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(54) Title: ANTIMICROBIAL COMPOSITION HAVING EFFICACY AGAINST ENDOSPORES

(57) Abstract: A sporicidal composition has a moderately low pH and includes at least one oxidizing acid and the dissociation product of at least one inorganic oxidizing agent. Very high effective solute concentrations can enhance the efficacy of the composition. Embodiments of the composition can be applied to a surface and allowed to absorb into the endospore, ultimately killing at least some of those bacteria in mature endospore form. The surface being treated can be an inanimate surface, particularly a hard surface, or a medical device.



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ANTIMICROBIAL COMPOSITION HAVING EFFICACY AGAINST ENDOSPORES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This international application claims the benefit of U.S. provisional patent application nos. 62/194,141 and 62/194,210, filed 17 July 2015 and 18 July 2015, respectively, the disclosures of which are incorporated herein by reference.

BACKGROUND INFORMATION

[0002] Unlike a true spore, an endospore is not an offspring of another living organism. Nevertheless, the terms “spore” and “endospore” are used interchangeably herein throughout.

[0003] A vegetative bacterium is one which can grow, feed and reproduce. When nutrients become scarce, certain vegetative bacteria begin a process referred to as “sporulation,” where they take on a reduced, dormant form which permits them to survive without nutrients and gives them resistance to UV radiation, desiccation, elevated temperatures, extreme freezing and chemical disinfectants. Soon after environmental conditions return to being favorable for vegetative growth, such bacteria can exit their dormant state (“spore germination”) with the spore core rehydrating, the cortex hydrolyzing, the coat being shed and, ultimately, DNA replication being initiated. For additional information on these complex processes, the interested reader is directed to any of a variety of texts such as, for example, J.C. Pommerville, *Fundamentals of Microbiology*, 10th ed. (Jones & Bartlett Learning; Burlington, Massachusetts).

[0004] At the outset of the sporulation step, a vegetative bacterium called a “mother cell” makes a copy of its DNA and then forms a membrane around the new copy of DNA, with the section of the cell having the copied DNA completely surrounded by a membrane being referred to as a “forespore.” The next morphological stage of sporulation is “engulfment” of the forespore by its mother cell, a process that is analogous to phagocytosis; when engulfment is complete, the forespore is entirely surrounded by its inner and outer membranes and free in the mother cell cytoplasm. Around this core of the spore is assembled a series of protective structures, completion of which results in a mature endospore, which is released after the mother cell is broken apart.

[0005] Endospores have a multi-layer structure, all of which protect the nucleus. The nucleus is protected by a number of layers, set forth below in Table 1 in order from inside-to-outside:

Table 1

Layer	Description
(1) core	protoplast that contains RNA, DNA, dipicolinic acid, low molecular weight basic proteins, and various minerals such as Ca, K, Mn and P
(2) cortical (outer) membrane	non-crosslinked or lightly crosslinked peptidoglycan-containing layer that develops into cell wall during germination
(3) cortex	peptidoglycan and manuronic acid residues
(4) inner spore coat	primarily acidic polypeptides
(5) outer spore coat	primarily protein with some carbohydrates and lipids and, in the case of <i>C. difficile</i> , a large amount of phosphorus

The inner spore coat is soluble in alkali solutions, but the outer spore coat is resistant to hydrolysis from alkalis, probably due in significant part to its numerous disulfide (-S-S-) linkages.

[0006] Spores are extremely difficult to eradicate and are involved in the spread of diseases such as *Clostridium difficile* (*C. diff*) infection and anthrax.

[0007] *C. difficile* is a Gram-positive, spore-forming bacterium often found in healthcare facilities and is the cause of antibiotic-associated diarrhea. *C. difficile* infection is a growing problem, affecting hundreds of thousands of people each year, killing a significant portion of those affected. *C. difficile* spores are resistant to most routine surface cleaning methods, remaining viable in the environment for long periods of time.

[0008] Anthrax is an acute, usually lethal, disease that affects both humans and animals, caused by the bacterium *Bacillus anthracis* (*B. anthracis*), which spores can be produced *in vitro* and used as a biological weapon. Anthrax does not spread directly from one infected animal or person to another, instead being spread by spores.

[0009] Sporostatic compounds are not sporicidal, i.e., they do not kill spores; instead, they inhibit germination of spores or cause germinated spores to grow abnormally. Spores can survive exposure to these compounds and then grow after sporostatic compounds no longer are present. Sporostatic compounds include phenols and cresols, organic acids and

esters, alcohols, quaternary ammonia compounds, biguanides, and organomercury compounds. Certain of these sporostatic compounds can be marginally sporicidal at high concentrations; see, e.g., A.D. Russell, "Bacterial Spores and Chemical Sporicidal Agents," *Clinical Microbiology Reviews*, pp. 99-119 (1990)) for the relative concentrations of certain sporostatic compounds needed to achieve any sporicidal efficacy.

[0010] Commonly employed spore treatment options include aldehydes, particularly gluteraldehyde and formaldehyde; chlorine-releasing agents including Cl₂, sodium hypochlorite, calcium hypochlorite, and chlorine reducing agents such as dichloroisocyanurate; iodine and iodophors; peroxygens including hydrogen peroxide and peracetic acid; gases such as ethylene oxide, propylene oxide and ozone; and β -propiolactone. The mechanisms of their activities against spores are not particularly well understood although, in all cases, the activity is rate-limited by the permeation of the active molecule(s) through the protective layers of the spore. This need to penetrate the various layers of protection means that spores must be exposed to these products for long periods of time at high concentrations.

[0011] U.S. Patent Nos. 8,940,792 and 9,314,017 as well as U.S. Pat. Publ. Nos. 2010/0086576, 2013/0272922, 2013/0079407 and 2016/0073628 describe antimicrobial compositions and various uses therefor. The core and cortex (including cortical membrane) of a spore are susceptible to dissolution and lysis by the types of high osmolarity compositions described in these documents. However, such compositions have not been found to be particularly effective against spores, likely due to a limited ability to break down and then penetrate the outer and inner spore coats.

[0012] That which remains desirable is a composition that is capable of penetrating the various defenses of endospores and killing bacteria therein. Such a composition preferably is effective against endospores of bacteria such as of *C. difficile* and *B. anthracis* while not presenting toxicity concerns toward humans who handle or contact it.

SUMMARY

[0013] In one aspect is provided a composition that can kill a variety of endospore-forming bacteria while in mature endospore form. Embodiments of the composition are effective against the various defenses of bacteria in endospore form, specifically, disulfide bonds can be cleaved, intrinsic hydrophobicity can be overcome, peptidoglycan can be disrupted, and the core and cortical membrane can be lysed.

[0014] The composition is acidic but has a $\text{pH} \geq 1.5$ and includes solvent and solute components, the latter including at least one oxidizing acid and the dissociation product of at least one electrolyte oxidizing agent. In certain embodiments, the composition also can include one or more of an organic liquid, a wetting agent (particularly an ionic surfactant), and any of a variety of non-oxidizing electrolytes. Advantageously, the composition need not include an active antimicrobial agent to be sporicidal.

[0015] The composition has an effective solute concentration of at least 1.0 Osm/L, typically at least 1.5 Osm/L and often even higher, up to the solubility limit of the solute component in the solvent component.

[0016] Also provided is a method of treating a surface. The method involves applying an embodiment of the foregoing composition to the surface and permitting the composition to be absorbed into the endospore and to kill at least some of those bacteria in mature endospore form. The surface being treated can be an inanimate surface, particularly a hard surface, and advantageously a hard surface in a healthcare facility.

[0017] Embodiments of the composition, when used in conjunction with tests such as ASTM standard E2197-11 and AOAC Official Method 966.04 can provide positive (passing) results within commercially relevant timeframes. For example, janitorial-type disinfecting treatment of (inanimate) hard surfaces can be effected in less than 20 minutes, while sterilizing treatment of medical instruments can be effected in less than 4 hours.

[0018] Other aspects of the invention will be apparent to the ordinarily skilled artisan from the detailed description that follows. To assist in understanding that description, certain definitions are provided immediately below, and these are intended to apply throughout unless the surrounding text explicitly indicates a contrary intention:

“comprising” means including, but not be limited to, the listed ingredients or steps;

“consisting of” means including only the listed ingredients (or steps) and minor amounts of inactive additives or adjuvants;

“consisting essentially of” means including only the listed ingredients (or steps), minor amounts (less than 5%, 4%, 3%, 2%, 1%, 0.5%, 0.25%, or 0.1%) of other ingredients that supplement sporicidal activity and/or provide a secondary effect (e.g., antifogging, soil removal, etc.) that is desirable in view of the intended end use, and/or inactive additives or adjuvants;

“polyacid” means a compound having at least two carboxyl groups and specifically includes dicarboxylic acids, tricarboxylic acids, etc.;

“pH” means the negative value of the base 10 logarithm of $[H^+]$ as determined by an acceptably reliable measurement method such as a properly calibrated pH meter, titration curve against a known standard, or the like;

“pK_a” means the negative value of the base 10 logarithm of a particular compound’s acid dissociation constant;

“E⁰_{red}” means the standard voltage for a reduction half-reaction in water at 25°C;

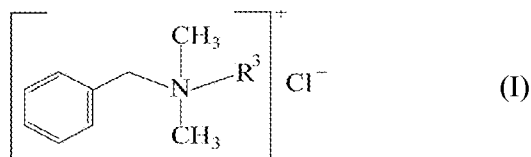
“buffer” means a compound or mixture of compounds having an ability to maintain the pH of a solution to which it is added within relatively narrow limits;

“buffer precursor” means a compound that, when added to a mixture containing an acid, results in a buffer;

“electrolyte” means a compound that exhibits some dissociation when added to water;

“non-oxidizing electrolyte” means an electrolyte other than one that can act as an oxidizing agent;

“benzalkonium chloride” refers to any compound defined by the following general formula



where R³ is a C₈-C₁₈ alkyl group, or any mixture of such compounds;

“effective solute concentration” is a measurement of the colligative property resulting from the number of moles of molecules (from nonelectrolyte) or ions (from electrolytes) present in a given volume solution, often presented in units of osmoles per liter;

“δ_p” is the dipolar intermolecular force Hansen Solubility Parameter (HSP), with the value for a solution or mixture of solvents being calculated by

$$\delta_p = \sum_{i=1}^n (\delta_{di} \times x_{di}) \quad (\text{I})$$

where δ_{di} is the energy from dipolar intermolecular force for solvent component i , x_{di} is the percentage of solvent component i relative to the total amount of solvent components, and n is the total number of solvent components;

“oxyacid” means a mineral acid that contains oxygen;

“substituted” means containing a heteroatom or functionality (e.g., hydrocarbyl group) that does not interfere with the intended purpose of the group in question;

“microbe” means any type of microorganism including, but not limited to, bacteria, viruses, fungi, viroids, prions, and the like;

“antimicrobial agent” means a substance having the ability to cause greater than a 90% (1 log) reduction in the number of one or more microbes;

“active antimicrobial agent” means an antimicrobial agent that is effective only or primarily during the active parts of the lifecycle, e.g., cell division, of a microbe;

“germicide” means a substance that is lethal to one or more types of harmful microorganisms;

“disinfectant” means a substance that is lethal to one or more types of bacteria;

“high level disinfectant” means a disinfectant that is capable of killing all bacteria except for small amounts of bacteria in endospore form;

“sterilant” means a substance capable of eliminating at least a 6 log (99.9999%) reduction of all microbes, regardless of form;

“contact time” means the amount of time that a composition is allowed to contact a surface and/or an endospore on such a surface;

“hard surface” means any surface that is non-porous to fluids and, in most cases, non-deformable; and

“healthcare” means involved in or connected with the maintenance or restoration of the health of the body or mind.

[0019] Throughout this document, unless the surrounding text explicitly indicates a contrary intention, all values given in the form of percentages are w/v, i.e., grams of solute per liter of composition. The relevant portion(s) of any specifically referenced patent and/or published patent application are incorporated herein by reference.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0020] The sporicidal composition is described first in terms of its properties and components, many of which are widely available and relatively inexpensive, and then in terms of certain uses.

[0021] The solvent component of the composition typically includes a significant amount of water. Relative to its overall volume, a composition often includes up to 20%, 25%, 30%, 33%, 35%, 40%, 45%, 50%, or even 55% (all v/v). On a per liter basis, a composition often includes from ~50 to ~500 mL, commonly from ~75 to ~475 mL, more commonly from ~100 to ~450 mL, usually from ~125 to ~425 mL, typically from ~150 to ~400 mL, and most typically 250 ± 50 mL water. The water need not be specially treated (e.g., distilled and/or deionized), although preference certainly can be given to water that does not interfere with the intended antimicrobial effect of the composition.

[0022] The solvent component of the composition often includes at least one organic liquid, and, in some embodiments, preference is given to those organic liquids with δ_p values lower than that of water ($\delta_p \approx 16.0 \text{ MPa}^{1/2}$). Where at least one organic liquid is present in the solvent component, the δ_p value of the overall solvent component generally is less than 16.0, no more than 15.6, no more than 15.2, no more than 15.0, no more than 14.6 or no more than 14.0 $\text{MPa}^{1/2}$. In some embodiments, the δ_p value of the overall solvent component can range from 13.1 to 15.7 $\text{MPa}^{1/2}$, from 13.3 to 15.6 $\text{MPa}^{1/2}$, from 13.5 to 15.5 $\text{MPa}^{1/2}$, and even from 13.7 to 15.4 $\text{MPa}^{1/2}$.

[0023] The organic liquid(s) often is/are present at concentrations of up to 60%, commonly 5 to 50%, more commonly 10 to 45%, even more commonly 15 to 40%, and typically 20 to 35% (all w/v, based on total volume of solvent component).

[0024] The amount of a given organic liquid (or mixture of organic liquids) to be added to water can be calculated using formula (I) if a targeted δ_p value is known. Similarly, a projected δ_p value can be calculated using formula (I) if the amount of organic liquid(s) and their individual δ_p values are known.

