

Use of aliphatic alcohols in the production of a
disinfectant for preventing contamination of the
disinfectant with bacterial spores

5 The present invention relates to the use of aliphatic
alcohols in the production of a disinfectant for
preventing contamination of the disinfectant with
bacterial spores. Furthermore, the invention relates to
a process for producing a disinfectant, to which
10 aliphatic alcohol is added to reduce or prevent
contamination with bacterial spores.

Controlling bacteria plays a decisive role in a
multitude of areas. Some bacteria form persistent
15 stages (so-called spores), in which the complete
metabolism is brought to a standstill. In this state,
the bacteria are able to withstand even extreme
environmental conditions and sometimes survive for
several years to decades. Suitable chemical active
20 ingredients for controlling spores are, inter alia,
peracetic acid, hydrogen peroxide, ozone, sodium
hypochlorite and mixtures of fruit acids with hydrogen
peroxide.

25 Spore-forming bacteria include bacteria of the genus
Bacillus, such as *Bacillus anthracis*, the pathogen of
anthrax. Members of the spore-forming genus *Clostridium*
are *Clostridium perfringens* (causes gaseous gangrene),
Clostridium botulinum (causes botulism) and *Clostridium*
30 *tetani* (causes tetanus). *Bacillus* spp. and *Clostridium*
spp. have in past years become the focus of attention,
particularly with regard to disinfection in the
hospital sector.

35 According to the list of disinfectants and disinfection
methods recognized and tested by the Robert Koch
Institute (the central facility in the Federal Republic
of Germany in the field of disease monitoring and

prevention) (status 31 May 2007, 15th edition), it is necessary, for example for killing spores of *Clostridium tetani*, to destroy the spores by combustion or special (chemo)thermal disinfection methods (e.g. at
5 superatmospheric pressure, 134°C, 1 h). However, for certain materials which may be carriers of bacterial spores, complete combustion, the use of the specified conventional chemical active ingredients or the (chemo)thermal treatment under superatmospheric
10 pressure is not an option, for example in the case of hand disinfection.

Hand disinfection is one of the essential methods for breaking chains of infection in hospitals and in the
15 form of "Surgical hand disinfection" (European Standard EN 12791), is an essential constituent of the measures for preventing wound infections in the case of surgical interventions. It is generally known that bacteria spores on the skin cannot be killed by customary
20 alcohol-based disinfectants. Also, according to Hübner, N-O., Belo, H. and Kramer, A., *GMS Krankenhaushyg. Interdiszip.* 2006; 1(1): Doc 13 (ISSN 1863-5245), sporicidal hand disinfectants are virtually nonexistent or are very aggressive. The
25 authors recommend that the surgeon removes loosely adhering spores from his hands by washing and then disinfects the hands with an alcoholic hand disinfectant. Hand washing leads to a lowering of the spore load.

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The Federal Institute for Drugs and Medical Devices in the Federal Republic of Germany (BfArM) requires that disinfectants for surgical hand disinfection be produced as a spore-free product so that when using the
35 surgical hand disinfectant (after hand washing), the hands are not recontaminated with spores. It is required to design the production process, by means of extensive measures, overall such that a spore-free product is obtained. The microbiological safety

measures should be observed in the entire production chain (i.e. from the starting materials to the finished drug). Because the microbiological quality of the finished disinfectant is also influenced by the production of the packaging, the production of the packaging should be included in the risk analysis. A mandatory part of production should also be a filtration step through a 0.2 μ m filter. Even during subsequent bottling, freedom from spores should be ensured. As a result, the method prescribed by the BfArM for the production of the required spore-free disinfectants for surgical hand disinfection is extraordinarily complex.

Setlow et al. (B. Setlow, C.A. Loshon, P.C. Genest, A.E. Cowan, C. Setlow and P. Setlow, Journal of Applied Microbiology 2002, 92, 362-365) describe the killing of spores of *Bacillus subtilis* by 70% strength ethanol at 65°C. In connection with mechanistic investigations described therein, reference is made to the killing of *B. subtilis* spores just by moist heat (cf. M. Paidhungat, B. Setlow, A. Driks, P. Setlow, Journal of Bacteriology 2000, 182, 5505-5512). An independent effect of ethanol on bacterial spores is not described.

It was an object of the invention to provide a process for the production of disinfectants, with which the disinfectants can be produced free from, or at least with a low content of, bacterial spores. This process should be as simple as possible, i.e. as far as possible require no alteration to the plants.

This object is achieved according to a first aspect of the invention by using aliphatic alcohols in the production of a disinfectant for preventing contamination of the disinfectant with bacterial spores. According to a second aspect, the invention relates to a process for producing a disinfectant, to

which aliphatic alcohol is added to reduce or prevent contamination with bacterial spores.

