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X Chemical Abstracts, Volume 70, no. 5, issued 1969, February 3 (Columbus, Ohio, US) C. Doudney: "Peroxyde effects on survival and mutation induction in UV light-exposed and photoreactivated bacteria", see page 1778, the abstract 17785c, Mutat. Res. volume 6, no. 3, 1968, pages 345-353, see the entire document
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A American Journal of hospital pharmacy no. 29, issued August 9, 1972 (Philadephia College of Pharmacy US) R.M.G. Boucher: "Advances in sterilization techniques" see page 663, lines 36-48; page 664, table I
A CH, A, 605421,
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(54) **Method of sterilisation**

(57) A method of sterilisation which comprises treating a microorganism with an ultraviolet irradiated solution of hydrogen peroxide, the wavelength of the ultraviolet radiation being wholly or predominantly below 325 nm and the concentration of the hydrogen peroxide being no greater than 10% by weight and such that the microorganism is rendered non-viable by synergism between the radiation and the hydrogen peroxide. The invention is particularly applicable to the treatment of spores contaminating food packaging.

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(54) Title: METHOD OF STERILISATION

(57) Abstract

A method of sterilisation which comprises treating a microorganism with an ultraviolet irradiated solution of hydrogen peroxide, the wavelength of the ultraviolet radiation being wholly or predominantly below 325nm and the concentration of the hydrogen peroxide being no greater than 10% by weight and such that the microorganism is rendered non-viable by synergism between the radiation and the hydrogen peroxide. The invention is particularly applicable to the treatment of spores contaminating food packaging.

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METHOD OF STERILISATION

This invention relates to a method of sterilisation in which a microorganism is rendered non-viable.

At present food packaging is sterilised by treatment with a solution of hydrogen peroxide. Certain strains of microorganisms are however resistant to such treatment and a small, though significant, number of spores survive with consequent risk of spoilage to the contents.

A method of sterilisation has now been found which reduces the number of surviving spores of resistant organisms more efficiently.

According to the present invention a method of sterilisation comprises treating a microorganism with an ultraviolet irradiated solution of hydrogen peroxide the wavelength of the radiation being wholly or predominantly below 325nm and the concentration of the hydrogen peroxide solution being no greater than 10% by weight and such that the microorganism is rendered non-viable by synergism between the radiation and hydrogen peroxide.

The concentration of the solution of hydrogen peroxide, which is usually aqueous, generally does not exceed 6% by weight and preferably does not exceed 3.0% by weight. The concentration is normally at least 0.01% by weight, a concentration at least 0.25% by weight being preferred especially when the microorganism is present in the form of spores, and a concentration at least 0.5% especially so.

In general the wavelength of the ultraviolet radiation is wholly or predominantly below 300nm and is usually at least 200nm. In practice radiation is normally provided from a source having a peak intensity below 325nm, particularly at 254nm. The energy of the radiation emitted at the source is normally at least 300 microwatts/cm^2, particularly at least 500 microwatts/cm^2. Although the intensity of the radiation with which the solution is treated may be established by simple experiment, it is normally at least 75 and preferably at least 150 microwatters per cm^2.

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Although spores of many microorganisms are destroyed by treatment as hereinbefore described at ambient temperatures, it may be desirable, particularly when treating especially resistant microorganisms, to maintain the solution at an elevated temperature either during irradiation or subsequent thereto. In general, such temperatures do not exceed 120°C and may not exceed 100°. The temperature, at least when treating resistant organisms, is usually however at least 85°C.

The method of the present invention is applicable to a wide variety of microorganisms, including moulds, yeasts, bacteria, viruses and protozoa and finds particular application in the destruction of spore-forming bacteria, especially those which are dairy contaminants.

Although microorganisms in vegetative form may be treated, the present invention is of particular interest for the destruction of spores, especially those of resistant Bacillus and Clostridium strains, e.g. strains of B. subtilis, and B. stearothermophilus such as B. subtilis (ATCC 9372) and B. stearothermophilus (NCDO 1096).