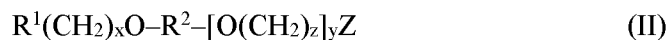
[0025] The solvent component can consist of, or consist essentially of, only water or only one or more organic liquids, with preference being given to mixtures of species of the same genus of organic liquids, e.g., two ethers or two alcohols rather than one ether and one alcohol. In certain preferred embodiments, the solvent component can consist of, or consist essentially of, water and an organic liquid, preferably one having a δ_p value less than 15.5

MPa^{1/2}. In yet other embodiments, the solvent component can consist of, or consist essentially of, water and two or more organic liquids, with the resulting solvent component having δ_p value that can be calculated using formula (I); again, preference is given to mixtures of species of the same genus of organic liquids, e.g., two ethers or two alcohols rather than one ether and one alcohol.

[0026] With respect to organic liquids that can be employed in the solvent component, those which are miscible with one another and/or water are preferred. Non-limiting examples of potentially useful organic liquids include ketones such as acetone, methyl butyl ketone, methyl ethyl ketone and chloroacetone; acetates such as amyl acetate, ethyl acetate and methyl acetate; (meth)acrylates and derivatives such as acrylamide, lauryl methacrylate and acrylonitrile; aryl compounds such as benzene, chlorobenzene, fluorobenzene, toluene, xylene, aniline and phenol; aliphatic alkanes such as pentane, isopentane, hexane, heptane and decane; halogenated alkanes such as chloroform, methylene dichloride, chloroethane and tetrachloroethylene; cyclic alkanes such as cyclopentane and cyclohexane; and polyols such as ethylene glycol, diethylene glycol, propylene glycol, hexylene glycol, and glycerol. When selecting such organic liquids for use in the solvent component of the composition, possible considerations include avoiding those which contain a functional group that will react with the acid(s) and optionally, salt(s) employed in the composition and favoring those which possess higher regulatory pre-approval limits.

[0027] Preferred organic liquids include ethers and alcohols due to their low tissue toxicity and environmentally friendliness. These can be added at concentrations up to the solubility limit of the other ingredients in the composition.

[0028] Ether-based liquids that can be used in the solvent component include those defined by the following general formula



where x is an integer of from 0 to 20 (optionally including, where $2 \leq x \leq 20$, one or more points of ethylenic unsaturation), y is 0 or 1, z is an integer of from 1 to 4, R^2 is a C₁-C₆ linear or branched alkylene group, R^1 is a methyl, isopropyl or phenyl group, and Z is a hydroxyl or methoxy group. Non-limiting examples of glycol ethers (formula (II) compounds where Z is OH) that can be used in the solvent component are set forth below in Table 2.

Table 2: Representative glycol ethers, with formula (II) variables and δ_p values

	R¹	x	R²	y	z	$\sim\delta_p$ (MPa ^{1/2})
ethylene glycol monomethyl ether	CH ₃	0	(CH ₂) ₂	0	-	9.2
ethylene glycol monoethyl ether	CH ₃	1	(CH ₂) ₂	0	-	9.2
ethylene glycol monopropyl ether	CH ₃	2	(CH ₂) ₂	0	-	8.2
ethylene glycol monoisopropyl ether	(CH ₃) ₂ CH	0	(CH ₂) ₂	0	-	8.2
ethylene glycol monobutyl ether	CH ₃	3	(CH ₂) ₂	0	-	5.1
ethylene glycol monophenyl ether	C ₆ H ₅	0	(CH ₂) ₂	0	-	5.7
ethylene glycol monobenzyl ether	C ₆ H ₅	1	(CH ₂) ₂	0	-	5.9
diethylene glycol monomethyl ether	CH ₃	0	(CH ₂) ₂	1	2	7.8
diethylene glycol monoethyl ether (DGME)	CH ₃	1	(CH ₂) ₂	1	2	9.2
diethylene glycol mono-n-butyl ether	CH ₃	3	(CH ₂) ₂	1	2	7.0
propylene glycol monobutyl ether	CH ₃	3	(CH ₂) ₃	0	-	4.5
propylene glycol monoethyl ether	CH ₃	1	(CH ₂) ₃	0	-	6.5
propylene glycol monoisobutyl ether	(CH ₃) ₂ CH	1	(CH ₂) ₃	0	-	4.7
propylene glycol monoisopropyl ether	(CH ₃) ₂ CH	0	(CH ₂) ₃	0	-	6.1
propylene glycol monomethyl ether	CH ₃	0	CH ₂ CH(CH ₃)	0	-	6.3
propylene glycol monophenyl ether	C ₆ H ₅	0	CH ₂ CH(CH ₃)	0	-	5.3
propylene glycol monopropyl ether (PGME)	CH ₃	2	CH ₂ CH(CH ₃)	0	-	5.6
triethylene glycol monomethyl ether	CH ₃	0	(CH ₂) ₂	2	2	7.6
triethylene glycol monooleyl ether	CH ₃	17*	(CH ₂) ₂	2	2	3.1

* includes unsaturation at the 9 position

[0029] Cyclic and C₁-C₁₆ acyclic (both linear and branched, both saturated and unsaturated) alcohols, optionally including one or more points of ethylenic unsaturation and/or one or more heteroatoms other than the alcohol oxygen such as a halogen atom, an amine nitrogen, and the like, can be employed as an organic liquid in the solvent component of the composition. Non-limiting examples of representative examples are compiled in the following table.

Table 3: Representative alcohols, with δ_p values

	$\sim\delta_p$ (MPa ^{1/2})
2-propenol	10.8
1-butanol	5.7
t-butyl alcohol	5.1
4-chlorobenzyl alcohol	7.5
cyclohexanol	4.1
2-cyclopentenyl alcohol	7.6
1-decanol	10.0
2-decanol	10.0
2,3-dichloropropanol	9.2
2-ethyl-1-butanol	4.3
ethanol	8.8
2-ethyl-hexanol	3.3
isooctyl alcohol	7.3
octanol	3.3
methanol	12.3
oleyl alcohol	2.6
1-pentanol	4.5
2-pentanol	6.4
1-propanol	6.8
2-propanol (IPA)	6.1

[0030] For further information on organic liquid-containing solvent components, the interested reader is directed to U.S. Pat. Publ. No. 2016/0073628.

[0031] The composition is acidic, more particularly having a pH of no more than 4, and certain embodiments can have a pH of no more than 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3.0, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3 or even 2.2. The composition has a pH of at least 1.5, generally at least 1.75, and typically at least 2.0. Ranges of pH values employing each of the lower limits in combination with each of the upper limits are envisioned. Embodiments of the composition can have pH values of 2.75 ± 1.15 , 2.70 ± 1.05 , 2.65 ± 1.0 , 2.60 ± 0.75 , 2.55 ± 0.60 , 2.50 ± 0.55 and 2.45 ± 0.45 .

[0032] Acidity can be achieved by adding to the solvent component (or vice versa) one or more acids. Strong (mineral) acids such as HCl, H₂SO₄, H₃PO₄, HNO₃, H₃BO₃, and the

like or organic acids, particularly organic polyacids, may be used. Examples of organic acids include monoprotic acids such as formic acid, acetic acid and substituted variants (e.g., hydroxyacetic acid, chloroacetic acid, dichloroacetic acid, phenylacetic acid, and the like), propanoic acid and substituted variants (e.g., lactic acid, pyruvic acid, and the like), any of a variety of benzoic acids (e.g., mandelic acid, chloromandelic acid, salicylic acid, and the like), glucuronic acid, and the like; diprotic acids such as oxalic acid and substituted variants (e.g., oxamic acid), butanedioic acid and substituted variants (e.g., malic acid, aspartic acid, tartaric acid, citramalic acid, and the like), pentanedioic acid and substituted variants (e.g., glutamic acid, 2-ketoglutaric acid, and the like), hexanedioic acid and substituted variants (e.g., mucic acid), butenedioic acid (both cis and trans isomers), iminodiacetic acid, phthalic acid, and the like; triprotic acids such as citric acid, 2-methylpropane-1,2,3-tricarboxylic acid, benzenetricarboxylic acid, nitrilotriacetic acid, and the like; tetraprotic acids such as prehnitic acid, pyromellitic acid, and the like; and even higher degree acids (e.g., penta-, hexa-, hepta-protic, etc.). Where a tri-, tetra-, or higher acid is used, one or more of the carboxyl protons can be replaced by cationic atoms or groups (e.g., alkali metal ions), which can be the same or different.

[0033] Because of the nature of some of the defenses resulting from the various layers present in endospores, the composition must include at least one oxidizing acid. Many oxy-acids, such as perchloric, chloric, chlorous, hypochlorous, persulfuric, sulfuric, sulfurous, hyposulfurous, pyrosulfuric, disulfurous, thiosulfurous, pernitric, nitric, nitrous, hyponitrous, perchromic, chromic, dichromic, permanganic, manganic, perphosphoric, phosphoric, phosphorous, hypophosphorus, periodic, iodic, iodous, etc., are considered to be oxidizing acids. Organic oxidizing acids include, but are not limited to, peracetic acid, peroxalic acid and diperoxalic acid.

[0034] Preferred oxidizing acids are those which have relatively high pK_a values (i.e., are not considered to be particularly strong acids) and positive standard potentials (E^0_{red}). The former permits production of a composition that has a pH value that is not too low, i.e., below ~ 1.5 , preferably not below ~ 1.75 , more preferably not below ~ 2 , and most preferably not below ~ 2.2 , so that the composition can be used without extreme protective measures by those charged with handling and applying them to surfaces and/or destroying components of articles to be treated. A positive standard potential permits the acid to have sufficient oxidizing capacity to permit overcoming or avoidance of certain endospore defenses such as,

for example, oxidation of disulfide linkages and protein polymers on the endospore coat, which allows the outer spore coat to be breached.

[0035] Preferred pK_a values are greater than ~ 1 , greater than ~ 1.5 , greater than ~ 2 , greater than ~ 2.5 , greater than ~ 3 , greater than ~ 3.5 , greater than ~ 4 , greater than ~ 4.5 , greater than ~ 5 , and even greater than ~ 5.5 . Acids with lower pK_a values can be used if other steps are taken to ensure compliance with required or desired properties of the composition such as pH range (discussed above) and effective solute concentration (discussed below).

[0036] Preferred E^0_{red} values are those which are at least +0.20 V, at least +0.25 V, at least +0.33 V, at least +0.40 V, at least +0.50 V, at least +0.60 V, at least +0.67 V, at least +0.75 V, at least +0.80 V, at least +0.90 V, at least +1.00 V, at least +1.10 V, at least +1.20 V, or even at least +1.25 V.

[0037] Some oxidizing acids are not particularly stable in aqueous solutions. Accordingly, providing a composition with an oxidizing acid prepared *in vitro* can be advantageous. For example, in one preferred embodiment, to a solvent component of a composition can be provided acetic acid and hydrogen peroxide which, when contacted, reversibly form peracetic acid.

[0038] The amount of any given acid employed can be determined from the target pH of a given composition and the pK_a value(s) of the chosen acids in view of the type and amounts of compound(s), if any, utilized to achieve the desired effective solute concentration in the composition. (More discussion of osmolarity and the types of osmolarity-adjusting compounds appears below.)

[0039] Also present in the solute component of the composition is an electrolyte oxidizing agent that does not contain any active hydrogen atoms when subjected to a Zerewitinoff determination. Non-limiting examples of potentially useful electrolyte, preferably inorganic, oxidizing agents include compounds which include anions such as manganate, permanganate, peroxochromate, chromate, dichromate, peroxymonosulfate, and the like. (Some of these electrolytes can impact pH, so a composition formulated to have a given pH might require adjustment after addition of the oxidizing agent(s).) Preferred are those compounds having E^0_{red} values of at least +1.25 V, preferably at least +1.5 V, more preferably at least +1.75 V, even more preferably at least +2.0 V and most preferably at least +2.25 V.

[0040] Electrolyte oxidizing agents generally can be added at up to their individual solubility limits, although the maximum amount generally is on the order of 30 g per liter of

total composition. Exemplary ranges of electrolyte oxidizing agent(s) are ~2 to ~25 g/L, ~3 to ~21 g/L, ~4 to ~18 g/L, ~5 to ~16 g/L and ~6 to ~14 g/L. Exemplary amounts of electrolyte oxidizing agent(s) are 17.5 ± 12 g/L, 15 ± 9 g/L, 12.5 ± 6 g/L and 10 ± 3 g/L.

[0041] Once the acid(s) and oxidizing agent(s) are added to a solvent component that contains water (or vice versa), each at least partially dissociates.

[0042] A composition that includes only a solvent component and a solute component that consists, or consists essentially of, one or more oxidizing acids and one or more electrolyte oxidizing agents can have efficacy against endospores, i.e., can result in some or all endospores being rendered incapable of returning to a vegetative state. Nevertheless, a composition that includes a solute component which includes additional subcomponents can have enhanced efficacy in certain circumstances.

[0043] In certain embodiments, the effective solute concentration of the composition can be relatively high. Often, efficacy increases as effective solute concentration (osmolarity) increases. The presence of an abundance of solutes ensures that a sufficient amount are present to induce a high osmotic pressure across the cortical membrane, leading to lysis.

[0044] This efficacy is independent of the particular identity or nature of individual compounds of the solute component, although smaller molecules are generally more effective than larger molecules due to solvent capacity (i.e., the ability to (typically) include more small molecules in a given amount of solvent component than an equimolar amount of larger molecules) and ease of transport across cortical membranes.

[0045] Any of a number of solutes can be used to increase the composition osmolarity.

[0046] One approach to achieving increased osmolarity of the composition is by adding large amounts of non-oxidizing electrolytes, particularly ionic compounds (salts); see, e.g., U.S. Pat. No. 7,090,882. Like the oxidizing acid and inorganic oxidizing agent, non-oxidizing electrolytes dissociate upon being introduced into a solvent component that includes water.

[0047] Where one or more organic acids are used in the composition, another approach to increasing osmolarity without increasing the pH of the composition past a desired target involves inclusion of salt(s) of one or more the acid(s) or the salt(s) of one or more other organic acids. Such compounds, upon dissociation, increase the effective amount of solutes in the composition without greatly impacting the molar concentration of hydronium ions while, simultaneously, providing a buffer system in the composition.

