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(54) **SYNBIOTIC SURFACE TREATMENT COMPOSITION**

SYNBIOTISCHE OBERFLÄCHENBEHANDLUNGSZUSAMMENSETZUNG

COMPOSITION DE TRAITEMENT DE SURFACE SYMBIOTIQUE

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**WO-A1-2016/022779**
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EP 3 686 266 B1

## Description

### Field of the invention

**[0001]** The present invention relates to methods for treating surfaces with compositions containing at least one prebiotic component and a probiotic component.

### Introduction

**[0002]** It is known that in some cases, traditional cleaning products and methods are not sufficient or efficient enough. In e.g. hospitals, equipment such as furniture, floors, patient beds and mattresses require regular and thorough cleaning to reduce the number of pathogenic microorganisms which may affect the health of recovering patients. Furthermore, for obvious reasons, shared facilities, such as industrial kitchens or HVAC installations, and their components as well as industrial facilities and components, such as filters, pumps and ducts, also need a permanent reduction and control of such pathogenic populations.

**[0003]** It is known that probiotics can be used in combating these harmful bacteria. WO 2006/125283 teaches that the colonizing of surfaces with non-pathogenic spore-forming bacteria via cleaning products has a favourable effect in reducing the number of pathogenic microorganisms on such surfaces. This decreases the risk of infections and has a beneficial effect on general health.

**[0004]** However, in view of the growing resistance of some bacterial strains against certain cleaning products, it is important to reduce the numbers of such pathogens as much as possible. There is therefore a need for products and methods that can reduce any unwanted, harmful or pathogenic microorganisms on a surface to a minimum.

**[0005]** Such compositions furthermore should be applicable to surfaces which are hard to treat, such as surfaces which are positioned in such a way that they are hard to reach via conventional cleaning techniques and/or surfaces which are situated in environmental conditions which make them hard to treat.

**[0006]** European patent application publication no. EP 3 210 612 A1 of the present applicant discloses a method for the treatment of intact or damaged human and animal skin, tissue and wounds with a synbiotic preparation, comprising a mixture with a combination of non-pathogenic spore-forming bacteria, prebiotic sugars, and optionally microcapsules. The spores of the non-pathogenic spore-forming bacteria germinate on the skin or in the tissue or wound in order to inhibit the growth of other microorganisms. The prebiotic sugars stimulate the activity of non-pathogenic microorganisms. The microcapsules provide prolonged release of additional non-pathogenic microorganisms and prebiotic sugars.

**[0007]** International patent application publication no. WO 2016/022779 A1 discloses a plant or plant part coated with a composition comprising a bacterial spore and a germinative compound. In another aspect, that disclosure is directed to a method of enhancing the growth of a plant or plant part comprising coating such plant or plant part with such a composition. In yet another aspect, that disclosure is directed to a composition comprising a bacterial spore and a germinative compound where such components are maintained in an inactive form. That disclosure also relates to use of the compositions in wastewater treatment, environmental remediation, oil recovery, aquaculture systems, and direct fed microbials.

**[0008]** European patent publication EP 0 245 560 B1 discloses a cleaning composition comprising a stable suspension of abrasive particles and viable microorganisms in a water solution containing a detergent.

**[0009]** United States patent application publication no. US 2004/191232 A1 discloses compositions and methods for inhibiting microbial infections associated with the use of sanitary products, such as diapers, bandages, sanitary napkins, tampons and the like. The disclosure comprises providing for use a sanitary product containing an effective amount of a viable non-pathogenic lactic-acid bacteria, such as Bacillus coagulans, or an extracellular product thereof, useful for inhibiting growth of parasites and pathogens on the epithelial tissue in contact with the sanitary product during use of the product. The disclosure also provides for enhancing biodegradation of sanitary products after use and disposal. Also described are methods using the product and systems containing the compositions.

**[0010]** United States patent no. US 6,387,874 B1 discloses an aqueous cleaning composition comprising: an effective mineral dissolving amount of an organic acid of at least 5% by weight of the cleaning compositions; an effective amount of a spore forming microbial composition; an effective cleaning amount of a blend of wetting agents, the first wetting agent having a hydrophobic/lipophobic number (HLB) of about 10 and higher and a second wetting agent having an HLB number of about 5 or less; an effective amount of a thickening agent and water. The composition is also available in powder form without the thickening agent.