The invention is based inter alia on the fact that it has surprisingly been found that the ability of bacterial spores to survive upon long term contact with aliphatic alcohols is limited (RF up to $> 2 \log$), and without it being necessary to use elevated temperature. This means that it is possible to at least partially dispense with the mentioned extensive safety measures during the production of low-spore and spore-free disinfectants. This was therefore not foreseeable because hitherto it has been assumed that the classic aggressive chemical active ingredients and/or thermal methods mentioned above are automatically required for the killing of spores. In this connection, it is not necessary in accordance with the invention to modify the formulation of the disinfectant (provided aliphatic alcohol is used in the production).

The killing of bacterial spores takes place here surprisingly at a temperature of less than 60°C , preferably less than 50°C , in particular less than 40°C , for example 15 to 35°C , such as 20 to 30°C .

The effect according to the invention, namely that spores in disinfectants can be controlled through the use of aliphatic alcohols and that disinfectants of this type are therefore autosterile offers advantages both for the production of the disinfectants and also for their use because opened packs are protected against attack by spores.

1. Aliphatic alcohol

The aliphatic alcohol is preferably a C_1 - to C_6 -alkyl alcohol, such as, for example, methanol, ethanol, propanol, butanol and mixtures thereof, in particular ethanol and propanol and mixtures thereof. The concentration of the aliphatic alcohol in the

disinfectant is typically at least 5 per cent by weight, preferably at least 30 per cent by weight, more preferably at least 40 per cent by weight, in particular 50-90 per cent by weight. In the case of n-propanol, even the low concentrations above 10 per cent by weight are very effective (in particular between 30 and 50 per cent by weight).

As is evident from the examples, the effect of different aliphatic alcohols differs as detailed:

(x) Longer-chain aliphatic alcohols have a better effect than short-chain ones. In one preferred embodiment, the aliphatic alcohol is accordingly propanol or butanol. Particular preference is given to using propanol. Ethanol is likewise suitable.

(y) Linear alcohols have a better effect than branched alcohols.

Accordingly, when using an aliphatic alcohol on its own, the use of n-propanol is preferred over the use of isopropanol, and the use of isopropanol is preferred over the use of ethanol.

1.1 Ethanol

If ethanol is used as aliphatic alcohol, a preferred amount in the disinfectant is at least 30% by weight, preferably at least 40% by weight, in particular at least 60% by weight, for example at least 80% by weight. If it is specifically about controlling aerobic spore-forming bacteria such as *Bacillus* spp., using the exemplary example of *Bacillus subtilis* demonstrated here, then the amount of ethanol in the disinfectant is preferably at most 90% by weight, more preferably at most 80% by weight, where an amount of ethanol of 60% by weight is most preferred for controlling aerobic spore-forming bacteria such as *Bacillus* spp., for

example *Bacillus subtilis*. When controlling anaerobic spore-forming bacteria such as *Clostridium* spp. using the exemplary example of *Clostridium sporogenes*, there is no preferred maximum concentration of ethanol in the disinfectant.

1.2 Isopropanol

When using isopropanol as aliphatic alcohol, the preferred amount is at least 10% by weight, more preferably at least 30% by weight.

1.3 n-Propanol

If n-propanol is used as aliphatic alcohol in the disinfectant, then the concentration is preferably at least 4% by weight, more preferably at least 10% by weight, in particular at least 20% by weight.

1.4 Mixtures of ethanol and isopropanol

In a further embodiment, the disinfectant comprises a mixture of ethanol and isopropanol as aliphatic alcohol. Preferred concentrations of ethanol are 60 to 85% by weight, more preferably 65 to 80% by weight, in particular 70 to 80% by weight. Preferred concentrations of isopropanol are 1 to 15% by weight, such as 2 to 12% by weight.

1.5 Mixtures of isopropanol and n-propanol

In a further preferred embodiment, the disinfectant comprises a mixture of isopropanol and n-propanol as aliphatic alcohol. The concentration of isopropanol is preferably 5 to 55% by weight, more preferably 15 to 50% by weight, in particular 25 to 50% by weight, such as 28 to 48% by weight. The amount of n-propanol is preferably 5 to 50% by weight, preferably 10 to 45% by weight, in particular 20 to 35% by weight.