[0048] For example, where the composition includes an acid, a fraction up to a many fold excess (e.g., 3× to 10×, at least 5× or even at least 8×) of one or more salts of that (or another) acid also can be included. The identity of the countercation portion of the salt is not believed to be particularly critical, with common examples including ammonium ions and alkali metals. Where a polyacid is used, all or fewer than all of the H atoms of the carboxyl groups can be replaced with cationic atoms or groups, which can be the same or different. For example, mono-, di- and trisodium citrate all constitute potentially useful buffer precursors, whether used in conjunction with citric acid or another organic acid. However, because trisodium citrate has three available basic sites, it has a theoretical buffering capacity up to 50% greater than that of disodium citrate (which has two such sites) and up to 200% greater than that of sodium citrate (which has only one such site).

[0049] Regardless of how achieved, the effective solute concentration of the composition is at least 1.0 Osm/L, generally at least 1.25 Osm/L, often at least 1.5 Osm/L, commonly at least 1.75 Osm/L, more commonly at least 2.0 Osm/L, typically at least 2.25 Osm/L, more typically at least 2.5 Osm/L. (As points of comparison, in biological applications, a 0.9% (by wt.) saline solution, which is ~0.3 Osm/L, typically is considered to have moderate tonicity, while a 3% (by wt.) saline solution, which is ~0.9 Osm/L, generally is considered to be hypertonic.) In some embodiments, the composition has an effective solute concentration of at least ~3.0, at least ~3.25, at least ~3.5, at least ~3.75, or even at least ~4.0 Osm/L, with the upper limit being defined by the solubility limit of the solutes in the solvent component; in some embodiments, the upper limit of effective solute concentration can range as high as 4.5, 4.75, 5.0, 5.25, 5.5, 5.75, 6.0, 6.25, 6.5, 6.75, 7.0, 7.2, 7.4, 7.6, 7.8, 8.0, 8.2, 8.4, 8.6, 8.8 or even ~9 Osm/L. Effective solute concentration ranges involving combinations of any of the lower and upper limits set forth in this paragraph also are envisioned. The effective solute concentrations of compositions according to the present invention, which are intended to be effective against (i.e., lethal to) endospores, generally are higher than those described in U.S. Patent Nos. 8,940,792 and 9,314,017 as well as U.S. Pat. Publ. Nos. 2010/0086576, 2013/0272922, 2013/0079407 and 2016/0073628, all of which are directed generally against planktonic bacteria and biofilms.

[0050] Effective solute concentration can be calculated using known techniques or, if desired, measured using any of a variety of colligative property measurements such as boiling point elevation, freezing point depression, osmotic pressure and lowering of vapor pressure.

[0051] Unlike many of the compositions described in the documents listed in the preceding paragraph, the present sporicidal composition does not require inclusion of surfactant in the solute component, although certain preferred embodiments include one or more wetting agents which include, but are not limited to, surfactants.

[0052] Essentially any material having surface active properties in water can be employed, regardless of whether water is present in the solvent component of the composition, although those surface active agents that bear some type of ionic charge are expected to have enhanced antimicrobial efficacy because such charges, when brought into contact with a bacteria, are believed to lead to more effective bacterial membrane disruption and, ultimately, to cell leakage and lysis.

[0053] Polar surfactants generally are more efficacious than non-polar surfactants, with ionic surfactants being most effective. For polar surfactants, anionic surfactants generally are the most effective, followed by zwitterionic and cationic surfactants, with smaller molecules generally being preferred over larger ones. The size of side-groups attached to the polar head can influence the efficacy of ionic surfactants, with larger size-groups and more side-groups on the polar head potentially decreasing the efficacy of surfactants.

[0054] Potentially useful anionic surfactants include, but are not limited to, ammonium lauryl sulfate, dioctyl sodium sulfosuccinate, perfluorobutanesulfonic acid, perfluorononanoic acid, perfluorooctanesulfonic acid, perfluorooctanoic acid, potassium laurylsulfate, sodium dodecylbenzenesulfonate, lauryl laureth sulfate, sodium lauryl sarcosinate, sodium myreth sulfate, sodium myreth sulfate, sodium pareth sulfate, sodium stearate, sodium chenodeoxycholate, *N*-laurylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate, sodium deoxycholate, sodium dodecyl sulfate (SDS), sodium glycodeoxycholate, sodium lauryl sulfate (SLS), and the alkyl phosphates set forth in U.S. Pat. No. 6,610,314. SLS is a particularly preferred material.

[0055] Potentially useful cationic surfactants include, but are not limited to, cetylpyridinium chloride (CPC), cetyl trimethylammonium chloride, benzethonium chloride, 5-bromo-5-nitro-1,3-dioxane, dimethyldioctadecylammonium chloride, cetrimonium bromide, dioctadecyldimethylammonium bromide, tetradecyltrimethyl ammonium borine, benzalkonium chloride (BK), hexadecylpyridinium chloride monohydrate and hexadecyltrimethylammonium bromide.

[0056] Potentially useful nonionic surfactants include, but are not limited to, sodium polyoxyethylene glycol dodecyl ether, *N*-decanoyl-*N*-methylglucamine, digitonin, *n*-dodecyl β -D-maltoside, octyl β -D-glucopyranoside, octylphenol ethoxylate, polyoxyethylene (8) iso-octyl phenyl ether, polyoxyethylene sorbitan monolaurate, and polyoxyethylene (20) sorbitan cholamidopropyl dimethylammonio]-2-hydroxy-1-propane sulfonate, 3-[(3-cholamidopropyl) dimethylammonio]-1-propane sulfonate, 3-(decyldimethylammonio) propanesulfonate inner salt, and *N*-dodecyl-*N,N*-dimethyl-3-ammonio-1-propanesulfonate.

[0057] Potentially useful zwitterionic surfactants include sulfonates (e.g. 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), sultaines (e.g. cocamidopropyl hydroxysultaine), betaines (e.g. cocamidopropyl betaine), and phosphates (e.g. lecithin).

[0058] For other potentially useful materials, the interested reader is directed to any of a variety of other sources including, for example, U.S. Pat. Nos. 4,107,328, 6,953,772, 7,959,943, and 8,940,792.

[0059] The amount(s) of wetting agent(s) to be added to the composition is limited to some extent by the target effective solute concentration and compatibility with other subcomponents of the solute component of the composition. The total amount of wetting agent present in the composition can range from at least 0.1%, from at least 0.25%, from at least 0.5%, from at least 0.75% or from at least 1% up to 5%, commonly up to 4%, more commonly up to 3%, and typically up to 2.5%. At times, maximum amounts of certain types of wetting agents, particularly surfactants, that can be present in a composition for a particular end use (without specific testing, review and approval) are set by governmental regulations.

[0060] If more than one type of surfactant is employed, the majority preferably is an ionic surfactant, with the ratio of ionic-to-nonionic surfactant generally ranging from ~2:1 to ~10:1, commonly from ~5:2 to ~15:2, and typically from ~3:1 to ~7:1. Additionally, as is known in the art, a composition should not include surfactant types that are incompatible, e.g., anionic with cationic or zwitterionic with either anionic or cationic.

[0061] The antimicrobial composition can include a variety of additives and adjuvants to make it more amenable for use in a particular end-use application with negatively affecting its efficacy in a substantial manner. Examples include, but are not limited to, emollients, fungicides, fragrances, pigments, dyes, defoamers, foaming agents, flavors, abrasives, bleaching agents, preservatives (e.g., antioxidants) and the like. A comprehensive listing of additives approved by the U.S. Food and Drug Administration is available as a zipped text

file at <http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm> (link active as of filing date of this application).

[0062] The composition's efficacy does not require the inclusion of an active antimicrobial agent (defined above) for efficacy, but such materials can be included in certain embodiments. Non-limiting examples of potentially useful active antimicrobial additives include C₂-C₈ alcohols (other than or in addition to any used as an organic liquid of the solvent component) such as ethanol, n-propanol, and the like; aldehydes such as glutaraldehyde, formaldehyde, and o-phthalaldehyde; formaldehyde-generating compounds such as noxythiolin, tauroline, hexamine, urea formaldehydes, imidazolone derivatives, and the like; anilides, particularly triclocarban; biguanides such as chlorhexidine and alexidine, as well as polymeric forms such as poly(hexamethylene biguanide); dicarboximidamides (e.g., substituted or unsubstituted propamidine) and their isethionate salts; halogen atom-containing or releasing compounds such as bleach, ClO₂, dichloroisocyanurate salts, tosylchloramide, iodine (and iodophors), and the like; silver and silver compounds such as silver acetate, silver sulfadiazine, and silver nitrate; phenols, bis-phenols and halophenols (including hexachlorophene and phenoxyphenols such as triclosan); and quaternary ammonium compounds.

[0063] The following tables provide ingredient lists for exemplary compositions according to the present invention, with amounts being provided in grams and with distilled water being added to bring the ingredients to a volume of 1 L.

Table 4: Formulations for exemplary compositions

	Formulation 1	Formulation 2
salt of organic acid	5-25	10-20
organic acid	125-200	140-180
ionic surfactant	5-30	15-25
nonionic surfactant	0-5	1-3
H ₂ O ₂ (30% by wt. in H ₂ O)	175-325	200-300
inorganic oxidizing agent	4-20	6-12
organic liquid	175-450	225-375

[0064] Various embodiments of the present invention have been provided by way of example and not limitation. As evident from the foregoing tables, general preferences regarding features, ranges, numerical limitations and embodiments are, to the extent feasible

and as long as not interfering or incompatible, envisioned as being capable of being combined with other such generally preferred features, ranges, numerical limitations and embodiments.

[0065] A composition according to the present invention is intended to be, and in practice is, aggressively antimicrobial. Its intended usages are in connection with inanimate objects such as, in particular, hard surfaces, particularly those commonly found in healthcare facilities.

[0066] The composition can be applied to inanimate objects, particularly hard surfaces, in a variety of ways including pouring, spraying or misting, via a distribution device (e.g., mop, rag, brush, textile wipe, etc.), and the like.

[0067] Alternatively, certain objects are amenable to being immersed in a composition. This is particularly true of medical equipment designed for use with multiple patients such as, for example, dialysis equipment, any of a variety of endoscopes, duodenoscopes, etc., endoscopic accessories such as graspers, scissors and the like, manual instruments such as clamps and forceps, laparoscopic surgical accessories, orthopedic and spinal surgery hardware such as clamps and jigs, and the like. Because the composition of the present invention has a more moderate $[H^+]$ than treatments such as peracetic acid and bleach, it can achieve disinfection, high level disinfection or even sterilization without negative effects such as, e.g., polymeric degradation, metal corrosion, glass or plastic etching, and the like.

[0068] Once applied to a surface or object, the various ingredients of the composition act on any endospores present and avoid or break down their various defenses. The contact time necessary for a composition to treat endospores (i.e., ensure that they cannot return to a vegetative state) can vary widely depending on the particular composition and its intended end use.

[0069] For example, embodiments of a composition intended to be applied to hard surfaces in a healthcare facility can achieve at least a 3.0, 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8 or 5.0 log reduction after a contact time of more than 1200 seconds, no more than 1050 seconds, no more than 900 seconds, no more than 840 seconds, no more than 780 seconds, no more than 720 seconds, no more than 660 seconds, or even no more than 600 seconds. When tested in accordance with ASTM E2197-11, certain embodiments can achieve at least a 4.5 log reduction after a contact time of 600 seconds.

[0070] These and/or other embodiments of a composition intended for use as a soaking bath for medical devices can achieve at least a 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, or

6.0 log reduction after a contact time of ~14,400 seconds, up to ~10,800 seconds, up to ~7200 seconds, up to ~5400 seconds, and on the order of ~3600 seconds. When tested in accordance with AOAC Official Method 966.04, certain embodiments can achieve a passing score after contact times as low as 1800 seconds.

[0071] Embodiments of the sporicidal composition may be able to be classified as high level disinfectants or even as sterilants.

[0072] After the composition has been allowed to contact a given object or surface for an appropriate time (in view of factors such as expected bacterial load, type of bacteria potentially present, importance of the object/surface, etc.), it can be left to evaporate or, preferably, rinsed away with water or a dilute saline solution.

[0073] The following non-limiting, illustrative examples provide detailed conditions and materials that can be useful in the practice of the present invention. Throughout those examples, any reference to room temperature refers to ~22°C.

EXAMPLES

Example 1 (comparative): *peracetic acid*

[0074] A widely recognized and recommended disinfection treatment where endospores are possible or suspected is application to the target surface and 10 minute contact time of 10% (w/v) peracetic acid. Accordingly, peracetic acid constitutes a good comparative for compositions of the present invention.

[0075] Two peracetic acid solutions were prepared by adding distilled water to a flask containing peracetic acid. The concentration of one of the solutions was 5% (w/v) while that of the other was 10% (w/v). The calculated effective solute concentrations for these solutions were 1.97 and 3.95 Osm/L, respectively.

[0076] To a 50 mL beaker was added 20 mL distilled water. A cleaned, rinsed and dried probe from a calibrated, temperature compensating pH meter was lowered into the beaker. Sequential aliquots of the peracetic acid solution then were added to the beaker, with pH readings after each. The titrations were performed at room temperature.

[0077] The results of these titrations are shown below in Table 5.

Table 5: Peracetic acid titrations

5% (w/v)		10% (w/v)	
Amt. acid, mL	pH	Amt. acid, mL	pH
0	5.91	0	5.31
0.25	2.42	0.1	2.74
0.50	2.17	0.2	2.48
0.75	2.00	0.3	2.33
1.00	1.89	0.4	2.22
1.25	1.80	0.5	2.14
1.50	1.74	0.6	2.07
1.75	1.68	0.7	2.00
2.00	1.59	0.8	1.95
2.25	1.56	0.9	1.91
2.50	1.53	1.0	1.86
2.75	1.48	1.1	1.83
3.00	1.45	1.2	1.79
3.25	1.42	1.3	1.76
3.50	1.41	1.4	1.73
3.75	1.38	1.5	1.70
4.00	1.34	1.6	1.67
4.25	1.30	1.7	1.65
4.50	1.28	1.8	1.62
4.75	1.26	1.9	1.60
5.00	1.25	2.0	1.58

[0078] The above data indicate, *inter alia*, that the pH of water is reduced to below 3 upon addition of even a tiny aliquot, i.e., less than 1% by volume, of either peracetic acid solution and that the addition of only 1 mL (5% by volume) of either solution has reduced the pH to below 2. Further, the asymptotic pH for either acid solution is on the order of 1.1 ± 0.1 .