In general, the irradiation period increases with the resistance of the organism, at least 10 sec., generally being required. Heating of the solution, which may, as hereinbefore indicated, be conducted synchronously with irradiation or subsequent thereto is normally carried out for at least 10 sec., and periods of at least 30 sec., e.g. 60 sec., or more may be desirable.

Although the present method may be applied to sterilisation of liquids, e.g. waste water and cannery cooling water, it is of particular interest for the sterilisation of surfaces, for example surfaces of walls and furniture in hospitals and the surfaces of food containers. The latter surfaces may be treated with the peroxide solution, for example by passing the container or material from which the container is fabricated through a

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tank containing the solution or by spraying the walls of the container or the material with solution. Irradiation may be carried out by a lamp so disposed that containers or packaging materials which have emerged from the tank or spray are subjected to the method of the present invention.

The synergistic action of the present method on the micro-organisms i.e. the action of the radiation and hydrogen peroxide attitional to their purely additive effect is illustrated by the following Examples when taken with the comparison experiments.

EXAMPLES 1 - 71

Organisms

The strains of Bacillus and Clostridium (Examples 1 - 40), of non-sporing bacteria (Examples 41 - 69) and of moulds (Examples 70 - 71) and where appropriate the origins thereof are listed in Table 1.

The following abbreviations apply:

NIRD - National Institute of Research in Dairying, Shinfield, Reading, UK.

NCDO - National Collection of Dairy Organisms

ATCC - American Type Culture Collection

NCIB - National Collection of Industrial Bacteria

FRI - Agricultural Research Council Food Research Institute, Colney Lane, Norwich, UK.

Table 1

Strain	Source
B. subtilis 713 (NCDO 2130)	Bulk milk tank, Iran
B. subtilis 738 (NCDO 738) (ATCC 9372)	
B. subtilis 706 (NDCO 2129)	Rinse of farm bulk milk tank

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Strain	Source
B. subtilis var. niger (NCIB 8058)	
B. subtilis SA22	R.T. Toledo et al, Appl. Microbiol. 26, 592-7 1973
B. globigii B17 (NCIB 8649)	
B. licheniformis 100	'In-line' milk
B. licheniformis 117	B. licheniformis T of O.Cerf & J. Hermier Le Lait 52, 1 - 20 1972
B. licheniformis 109 2A0	UHT spoiled milk, O. Cerf & F. Metro J. Appl. Bacteriol. 42, 405-415, 1977
B. cereus 818	'In can' milk
B. cereus T	G.J. Dring & G.W.Gould in Spores VI pp 488-494 Ed. Gerhardt P. Costilow R.N. & Sadoff, H. Washington DC American Society for Microbiology 1975
B. pumilus 312	B. pumilus EJ of O.Cerf & J. Hermier Le Lait 52, 1 - 20 1972
B. stearothermophilus 202 (NCDO 1096)	
G 12	Pond mud
Clostridium sporogenes PA 3679 (NCIB 8053)	
Escerichia coli K12	FRI
Streptococcus faecalis ss liquefaciens EB/F/30/39	Chicken gut

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Strain	Source
Serratia marcescens (NCTC 10211)	
Penicillium chrysogenum	UHT spoiled milk

Spore preparation and maintenance of cultures

C. sporogenes is maintained in Robertson's cooked meat medium; the other strains used are maintained on slopes of Oxoid nutrient agar. Spores are produced by growth on one of the following:

i) the potato agar of Gould, Stubbs & King (1970) for B. subtilis var. niger ii) Oxoid nutrient agar for B. stearothermophilus 202, iii) the agar medium of Wang, Scharer & Humphrey (1964) for B. licheniformis 109 2AO, iv) a medium containing (g/1), Oxoid nutrient broth No. 2 (3.1), $MnSO_4.4H_2O$ (0.03), K_2HPO_4 (0.25) and New Zealand Agar (15) for strain G12, v) a medium containing (g/1); Oxoid skim milk powder (50), Difco yeast extract (3.0), Oxoid peptone (5.0), BBL trypticase (5.0), $MnCl_2.4H_2O$ (0.072) BDH cysteine hydrochloride (0.5), Davis agar (15); the medium is adjusted to a final pH of 7.0 to 7.2 with 1M-NaOH and spores produced under H_2 : CO_2 (9:1) for C. sporogenes PA 3679, vi) Bacillus spore agar as described by Franklin et al (1970) with Oxoid Lab Lemco added at 0.1% (w/v) for all other strains. Growth is at 30°C for B. subtilis var. niger, B. subtilis 738, B. globigii B17, strain G12, B. cereus T and B. cereus 818, at 33°C for C. sporogenes PA 3679, at 37°C for B. pumilus 312, B. subtilis SA22, B. subtilis 713, B. subtilis 706, B. licheniformis 100, B. licheniformis 117 and B. licheniformis 109 2AO or at 55°C for B. stearothermophilus 202.

Spores of B. stearothermophilus 202 are produced on 30 ml slopes in 100 ml screw-capped bottles; those of other strains on agar in Petri dishes. Sporulation is detected by the appearance of bright spores by phase contrast microscopy and incubation

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continued until the highest percentage of free spores is observed (after one to nine days, depending on strain). The cultures are harvested and washed 5 times with sterile glass-distilled water before storage at -18°C.

Preparation of cells of non-sporing bacteria and maintenance of cultures

Non-sporing bacteria are grown at 33°C in i) heart infusion broth for E. coli and S. faecalis, ii) glycerol-salts medium as described by Dimmick (1965) for S. marcescens on a gyratory shaker (Gallenkamp, 150 rev/min) for 18 hours. The cells are harvested and washed with 50 ml sterile 25mM-potassium phosphate buffer pH 7.0 by centrifugation before resuspension at a density of about 1×10^9 viable units/ml and were used within 4 hours. Cultures are maintained on slopes of Heart Infusion Agar (Difco).

Preparation of mould spores and maintenance of culture

Spores of Penicullium chrysogenum are grown on slopes of Czapek Dox Agar containing (g/l); sucrose (30), K_2HPO_4 (1.0), $NaNO_3$ (2.0), $MgSO_4.7H_2O$ (0.5), KCl (0.5), $FeSO_4.7H_2O$ (0.01), and New Zealand Agar (20) at 20°C for at least 7 days before harvesting in sterile glass distilled water containing 0.1% Tween 80 and washing by centrifuging with sterile glass distilled water. Cultures were also maintained on Czapek Dox Agar.

U.V. irradiation of bacterial spores, vegetative cells and mould spores

Bacterial spores or vegetative cells at 3×10^7 to 5×10^8/ml and mould spores at 1×10^6ml are suspended in 0.1M sodium phosphate buffer pH 7.0 and up to 2.5g/100ml H_2O_2 (Analar, B.D.H.) Volumes of 4ml are rocked gently to and fro for 30 sec. at 20°C in a Petri dish base with a diameter of 9 cm at distances of 5.5, 30cm or 33cm from an Hanovia Chromatolite low-pressure Hg lamp (Hanovia Ltd., Slough, England) from which the filter is removed as described by W.M. Waites & B.A. Fry J. Gen. Microbiol 34, 413-426 (1964). The lamp radiation in the range 185-579nm

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with a peak intensity at 254nm; the latter radiation is the only radiation of significant practical value produced by the lamp, the other spectral lines and groups being of much lower intensity. The energy emitted is 300-500 microwatts/sq. cm. at source. Samples (2ml) are removed and mixed with a filter sterilised solution (2ml) of catalase (Sigma Ltd) at 7650 units/ml at 20°C before storing in ice for at least 5 min., dilution and plating.

Heat treatment

(Examples 2-16, 33-35, 31-41, 61 and 71)

Samples (2ml) are heated to 85°C in 60 sec., (bacterial spores), 65°C in 30 sec. (vegetative bacteria) or 54° in 15 sec. (mould spores) by adding to pre-heated screw-capped bottles before adding 2 ml of catalase pre-cooled to +1°C and containing 7650 units/ml and plunging into ice. The suspensions are stored in ice for at least 5 min. before diluting and plating.