1.6 Mixtures of ethanol and n-propanol

In a further preferred embodiment, mixtures of ethanol and n-propanol are used as aliphatic alcohol in the

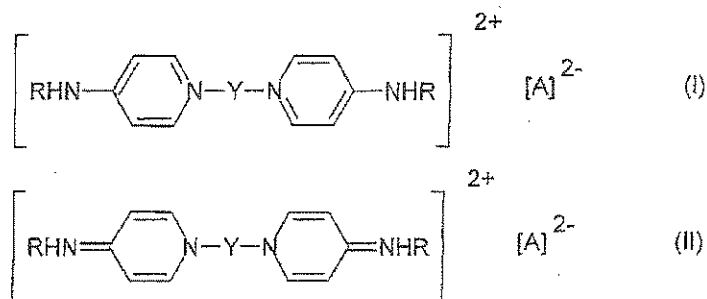
disinfectant. A preferred concentration of ethanol in the disinfectant is 10 to 50% by weight, more preferably 20 to 30% by weight, such as, for example, about 25% by weight. A preferred concentration of n-propanol is 30 to 50% by weight, preferably 35 to 45% by weight.

1.7 Mixtures of ethanol and n-propanol and isopropanol

Finally, it is also possible to use mixtures of ethanol with isopropanol and n-propanol as aliphatic alcohol in the disinfectant. A preferred concentration of ethanol is 10 to 30% by weight, such as about 20% by weight. A preferred amount of isopropanol is 20 to 40% by weight, such as about 30% by weight. Preferably, the amount of n-propanol is 15 to 35% by weight, such as about 25% by weight.

In all embodiments of the invention, the aliphatic alcohol is preferably propanol, i.e. n-propanol, isopropanol and mixtures thereof (see the above statements under 1.2, 1.3, 1.5 and 1.7).

The disinfectant obtained according to the invention can comprise, for example, one or more bispyridiniumalkanes as antimicrobial active ingredient. According to the invention, all suitable bispyridiniumalkanes can be used. The bispyridinium-alkane used according to the invention is preferably a bis[4-(substituted-amino)-1-pyridinium]alkane of the general formula I or II



where Y is an alkylene group having 4 to 18 carbon atoms, R is an alkyl group having 6 to 18 carbon atoms or a cycloalkyl group having 5 to 7 carbon atoms or the phenyl radical, which may be substituted by a halogen atom, and A is one or more anions. In a particularly preferred embodiment, the bispyridiniumalkane used according to the invention is N,N'-(1,10-decanediyl-di-1[4H]-pyridinyl-4-ylidene)bis(1-octanamine) dihydrochloride (octenidine dihydrochloride, hereinbelow octenidine). Moreover, octenidine disulphate is also suitable.

Alternatively to bispyridiniumalkane, such as octenidine, 2-biphenylol may also be present.

A typical amount of bispyridiniumalkane or 2-biphenylol is 0.02 to 5% by weight, preferably 0.03 to 3% by weight, in particular 0.04 to 0.5% by weight, for example 0.06 to 0.2% by weight, such as about 0.1% by weight.

Besides the obligatorily prescribed aliphatic alcohol and optionally bispyridiniumalkane or 2-biphenylol as antimicrobial active ingredient, the disinfectant produced according to the invention comprises, in a preferred embodiment, one or more of the following optional components:

- one or more surfactants,
- one or more solvents and/or
- one or more active ingredients and/or auxiliaries.

2. Surfactant

As optional constituent in the disinfectants produced according to the invention, cationic, anionic, amphoteric and/or nonionic surfactants are present, preferably amphoteric or nonionic surfactants.

As nonionic surfactant, all suitable nonionic surfactants can be used, preference being given to (i) (fatty) alcohol ethoxylates, (ii) sorbitan esters, (iii) alkyl glycosides (in particular alkyl polyglucosides), (iv) amine oxides and (v) ethylene oxide/propylene oxide block copolymers.

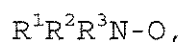
The (i) alcohol polyalkoxylates include fatty alcohol alkoxyates, e.g. isodecyl ethoxylates with different fractions of ethylene oxide, isotridecyl ethoxylates, polyethylene glycol ethers of stearyl, lauryl and cetyl and oleyl alcohol. Here, the alcohols may have been alkoxyated with ethylene oxide, propylene oxide or any desired mixtures of ethylene oxide and propylene oxide. Alcohol polyalkoxylates are known inter alia under the names Lutensol®, Marlipal®, Marlox®, Brij® and Plurafac®. As nonionic surfactant, particular preference is given to lauryl alcohol ethoxylates.

Furthermore, the nonionic surfactants (ii) used are sorbitan esters, which are mostly present as oleates, stearates, laurates and palmitates and which are referred to as polysorbates (e.g. Tween®).

Moreover, the nonionic surfactant may be a (iii) alkyl glycoside, such as an alkyl glucoside (i.e. an alkyl glycoside of glucose), more preferably a C₈- to C₂₀-alkyl polyglucose, in particular a C₈- to C₁₆-alkyl polyglucose of a fatty alcohol, with a laurylpolyglucose, a decylpolyglucose or a mixture thereof being preferred. The carbon chain length is 8 to 16 atoms in the case of cocoylpolyglucose, 12 to 16 carbon atoms in the case of laurylpolyglucose and likewise 8 to 16 atoms in the case of decylpolyglucose.