### Summary of the invention

**[0011]** According to an aspect of the present invention, there is provided a method for treating surfaces with a surface treatment composition, said composition comprising spores of non-pathogenic species of the genus *Bacillus* and a prebiotic component, in accordance with claim 1.

**[0012]** It is an advantage of the present invention that surfaces can be substantially purified of pathogenic micro-organisms by inoculating said surfaces with said composition. The non-pathogenic probiotics have as an effect that nutrients on the surface become less available for other micro-organisms through the mechanism of nutrient depletion. It has thereby been advantageously been found that the combination of non-pathogenic pro-

biotics with a prebiotic component further amplifies this effect. A further advantage is that both probiotic and prebiotic components place a lighter burden on the environment, in comparison with other compounds used in detergents, such as alcohols etc. It is a further advantage that said composition can be used in products for the purification of surfaces, said products having a shelf life of at least three years.

**[0013]** It is an advantage of the invention that each of the selected *Bacillus spp.* are non-pathogenic microorganisms.

**[0014]** It is an advantage of the invention that the selected *Bacillus spp.* have different germination rates. As a result, said *Bacillus spp.* will have a different concentration profile in time, thereby ensuring a constant presence of a probiotic component on the surface. Furthermore, said selection results in an optimized diversity of extracellular enzymes. It is a further advantage that said presence of probiotic components on the surface can be guaranteed when each of said four *Bacillus* species has a number of spores which is between 10 % and 70 %, with reference to the total number of spores of said four *Bacillus spp.*

**[0015]** It is an advantage of the invention that the presence of a prebiotic sugar further amplifies the effect of the probiotic component. More in particular, it has been found that said prebiotic component accelerates in time the activity of the probiotic component and that a combination of a probiotic component and a prebiotic component according to the invention reduces even further the concentration of pathogens.

**[0016]** In an embodiment of the method according to the present invention, said at least one non-pathogenic species is present in a concentration of $1 \times 10^6$ to $1 \times 10^{10}$ cfu per gram composition.

**[0017]** It is an advantage of this embodiment that said concentration range allows both treating surfaces by use of a detergent or cleaning product comprising said composition, as well as treating surfaces by water dosing, wherein products for the latter typically have an initially higher cfu concentration per gram composition due to the fact that these products tend to be diluted upon use.

**[0018]** In an embodiment of the method according to the present invention, said prebiotic component is present in a concentration of 100 mg to 100 g per kg composition.

**[0019]** It is an advantage of this embodiment that said amplifying effect on the probiotic component is thereby maximized.

**[0020]** In an embodiment of the method according to the present invention, said spores of at least one species and said prebiotic component are at least partly encapsulated in microcapsules.

**[0021]** It is an advantage of this embodiment that the use of microcapsules further allows to spread germination in time, thereby ensuring a constant presence and a persistent activity of *Bacillus spp.* on the treated surface.

**[0022]** In an embodiment of the method according to the present invention, said microcapsule comprises a brittle shell and is furthermore filled with a non-aqueous solution.

**[0023]** In an embodiment of the method according to the present invention, the diameter of the microcapsules is 1 to 300 μm.

**[0024]** In an embodiment of the method according to the present invention, said composition is formulated as a detergent, cleaning product, liquid, emulsion, cream, ointment, lotion, gel, oil, solution, trigger spray, aerosol spray, powder, or semi-solid formulation.

**[0025]** It is an advantage of this embodiment that said composition can be used as part of or formulated as the above-mentioned products, thereby increasing the field of application of the composition. Advantageously, said products typically have a shelf life of at least three years.

**[0026]** It is an advantage of the inveniton that said composition can be applied on a variety of surfaces, which are typically abiotic. However, with the exception of human or animal skin, said composition can also be applied on some biotic materials.

## Brief description of the figures

**[0027]**

Figure 1 shows the germination speed of probiotic and synbiotic compositions as a function of time.

Figures 2A-2C show the effect of two cleaning products on a stone tile floor.

Figure 3 shows the effect of cleaning products on the count of Staphylococcus aureus populations.

Figures 4A-4D show the effect in time of ultrasound fogging of a synbiotic preparation by use of probiotic counts

## Detailed Description of Embodiments

**[0028]** The invention described herein refers to a method for treating surfaces with a surface treatment composition, as defined in claim 1.

**[0029]** The composition used in the method according to claim 1 is a composition for the treatment of surfaces of non-living things. It is thereby noted that the term "non-living thing" can also refer to that which used to be part of a living thing, such as wood, rubber, paper and the like. However, the term "surface" does not refer herein to the surface of living beings, thereby excluding human skin, animal skin or fur and the like.