Determination of survivors

Diluted spore suspensions are plated on (i) Bacillus spore agar and incubated for 2 days at 30°C (B. subtilis 713, B. subtilis 706, B. cereus T and B. cereus 818) or 37°C (B.licheniformis 109 2AO, B. licheniformis 100, B. licheniformis 117 and B. pumilus 312); (ii) plate count agar (Oxoid) and incubated for 2 days at 33°C (B. subtilis SA 22) or 2 days at 37°C (B. globigii B17, B. subtilis 738 and B. subtilis var. niger); (iii) a medium containing (g/1); tryptone (Oxoid) (5); yeast extract (Oxoid) (2.5), glucose (10) and New Zealand Agar (28) and incubated for 3 days at 55°C (B. stearothermophilis 202) (iv) a medium containing (g/1); tryptone (Oxoid) (10), glucose (5.0) and New Zealand Agar (12) and incubated for 2 days at 30°C (G12) or (v) reinforced Clostridial medium agar of Hirsch & Grinsted (1954) but with 1.6% New Zealand Agar in place of 1.2% (C. sporogenes PA 3679).

Suspensions of vegetative cells are enumerated by diluting 100-fold in maintenance medium (Difco Bacto-peptone), 1.0g/l;

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NaCl, 5.0 g/l; adjusted to pH 7.0-7.1) and plating on Heart Infusion Agar (Difco) (E. coli and S. faecalis) or Trypticase Soy agar (BBL) (S. marcescens) using a Spiral Plate Maker (Spiral Systems, Cincinnati, Ohio, USA) (Gilchrist et al 1973, Jarvis et al 1977) and colonies counted after 3 days incubation at 33°C (S. faecalis), 2 days incubation at 30°C (E. coli) or 33°C (S. marcescens).

Suspension of mould spores are enumerated by diluting in sterile glass distilled water, plating on Czapek Dox Agar and colonies counted after 9 days incubation at 20°C.

Full references to the publications hereinbefore cited are as follows:-

Dimmick R.C. (1965) J. Bacteriology 89, 791-798.

Gilchrist, J.E., Campbell, J.E., Donnelly, C.B., Peeler, J.T. & Delaney, J.M. (1973) Applied Microbiol. 25, 244-252

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Franklin J.G., Underwood, H.M., Perkin A.G. and Burton, H (1970) J. Dairy Res. 37, 219-226.

Hirsch, A, Grinstead, E. (1954) J. Dairy Res. 21, 101-110

Example 1

Rate of kill of spores of Bacillus subtilis 706 by U.V. irradiation and hydrogen peroxide

Spores of Bacillus subtilis 706 are irradiated as described above but in the presence or, for purposes of comparison, in the absence of a concentration of H_2O_2 (ig/100ml) which was previously found not to kill spores at comparable temperatures. In the absence of peroxide, irradiation produces a logarithmic rate of kill which results in 19 and 2% survivors after 30 and

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60 sec. respectively. In the presence of peroxide, irradiation produces a much more rapid logarithmic kill so that after 30 sec. only 0.01% of spores survive.

Examples 2-16

Several strains of Bacillus and Clostridium are irradiated for 30 sec. In the presence of 2.5g/100ml H_2O_2 as described above followed by heating to 85°C during 60 sec. The results are shown in Table 2 which also shows the results obtained from irradiation with U.V. alone and with U.V. followed by heat treatment.

Table 2

Example	Strain	Survivors (%) U.V. alone*	U.V. and heat**	U.V. plus H_2O_2*** and heat
2	B. subtilis SA 22	1.44	0.23	0.0004
3	B. licheniformis 109 2AO	0.045	0.73	0.004
4	B. globigii B17 (NCIB 8649)	0.44	0.47	0.008
5	B. stearothermophilus 202 (NCDO 1096)	0.64	0.14	0.004
6	B. pumilus 312	0.034	0.031	0.001
7	B. subtilis 738 (NCDO 738:ATCC 9372)	0.0053	0.022	0.002
8	G12	0.067	0.00005	0.0009
9	B. licheniformis 117	0.19	0.019	0.006
10	B. subtilis 713 (NCDO 2130)	0.89	0.005	0.006