A typical amount of alkyl glycoside is 0.03 to 10% by weight, preferably 0.06 to 5% by weight, in particular 0.1 to 2% by weight.

According to the invention, as (iv) amine oxide it is in principle possible to use all suitable amine oxides. The amine oxides, which are N-oxides of tertiary amines, include aliphatic amine oxides, cyclic amine oxides (such as N-alkylmorpholine oxide) and aromatic amine oxides (such as pyridine N-oxides). In a preferred embodiment, the amine oxide has the general formula



in which R^1 is methyl, ethyl or 2-hydroxyethyl, R^2 is methyl, ethyl or 2-hydroxyethyl, R^1 and R^2 together may be morpholine, R^3 is alkyl having 8 to 18 carbon atoms or $R^4CONH(CH_2)_n$, where R^4 is alkyl having 8 to 18 carbon atoms and n is in the range from 1 to 10, preferably 1 to 5, more preferably 2 to 4, and in particular 3, and may be 2-hydroxyethyl condensed with 1 to 2000 ethylene oxide, ethylene oxide/propylene oxide or propylene oxide units.

Exemplary amine oxides are cocamidopropylamine oxide, N-cocomorpholine oxide, decyldimethylamine oxide, dimethylcetylamine oxide, dimethylcocamine oxide, dimethyl-hydr. tallow-amine oxide, dimethylaurylamine oxide, dimethylmyristylamine oxide, (2-hydroxyethyl)-cocamine oxide and oleamine oxide. See also "International Cosmetic Ingredient Dictionary and Handbook", 10th edition 2004, Volume 3, pages 2268-2275 (Surfactants-Cleansing Agents).

In a preferred embodiment, the amine oxide is cocamidopropylamine oxide, i.e. R^4CO is the acyl radical derived from the fatty acids of coconut oil, $n = 3$, and R^1 and R^2 are methyl. This product is sold as Rewominox B 204 by Goldschmidt, Federal Republic of Germany.

A typical amount of amine oxide is 0.03 to 10% by weight, preferably 0.06 to 5% by weight, in particular 0.1 to 2% by weight.

5 Amphoteric surfactants are likewise suitable as surfactant, for example betaines. Suitable betaines are described in EP 560 114 A2. Particular preference is given to cocamidopropylbetaine. A typical amount of betaine is 0.03 to 10% by weight, preferably 0.06 to 5%
10 by weight, in particular 0.1 to 2% by weight.

Also suitable as surfactant are cationic surfactants, such as quaternary ammonium salts. In principle, according to the invention, it is possible to use all
15 suitable quaternary ammonium compounds. Preferably, the quaternary ammonium compound is a dialkyldimethyl-ammonium salt.

Quaternary ammonium salts used according to the
20 invention are represented by the formula $[R^1R^2R^3(CH_3)N]^+[X]^-$, where R^1 to R^3 may be identical or different and are selected from C_1 - to C_{30} -alkyl, aralkyl, alkenyl and mixed groups, which may have one or more atoms selected from O, S, N and P, where R^1 to
25 R^3 are for example C_8 - to C_{18} -alkyl, benzyl or methyl, preferably C_9 - to C_{18} -alkyl, benzyl or methyl, such as C_{16} -alkyl, benzyl or methyl. X is an anion (of an inorganic or organic acid). Here, both anion and cation of the quaternary ammonium salt may be polyvalent ions,
30 from which a stoichiometry $[A^{(n+)}]_m[K^{(m+)}]_n$ arises.

Suitable quaternary ammonium salts according to the invention are all quaternary ammonium salts of the aforementioned formula known in the prior art, as are
35 disclosed, for example, in WO 00/63337, to which reference is hereby made. However, preference is given to using dialkyldimethylammonium salts, for example dialkyldimethylammonium chlorides, the alkyl chains of which are selected independently of one another from C_8 -

to C₁₈-alkyl, preferably C₉- to C₁₈-alkyl, such as C₁₆-alkyl. In the dialkyldimethylammonium salts, one of the methyl groups may be an alkoxyated, for example ethoxyated, hydromethyl group.

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Quaternary ammonium salts used preferably according to the invention are compounds of the formulae $[R^1N(CH_3)_3]^+[X]^-$, $[R^1R^2N(CH_3)_2]^+[X]^-$ and $[R^1R^2R^3(CH_3)N]^+[X]^-$, where R¹ to R³, independently of one another, are selected from C₈- to C₁₈-alkyl and $-(CH_2-CHR^4O)_n-R^5$, where n is a number from 1 to 20, preferably 1 to 5, and R⁴ and R⁵, which may be identical or different, are H and/or C₁- to C₄-alkyl, preferably H.