[0079] Further, U.S. EPA recommendations are for peracetic acid solutions of at least 2.5% (w/v) which, according to the tabulated data, has a pH of no more than 1.25.

[0080] Thus, any worker performing this recommended disinfection procedure (i.e., application of a 2.5-10% peracetic acid solution) should employ the types of precautions

appropriate for handling strong acids such as, e.g., protective gloves, protective eyewear, breathing masks, etc.

Example 2 (comparative): *bleach*

[0081] Another widely recognized and recommended disinfection treatment where endospores are possible or suspected is application to the target surface and 10 minute contact time of a bleach solution.

[0082] To a 50 mL beaker was added 5 mL of a bleach solution, i.e., 8.25% (w/v) sodium hypochlorite. A cleaned, rinsed and dried probe from a calibrated, temperature compensating pH meter was lowered into the beaker. Sequential aliquots of distilled water then were added to the beaker, with pH readings after each.

[0083] The data from this titration are shown below in Table 6.

Table 6: Titration of household bleach with water

Water, mL	[ClO ⁻], % (w/v)	pH
0	8.25	12.56
2	5.89	12.46
4	4.58	12.33
6	3.75	12.23
8	3.17	12.14
10	2.75	12.06
12	2.43	12.00
14	2.17	11.94
16	1.96	11.89
18	1.79	11.84
20	1.65	11.79
22	1.53	11.74
24	1.42	11.70
25	1.38	11.68

[0084] The data of this table indicate, *inter alia*, that an undiluted 8.25% bleach solution has a pH of almost 13 and that reducing the ClO⁻ concentration by three-fourths reduces this only to ~11.9. Conversely, reducing the concentration from 8.25% to 5% (both

w/v) results in the calculated effective solute concentration being reduced by almost half, i.e., from 2.28 to 1.34 Osm/L.

[0085] Thus, any worker performing this recommended disinfection procedure (i.e., application of a bleach solution) should employ the types of precautions appropriate for handling strong bases, e.g., protective gloves, protective eyewear, breathing masks, etc.

Examples 3-10: *in vitro* time-to-kill

[0086] Efficacy of certain sporicidal compositions was performed against *Clostridium difficile* (ATCC# 43598). In this testing, reduction of bacteria is determined by comparison against untreated controls (employing phosphate buffered saline as liquid) at various time test points, typically equal increments such as 15 minutes (900 seconds).

[0087] A 9.9 mL aliquot of the solution to be tested was placed in a 20 mL test tube. A 0.1 mL volume of the test culture ($\sim 10^6$ colony forming units (CFU) of *C. Diff* per mL when diluted) was added to the test tube, which then was vortexed. After a predetermined amount of contact time, 1.0 mL of the sample/test culture suspension was transferred into sterile test tubes containing 9.0 mL of an appropriate neutralization solution, followed by additional vortexing.

[0088] Serial tenfold dilutions then were prepared by transferring 0.5 mL aliquots of test solution into 4.5 mL of neutralizing solution, with vortex mixing between dilutions. From these dilutions, duplicate 1.0 mL aliquots were spread-plated onto brain-heart agar plates, which then were incubated anaerobically at $35^\circ \pm 2^\circ\text{C}$ for ~ 72 hours.

[0089] Following incubation, the colonies on the plates were counted, with counts in the 20 to 200 CFU range used in data calculations. The log reduction from this testing is performed by subtracting the CFU/mL recovered treatment value from the CFU/mL recovered control sample.

[0090] A number of compositions were tested in this manner, with the time to achieve 6 log reductions in spores shown in the last column of Table 7 below. Each of the compositions was prepared based on a targeted ~ 2.3 Osm/L effective solute concentration and a target pH of 4. (In the buffer system column, “A” represents acetic acid/sodium acetate, while “C” represents citric acid/trisodium citrate. In the oxidant column, “PPOMS” represents peroxy-monosulfate, all at 0.22% (w/v), and “PAA” represents peracetic acid at the noted concentration.) Those compositions designated as employing BK as a surfactant included 0.21% (w/v),

while those designated as employing SDS included 0.175% (w/v). For those compositions showing inclusion of an organic liquid, an isopropanol solution (70% in water) was employed.

Table 7

Example	Buffer system	Oxidant	Surfactant	Org. liquid (% w/v)	Time (min.)
3	A	PPOMS	BK	--	30
4	A	PPOMS	SDS	--	30
5	A	PPOMS	BK	10.0	60
6	C	PAA (0.1%)	BK	20.0	30
7	C	PAA (1.0%)	BK	20.0	30
8	A	PPOMS	BK	--	30
9	A	PPOMS	SDS	--	30
10	A	PPOMS	SDS	10.0	15

[0091] The times shown in the foregoing table are better than those which can be achieved with most commercially available sporicidal products, which typically require 240 to 2160 minutes for *C. Diff* disinfection. Additionally, exposure to each of these compositions is far less dangerous than exposure to such commercial products.

Examples 11-22

[0092] The data from Table 7 above seem to indicate that compositions employing an anionic surfactant (SDS) might provide better results than those employing a cationic surfactant (BK).

[0093] To further investigate efficacy, additional compositions were prepared, each of which employed the same amount of SDS as was used in Examples 4 and 9-10 plus 0.02% (w/v) of a polysorbate-type nonionic surfactant. Each also included 250 g/L of a 30% H₂O₂ solution and 300 g/L of an organic liquid. The electrolyte oxidizing agent (EOA) for each composition was PPOMS. The citric acid-containing compositions included 140 g/L citric acid and 17.5 g/L trisodium citrate dihydrate (along with the noted amounts of NaCl to raise the effective solute concentration to a predetermined target), while the acetic acid-containing compositions included the noted amounts of acetic acid (AA) and sodium acetate (SA).

[0094] Quantitative carrier testing was performed in substantial accord with ASTM standard E2197-11, with efficacy against spores being shown in the last columns of the following tables.

Table 8a: citric acid compositions

Example	Target pH	Amt. NaCl (g/L)	Amt. EOA (g/L)	Organic liquid	Log reduction
11	1.5	54.0	12.0	IPA	5.6
12	3.5	54.0	4.0	IPA	3.2
13	1.5	178.2	12.0	DGME	< 3
14	3.5	178.2	4.0	DGME	< 3
15	2.5	116.0	8.0	DGME	3.9
16	2.5	116.0	8.0	IPA	3.9

Table 8b: acetic acid compositions

Example	Target pH	Amts. AA/SA (g/L)	Amt. EOA (g/L)	Organic liquid	Log reduction
17	1.5	82.4 / 7.4	4.0	DGME	5.0
18	3.5	82.4 / 7.4	12.0	DGME	3.3
19	1.5	164.9 / 14.8	4.0	IPA	5.1
20	3.5	164.9 / 14.8	12.0	IPA	4.7
21	2.5	123.6 / 11.1	8.0	DGME	5.3
22	2.5	123.6 / 11.1	8.0	IPA	5.0

[0095] Statistical analysis of the data from these tables suggested that the type of acid has the greatest impact on efficacy followed by, in order, the pH (lower being better), type of solvent, and effective solute concentration. The amount of electrolyte oxidizing agent appears to have a lesser effect.

Examples 23-31

[0096] Using Example 22 as a center point (rerun below as Example 23), additional quantitative carrier tests were conducted on another round of prepared compositions in which

the targeted pH (2.5), effective solute concentration (~6.4 Osm/L) and amount of PPOMS (8 g/L) were held constant. The anionic surfactant was SDS, while the nonionic surfactant was a polysorbate. The organic liquid for each was a 70% (v/v) IPA solution.

Table 9

Example	Org. liquid (g/L)	H₂O₂ soln. (g/L)	Anionic surf. (g/L)	Nonionic surf. (g/L)	Log reduction
23	250	250	17.5	2.0	4.6
24	200	200	15.0	1.5	4.6
25	400	300	15.0	1.5	4.7
26	200	300	20.0	1.5	4.6
27	400	200	20.0	1.5	4.6
28	200	300	15.0	2.5	4.7
29	400	200	15.0	2.5	4.6
30	200	200	20.0	2.5	4.5
31	400	300	20.0	2.5	4.7

[0097] Analysis of the data for Examples 11-31 indicated that pH and type of acid had the greatest impact, followed by effective solute concentration and type of solvent.

Examples 32-40

[0098] Additional quantitative carrier testing was performed on compositions in which the pH (2.5) and effective solute concentration (~6.4 Osm/L) were held constant. This set varied the amount of electrolyte oxidizing agent (PPOMS), the amount and type of solvent (with E representing absolute ethanol), the amount of hydrogen peroxide solution, and the amounts of anionic (SDS) and nonionic (polysorbate-type) surfactants.

Table 10

Example	Org. liquid (g/L)	H ₂ O ₂ soln. (g/L)	PPOMS (g/L)	Anionic surf. (g/L)	Nonionic surf. (g/L)	Log reduction
32	E, 250	200	12	15.0	1.5	4.7
33	E, 250	300	20	15.0	1.5	4.7
34	DGME, 250	300	12	20.0	1.5	4.5
35	DGME, 250	200	20	20.0	1.5	3.9
36	E, 350	300	12	15.0	2.5	3.8
37	E, 350	200	20	15.0	2.5	4.5
38	DGME, 350	200	12	20.0	2.5	4.6
39	DGME, 350	300	20	20.0	2.5	4.7
40	IPA, 300	250	16	17.5	2.0	4.7

[0099] Analysis of this data suggests that, when type and amount of acid is held constant, the most statistically significant factors might be two-way combinations type of solvent, solvent concentration, and amount of electrolyte oxidizing agent.

CLAIMS

That which is claimed is:

1. A method for treating a surface susceptible to the presence of one or more types of endospores, said method comprising contacting said surface with an acidic sporicidal composition having a pH of no less than 1.5 and an effective solute concentration of from 1.5 to 9 Osm/L, said composition comprising (1) a solvent component that comprises water and (2) a solute component that comprises dissociation products of at least one oxidizing acid and one or more electrolyte oxidizing agents.
2. The method of claim 1 wherein said solvent component of said composition further comprises an organic liquid having a δ_p value less than 16.0 MPa^{1/2}.
3. The method of claim 2 wherein said organic liquid is a glycol ether.
4. The method of any of claims 1 to 3 wherein said solute component of said composition further comprises anionic surfactant.
5. The method of claim 4 wherein said solute component of said composition further comprises nonionic surfactant.
6. The method of claim 4 wherein said solute component of said composition further comprises dissociation product of one or more non-oxidizing electrolytes.
7. The method of any of claims 1 to 3 wherein said solute component of said composition further comprises dissociation product of one or more non-oxidizing electrolytes.
8. The method of claim 7 wherein said one or more non-oxidizing electrolytes comprises at least one salt of an organic acid.
9. The method of claim 7 wherein said composition has an effective solute concentration of at least 2 Osm/L.
10. The method of claim 7 wherein said composition has an effective solute concentration of at least 3 Osm/L.
11. The method of any of claims 1 to 3 wherein said composition has a pH of no more than 4.

12. The method of any of claims 1 to 3 wherein said solvent component of said composition has a δ_p value of from 13.5 to 15.5 MPa^{1/2}.
13. The method of any of claims 1 to 3 wherein said contacting involves applying said composition to an inanimate hard surface for a contact time of no more than 1200 seconds.
14. The method of claim 13 wherein said contact time is no more than 600 seconds.
15. The method of any of claims 1 to 3 wherein said contacting involves immersing said surface in said composition for a contact time of no more than 14,400 seconds.
16. The method of claim 13 wherein said contact time is no more than 1800 seconds.
17. A sporicidal composition having a pH of from 1.5 to 4, inclusive, and an effective solute concentration of from 1.5 to 9 Osm/L, inclusive, said composition comprising, on a per liter basis:
 - a) a solvent component having a δ_p value less than 15.6 MPa^{1/2} that comprises
 - 1) 50-500 mL water and
 - 2) at least one organic liquid,
 - b) a solute component that comprises
 - 1) dissociation product of an oxidizing acid having a pK_a value of greater than 3 and a standard potential of at least +0.5 V,
 - 2) dissociation product of from 4 to 20 g of an electrolyte oxidizing agent having a standard potential of at least +1.5 V,
 - 3) dissociation product of at least one non-oxidizing electrolyte, and
 - 4) optionally, wetting agent.
18. The sporicidal composition of claim 17 wherein said solute component comprises wetting agent which comprises anionic surfactant and, optionally, nonionic surfactant.
19. The sporicidal composition of any of claims 17 to 18 wherein said oxidizing acid is the reaction product of an organic acid and a peroxide.
20. The sporicidal composition of any of claims 17 to 18 having a pH of no more than 3 and an effective solute concentration of at least 2 Osm/L.



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(54) Title: ANTIMICROBIAL COMPOSITION HAVING EFFICACY AGAINST ENDOSPORES

(57) Abstract: A sporicidal composition has a moderately low pH and includes at least one oxidizing acid and the dissociation product of at least one inorganic oxidizing agent. Very high effective solute concentrations can enhance the efficacy of the composition. Embodiments of the composition can be applied to a surface and allowed to absorb into the endospore, ultimately killing at least some of those bacteria in mature endospore form. The surface being treated can be an inanimate surface, particularly a hard surface, or a medical device.