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Example	Strain	Survivors (%) U.V. alone*	U.V. and heat**	U.V. plus H_2O_2 and heat***
11	B. licheniformis 100	0.67	1.1	0.0003
12	B. cereus 818	0.022	0.038	<.0001
13	B. cereus T	0.020	0.0061	0.0001
14	B. subtilis var. niger (NCIB 8058)	0.14	0.61	0.002
15	B. subtilis 706 (NCDO 2129)	0.18	0.42	0.0008
16	Clostridium sporogenes PA 3679 (NCIB 8053)	0.21	0.0097	< 0.0001

* Lamp 5.5cm above spore suspension for 30 sec.

** Samples (2ml) removed and heated to 85°C during 60 sec.

***Lamp 5.5cm above suspension with 2.5g peroxide/100ml for 30 sec. followed by heating to 85°C during 60 sec.

Examples 17-24

B. subtilis 713 is irradiated with U.V. in the presence of varying concentrations of hydrogen peroxide at two different spore concentrations. The conditions and results are shown in Table 3.

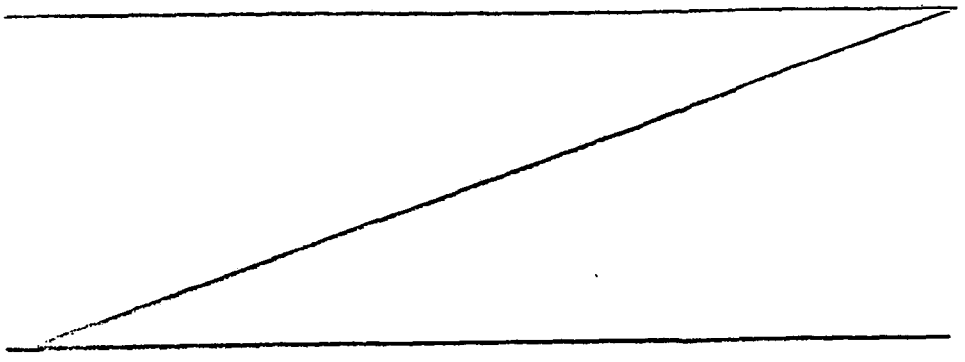

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Table 3

Example	Hydrogen peroxide (g/100ml)	Survivors (%) after U.V. irradiation*	
		1.2 x 10^7/ml	12 x 10^7/ml
17	0	0.43	
18	0		0.82
19	0.5	0.0073	
20	0.5		0.39
21	1.0	0.0089	
22	1.0		0.44
23	2.5	0.22	
24	2.5		1.6

* Spores are suspended at 1.2 x 10^7 or 12 x 10^7/ml with hydrogen peroxide before irradiation with the U.V. lamp at 5.5cm above the suspension.

Examples 25-28

Four Bacillus strains are irradiated with U.V. in the presence of hydrogen peroxide and in two cases are subsequently heated to 85°C over 60 sec. The conditions and results are shown in Table 4 which also shows for comparison purposes the results obtained from prior work using H_2O_2 without U.V. irradiation.

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Table 4

Comparison of spore destruction by H_2O_2 and U.V. irradiation and by H_2O_2 alone.

Strain	Example	H_2O_2 (g/100ml)	Temperature (°C)	Time* (sec)	
B. subtilis	-	10	25	1800	Prior work
var. niger	25	1.0 + U.V.	20	30	
B. subtilis 738	-	25.8	24	660	Prior work
	26	1.0 + U.V.	20	30	
B. subtilis 713	-	3.0	90	600	Prior work
	27	2.5 + U.V.	20 + 85**	30 + 60**	
B. licheniformis	-	15	80	126	Prior work
109 2AO	28	2.5 + U.V.	20 + 85**	30 + 60**	

* Time is that required to produce a kill of 99.99%

**Spores irradiated at 20°C for 30 sec. and then heated to 85°C over 60 sec.

EXAMPLE 28

Spores of B. subtilis 706 are irradiated with U.V. for 60 sec. in the presence of H_2O_2 (1g/100ml). The U.V. lamp is located 30cm above the spore suspension. For comparison purposes the strain is also irradiated with U.V. in the absence of H_2O_2. Samples are removed diluted and plated as hereinbefore described. The results are shown in Table 5.