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Exemplary anions and classes of anions of the quaternary ammonium salts used according to the invention are hydroxide, sulphate, hydrogensulphate, methosulphate, ethosulphate, lauryl sulphate, lauryl ether sulphate, cellulose sulphate, sulfamate, halide (fluoride, chloride, bromide, iodide), nitrite, nitrate, carbonate, hydrogencarbonate, phosphate, alkyl phosphate, metaphosphate, polyphosphate, thiocyanate (rhodanide), carboxylic acid salt such as benzoate, lactate, acetate, propionate, citrate, succinate, glutarate, adipate, toluenesulfonate (tosylate) and salicylate. Particularly preferred anions are chloride and propionate.

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Particular preference is given to using the quaternary ammonium salts mecetronium etilsulphate (hexadecyl(ethyl)dimethylammonium ethyl sulphate) and benzalconium chloride.

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35 3. Solvents

Moreover, the disinfectant used optionally comprises 3) solvents. Preferred solvents are glycols and water and mixtures thereof. A preferred solvent is water.

4. Active ingredients and/or auxiliaries

Exemplary active ingredients and/or auxiliaries which may optionally be present in disinfectants produced according to the invention are skincare additives, refatting agents, perfumes, fragrances, thickeners, pH regulators, humectants and dyes. These are inter alia:

- polyols which act as skincare additives, refatting agents and humectants, such as glycerol, erythritol, 1,2,6-hexanetriol, inositol, lactitol, maltitol, mannitol, methylpropanediol, phytantriol, polyglycerols, sorbitol and xylitol, with glycerol being particularly preferred,
- glycerol esters, preferably glycerol cocoate, isopropyl myristate, isopropyl palmitate, and triglycerides, which act as refatting agents, and/or
- allantoin and sodium gluconate, which act as skincare additive.

The mentioned effect, namely that aliphatic alcohols lead over time to the death of bacterial spores, is present at the typical pH values of disinfectants which are contemplated according to the invention. Preferred pH values of the disinfectants are in the range from 3 to 9, more preferably 4 to 8, such as 4.5 to 7, for example about 5.5. The desired pH can be adjusted for example with sodium lactate, citric acid or NaOH.

The disinfectants produced according to the invention are used in a customary manner on animate surfaces, in particular human skin.

Typically, disinfectants produced according to the invention are in the form of (alcohol-containing) aqueous solution. According to the invention, however, it is also possible to produce (alcohol-containing)

semisolid disinfectants which are referred to below as semisolid preparations.

5. Semisolid preparations

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Ointments (Latin unguenta) are spreadable preparations which are intended for use by application to or rubbing into the skin. They consist of one or more ointment bases (such as vaseline, wool grease, lanolin, etc.),
10 into which the active ingredient is incorporated. The active ingredient should be dissolved or finely dispersed. In order to increase the solubility, ointments often comprise water or oils. In an ointment, however, the fat/oil fraction is higher than the water
15 fraction.

In ointments according to the invention, the viscosity is generally 500 to 15 000 mPa·s, preferably 1000 to 10 000 mPa·s, measured using a rotary viscometer at
20 95 s⁻¹ and 20°C.

The disinfectants according to the invention are present for example as aqueous-alcoholic gels (hydrogels). Hydrogels are valued due to their
25 transparency and the non-greasy character. Lipophilic gels (oleogels) are likewise used on account of their aesthetic appearance and their consistency-imparting properties. Gels are intended predominantly for external use and should be applied thinly.

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A hydrogel is a mostly translucent mass which is produced with the help of gelatin, tragacanth, carbopol or similar swelling substances with the addition of water and glycerol. As a result of the evaporation of
35 the water, they have a cooling effect.

Emulsion is understood as meaning preparations which are composed of immiscible liquids, e.g. oil and water. A distinction is made between W/O (water in oil)

emulsions or O/W (oil in water) emulsions and ambiphilic emulsions. These have to be shaken vigorously prior to use. By adding an emulsifier, the very fine distribution of the liquids in one another is possible and the emulsions are stable as a result, i.e. the oil and the water do not separate again. Depending on the type of use, emulsions are intended for internal or external use. Emulsions for external use are often referred to as lotions. Here, an oil-in-water emulsion is present.

Preferably, the composition is present as hydrogel. Here, preference is given to an embodiment in which the composition is formulated as a composition that is to be applied topically (externally).

