)
pH7 @ 20°C						
Eb. Typhosa	0.04	0.025	0.025	0.075	0.06	0.02
Sh. dysenteriae	0.025	0.02	0.02	0.05	0.035	0.015
S. paratyphi B	0.075	0.06	0.055	0.05	0.03	0.01
Ps. aeruginos	0.05	0.04	0.04	0.10	0.075	0.04
Staph. aureus	0.20	0.15	0.14	0.20	0.16	0.07
Esch. Coli	0.10	0.10	0.09	0.1	0.08	0.05
Aer. aerogenes	0.04	0.025	0.02	0.04	0.015	Traces
pH7 @ 5°C						
Eb. Typhosa	0.075	0.065	0.06			
Sh. dysenteriae	0.05	0.05	0.05			
S. paratyphi B	0.075	0.06	0.055			
Ps. aeruginos	0.10	0.095	0.095			
Staph. aureus	>0.25	>0.19	>0.19			
Esch. Coli	0.10	0.10	0.09	0.10	0.075	0.05
Aer. aerogenes	0.10	0.09	0.08	0.075	0.04	0.02
pH9.5 @ 20°C						
Eb. Typhosa	0.05	0.025	0.025			
Sh. dysenteriae	0.015	0.012	0.005			
S. paratyphi B	0.04	0.015	0.015			
Ps. aeruginos	0.04	0.018	0.01			
Esch. Coli	0.10	0.085	0.08	>0.25	>0.20	>0.175
Aer. aerogenes	0.04	0.022	0.01	>0.25	>0.18	>0.12
pH9.5 @ 5°C						
Eb. Typhosa	0.04	0.025	0.02			
Sh. dysenteriae	0.02	0.012	0.01			
S. paratyphi B	0.05	0.025	0.02			
Ps. aeruginos	0.04	0.03	0.03			
Staph. aureus	0.10	0.075	0.07			
Esch. Coli	0.05	0.035	0.032	>0.50	>0.45	>0.335
Aer. aerogenes	0.03	0.01	0.008	>0.50	>0.38	>0.30

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Comparison of the Activities of Chlorine Dioxide and Peracetic Acid

Comparative testing of Chlorine Dioxide and Peracetic Acid were conducted as previously described (Tanner R.S. 1989 Comparative testing and evaluation of hard-surface disinfectants. J. Indust.Microbiol_4:145-154) with the following exceptions:-

- a. Cultures of a strain of *Pediococcus* and a mixed culture of *Lactobacillus* sp. were obtained from a brewing company.
- b. 10 mM potassium phosphate was included in the medium to stimulate the growth and viability of the lactic acid bacteria which are dependent on substrate level phosphorylation for metabolism and growth).
- c. Test cultures were incubated for 48 hr at 30°C. enumeration plates were incubated for 72 hr.
- d. Sodium thiosulphate (5,000 ppm) was used to neutralize each of the oxidising biocides tested.
- e. Tile tests containing Peracetic Acid (100, 200, and 500 ppm) also contained hydrogen peroxide (620, 1,200, and 3,100 ppm, respectively).

Condition	ppm	Viable Cells/ml (60 second exposure)	
		<i>Pediococcus</i>	<i>Lactobacillus</i>
Control		2.5×10^6	1.6×10^6
Chlorine Dioxide	20	$<2 \times 10^0$	$<2 \times 10^0$
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DR RALPH S TANER
 SEPTEMBER 14 1991

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