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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/042780**A. CLASSIFICATION OF SUBJECT MATTER****A01N 59/00(2006.01)i, A01N 25/04(2006.01)i, A61L 2/18(2006.01)i, A01N 25/30(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01N 59/00; A61K 31/11; A01N 41/04; A01N 33/12; A61K 36/53; A61K 33/40; A61K 31/045; A61K 47/00; C09D 5/16; A01N 25/04; A61L 2/18; A01N 25/30

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & keywords: endospore, acidic sporidial composition, pH, oxidizing acid, electrolyte oxidizing agent, non-oxidizing electrolyte

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013-0272922 A1 (MICROBIAL DEFENSE SYSTEMS, LLC) 17 October 2013 See paragraphs [0008], [0035], [0039], [0044], [0049]; claims 1, 9.	1-20
Y	US 6656919 B1 (BAUGH et al.) 02 December 2003 See column 10, line 60 - column 11, line 10; claim 12.	1-20
Y	US 2006-0134239 A1 (WEIDE et al.) 22 June 2006 See abstract; paragraphs [0038], [0222].	1-20
Y	WO 2014-179754 A2 (NEXT SCIENCE, LLC) 06 November 2014 See paragraphs [0010], [0056]-[0057]; claim 1; table 1.	2-3, 12, 17-20
A	US 9034390 B2 (KIELBANIA, JR.) 19 May 2015 See abstract; claims 1-2, 5.	1-20

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

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

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published 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; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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权利要求书2页 说明书17页

(54)发明名称

具有抗内生孢子功效的抗微生物组合物

(57)摘要

一种杀孢子组合物具有适度低的pH并且包含至少一种氧化酸和至少一种无机氧化剂的离解产物。非常高的有效溶质浓度可以提高组合物的功效。可以将组合物的实施方案施用于表面并允许吸收到内生孢子中,最终杀死成熟内生孢子形式的那些细菌中的至少一些。经处理的表面可以是无生命的表面,尤其是硬表面或医疗设备。

1. 一种用于处理易于存在一种或多种类型内生孢子的表面的方法,所述方法包括使所述表面与酸性杀孢子组合物接触,所述酸性杀孢子组合物具有不小于1.5的pH和1.5至90sm/L的有效溶质浓度,所述组合物包含(1)包含水的溶剂组分和(2)包含至少一种氧化酸和一种或多种电解质氧化剂的离解产物的溶质组分。

2. 根据权利要求1所述的方法,其中,所述组合物的所述溶剂组分还包含具有小于 $16.0\text{MPa}^{1/2}$ 的 δ_p 值的有机液体。

3. 根据权利要求2所述的方法,其中,所述有机液体是二醇醚。

4. 根据权利要求1至3中的任一项所述的方法,其中,所述组合物的所述溶质组分还包含阴离子型表面活性剂。

5. 根据权利要求4所述的方法,其中,所述组合物的所述溶质组分还包含非离子型表面活性剂。

6. 根据权利要求4所述的方法,其中,所述组合物的所述溶质组分还包含一种或多种非氧化电解质的离解产物。

7. 根据权利要求1至3中的任一项所述的方法,其中,所述组合物的所述溶质组分还包含一种或多种非氧化电解质的离解产物。

8. 根据权利要求7所述的方法,其中,所述一种或多种非氧化电解质包含至少一种有机酸的盐。

9. 根据权利要求7所述的方法,其中,所述组合物具有至少20sm/L的有效溶质浓度。

10. 根据权利要求7所述的方法,其中,所述组合物具有至少30sm/L的有效溶质浓度。

11. 根据权利要求1至3中的任一项所述的方法,其中,所述组合物具有不超过4的pH。

12. 根据权利要求1至3中的任一项所述的方法,其中,所述组合物的所述溶剂组分具有 13.5 至 $15.5\text{MPa}^{1/2}$ 的 δ_p 值。

13. 根据权利要求1至3中的任一项所述的方法,其中,所述接触包括将所述组合物施用于无生命的硬表面,接触时间不超过1200秒。

14. 根据权利要求13所述的方法,其中,所述接触时间不超过600秒。

15. 根据权利要求1至3中的任一项所述的方法,其中,所述接触包括将所述表面浸入所述组合物中,接触时间不超过14,400秒。

16. 根据权利要求13所述的方法,其中,所述接触时间不超过1800秒。

17. 一种杀孢子组合物,具有包括端值的1.5至4的pH值,以及包括端值的1.5至90sm/L的有效溶质浓度,以每升为基础,所述组合物包含:

a) 具有小于 $15.6\text{MPa}^{1/2}$ 的 δ_p 值的溶剂组分,所述溶剂组分包含

1) 50-500mL水以及

2) 至少一种有机液体,

b) 溶质组分,所述溶质组分包含

1) pKa值大于3且标准电位为至少+0.5V的氧化酸的离解产物,

2) 具有至少+1.5V的标准电位的4至20g电解质氧化剂的离解产物,

3) 至少一种非氧化电解质的离解产物,以及

4) 任选的润湿剂。

18. 根据权利要求17所述的杀孢子组合物,其中,所述溶质组分包含润湿剂,所述润湿

剂包含阴离子型表面活性剂和任选的非离子型表面活性剂。

19. 根据权利要求17至18中的任一项的杀孢子组合物, 其中, 所述氧化酸是有机酸和过氧化物的反应产物。

20. 根据权利要求17至18中的任一项所述的杀孢子组合物, 具有不超过3的pH和至少20sm/L的有效溶质浓度。

具有抗内生孢子功效的抗微生物组合物

[0001] 相关申请的交叉引用

[0002] 本国际申请要求分别于2015年7月17日和2015年7月18日提交的美国临时专利申请No.62/194,141和No.62/194,210的权益,其公开内容通过引用并入本文。

背景技术

[0003] 不同于真孢子,内生孢子(endospore,芽孢)不是另一活体的后代。尽管如此,术语“孢子”和“内生孢子”在本文中能互换使用。

[0004] 营养细菌(vegetative bacterium)是可以生长、饲养和繁殖的细菌。当营养物质变得稀少时,某些营养细菌开始被称为“孢子形成”的过程,其中它们呈现一种减少的休眠形式,允许它们在没有营养的情况下生存,并使它们抵抗紫外线辐射、干燥、高温、极端严寒和化学消毒剂。在环境条件恢复到有利于营养生长之后不久,这种细菌可以退出它们的休眠状态(“孢子萌发”),孢子核复水,皮质水解,孢壳脱落,并最终开始DNA复制。有关这些复杂过程的更多信息,感兴趣的读者可以参考各种文本中的任一个,诸如例如J.C.Pommerville,Fundamentals of Microbiology,10th ed.(第10版)(Jones&Bartlett Learning;Burlington,Massachusetts(马萨诸塞州伯灵顿))。

[0005] 在孢子形成步骤开始时,称为“母细胞”的营养细菌制造其DNA的副本,然后在DNA的新副本周围形成质膜,其中具有复制的DNA的细胞部分完全被称为“前孢子”的质膜包围。孢子形成的下一个形态阶段是通过其母细胞“吞噬”前孢子,类似于吞噬作用的过程;当吞噬完成时,前孢子完全被其内膜和外膜所包围,并在母细胞细胞质中游离。在这个孢子核周围,组装了一系列保护性结构,其完成导致了一个成熟的内生孢子,该成熟的内生孢子在母细胞裂解后释放。

[0006] 内生孢子具有多层结构,其全部保护核仁。核仁被在表1中按从内到外的顺序列出的多层保护:

[0007] 表1

	层	描述
	(1) 核心	包含 RNA、DNA、吡啶二羧酸、低分子量碱性蛋白质以及各种矿物质(诸如 Ca、K、Mn 和 P)的原生质体
[0008]	(2) 皮质(外)膜	非交联的或轻度交联的含肽聚糖层,其在萌发期间发育成细胞壁
	(3) 皮质	肽聚糖和甘露糖醛酸残基
	(4) 孢子内壳	主要是酸性多肽
	(5) 孢子外壳	主要是蛋白质与一些碳水化合物和脂质以及大量的磷(在艰难梭菌(<i>C. difficile</i>)的情况下)

[0009] 孢子内壳能溶于碱性溶液,但孢子外壳耐碱性水解,可能主要是由于其众多的二硫(-S-S-)键。

[0010] 孢子极其难以根除,并且涉及诸如艰难梭菌(*C.diff*)感染和炭疽等疾病的传播。

[0011] 艰难梭菌是在医疗保健设施中常见的革兰氏阳性产孢细菌,并且是抗生素相关性腹泻的成因。艰难梭菌感染是日益严重的问题,每年感染成千上万的人,使相当大一部分受感染的人死亡。艰难梭菌孢子对大多数常规表面清洁方法具有抗性,在环境中长时间保持存活。

[0012] 炭疽是一种由炭疽杆菌(*B.anthraxis*)细菌引起的通常是致命的急性疾病,其影响人和动物,其孢子可以在体外产生并用作生物武器。炭疽不是直接从一个被感染的动物或人传播到另一个,而是通过孢子传播。

[0013] 孢子抑制化合物不是杀孢子的,即它们不杀死孢子;相反,它们抑制孢子的萌发或使萌发的孢子生长异常。孢子可以在暴露于这些化合物的情况下存活,并且然后在孢子抑制化合物不再存在之后生长。孢子抑制化合物包括酚和甲酚、有机酸和酯、醇、季铵化合物、双胍以及有机汞化合物。这些孢子抑制化合物中的某些在高浓度下可以是少量地杀孢子的;对于实现任何杀孢子功效所需的某些孢子抑制化合物的相对浓度,参见例如A.D.Russell的“Bacterial Spores and Chemical Sporicidal Agents”,*Clinical Microbiology Reviews*,第99-119页(1990))。

[0014] 通常使用的孢子处理选择包括醛,尤其是戊二醛和甲醛;释氯剂,包括 Cl_2 、次氯酸钠、次氯酸钙和氯还原剂诸如二氯异氰尿酸酯;碘和碘伏;过氧化物,包括过氧化氢和过乙酸;气体,诸如环氧乙烷、氧化丙烯和臭氧;以及 β -丙内酯。尽管它们对抗孢子的活性机制不是特别好理解,但是在所有情况下,活性受到一种或多种活性分子穿过孢子的保护层渗透的速率限制。这种穿透不同保护层的需要意味着孢子必须长时间暴露在高浓度的这些产物下。

[0015] 美国专利No.8,940,792和No.9,314,017以及美国公布号2010/0086576、2013/0272922、2013/0079407和2016/0073628描述了抗微生物组合物及其各种用途。孢子的核心和皮质(包括皮质膜)易于通过这些文献中描述的这类高渗透压摩尔浓度(osmolarity)组合物分解和溶解。然而,并未发现这些组合物对抗孢子特别有效,可能是由于分解并且然后穿透孢子内壳和孢子外壳的能力有限。

[0016] 仍然需要的是能够穿透内生孢子的各种防御并杀死细菌的组合物。这种组合物优选地对抗细菌诸如艰难梭菌和炭疽杆菌的内生孢子是有效的,同时对处理或接触它的人没有毒性问题。

发明内容

[0017] 一方面提供了一种组合物,其可以杀死成熟的内生孢子形式的形成内生孢子的多种细菌。该组合物的实施方案对抗内生孢子形式的细菌的各种防御是有效的,具体而言,可以断开二硫键,可以克服内在疏水性,可以破坏肽聚糖,并且可以溶解核心和皮质膜。

[0018] 该组合物是酸性的,但具有 $\text{pH} \geq 1.5$,并且包括溶剂组分和溶质组分,后者包括至少一种氧化酸和至少一种电解质氧化剂的离解产物。在某些实施方案中,该组合物还可以包括有机液体、润湿剂(尤其是离子型表面活性剂)和任何各种非氧化电解质中的一种或多

种。有利地,该组合物不需要包含杀孢子的活性抗微生物剂。

[0019] 该组合物具有至少1.00sm/L,典型地至少1.50sm/L且通常甚至更高的直到溶质组分在溶剂组分中的溶解度极限的有效溶质浓度。

[0020] 还提供了一种处理表面的方法。该方法涉及将前述组合物的实施方案施用(apply)于表面,并允许组合物被吸收到内生孢子中并杀死成熟内生孢子形式的那些细菌中的至少一些。经处理的表面可以是无生命的表面,尤其是硬表面,并且有利地是医疗保健设施的硬表面。

[0021] 当与诸如ASTM标准E2197-11和A0AC官方方法966.04的测试结合使用时,组合物的实施方案可以在商业相关的时间框架内提供正面的(合格的)结果。例如,(无生命的)硬表面的保洁用品类消毒处理可以在不到20分钟的时间内生效,而医学仪器的杀菌处理可以在不到4个小时的时间内生效。

[0022] 对于普通技术人员来说,本发明的其他方面将从下面的详细描述显而易见。为了帮助理解描述,下面立即将提供一些定义,并且这些定义旨在适用于全文,除非上下文明确指出相反的意图:

[0023] “包含(comprising)”是指包括但不限于列出的成分或步骤;

[0024] “由.....组成”是指仅包括列出的成分(或步骤)和少量的非活性添加剂或助剂;

[0025] “基本上由.....组成”是指仅包括列出的成分(或步骤),鉴于所需最终用途补充杀孢子活性和/或提供期望的次要效果(例如防雾、去污等)的少量(少于5%、4%、3%、2%、1%、0.5%、0.25%或0.1%)的其他成分,和/或非活性添加剂或助剂;

[0026] “多元酸”是指具有至少两个羧基的化合物,尤其包括二羧酸、三羧酸等;

[0027] “pH”是指由能接受的可靠测量方法,诸如正确校准的pH计,根据已知标准溶液的滴定曲线或类似方法测定的[H⁺]的以10为底的对数的负值;

[0028] “pK_a”是指特定化合物的酸离解常数的以10为底的对数的负值;

[0029] “E⁰_{red}”是指在25°C水中还原半反应的标准电压;

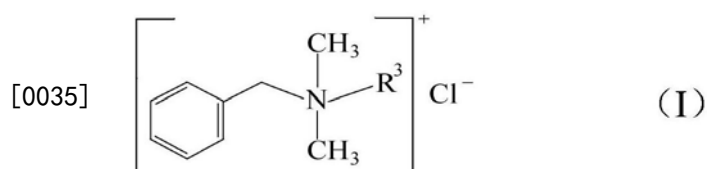
[0030] “缓冲剂”是指具有将其加入其中的溶液的pH保持在相对窄的范围内的能力的化合物或化合物的混合物;

[0031] “缓冲剂前体”是指当加入到含有酸的混合物中时产生缓冲剂的化合物;

[0032] “电解质”是指加入水中时表现出一些离解的化合物;

[0033] “非氧化电解质”是指除可以用作氧化剂的电解质以外的电解质;

[0034] “苯扎氯铵”是指由以下通式限定的任何化合物



[0036] 其中R³是C₈-C₁₈烷基,或这种化合物的任何混合物;

[0037] “有效溶质浓度”是由给定体积溶液中存在的分子(来自非电解质)或离子(来自电解质)的摩尔数产生的依数性质(colligative property)的量度,通常以渗透压摩尔每升为单位表示;