According to the invention, the composition can comprise further active ingredients which supplement the activity of, for example, octenidine dihydrochloride or 2-biphenylol and can possibly be used in a significantly lower concentration than in the known commercial products if they are combined with octenidine dihydrochloride or 2-biphenylol. As a result, the disadvantages present can sometimes be significantly reduced. These further active ingredients include: clotrimazole and other locally effective antimycotics, cortisones, tretinoin, benzoyl peroxide, acyclovir, local anaesthetics (e.g. benzocaine, lidocaine, polidocanol etc.), antibiotics, bufexamac etc.

6. Use

Disinfectants produced according to the present invention can be used for wound antiseptics and surgical hand disinfection. Alternatively, the disinfectants are used for surface disinfection.

According to one embodiment, the disinfectant produced according to the invention is used for the treatment of

wounds. Here, preference is given to selecting an emulsifier-free formulation which includes a high degree of moisturizing factors (e.g. a gel).

5 The invention therefore offers the following advantages:

- 10 1. If contamination of a product with spores has occurred during a production process, it may suffice to eliminate the cause of the contamination and to leave the production plant in contact with the (alcohol-containing) disinfectant for a certain time; complete removal of spores from the production plant (by
15 using the specified conventional chemical active ingredients or (chemo)thermal treatment under superatmospheric pressure) is not necessary; instead, the production plant can be operated again after a certain time.
- 20 2. Because spores of the medically more relevant anaerobic spore-forming bacteria such as, for example, *Clostridium* spp. are even more sensitive towards aliphatic alcohols than
25 spores of aerobic spore-forming bacteria such as, for example, *Bacillus* spp., the spores which are more hazardous from a medically infectiological point of view are controlled even more easily.
- 30 3. In the examples below, it is shown that according to the invention it is also possible to control spores which are adapted on account of their storage in aliphatic alcohols.
- 35 4. According to the invention, the production of disinfectants can take place by formulating in the aliphatic alcohol during one of the first production process steps in order to keep the

production plant low-spore or spore-free, although it is typically not absolutely necessary to alter the production process.

5 7. Refatting system

According to the invention, it has also been found that a specific combination of substances which act as refatting system further intensifies the effect of aliphatic alcohols towards bacterial spores. In a preferred embodiment, the refatting system consists of a combination of cetearyl octanoate and isopropyl myristate, or consists of cetearyl octanoate and myristyl alcohol. The preferred concentration of cetearyl octanoate in the disinfectant is 0.05 to 5% by weight, more preferably 0.1 to 1% by weight, in particular 0.2 to 0.4% by weight, such as about 0.3% by weight. The preferred amount of isopropyl myristate (or myristyl alcohol) is 0.2 to 5% by weight, in particular 0.5 to 3% by weight, such as 0.8 to 1.2% by weight, for example about 1.0% by weight.

The advantages of the present invention arise in particular from the following examples.

25 Examples

1. Method A: Concentration test spores of *Bacillus subtilis*/*Clostridium sporogenes*

30 1.1 Instruments, nutrient media and spores used:

- anaerobic jar
- fixed pipettes 1 ml; 500 μ l and 100 μ l
- sterile glass pipettes 10 ml
- 35 - sterile spatulas
- sterile Schott bottles
- water bath 30°C
- CSA agar for *B. subtilis*
- Columbia agar for *C. sporogenes*

- *Bacillus subtilis* ATCC 6633 stored in a) H₂O or b) ethanol

Spore samples are provided commercially, sometimes as
 5 ethanolic solution (40% ethanol in water). Spores of
 this type are already adapted to ethanol and therefore
 more difficult to control, as can be seen from the
 examples below. Spores of this type adapted to ethanol
 are in practice also of less relevance than spore
 10 samples stored in water.

- *Clostridium sporogenes* ATCC 19404 stored in H₂O

On account of the high resistance of the *Bacillus*
 15 *subtilis* and *Clostridium sporogenes* spores, these were
 used as indicator.

1.2 Diluents:

20 Germination medium:

	For 1 litre:
0.3 M Saccharose	102.7 g
10 mM Tris-HCl (pH 7.5)	10 ml of the 1 M stock solution
10 mM MgSO ₄ × 7 H ₂ O	2.46 g
30 mM CaCl ₂ × 6 H ₂ O	6.57 g
8 mM L-alanine	0.71 g
(25 µg/ml Lysozyme)	100 µl of the 250 mg/ml stock

1.3 Test suspension:

The microbial count of the ordered spore solution is
 25 ascertained prior to the start of the experiment by
 plating out and counting and, if appropriate, the spore
 suspension is diluted. On the day of the experiment an
 initial microbial count (IGC) is ascertained again from
 the spore suspension used. By means of a counting of

spores under the microscope in the Helber chamber, the comparison is drawn between spores which are actually present and the number of germinating spores. The initial spore number should be ca. 1×10^7 CFU/ml.

