S6P05.WP

THE IN VITRO AND IN VIVO BACTERICIDAL ACTIVITY OF STABILISED CHLORINE DIOXIDE (Chlortech)

J.F. DEMPSTER[1], H. POMEROY[2] and R.J. RUSSELL[2]

1 "Lincoln House", Adelaide Road, Glenageary, Dublin, Ireland
2 Moyne Institute, University of Dublin, Trinity College, Dublin 2, Ireland

INTRODUCTION

For many years chlorine dioxide gas has been known to be a powerful antimicrobial agent because of its strong oxidising properties. Its use in the food industry has been restricted since the gas is highly explosive, particularly in the presence of oxidisable substances. This problem has now been solved through development of a patented process which enables a complex of chlorine gas to be produced in liquid form. When this is acidified with citric acid it yields a stabilised form of chlorine dioxide called *Chlortech*. This is the trade name assigned to the stabilised form by the patenting company (Alltech Corp., Nicholasville, Kentucky 40356, USA).

This study assesses the efficacy of Chlortech in vitro against four bacterial species isolated from a food plant and also its in-use efficacy in cleaning four different food plants compared with their normal cleaning programmes.

MATERIALS AND METHODS

In vitro tests

Four bacterial species (*Escherichia coli, Salmonella enteritidis, Staphylococcus aureus and Pseudomonas aeruginosa*) were isolated from food plant samples and were grown in nutrient broth (Oxoid CM67) at 37°C for 48 hours. Test suspensions were made using quarter-strength Ringer solution (Oxoid BR52) and counts estimated by spread plate technique on Plate Count Agar (Oxoid CM325), incubating at 37°C for 48 hours according to the method of Sykes (1967).

Chlortech was constituted as a stock solution of 500mg/l by adding 10g citric acid to 100ml Chlortech and diluting to 4.5l using sterile distilled water. In vitro efficacy tests were carried out by mixing equal volumes of serially 10-fold diluted stock solution to overnight bacterial suspensions diluted to 10^{-5}. After 60 seconds exposure time at room temperature the mixtures were serially diluted in quarter strength Ringer solution and sampled onto well dried spread plates for counting.

In situ tests

Comparative trials of cleansing efficacy were undertaken in four different food plants. Chlortech was used in a cleaning protocol, referred to as the Alltech method. This used cold water hosing followed by application of the Chlortech solution at 500ppm applied by spraying. No rinse was used.This method was compared with 'in-house' cleaning protocols as detailed in Table 1.

RESULTS AND DISCUSSION

In vitro testing

E. coli and *S. enteritidis* were inactivated at all concentrations of Chlortech tested down to and including 0.5mg/l after 60 seconds exposure at room temperature (see Table 2). *S. aureus* was killed at concentrations down to 0.5mg/l but at that level approximately 0.2% of the organisms survived. *Pseudomonas aeruginosa* was more resistant, with 0.1% surviving at 50mg/l. These results agree with the observations of Tanner (1989) who showed chlorine dioxide at 43mg/l to cause a 99.9% reduction of *Pseudomonas aeruginosa* and *Staphylococcus aureus* after 60 seconds exposure.

In situ testing

Efficacy of Chlortech was tested in four different food processing premises, namely a poultry processing plant, a pork cutting plant, a pork sausage and bacon plant and a beef cutting and packing plant against regular in-house cleaning schedules on a number of occasions. The results are presented in Tables 3 through 6.

Table 3 shows results from three separate trials using Chlortech during an elapsed period of EIGHT weeks in a poultry plant compared with the regular cleaning method which was the method outlined in Table 1. In all, 30 locations were sampled during the trials for each cleaning method. In all but three of these the Chlortech method was the most effective. It should also be noted that in two of these three cases the surface sampled was a rubber conveyor belt, a surface notoriously difficult to sanitise due to deep-seated foci of contamination in knife cuts in the rubber. The method of application may also have a role to play.

Table 4 shows the results for cleaning surfaces in a pork cutting plant. While four locations were satisfactorily cleaned (less that 100 bacteria per cm^2) by the Alltech method, three other surfaces were not. It is suggested that these results may have been due to the heavily contaminated state of the sites before cleaning began and highlights the importance of regular and efficient cleaning. The `in-house' method was completely unsatisfactory in cleaning any of the locations sampled.

In Table 5 it is evident that the Alltech method cleaned all surfaces sampled in the sausage/cooked meats factory in an extremely satisfactory manner while the in-house method failed in two cases.

Table 6 presents the results from trials in a pork processing plant. Here, Chlortech cleaned seven out of ten sites satisfactorily whereas only one satisfactory result was obtained using the `in-house' method. It is notable that one of the unsatisfactory results for cleaning using Chlortech came from a wooden cutting block. This agrees with results from earlier studies (Dempster, 1972).