Table 5

Destruction of spores of B. subtilis 706 by U.V.

Example: U.V. + H_2O_2

Comparison: U.V. only

Irradiation Time (sec.)	Example Survivors %	Comparison Survivors %
15	1.0	-
30	0.009	20
45	0.002	8
60	0.001	2

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EXAMPLES 30-35

Spores of B. pumilus 312 are irradiated with U.V. for 30 sec. in the presence of H_2O_2 both with and without subsequent heating to 85°C over 60 sec. The lamp is 5cm above the suspension. For comparison purposes spores are incubated: (i) at 20°C with hydrogen peroxide for 30 sec., (ii) with hydrogen peroxide for 30 sec. followed by heating to 85°C over 60 sec. Samples are removed at intervals, diluted and plated as hereinbefore described. The results are shown in Table 6.

Table 6

Conditions:

Examples 30-32: U.V. + H_2O_2

(Comparison: H_2O_2)

Examples 33-35: U.V. + H_2O_2 + Heat

(Comparison: H_2O_2 + Heat)

Example	Concentration of H_2O_2	Survivors (%) (Examples)	Survivors (%) (Comparisons)
30	0.25	0.04	90
30a	0.50	0.02	80
30b	0.75	0.01	100
31	1.0	0.01	100
32	1.75	0.3	80
32a	2.50	0.5	100
33	0.25	0.01	100
33a	0.50	0.002	80
33b	0.75	0.001	50
34	1.0	0.0002	14
35	1.75	0.0002	0.13
35a	2.50	0.0003	0.01

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Examples 36-41

The procedure of Examples 30-35 is repeated using B. subtilus 713 in place of B. pumilus 312. The results are shown in Table 7.

Table 7

Conditions:

Examples 36-38: U.V. + H_2O_2

(Comparison: H_2O_2 only)

Examples 39-41: U.V. + H_2O_2 + Heat

(Comparison: H_2O_2 + Heat)

Example	Concentration of H_2O_2	Survivors (%) (Examples)	Survivors(%) (Comparisons)
36	0.5	0.014	100
37	1.0	0.17	100
38	2.5	2.0	100
39	0.5	0.005	40
40	1.0	0.005	35
41	2.5	0.005	0.7

Examples 42-49

E. coli K-12 is irradiated with U.V. for 30 sec. in the presence of varying concentrations of hydrogen peroxide. The lamp is 30 cm above the suspension. For comparison purposes cells are also incubated for 30 sec. with 1g/100ml H_2O_2 in the absence of U.V. irradiation. Samples are removed, diluted and plated as hereinbefore described. The results are shown in Table 8.

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Table 8

Conditions:

Examples 42-49: U.V. + H_2O_2

Comparison Experiment 50: H_2O_2 only

Example	Concentration of H_2O_2	Survivors (%) (Examples)
42	0.001	0.41
43	0.005	0.26
44	0.01	0.14
45	0.025	0.08
46	0.05	0.044
47	0.075	0.017
48	0.01	0.0085
49	0.25	0.0026
50	1.0	74

Examples 51-59

S. faecalis ss liquefaciens EB/F/30/39 is irradiated with U.V. for 30 sec. in the presence of varying concentrations of hydrogen peroxide. The lamp is 30cm above the suspension. For comparison purposes cells are also incubated for 30 sec. with 1.0g/100ml H_2O_2 in the absence of U.V. irradiation. Samples are removed, diluted and plated as hereinbefore described. The results are shown in Table 9.