[0038] “δ_p”是偶极分子间力汉森溶解度参数(Hansen Solubility Parameter) (HSP),其

中溶剂的混合物或溶液的值通过下式计算：

$$[0039] \quad \delta_p = \sum_{i=1}^n (\delta_{di} \times x_{di}) \quad (I)$$

[0040] 其中 δ_{di} 是来自溶剂组分*i*的偶极分子间力的能量， x_{di} 是溶剂组分*i*相对于溶剂组分总量的百分比，并且*n*是溶剂组分的总数；

[0041] “含氧酸”是指含氧的无机酸；

[0042] “取代的”是指含有不干扰讨论中的基团的预期目的的杂原子或官能团(例如烃基基团)；

[0043] “微菌(microbe)”是指任何类型的微生物(microorganism)，包括但不限于细菌、病毒、真菌、类病毒、朊病毒等；

[0044] “抗微生物剂”是指具有能够使一种或多种微菌的数量减少大于90% (1个对数值)的物质；

[0045] “活性抗微生物剂”是指仅在或主要在微菌的生命周期的活性片段(例如细胞分裂)有效的抗微生物剂；

[0046] “杀菌剂”是指对一种或多种类型有害微生物致死的物质；

[0047] “消毒剂”是指对一种或多种类型细菌致死的物质；

[0048] “高水平消毒剂”是指能杀死除内生孢子形式的少量细菌以外的所有细菌的消毒剂；

[0049] “灭菌剂”是指能够消除所有微菌(不管形式如何)的至少6个对数值(99.9999%)减少的物质；

[0050] “接触时间”是指允许组合物接触表面和/或这种表面上的内生孢子的时间量；

[0051] “硬表面”是指不渗透流体并且在大多数情况下不能变形的任何表面；以及

[0052] “医疗保健”是指涉及维持或恢复身心健康或与之有关。

[0053] 贯穿本文件，除非上下文明确地指示相反的意图，否则以百分比形式给出的所有值均是w/v，即每升组合物的溶质克数。任何具体引用的专利和/或公开的专利申请的一个或多个相关部分均通过引用并入本文。

具体实施方式

[0054] 首先描述杀孢子组合物的性质和组分(许多组分是广泛可获得的并且相对便宜的)，然后描述某些用途。

[0055] 该组合物的溶剂组分通常包含大量的水。相对于其总体积，组合物通常包含最高达20%、25%、30%、33%、35%、40%、45%、50%或甚至55% (均为v/v)。以每升为基础，组合物通常包含~50至~500mL，通常~75至~475mL，更通常地~100至~450mL，经常~125至~425mL，典型地~150至~400mL，最典型地250±50mL的水。不需要对水进行特殊处理(例如蒸馏和/或去离子)，但是对于不干扰组合物的所需抗微生物作用的水当然是优选的。

[0056] 组合物的溶剂组分通常包含至少一种有机液体，并且在一些实施方案中， δ_p 值低于水($\delta_p \approx 16.0 \text{ MPa}^{1/2}$)的那些有机液体是优选的。在溶剂组分中存在至少一种有机液体的情况下，总溶剂组分的 δ_p 值通常小于16.0，不大于15.6，不大于15.2，不大于15.0，不大于

14.6或不大于14.0MPa^{1/2}。在一些实施方案中,总溶剂组分的 δ_p 值可以在13.1至15.7MPa^{1/2},13.3至15.6MPa^{1/2},13.5至15.5MPa^{1/2},甚至13.7至15.4MPa^{1/2}的范围内。

[0057] 有机液体通常以最高达60%,通常5至50%,更通常地10至45%,甚至更通常地15至40%,以及典型地20至35%的浓度(均为w/v,基于溶剂组分的总体积)存在。

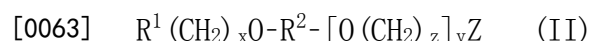
[0058] 如果目标 δ_p 值是已知的,则可以使用公式(I)计算待添加到水中的给定有机液体(或有机液体的混合物)的量。类似地,如果一种或多种有机液体的量及其各自的 δ_p 值是已知的,则可以使用公式(I)计算预计的 δ_p 值。

[0059] 溶剂组分可以仅由水或仅由一种或多种有机液体组成,或者基本上仅由水或仅由一种或多种有机液体组成,优选地是相同属性的有机液体的物质的混合物,例如两种醚或两种醇,而不是一种醚和一种醇。在某些优选的实施方案中,溶剂组分可以由水和有机液体组成,或者基本上由水和有机液体组成,溶剂组分优选地具有小于15.5MPa^{1/2}的 δ_p 值。在其他实施方案中,溶剂组分可以由水和两种或更多种有机液体组成,或者基本上由水和两种或更多种有机液体组成,所得的溶剂组分具有可以使用式(I)计算的 δ_p 值;相同属性的有机液体的物质的混合物也是优选的,例如两种醚或两种醇,而不是一种醚和一种醇。

[0060] 关于可以在溶剂组分中使用的有机液体,优选地是能彼此混溶和/或与水混溶的那些有机液体。潜在有用的有机液体的非限制性示例包括酮类,诸如丙酮、甲基丁基甲酮、甲基乙基酮和氯丙酮;乙酸酯,诸如乙酸戊酯、乙酸乙酯和乙酸甲酯;(甲基)丙烯酸酯和衍生物,诸如丙烯酰胺、甲基丙烯酸月桂酯和丙烯腈;芳基化合物,诸如苯、氯苯、氟苯、甲苯、二甲苯、苯胺和苯酚;脂族烷烃,诸如戊烷、异戊烷、己烷、庚烷和癸烷;卤代烷烃,诸如氯仿、二氯甲烷、氯乙烷和四氯乙烯;环烷烃,诸如环戊烷和环己烷;以及多元醇,诸如乙二醇、二甘醇、丙二醇、己二醇和甘油。当选择这些有机液体用于组合物的溶剂组分时,可能的考虑包括避免含有与组合物中使用的一种或多种酸和任选的一种或多种盐反应的官能团的那些有机液体,并且倾向于具有更高的前置审批监管限制的那些有机液体。

[0061] 优选的有机液体包括醚和醇,因为它们具有低组织毒性和环境友好性。这些可以以最高达到组合物中其他成分的溶解度极限的浓度加入。

[0062] 可以在溶剂组分中使用的醚基液体包括由以下通式定义的那些



[0064] 其中x是0至20的整数(任选地包括一个或多个烯属不饱和点,其中 $2 \leq x \leq 20$),y是0或1,z是1至4的整数, R^2 是C₁-C₆直链或支链亚烷基, R^1 是甲基、异丙基或苯基,Z是羟基或甲氧基。可以在溶剂组分中使用的二醇醚(式(II)化合物,其中Z为OH)的非限制性示例在下表2中列出。

[0065] 表2:代表性二醇醚、式(II)变量和 δ_p 值

[0066]

	R ¹	x	R ²	y	z	$\sim\delta_p$ (MPa ^{1/2})
乙二醇单甲醚	CH ₃	0	(CH ₂) ₂	0	-	9.2
乙二醇单乙醚	CH ₃	1	(CH ₂) ₂	0	-	9.2
乙二醇单丙醚	CH ₃	2	(CH ₂) ₂	0	-	8.2
乙二醇单异丙醚	(CH ₃) ₂ CH	0	(CH ₂) ₂	0	-	8.2
乙二醇单丁醚	CH ₃	3	(CH ₂) ₂	0	-	5.1
乙二醇单苯醚	C ₆ H ₅	0	(CH ₂) ₂	0	-	5.7
乙二醇单苄醚	C ₆ H ₅	1	(CH ₂) ₂	0	-	5.9
二甘醇单甲醚	CH ₃	0	(CH ₂) ₂	1	2	7.8
二甘醇单乙醚 (DGME)	CH ₃	1	(CH ₂) ₂	1	2	9.2
二甘醇单正丁基醚	CH ₃	3	(CH ₂) ₂	1	2	7.0
丙二醇单丁醚	CH ₃	3	(CH ₂) ₃	0	-	4.5
丙二醇单乙醚	CH ₃	1	(CH ₂) ₃	0	-	6.5
丙二醇单异丁基醚	(CH ₃) ₂ CH	1	(CH ₂) ₃	0	-	4.7
丙二醇单异丙基醚	(CH ₃) ₂ CH	0	(CH ₂) ₃	0	-	6.1
丙二醇单甲醚	CH ₃	0	CH ₂ CH (CH ₃)	0	-	6.3
丙二醇单苯醚	C ₆ H ₅	0	CH ₂ CH (CH ₃)	0	-	5.3
丙二醇单丙醚 (PGME)	CH ₃	2	CH ₂ CH (CH ₃)	0	-	5.6
三甘醇单甲醚	CH ₃	0	(CH ₂) ₂	2	2	7.6
三甘醇单油基醚	CH ₃	17*	(CH ₂) ₂	2	2	3.1

[0067] *包括9位处的不饱和点

[0068] 环状和C₁-C₁₆无环(直链和支链,饱和的和饱和的)醇,可选地包括一个或多个烯属不饱和点和/或除醇氧之外的一个或多个杂原子,诸如卤素原子、胺氮等,可以用作组合物的溶剂组分中的有机液体。代表性示例的非限制性示例汇编于下表中。

[0069] 表3:代表性醇以及 δ_p 值

[0070]		$\sim\delta_p \text{MPa}^{1/2}$
	2-丙烯醇	10.8
	1-丁醇	5.7
	叔丁醇	5.1
	4-氯苄醇	7.5
	环己醇	4.1
	2-环戊烯醇	7.6
	1-癸醇	10.0
	2-癸醇	10.0
	2,3-二氯丙醇	9.2
	2-乙基-1-丁醇	4.3
	乙醇	8.8
	2-乙基己醇	3.3
	异辛醇	7.3
	辛醇	3.3
	甲醇	12.3
	油醇	2.6
	1-戊醇	4.5
	2-戊醇	6.4
	1-丙醇	6.8
	2-丙醇 (IPA)	6.1

[0071] 关于含有机液体的溶剂组分的进一步信息,感兴趣的读者可以参考美国专利公布号为2016/0073628。

[0072] 该组合物是酸性的,更具体地具有不超过4的pH,并且某些实施方案可以具有不超过3.8、3.7、3.6、3.5、3.4、3.3、3.2、3.1、3.0、2.9、2.8、2.7、2.6、2.5、2.4、2.3或者甚至2.2的pH。该组合物具有至少1.5,通常至少1.75,并且典型地至少2.0的pH。可以设想使用每个下限与每个上限结合的pH值的范围。组合物的实施方案可以具有 2.75 ± 1.15 、 2.70 ± 1.05 、 2.65 ± 1.0 、 2.60 ± 0.75 、 2.55 ± 0.60 、 2.50 ± 0.55 和 2.45 ± 0.45 的pH值。

[0073] 酸性可以通过向溶剂组分(或反之亦然)中添加一种或多种酸来实现。可以使用强(无机)酸,诸如 HCl 、 H_2SO_4 、 H_3PO_4 、 HNO_3 、 H_3BO_3 等或有机酸,尤其是有机多元酸。有机酸的示例包括一元酸,诸如甲酸、乙酸和取代的变体(例如羟基乙酸、氯乙酸、二氯乙酸、苯乙酸等)、丙酸和取代的变体(例如乳酸、丙酮酸等)、各种苯甲酸(例如扁桃酸、氯扁桃酸、水杨酸等)中的任何一种、葡糖醛酸等;二元酸,诸如草酸和取代的变体(例如草氨酸)、丁二酸和取代的变体(例如苹果酸、天冬氨酸、酒石酸、柠草酸等)、戊二酸和取代的变体(例如谷氨酸、2-酮戊二酸等)、己二酸和取代的变体(例如粘酸)、丁烯二酸(顺式和反式异构体)、亚氨基二乙酸、邻苯二甲酸等;三元酸,诸如柠檬酸、2-甲基丙烷-1,2,3-三羧酸、苯三羧酸、次氨基三乙酸等;四元酸,诸如连苯四酸、均苯四甲酸等;以及甚至更高阶的酸(例如,五元、六元、七元等)。在使用三元、四元或更高酸的情况下,一个或多个羧基质子可以被阳离子原子或基

团(例如碱金属离子)替代,阳离子原子或基团可以相同或不同。

[0074] 由于内生孢子中存在的各层产生的一些防御的性质,组合物必须包含至少一种氧化酸。许多含氧酸,诸如高氯酸、氯酸、亚氯酸、次氯酸、过硫酸、硫酸、亚硫酸、次硫酸(hyposulfurous)、焦硫酸(pyrosulfuric)、重硫酸(disulfurous),硫代亚硫酸、高硝酸(pernitric)、硝酸、亚硝酸、连二次硝酸(hyponitrous)、过铬酸、铬酸、重铬酸、高锰酸、锰酸、过磷酸、磷酸、亚磷酸、次磷酸、高碘酸、碘酸、亚碘酸等均被认为是氧化酸。有机氧化酸包括但不限于过乙酸、过草酸和二过草酸。

[0075] 优选的氧化酸是具有相对较高的 pK_a 值(即不被认为是特别强的酸)和正标准电位(E_{red}^0)的那些酸。前者允许产生具有不太低的pH值,即低于 ~ 1.5 ,优选地不低于 ~ 1.75 ,更优选地不低于 ~ 2 ,最优选地不低于 ~ 2.2 的组合物,使得组合物可以在没有极端保护措施的情况下被负责处理并将其施用于表面和/或销毁待处理物品的部件的那些人使用。正标准电位允许酸具有足够的氧化能力,以允许克服或消除某些内生孢子防御,诸如例如,二硫键和蛋白质聚合物在内生孢子壳上的氧化,其允许破坏孢子外壳。

[0076] 优选的 pK_a 值大于 ~ 1 ,大于 ~ 1.5 ,大于 ~ 2 ,大于 ~ 2.5 ,大于 ~ 3 ,大于 ~ 3.5 ,大于 ~ 4 ,大于 ~ 4.5 ,大于 ~ 5 ,并且甚至大于 ~ 5.5 。如果采取其他步骤以确保符合组合物的所需或所期望的性质,诸如pH范围(上面讨论的)和有效溶质浓度(下面讨论的),则可以使用具有较低 pK_a 值的酸。

[0077] 优选的 E_{red}^0 值是至少+0.20V,至少+0.25V,至少+0.33V,至少+0.40V,至少+0.50V,至少+0.60V,至少+0.67V,至少+0.75V,至少+0.80V,至少+0.90V,至少+1.00V,至少+1.10V,至少+1.20V或者甚至至少+1.25V的那些值。