Evidence is presented that chlorine dioxide had a high biocidal activity against four species of typical food-related pathogens. Similar results were presented by Tanner (1989) in a comparative study of 11 disinfectants where 500mg/l of Chlortech was shown to be capable of reducing viable cell counts of *S.aureus, Ps. aeruginosa* and *S. cerevisiae* by 99.99%. The in-use tests on food preparation surfaces and equipment in this present study showed Chlortech to be superior to any of the regular in-house cleaning methods with which it was compared. Among the advantages of Chlortech is that in solution it decomposes to a mixture of chlorine, perchloric acid and oxygen, together more bactericidal for coliform bacteria than chlorine alone (Burrows, 1963).

Chlortech now has EPA and FDA clearance for many applications including water sterilisation. For example, the EPA has cleared it for treatment of municipal water supplies at 1mg/l (1ppm) and for stored potable drinking water at 5ppm (Jaques 1989, unpublished data). These considerations have obvious advantages for any Irish food company wishing to market its goods in the United States.

REFERENCES

BURROWS, W. 1979. *Textbook of Microbiology.* 21st edition. W.B. Saunders Co. Philadelphia.

DEMPSTER, J.F. 1972. An Evaluation of the Efficiency of Cleaning Methods in a Bacon Factory. *J. Hygiene (Camb).* 69:133-140.

JAQUES, K. 1989. Internal Memo. Alltech Biotechnology Centre, 3031 Catnip HillPike, Nicholasville, KY.

SYKES, G. 1967. Disinfection and Sterilisation. 2nd edition. E.&F.E. Spon. London.

TANNER, R.S. 1989. Comparative testing and evaluation of hard surface disinfectants. *J. Indus. Micro.* 4:145.

Table 1. In-House Cleaning Protocols.

Establishment	Method
Beef/Pork Cutting	Cold water hosing daily using heavy duty alkaline foam (10% for heavy soil, 4% for light soil)
Pork Abattoir	Cold water pre-cleaning. Tables:1% Sterbrite (10-16% NaOCl) for 10 minutes, hot high pressure rinse. Belts: High foam alkaline detergent applied at high pressure, contact time 10 minutes, cold high pressure rinse. Walls: As for belts but scrubbed before rinse.
Pork Cutting	All surfaces steam hosed at 80°C (176°F), sanitised with Stertone Blue (quaternary ammonium + alkaline detergent) by spraying. No rinse.
Poultry Processing	Coarse soil removed by brushing. High pressure wash using unspecified detergent. High pressure rinse.

Table 2. Bactericidal efficacy of Chlortech against four species of bacteria using 60 seconds exposure time

Chlortech (mg/l)	% Kill			
	E. coli	Salmonella	Pseudomonas	S.aureus
500	100	100	N.T.	100
50	100	100	99.9	100
5	100	100	99.2	100
0.5	100	100	N.T.	99.8

Table 3. Comparison of Alltech cleaning method (A) with in-house method (B) in a poultry processing plant.

	Count per cm^2 after cleaning					
	Method A			Method B		
Location	Trial1	Trial2	Trial3	Trial1	Trial2	Trial3
Plastic table	162	72	14	2090	8000	34
Steel table	6	6	1000	3800	100	2800
Weigh pan	<2	6	-	1170	180	-
Rubber conveyor belt	292	20	78	36	7000	10
Evisceration table	28	<2	128	436	200	-
Bleeding trough	14	8	16	218	120	30
Evisceration trough	12	12	10	2900	660	54
Shackle	18	2	<2	18	54	8
Steel barrow	72	<2	12	122	2	22
Scald tank	4	4	8	155	2	340
Means	61	17	127	1094	1632	412
Overall Means	68 for Method A			1046 for Method B		

Table 4. Comparison of Alltech cleaning method (A) with in-house cleaning method (B) at a pork cutting plant.

Sample Location	Count per cm^2 after cleaning Method A	Method B
Steel conveyor belt	59	$>10^6$
Cutting table No. 1	$>10^6$	$>10^6$
Cutting table No. 2	7236	$>10^6$
Cutting table No. 3	49	$>10^6$
Cutting table No. 4	6696	$>10^6$
Barrow	<2	$>10^6$
Steel chute	9	$>10^6$
Mean values	2342	$>10^6$

Table 5. Comparison of Alltech cleaning method (A) with in-house cleaning method (B) at sausage/cooked meats processing plant.

Sampling Location	Count per cm^2 after cleaning Method A	Method B
Trimming table	<2	34
Sausage table	2	32400
Sausage filler	4	16
Bowl chopper	2	2
Mincer worm	64	1300
Mean values	15	6750

Table 6. Comparison of Alltech cleaning method (A) with in-house cleaning method (B) at a pork processing plant.

Sampling Location	Count per cm^2 after cleaning Method A	Method B
Steel table	26	19840
Cutting board	3200	7920
Slicer platform	80	$>10^6$
Slicer blade	248	$>10^6$
Steel table	<2	19040
Wooden cutting block	$>10^6$	$>10^6$
Steel table	64	3320
Steel table	<2	$>10^6$
Sausage filler	<10	72
Mincer tray	<3	1400
Mean values	>100364	>405159