Table 9

Conditions:

Examples 51-59: U.V. + H_2O_2

(Comparison: 1g/100ml H_2O_2 only)

Example	Concentration of H_2O_2	Survivors (%) (Examples)	Survivors (%) (Comparison)
51	0.001	1.0	*
52	0.005	0.7	*
53	0.01	0.6	*
54	0.025	0.32	*

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Table 9 (continued)

Example	Concentration of H_2O_2	Survivors (%) (Examples)	Survivors (%) (Comparison)
55	0.05	0.29	*
56	0.1	0.11	*
57	0.5	0.026	*
58	0.75	0.024	*
59	1.0	0.011	100

* Not tested

Examples 60-61

Cells of E. coli K12 are irradiated with U.V. for 30 sec. in the presence of 1.0g/100ml H_2O_2 both with and without subsequent heating to 65°C over 30 sec. The lamp is 30cm above the suspension. For comparison purposes cells are (i) irradiated with U.V. for 30 sec. followed by incubation with H_2O_2 for 30 sec. (ii) heated to 65°C over 30 sec. Samples are removed, diluted and plated as hereinbefore described. The results are shown in Table 10.

Table 10

Conditions:

Example 60: U.V. + H_2O_2

(Comparison: U.V. followed by H_2O_2)

Example 61: U.V. + H_2O_2 + heat

(Comparison: heat)

Example	Concentration of H_2O_2	Survivors(%) (Examples)	Survivors (%) (Comparisons)
60	1.0	0.0044	0.14
61	1.0	< 0.00005	0.0013

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Examples 62-69

Cells of S. marcescens are irradiated with U.V. for 30 sec. in the presence of varying concentrations of hydrogen peroxide. The lamp is 33 cm above the suspension. For comparison purposes cells are also incubated for 30 sec. with 1.0g/100 ml H_2O_2 in the absence of U.V. irradiation. Samples are removed, diluted and plated as hereinbefore described. The results are shown in Table 11.

Table 11

Conditions:

Examples 62-69: U.V. + H_2O_2

(Comparison: 1.0g/100ml H_2O_2 only)

Example	Concentration of H_2O_2	Survivors (%) (Examples)	Survivors (%) (Comparison)
62	0.01	0.046	*
63	0.025	0.022	*
64	0.05	0.014	*
65	0.1	0.011	*
66	0.25	0.0014	*
67	0.5	0.00095	*
68	0.75	0.00016	*
69	1.0	< 0.0001	86

* Not tested

Examples 70-71

Spores of Penicillium chrysogenum are irradiated with U.V. for 30 sec. in the presence of 0.5g/100ml H_2O_2 both with and without subsequent heating to 54°C over 15 sec. The lamp is 5cm above the suspension. For comparison purposes spores are (i) irradiated with U.V. for 30 sec. followed by incubation with H_2O_2 for 30 sec. (ii) heated to 54°C over 15 sec. Samples are removed, diluted and plated as hereinbefore described. The results are shown in Table 12.

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Table 12

Conditions:

Example 70: U.V. + H_2O_2

(Comparison: U.V. followed by H_2O_2)

Example 71: U.V. + H_2O_2 + heat

(Comparison: heat)

Example	Concentration of H_2O_2	Survivors (%)	Survivors (%)
70	0.5	0.95	3.3
71	0.5	0.0033	9.6

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CLAIMS

1. A method of sterilisation which comprises treating a micro-organism with an ultraviolet irradiated solution of hydrogen peroxide, the wavelength of the ultraviolet radiation being wholly or predominantly below 325nm and the concentration of the hydrogen peroxide being no greater than 10% by weight and such that the micoorganism is rendered non-viable by synergism between the radiation and the hydrogen peroxide.

2. A method according to Claim 1 in which the wavelength of the ultraviolet radiation is wholly or predominantly below 300nm.

3. A method according to Claim 1 in which the radiation has a peak intensity below 325nm.

4. A method according to any of the preceding claims in which the wavelength of the ultraviolet radiation is at least 200nm.

5. A method according to any of the preceding claims in which the ultraviolet radiation has a peak intensity at 254nm.

6. A method according to any of the preceding claims in which the concentration of the hydrogen peroxide solution is at least 0.01% by weight.

7. A method according to any of the preceding claims in which the concentration of the hydrogen peroxide solution is at least 0.5% by weight.

8. A method according to any of the preceding claims in which the concentration of the hydrogen peroxide solution is no greater than 6% by weight.