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1.4 Concentration of alcohols in % by volume:

Ethanol	Isopropanol	n-Propanol
40%	10%	10%
60%	30%	30%
80%	50%	50%
95%	70%	70%

1.5 Contact times:

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Samples are taken every Tuesday and Friday over 7 weeks. The inoculated alcohols stand in the dark at room temperature during the contact times.

1.6 Procedure:

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Each 50 ml per alcohol and concentration are pipetted into a sterile Schott flask, inoculated with 50 μ l of spore suspension and incubated at room temperature.

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Additionally, per test suspension, a Schott flask containing 50 ml of H₂O is inoculated with 50 μ l of test suspension. (42 flasks (13 alcohol conc. + 1 H₂O control $\times$ 3 germ suspension)).

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As blank value, after brief mixing, in each case 1 ml of the mixture is immediately pipetted to 9 ml of germination medium with lysozyme and 9 ml of germination medium without lysozyme. These tubes are incubated in a water bath for 15 min at 30°C and then

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further diluted in germination medium without lysozyme such that 0.5 ml of the 10^0 and in each case 0.1 ml of the 10^0 , 10^{-1} dilution can be streaked out. The other samplings are made similarly on the days stated above.

1.7 Incubation and evaluation:

The plates are incubated at 36°C for 48 h (*B. subtilis*
5 24 h).

C. sporogenes - plates are incubated under anaerobic conditions.

10 1.8 Validation:

To check the nontoxicity of the diluent, 0.1 ml of the
10⁻⁴ dilution of the spore suspension is pipetted into
9.9 ml of germination medium and incubated in the water
15 bath for 15 min at 30°C. 0.5 ml and 0.1 ml are then
streaked out.

1.9 Lysozyme test:

20 In order to exclude false negative results, a lysozyme
test is carried out in parallel (according to
D.L. Popham, J. Helin, C.E. Costello and P. Setlow,
Proc. Natl. Acad. Sci. USA, Volume 93, pages 15405 to
15410, December 1996).

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2. Method B: Recovery rate of spores (*Bacillus subtilis* and *Clostridium sporogenes*)

2.1 Instruments, nutrient media and spores used

- 30
- glass pipettes 10 ml
 - piston pipette 0.1-1.0 ml with tips
 - Erlenmeyer flask
 - stainless steel filter strip
 - annealable tweezers

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 - sterile membrane filter 0.45 µm
 - vacuum pump
 - anaerobic jar
 - generator and oxygen indicator
 - incubator 30-35°C

- CASO agar
- Columbia agar base
- NaCl peptone buffer solution
- 5 - NaCl peptone buffer solution with LTH

- *Bacillus subtilis* ATCC 6633
- *Clostridium sporogenes* ATCC 19404

10 2.2 Procedure

The described procedure (mostly over 50 days) refers to a single determination, and is then reproduced 2x. The data from one experiment are shown.

15 2.2.1 Preparation of the reference suspensions

With the help of NaCl peptone buffer solution, the spore suspensions of *Bacillus subtilis* and *Clostridium sporogenes* are adjusted to a microbial count of 100 CFU/ml.

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2.2.2 Sample preparation

Per product, for each determination, in each case 10 ml are removed aseptically and transferred in each case to a sterile Erlenmeyer flask.

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Depending on the product, the respective flasks are inoculated in each case with 0.1 ml of a reference suspension *Bacillus subtilis*. Depending on the product, the respective flasks are inoculated with in each case
30 0.1 ml of a reference suspension of *Clostridium sporogenes*.

2.2.3 Filtration

Directly after inoculation, 2 flasks per product (1x
35 inoculated with *Bacillus subtilis*, 1x inoculated with *Clostridium sporogenes*) are inhibited with 90 ml of buffer/inhibitor for 15 minutes:

Disinfectant 1 and 2: NaCl peptone buffer solution with LTH

Disinfectant 3: NaCl peptone buffer solution

- 5 After the inhibition, the samples are filtered (1 membrane filter per flask) and applied to agar:
Bacillus subtilis: CASO agar; incubation: 24 h at 30-35°C
Clostridium sporogenes: Columbia agar; incubation: 48 h
 10 at 30-35°C under anaerobic conditions

On the following days, in each case 2 flasks per day and product (1x inoculated with *Bacillus subtilis*, 1x inoculated with *Clostridium sporogenes*) are treated
 15 repeatedly according to the described directions.

Figures 1 and 2 show the effectiveness of 40 to 80% strength ethanol against *Bacillus subtilis*, with 95% strength ethanol showing no effectiveness.
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Figure 3 shows the effectiveness of 40% strength, 60% strength, 80% strength and 95% strength ethanol against *Clostridium sporogenes*.