[0078] 一些氧化酸在水溶液中不是特别稳定的。因此,提供具有体外制备的氧化酸的组合物可能是有利的。例如,在一个优选的实施方案中,可以向组合物的溶剂组分提供在接触时可逆地形成过乙酸的乙酸和过氧化氢。

[0079] 考虑到用于实现组合物中所期望的有效溶质浓度的一种或多种化合物(如果有的话)的类型和用量,可以从给定组合物的目标pH和所选择的酸的一个或多个 pK_a 值来确定所使用的任何给定的酸的用量。(更多关于渗透压摩尔浓度和渗透压摩尔浓度调整化合物的类型的讨论在下文呈现。)

[0080] 还存在于组合物的溶质组分中是电解质氧化剂,其在进行泽尔维季诺夫测定(Zerewitinoff determination)时不含任何活性氢原子。潜在有用的电解质(优选地无机氧化剂)的非限制性示例包括包含阴离子,诸如锰酸根、高锰酸根、过氧铬酸根、铬酸根、重铬酸根、过氧单硫酸根等的化合物。(这些电解质中的一些可能影响pH,因此配制成的具有给定pH的组合物可能需要在加入一种或多种氧化剂之后进行调整。)优选的是具有至少+1.25V,优选地至少+1.5V,更优选地至少+1.75V,甚至更优选地至少+2.0V,而且最优选地至少+2.25V的 E_{red}^0 值的那些化合物。

[0081] 电解质氧化剂通常可以以最高达其各自的溶解度极限加入,但是最大量通常为每升总组合物30g的数量级。一种或多种电解质氧化剂的示例性范围是 ~ 2 至 ~ 25 g/L, ~ 3 至 ~ 21 g/L, ~ 4 至 ~ 18 g/L, ~ 5 至 ~ 16 g/L以及 ~ 6 至 ~ 14 g/L。一种或多种电解质氧化剂的示例性用量为 17.5 ± 12 g/L、 15 ± 9 g/L、 12.5 ± 6 g/L和 10 ± 3 g/L。

[0082] 一旦将一种或多种酸和一种或多种氧化剂添加到含有水的溶剂组分中(反之亦

然),则各自至少部分地离解。

[0083] 仅包含溶剂组分以及由一种或多种氧化酸和一种或多种电解质氧化剂组成或基本上由一种或多种氧化酸和一种或多种电解质氧化剂组成的溶质组分可以具有抗内生孢子的功效,即可以导致一些或所有内生孢子不能恢复到营养状态。尽管如此,包括包含附加子组分的溶质组分的组合物在某些情况下可以具有增强的功效。

[0084] 在某些实施方案中,组合物的有效溶质浓度可以相对高。功效常常随着有效溶质浓度(渗透压摩尔浓度)的增加而增加。大量溶质的存在确保存在足够的量,以诱导跨皮质膜高渗透压力,导致溶解。

[0085] 这种功效与溶质组分的单个化合物的具体特性或性质无关,但是由于溶剂容量(即,(典型地)在给定量溶剂组分中包含比等摩尔量的较大分子更多的小分子的能力)和跨皮质膜的运输容易,较小的分子通常比较大的分子更有效。

[0086] 可以使用许多溶质中的任何溶质来增加组合物的渗透压摩尔浓度。

[0087] 实现组合物的增加的渗透压摩尔浓度的一个途径是通过加入大量的非氧化电解质,尤其是离子化合物(盐);参见,例如,美国专利No.7,090,882。像氧化酸和无机氧化剂一样,非氧化电解质在引入包含水的溶剂组分中时离解。

[0088] 在组合物中使用一种或多种有机酸的情况下,增加渗透压摩尔浓度而不使组合物的pH增加超过所期望的目标的另一个途径包括包含一种或多种酸的盐或一种或多种其他有机酸的盐。这种化合物在离解时增加组合物中溶质的有效量,而不显著影响水合氢离子的摩尔浓度,同时在组合物中提供缓冲体系。

[0089] 例如,在组合物包含酸的情况下,也可以包含份数最高达多倍(例如,3倍至10倍,至少5倍或甚至至少8倍)的该(或其他)酸的一种或多种盐。盐的抗衡离子部分的特性被认为不是特别重要的,常见的示例包括铵离子和碱金属。在使用多元酸的情况下,全部或少于羧基的全部H原子可以被相同的或不同的阳离子原子或基团替代。例如,柠檬酸一钠、柠檬酸二钠和柠檬酸三钠均构成潜在有用的缓冲剂前体,无论是与柠檬酸还是另外的有机酸一起使用。然而,由于柠檬酸三钠具有三个可用的碱性位点,所以其理论缓冲能力最高比柠檬酸二钠(其具有两个这种位点)高50%,并且最高比柠檬酸钠(其仅具有一个这种位点)高200%。

[0090] 无论如何实现,组合物的有效溶质浓度为至少1.00sm/L,通常至少1.250sm/L,通常至少1.50sm/L,通常至少1.750sm/L,更通常地至少2.00sm/L,典型地至少2.250sm/L,更典型地至少2.50sm/L。(作为比较点,在生物应用中,0.9%(以wt.计)的盐水溶液,其是~0.30sm/L,典型地被认为具有适中的张力,而3%(以wt.计)的盐水溶液,其是~0.90sm/L,通常被认为是高张的)。在一些实施方案中,组合物具有至少~3.0,至少~3.25,至少~3.5,至少~3.75,或者甚至至少~4.00sm/L的有效溶质浓度,上限由溶剂组分中溶质的溶解度极限限定;在一些实施方案中,有效溶质浓度的上限范围可以高达4.5、4.75、5.0、5.25、5.5、5.75、6.0、6.25、6.5、6.75、7.0、7.2、7.4、7.6、7.8、8.0、8.2、8.4、8.6、8.8或甚至~90sm/L。也可以设想包括本段中提出的任何下限和上限的组合的有效溶质浓度范围。旨在对内生孢子有效(即致死的)的根据本发明的组合物的有效溶质浓度通常高于美国专利No.8,940,792和No.9,314,017以及美国公布号2010/0086576、2013/0272922、2013/0079407和2016/0073628中所描述的那些,其全部通常针对浮游细菌和生物膜。

[0091] 可以使用已知技术来计算有效溶质浓度,或者如果期望的话,可以使用诸如沸点升高、凝固点降低、渗透压和降低蒸汽压的各种依数性能测量中的任何一种来测量。

[0092] 与前面段落中列举的文献中描述的许多组合物不同,本杀孢子组合物不要求在溶质组分中包含表面活性剂,但是某些优选的实施方案包括一种或多种润湿剂,其包括但不限于表面活性剂。

[0093] 基本上任何在水中具有表面活性的材料均可以使用,而不管在组合物的溶剂组分中是否存在水,但是期望那些带有某种类型的离子电荷的表面活性剂具有增强的抗微生物功效,因为这些电荷在与细菌接触时被认为导致更有效的细菌膜破裂,并最终导致细胞渗漏和溶解。

[0094] 极性表面活性剂通常比非极性表面活性剂更有效,离子型表面活性剂是最有效的。对于极性表面活性剂,阴离子型表面活性剂通常是最有效的,其次是两性离子型表面活性剂和阳离子型表面活性剂,较小的分子通常比较大的分子更优选。附连在极性头上的侧基的大小可能影响离子型表面活性剂的功效,在极性头上具有较大尺寸的基团和更多的侧基可能会降低表面活性剂的功效。

[0095] 潜在有用的阴离子型表面活性剂包括但不限于美国专利No.6,610,314中提出的十二烷基硫酸铵、磺基琥珀酸二辛酯钠、全氟丁磺酸、全氟壬酸、全氟辛烷磺酸、全氟辛酸、月桂基硫酸钾、十二烷基苯磺酸钠、月桂醇聚醚硫酸钠、月桂酰肌氨酸钠、肉豆蔻醇聚醚硫酸钠(sodium myreth sulfate)、豆蔻醇聚醚硫酸钠、烷醇聚醚硫酸钠(sodium pareth sulfate)、硬脂酸钠、鹅脱氧胆酸钠、N-月桂酰肌氨酸钠盐、十二烷基硫酸锂、1-辛烷磺酸钠盐、胆酸钠水合物、脱氧胆酸钠、十二烷基硫酸钠(SDS)、甘氨脱氧胆酸钠、月桂基硫酸钠(SLS)以及磷酸烷基酯。SLS是特别优选的材料。

[0096] 潜在有用的阳离子型表面活性剂包括但不限于氯化十六烷基吡啶鎓(CPC)、十六烷基三甲基氯化铵、苜蓿氯铵、5-溴-5-硝基-1,3-二噁烷、二甲基双十八烷基氯化铵、十六烷基三甲基溴化铵、双十八烷基二甲基溴化铵、十四烷基三甲基硼化铵、苯扎氯铵(BK)、氯化十六烷基吡啶鎓单水合物和十六烷基三甲基溴化铵。

[0097] 潜在有用的非离子型表面活性剂包括但不限于聚氧乙二醇十二烷基醚钠(sodium polyoxyethylene glycol dodecyl ether)、N-癸酰基-N-甲基葡萄糖胺、毛地黄皂苷(digitonin)、正十二烷基β-D-麦芽糖苷、辛基β-D-吡喃葡萄糖苷、辛基酚乙氧基化物、聚氧乙烯(8)异辛基苯基醚、聚氧乙烯山梨糖醇酐单月桂酸酯和聚氧乙烯(20)脱水山梨糖醇酐酰胺丙基二甲基氨基]-2-羟基-1-丙磺酸盐、3-[(3-胆酰胺丙基)二甲基氨基]-1-丙磺酸盐、3-(癸基二甲基氨基)丙烷磺酸内盐以及N-十二烷基-N,N-二甲基-3-氨基-1-丙磺酸盐。

[0098] 潜在有用的两性离子型表面活性剂包括磺酸盐(例如3-[(3-胆酰胺丙基)二甲基氨基]-1-丙磺酸盐)、磺基甜菜碱(例如椰油酰胺丙基羟基磺基甜菜碱)、甜菜碱(例如椰油酰胺丙基甜菜碱)以及磷酸酯(例如卵磷脂)。

[0099] 对于其他潜在有用的材料,感兴趣的读者可以参考多个其他来源中的任何一个,例如,美国专利No.4,107,328、6,953,772、7,959,943和8,940,792。

[0100] 待加入到组合物中的一种或多种润湿剂的量在一定程度上受到目标有效溶质浓度以及与组合物的溶质组分的其他子组分的相容性的限制。存在于组合物中的润湿剂的总量可以在从至少0.1%,至少0.25%,至少0.5%,至少0.75%或至少1%最高至5%,通常最

高至4%，更通常地是最高至3%，典型地最高至2.5%的范围内。有时，组合物中可能存在的用于特定最终用途(无特定测试、审查和批准)的某些类型的润湿剂(尤其是表面活性剂)的最大用量由政府法规设定。

[0101] 如果使用多于一种类型的表面活性剂，则大多数优选的是离子型表面活性剂，其中离子型与非离子型表面活性剂的比例范围通常为~2:1至~10:1，通常~5:2至~15:2，典型地~3:1至~7:1。此外，如本领域已知的，组合物不应包括不相容的表面活性剂类型，例如阴离子与阳离子或两性离子与阴离子或阳离子。

[0102] 抗微生物组合物可以包括各种添加剂和助剂，以使其更适合用于特定的最终用途应用，以实质方式对其功效产生负面影响。示例包括但不限于润肤剂、杀真菌剂、香精、颜料、染料、消泡剂、发泡剂、香料、研磨剂、漂白剂、防腐剂(例如抗氧化剂)等。美国食品和药物管理局批准的添加剂的综合列表可以以压缩文本形式在<http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm>(自本申请的申请日期起有效的链接)获得。

[0103] 该组合物的功效不要求为了功效包括活性抗微生物剂(上文定义的)，但是这些材料可以包括在某些实施方案中。潜在有用的活性抗微生物添加剂的非限制性示例包括C₂-C₈醇(除了任何用作溶剂组分的有机液体之外或另外)，诸如乙醇、正丙醇等；醛类，诸如戊二醛、甲醛和邻苯二甲醛；甲醛生成化合物，诸如羟甲基甲硫脲(noxythiolin)、牛磺酸、六胺、脲甲醚、咪唑啉酮衍生物等；苯胺类，尤其是三氯卡班；双胍类，诸如洗必泰和阿来西定以及聚合物形式诸如聚(六亚甲基双胍)；二羧酰亚胺(例如取代或未取代的丙烷脒)及其羟乙基磺酸盐；含卤原子或释放卤原子化合物，诸如漂白剂、ClO₂、二氯异氰尿酸盐、甲苯磺酰氯、碘(和碘伏)等；银和银化合物，诸如乙酸银、磺胺嘧啶银和硝酸银；苯酚、双酚和卤代酚(包括六氯酚和苯氧基苯酚，诸如三氯生)；以及季铵化合物。

[0104] 下表提供了根据本发明的示例性组合物的成分列表，其中用量以克为单位提供并加入蒸馏水以使成分达到1L的体积。

[0105] 表4:用于示例性组合物的制剂

[0106]		制剂 1	制剂 2
	有机酸的盐	5-25	10-20
[0107]	有机酸	125-200	140-180
	离子型表面活性剂	5-30	15-25
	非离子型表面活性剂	0-5	1-3
	H ₂ O ₂ (在H ₂ O中按重量为30%)	175-325	200-300
	无机氧化剂	4-20	6-12
	有机液体	175-450	225-375

[0108] 已经通过举例而非限制的方式提供了本发明的各种实施方案。从上述表格中显而易见的是，关于特征、范围、数值极限和实施方案的一般偏好，在可行的范围内，只要不干扰或不相容，就可以设想为能够与其他这种通常优选的特征、范围、数值极限和实施方案相结合。