9. A method according to any of the preceding claims in which the concentration of the hydrogen peroxide solution is no greater than 3% by weight.

10. A method according to any of the preceding claims in which the microorganism is present in the form of spores and the concentration of hydrogen peroxide is at least 0.25%.

11. A method according to any of the preceding claims in which the microorganism is a mould, yeast, bacterium, virus or protozoa.

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12. A method according to any of the preceding claims in which the microorganism is a dairy contaminant.

13. A method according to Claim 11 in which the mocroorganism is present in the form of spores of B. subtilis or B. stearothermophilus.

14. A method according to any of the preceding claims in which the hydrogen peroxide is maintained at an elevated temperature during or subsequent to irradiation.

15. A method according to any of the preceding claims in which the hydrogen peroxide solution is maintained at at least 85°C during or subsequent to irradiation.

16. A method according to any of the preceding claims in which the hydrogen peroxide solution is heated for at least 10 seconds during or subsequent to irradiation.

17. A method according to any of the preceding claims in which the microorganism is a contaminant on the surface of food packaging material.

18. Food packaging material when sterilised according to the method of Claim 17.

19. A method of sterilisation substantially as described in any one of the Examples.

20. A method of sterilisation which comprises treating spores of a microorganism with an ultraviolet irradiated solution of hydrogen peroxide, the wavelength of the ultraviolet radiation having a peak intensity within the range 200-325nm and the concentration of the hydrogen peroxide being at least 0.5% and no greater than 3% by weight, whereby the microorganism is rendered non-viable by synergism between the radiation and hydrogen peroxide.

21. Food packaging material when sterilised according to the method of the preceding claim.

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INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 80/00003

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.[3]: A 61 L 2/18; A 61 L 2/10; A 01 N 59/00; A 23 L 3/34; A 23 C 7/02; B 65 B 55/10

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
Int.Cl.[3]	A 61 L 2/18; A 61 L 2/10; A 01 N 59/00; A 23 L 3/28; A 23 L 3/34; A 23 C 7/02

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X	Chemical Abstracts, Volume 70, no. 5, issued 1969, February 3 (Columbus,Ohio, US) C. Doudney: "Peroxyde effects on survival and mutation induction in UV light-exposed and photoreactivated bacteria", see page 1778, the abstract 17785c, Mutat.Res.volume 6, no. 3,1968, pages 345-353, see the entire document	1-21
X	Chemical Abststracts, Volume 77, no. 25, issued 1972, December 18, (Columbus, Ohio, US) E.Powers et al.: "Hydrogen peroxyde and hydroxy radicals in radiation inactivation of bacterial spores" see page 153, the abstract 161570r, Int.J.Radiat.Biol.volume 22, no. 3, 1972, pages 237-243, see the entire document	1-21
	CH, A, 361636, published June 15, 1962, see page 2, lines 3-5, 91-97 and 110-120, page 3, line 92- page 4, line 27,Alpura.	1-21
	FR, A, 2176504, published November 2, 1973, see page 2, lines 28-36;page 3, line 8, Prepac ./.	1-21

* Special categories of cited documents: [15]

"A" document defining the general state of the art

"E" earlier document but published on or after the international filing date

"L" document cited for special reason other than those referred to in the other categories

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but on or after the priority date claimed

"T" later document published on or after the international filing date or priority date and not in conflict with the application, but cited to understand the principle or theory underlying the invention

"X" document of particular relevance

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21st March 1980	28th March 1980

International Searching Authority [1]	Signature of Authorized Officer [20]
EUROPEAN PATENT OFFICE	G.L.M.KRUYDENBERG

Form PCT/ISA/210 (second sheet) (October 1977)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	American Journal of hospital pharmacy no. 29, issued August 9, 1972 (Philadelphia College of Pharmacy US) R.M.G. Boucher: "Advances in sterilization techniques" see page 663, lines 36-48; page 664, table I	1
A	CH, A, 605421, published September 29, 1978, see column 4, lines 43-51, Schweizerische Sodafabrik	1

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [10]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers ....., because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [11]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.