25 Figures 4 and 5 show the effectiveness of isopropanol against *Bacillus subtilis* above a concentration of just 10% by weight.

Figure 6 shows the effectiveness of isopropanol even in
 30 10% strength concentration against *Clostridium sporogenes*.

Figures 7 and 8 show that n-propanol even in a concentration of 10% by weight is more effective
 35 against *Bacillus subtilis* than isopropanol.

Figure 9 shows the effectiveness of n-propanol above a concentration of just 10% by weight against *Clostridium sporogenes*.

Figure 10 shows the spore count in composition X, which consists of 79% by weight of ethanol (denatured with 1% methyl ethyl ketone), 8.9% by weight of purified water, 0.5% by weight of sorbitol solution (70%), 0.2% by weight of Povidone K30, 1.0% by weight of isopropyl myristate, 0.3% by weight of cetearyl octanoate, 10.0% by weight of isopropanol and 0.1% by weight of 2-biphenylol.

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Figure 11 shows the spore count in composition Y, which consists of 28.0% by weight of isopropanol, 45.0% by weight of n-propanol, 0.3% by weight of lactic acid, 1.0% by weight of myristyl alcohol, 0.09% by weight of cetearyl octanoate, 0.5% by weight of Macrogol 4000, 0.75% by weight of sodium lactate solution (50% strength), 24.2599% by weight of purified water, 0.1% by weight of perfume and 0.06% by weight of dye.

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Patent claims

1. Use of aliphatic alcohols in the production of a
5 disinfectant for preventing contamination of the
disinfectant with bacterial spores.
2. Use according to Claim 1, characterized in that
the use takes place at a temperature of less than 60°C,
10 preferably less than 50°C, in particular less than
40°C, for example 15 to 35°C, such as 20 to 30°C.
3. Use according to Claim 1 or 2, characterized in
that the aliphatic alcohol is selected from n-propanol,
15 isopropanol and ethanol, and mixtures thereof.
4. Use according to one of the preceding claims,
characterized in that the concentration of the
aliphatic alcohol in the disinfectant is at least
20 5 per cent by weight, preferably at least 30 per cent
by weight, more preferably at least 40 per cent by
weight, in particular 50-90 per cent by weight.
5. Use according to one of the preceding claims,
25 characterized in that the aliphatic alcohol is ethanol,
preferably in a concentration of at least 30 per cent
by weight, more preferably at least 40 per cent by
weight, in particular at least 60 per cent by weight,
where a maximum concentration of 90 per cent by weight
30 and preferably at most 80 per cent by weight is
preferred.
6. Use according to one of Claims 1 to 4,
characterized in that isopropanol is used as aliphatic
35 alcohol, preferably in an amount of at least 10 percent
by weight, more preferably at least 30 per cent by
weight.

7. Use according to one of Claims 1 to 4, characterized in that n-propanol is used as aliphatic alcohol, preferably in an amount of at least 4 per cent by weight, more preferably at least 10 per cent by weight, in particular at least 20 per cent by weight.

8. Use according to one of Claims 1 to 4, characterized in that a mixture of ethanol and isopropanol is used as aliphatic alcohol, preferably in an amount of from 60 to 85 per cent by weight of ethanol and 1 to 15 per cent by weight of isopropanol.

9. Use according to one of Claims 1 to 4, characterized in that a mixture of isopropanol and n-propanol is used as aliphatic alcohol, preferably in an amount of from 5 to 55 per cent by weight of isopropanol and 5 to 50 per cent by weight of n-propanol.

10. Use according to one of Claims 1 to 4, characterized in that a mixture of ethanol and n-propanol is used as aliphatic alcohol, preferably in an amount of from 10 to 50 per cent by weight of ethanol and 30 to 50 per cent by weight of n-propanol.

11. Use according to one of Claims 1 to 4, characterized in that a mixture of ethanol, isopropanol and n-propanol is used as aliphatic alcohol, preferably in an amount of from 10 to 30 per cent by weight of ethanol, 20 to 40 per cent by weight of isopropanol and 15 to 35 per cent by weight of n-propanol.

12. Use according to one of the preceding claims, characterized in that the disinfectant also comprises a refatting system, where the refatting system consists of a combination of (i) cetearyl octanoate with isopropyl myristate or (ii) cetearyl octanoate with myristyl alcohol.

13. Use according to one of the preceding claims, characterized in that the spores are selected from *Clostridium sporogenes* and *Bacillus subtilis*.
- 5 14. Process for producing a disinfectant, in which aliphatic alcohol is added to prevent contamination with spores.