[0109] 根据本发明的组合物旨在并且实际上是积极抗微生物的。其预期用途是与无生命

的物体,诸如尤其是硬表面,尤其是在医疗保健设施中常见的硬表面相关。

[0110] 该组合物可以通过分配装置(例如,拖把、抹布、刷子、织物擦拭物等)等以各种方式包括倾倒、喷施或雾化施用于无生命的物体,尤其是硬表面。

[0111] 或者,某些物体能够浸入组合物中。对于设计成用于多个患者的医疗设备尤其如此,诸如例如透析设备、各种内窥镜、十二指肠镜等中的任何一种、内窥镜配件(诸如抓紧器、剪刀等)、手动仪器(诸如夹钳和镊子),腹腔镜手术配件、整形外科和脊柱手术硬件(诸如夹钳和夹具等)。由于本发明的组合物比诸如过氧乙酸和漂白剂的处理剂具有更为适中的 $[H^+]$,其可以实现消毒、高水平消毒或者甚至灭菌,而不具有诸如例如聚合物降解、金属腐蚀、玻璃或塑料蚀刻等负面效果。

[0112] 一旦施用于表面或物体,组合物的各种成分就在存在的任何内生孢子上作用并消除或破坏其各种防御。组合物处理内生孢子(即,确保它们不能恢复到营养状态)所需的接触时间可以根据具体的组合物及其预期的最终用途而大幅度变化。

[0113] 例如,旨在施用于医疗保健设施的硬表面的组合物的实施方案可以在超过1200秒、不超过1050秒、不超过900秒、不超过840秒、不超过780秒、不超过720秒、不超过660秒或者甚至不超过600秒的接触时间之后实现至少3.0、3.2、3.4、3.6、3.8、4.0、4.2、4.4、4.6、4.8或5.0的对数减少。当根据ASTM E2197-11测试时,某些实施方案在600秒的接触时间之后可以实现至少4.5的对数减少。

[0114] 旨在用作医疗设备的浸泡浴的组合物的这些和/或其他实施方案可以在 $\sim 14,400$ 秒,最高达 $\sim 10,800$ 秒,最高达 ~ 7200 秒,最高达 ~ 5400 秒和 ~ 3600 秒的数量级的接触时间之后实现至少5.0、5.1、5.2、5.3、5.4、5.5、5.6、5.7、5.8、5.9或6.0的对数减少。当根据AOAC官方方法966.04进行测试时,某些实施方案可以在接触时间低至1800秒后达到合格分数。

[0115] 杀孢子组合物的实施方案可能能够被分类为高水平的消毒剂或者甚至分类为灭菌剂。

[0116] 在允许组合物接触给定的物体或表面适当的时间(鉴于诸如预期的细菌负载、可能存在的细菌的类型,物体/表面的重要性等因素)之后,其可以留待蒸发,或者优选地用水或稀盐溶液冲洗掉。

[0117] 以下非限制性的说明性实施例提供了可以用于实施本发明的详细条件和材料。贯穿这些实施例,提到的任何室温是指 $\sim 22^\circ\text{C}$ 。

[0118] 实施例

[0119] 实施例1(比较):过乙酸

[0120] 在可能或怀疑有内生孢子的情况下,广泛认可和推荐的消毒处理是将10% (w/v) 过乙酸施用到目标表面并接触10分钟。因此,过乙酸构成本发明的组合物的良好比较。

[0121] 通过将蒸馏水加入到含有过乙酸的烧瓶中来制备两种过乙酸溶液。其中一种溶液的浓度是5% (w/v),而另一种的浓度是10% (w/v)。这些溶液的所计算的有效溶质浓度分别为1.97和3.950sm/L。

[0122] 向50mL烧杯中加入20mL蒸馏水。将来自校准的温度补偿pH计的干净的、冲洗并干燥的探针放入烧杯中。然后将过乙酸溶液的连续等分试样加入到烧杯中,每次加入后进行pH读数。在室温下进行滴定。

[0123] 这些滴定的结果在下表5中示出。

[0124] 表5:过乙酸滴定

5% (w/v)		10% (w/v)	
酸用量 mL	pH	酸用量 mL	pH
0	5.91	0	5.31
0.25	2.42	0.1	2.74
0.50	2.17	0.2	2.48
0.75	2.00	0.3	2.33
1.00	1.89	0.4	2.22
1.25	1.80	0.5	2.14
1.50	1.74	0.6	2.07
1.75	1.68	0.7	2.00
2.00	1.59	0.8	1.95
2.25	1.56	0.9	1.91
2.50	1.53	1.0	1.86
2.75	1.48	1.1	1.83
3.00	1.45	1.2	1.79
3.25	1.42	1.3	1.76
3.50	1.41	1.4	1.73
3.75	1.38	1.5	1.70
4.00	1.34	1.6	1.67
4.25	1.30	1.7	1.65
4.50	1.28	1.8	1.62
4.75	1.26	1.9	1.60
5.00	1.25	2.0	1.58

[0126] 上述数据尤其表明,即使加入很小的等分试样(即以体积计小于1%)的任一种过乙酸溶液,水的pH也降低至低于3,并且仅加入1mL(以体积计5%)的任一种溶液都将pH降低到低于2。另外,任一种酸溶液的渐近pH都在 1.1 ± 0.1 的数量级上。

[0127] 此外,美国环保署的建议是至少2.5% (w/v) 的过乙酸溶液,其根据表格数据具有不超过1.25的pH。

[0128] 因此,进行这种推荐的消毒程序(即施用2.5-10%的过乙酸溶液)的任何工人应采用适合于处理强酸的预防措施类型,例如防护手套、防护眼镜、呼吸面罩等。

[0129] 实施例2(比较):漂白剂

[0130] 在可能或怀疑有内生孢子的情况下,另一种广泛认可和推荐的消毒处理是将漂白剂溶液施用到目标表面并接触10分钟。

[0131] 向50mL烧杯中加入5mL漂白剂溶液,即8.25% (w/v) 次氯酸钠。将来自校准的温度补偿pH计的干净的、冲洗并干燥的探针放入烧杯中。蒸馏水的连续等分试样加入到烧杯中,每次加入后进行pH读数。

[0132] 该滴定的数据在下表6中示出。

[0133] 表6:用水滴定家用漂白剂

[0134]

水 mL	[ClO ⁻] % (w/v)	pH
0	8.25	12.56
2	5.89	12.46
4	4.58	12.33
6	3.75	12.23
8	3.17	12.14
10	2.75	12.06
12	2.43	12.00
14	2.17	11.94
16	1.96	11.89
18	1.79	11.84
20	1.65	11.79
22	1.53	11.74
24	1.42	11.70
25	1.38	11.68

[0135] 该表的数据尤其表明,未稀释的8.25%漂白剂溶液具有的pH几乎为13,并且将ClO⁻浓度降低四分之三将其仅降低至~11.9。相反,将浓度从8.25%降低到5% (均为w/v) 导致所计算的有效溶质浓度降低了几近一半,即从2.28到1.340sm/L。

[0136] 因此,进行这种推荐的消毒程序(即施用漂白剂溶液)的任何工人应采用适合于处理强碱的预防措施类型,例如防护手套、防护眼镜、呼吸面罩等。

[0137] 实施例3-10:体外致死时间

[0138] 针对艰难梭菌(ATCC#43598)实施某些杀孢子组合物的功效。在该测试中,通过在不同的时间测试点(通常是相等的增量,诸如15分钟(900秒))与未经处理的对照(采用磷酸盐缓冲盐水作为液体)进行比较来确定细菌的减少。

[0139] 将待测试溶液的9.9mL等分试样放入20mL试管中。将0.1mL体积的测试培养物(稀释时,每毫升艰难梭菌的~10⁶菌落形成单位(CFU))加入到试管中,然后对其进行涡旋。在预定量的接触时间之后,将1.0mL的样品/测试培养物悬浮液转移到含有9.0mL适当中和溶液的无菌试管中,然后再进行涡旋。

[0140] 然后通过将0.5mL等分试样的测试溶液转移到4.5mL中和溶液中来制备系列十倍稀释液,在稀释之间进行涡旋混合。从这些稀释液中,将双份的1.0mL等分试样平板涂布在脑心琼脂平板上,然后在35±2℃下厌氧培养~72小时。

[0141] 培养后,对平板上的菌落进行计数,在数据计算中使用20至200CFU范围内的计数。通过从CFU/mL回收的对照样品中减去CFU/mL回收的处理值来进行该测试的对数减少。

[0142] 以这种方式测试许多组合物,在下表7的最后一列中显示了孢子达到6个对数减少的时间。基于目标~2.30sm/L有效溶质浓度和目标pH4制备每种组合物。(在缓冲体系列中,“A”代表乙酸/乙酸钠,而“C”代表柠檬酸/柠檬酸三钠。在氧化剂列中,“PPOMS”代表过氧化

单硫酸盐,均为0.22% (w/v),“PAA”代表指定浓度的过氧乙酸。)指定为使用BK作为表面活性剂的那些组合物包括0.21% (w/v),而指定为使用SDS的那些组合物包括0.175% (w/v)。对于显示包含有机液体的那些组合物,使用异丙醇溶液(70%的水溶液)。

[0143] 表7

[0144]

实施例	缓冲体系	氧化剂	表面活性剂	有机液体 (%, w/v)	时间 (分钟)
3	A	PPOMS	BK	--	30
4	A	PPOMS	SDS	--	30
5	A	PPOMS	BK	10.0	60
6	C	PAA (0.1%)	BK	20.0	30
7	C	PAA (1.0%)	BK	20.0	30
8	A	PPOMS	BK	--	30
9	A	PPOMS	SDS	--	30
10	A	PPOMS	SDS	10.0	15

[0145] 上表中所示的时间好于用大多数商购的杀孢子产品所能达到的时间(其典型地需要240至2160分钟用于艰难梭菌消毒)。此外,暴露于这些组合物中的每一种的危险远远低于暴露于这种商业产品。

[0146] 实施例11-22

[0147] 上表7的数据看来表明,使用阴离子型表面活性剂(SDS)的组合物可能比使用阳离子型表面活性剂(BK)的组合物提供更好的结果。

[0148] 为了进一步研究功效,制备另外的组合物,其中每一种使用与实施例4和9-10中使用的相同量的SDS,加上0.02% (w/v) 聚山梨酯型非离子型表面活性剂。每一种还包括250g/L的30% H₂O₂溶液和300g/L有机液体。每种组合物的电解质氧化剂(EOA)是PPOMS。含柠檬酸的组合物包含140g/L柠檬酸和17.5g/L柠檬酸三钠二水合物(以及所述用量的NaCl,以将有效溶质浓度提高至预定目标),而含有乙酸的组合物包含所述用量的乙酸(AA)和乙酸钠(SA)。

[0149] 定量载体测试基本上按照ASTM标准E2197-11进行,对孢子的功效在下表的最后一列中示出。

[0150] 表8a:柠檬酸组合物

[0151]

实施例	目标pH	NaCl用量 (g/L)	EOA用量 (g/L)	有机液体	对数减少
11	1.5	54.0	12.0	IPA	5.6
12	3.5	54.0	4.0	IPA	3.2
13	1.5	178.2	12.0	DGME	<3
14	3.5	178.2	4.0	DGME	<3
15	2.5	116.0	8.0	DGME	3.9

16	2.5	116.0	8.0	IPA	3.9
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[0152] 表8b:乙酸组合物

[0153]

实施例	目标 pH	AA/SA 用量 (g/L)	EOA 用量(g/L)	有机液体	对数减少
17	1.5	82.4 / 7.4	4.0	DGME	5.0
18	3.5	82.4 / 7.4	12.0	DGME	3.3
19	1.5	164.9 / 14.8	4.0	IPA	5.1
20	3.5	164.9 / 14.8	12.0	IPA	4.7
21	2.5	123.6 / 11.1	8.0	DGME	5.3
22	2.5	123.6 / 11.1	8.0	IPA	5.0

[0154] 这些表的数据的统计分析表明,酸的类型对功效影响最大,依次为pH(越低越好)、溶剂类型和有效溶质浓度。电解质氧化剂的用量似乎具有较小的影响。

[0155] 实施例23-31

[0156] 使用实施例22作为中心点(下面重新作为实施例23),对其中目标pH(2.5)、有效溶质浓度(~6.40sm/L)和PPOMS的用量(8g/L)保持恒定的另一轮制备的组合物进行额外的定量载体测试。阴离子型表面活性剂是SDS,而非离子型表面活性剂是聚山梨酯。用于每种的有机液体是70% (v/v) IPA溶液。

[0157] 表9

[0158]

实施例	有机液体 (g/L)	H ₂ O ₂ 溶液 (g/L)	阴离子型表面 活性剂 (g/L)	非离子型表面活 性剂 (g/L)	对数减少
23	250	250	17.5	2.0	4.6
24	200	200	15.0	1.5	4.6
25	400	300	15.0	1.5	4.7
26	200	300	20.0	1.5	4.6
27	400	200	20.0	1.5	4.6
28	200	300	15.0	2.5	4.7
29	400	200	15.0	2.5	4.6
30	200	200	20.0	2.5	4.5
31	400	300	20.0	2.5	4.7

[0159] 实施例11-31的数据分析表明,酸的pH和类型具有最大的影响,其次是有效溶质浓度和溶剂类型。

[0160] 实施例32-40

[0161] 对其中pH(2.5)和有效溶质浓度(~6.40sm/L)保持恒定的组合物进行另外的定量载体测试。该组测试改变了电解质氧化剂(PPOMS)的用量、溶剂的用量和类型(其中E代表无

水乙醇)、过氧化氢溶液的用量以及阴离子型 (SDS) 和非离子型 (聚山梨酯型) 表面活性剂的用量。

[0162] 表10

[0163]

实施例	有机液体 (g/L)	H ₂ O ₂ 溶液 (g/L)	PPOMS (g/L)	阴离子型表面 活性剂 (g/L)	非阴离子型表面 活性剂 (g/L)	对数减少
32	E, 250	200	12	15.0	1.5	4.7
33	E, 250	300	20	15.0	1.5	4.7
34	DGME, 250	300	12	20.0	1.5	4.5
35	DGME, 250	200	20	20.0	1.5	3.9
36	E, 350	300	12	15.0	2.5	3.8
37	E, 350	200	20	15.0	2.5	4.5
38	DGME, 350	200	12	20.0	2.5	4.6
39	DGME, 350	300	20	20.0	2.5	4.7
40	IPA, 300	250	16	17.5	2.0	4.7

[0164] 该数据的分析表明,当酸的类型和用量保持恒定时,最显著的统计学因素可能是溶剂的双向组合类型、溶剂浓度以及电解质氧化剂的用量。