**[0030]** The surface is wood, paper, leathei rubber or textile; or the surface is an abiotic material, such as stone, metal (and alloys thereof), plastic and other polymeric materials, glass, ceramic materials, bricks and the like. Furthermore, said surface can be a surface of composite material, the term "composite material" referring herein to material made from two or more constituent materials. Said surface can further be a mixture of the aforemen-

tioned materials.

**[0031]** According to embodiments, said surface can relate to a surface which is exposed to air, or which is partially or completely submerged in water or in another liquid.

**[0032]** For the purpose of the invention, the term "surface treatment" refers herein to acting on a surface with the purpose of improving or altering a condition or parameter of said surface. A specific application of embodiments of the invention is for the purpose of purification of the surface, meaning the reduction of unwanted, harmful or pathogenic microorganisms on the surface, such as bacteria, fungi, yeasts, viruses and the like.

**[0033]** For this purpose, the surface treatment comprises the act of cleaning the surface with said composition, or applying said composition to said surface through the act of spraying ultrasound fogging, painting or water dosing.

**[0034]** For the purpose of the invention, the term "water dosing" refers herein to the purification of surfaces in installations and/or surfaces of components thereof by addition of said composition to a liquid, such as a cooling liquid, a rinsing liquid or a circulating liquid, wherein said liquid is in contact with the surface to be treated. Surfaces of pools and ponds, as well as surfaces of industrial installations, such as the inside surface of ducts, pumps and filters may be subject to biofilm growth and accumulation of organic waste. Said composition may thus be added to the liquid which is in contact with said surface.

**[0035]** For the purpose of the present invention, the term "synbiotic" refers to a combination of a probiotic and a prebiotic.

**[0036]** The prebiotic contains one or more from the group consisting of galacto-oligosaccharides, fructo-oligosaccharides and inulin.

**[0037]** According to preferred embodiments, the prebiotic is inulin.

**[0038]** According to preferred embodiments of the invention, the prebiotic component has a mass fraction which is at least 0.5 mg, preferably at least 1 mg, more preferably at least 10 mg, even more preferably at least 100 mg, and most preferably at least 1g, per kg of the composition. It will be understood that the prebiotic component has a mass fraction which is at most 300 g, preferably at most 200 g, more preferably at most 100 g and most preferably at most 80 g, per kg of the composition.

**[0039]** For the purpose of the present invention, the term "probiotic" or "probiotic component" refers to spores of at least one non-pathogenic species of the genus *Bacillus.*

**[0040]** According to the invention, the probiotic component comprises a mixture of spores of *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens.* For the mixture comprising spores of *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens,* each of said four *Bacillus* species has a number of spores which is at least 10%, preferably at least 15%, and more preferably at least 20%, with reference to the total number of spores of said four *Bacillus spp.* in the mixture. Each of said four *Bacillus* species has a number of spores which is at most 70%, preferably at most 60%, more preferably at most 60%, even more preferably at most 50%, preferably at most 40%, more preferably at most 35%, and most preferably at most 30%, with reference to the total number of spores of said four *Bacillus spp.* in the mixture. Preferably, spores of said four species are present in substantially or exactly equal amounts in the mixture.

**[0041]** According to particular embodiments of the invention, said mixture further comprises *Bacillus polymyxa.*

**[0042]** According to particular embodiments of the invention, said mixture does not comprise *Bacillus coagulans.*

**[0043]** According to preferred embodiments, the total amount of spores in said aforementioned mixtures ranges from $1\times10^6$ to $1\times10^{10}$ per gram of the composition. Such an amount is expressed as cfu (colony-forming units). It is thereby understood that this value refers to the total number of cfu of all the *Bacillus spp.* together.

**[0044]** According to particular embodiments, the probiotic component is a mixture consisting of spores of *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens.*

**[0045]** The transformation of spores to vegetative cells is the result of absorption of moisture from the surrounding surface or from the biofilm to which the preparation is applied [Knudsen et al. (2015) J. Bacteriol. 19;198(1): 168-77]. This moisture absorption decreases the absolute moisture content of the surface or biofilm, thus exerting an inhibitory action on moisture availability to other microorganisms. The vegetative *Bacillus* cells produce a variety of extracellular enzymes on surfaces and in biofilm that reduce the amount of organic nutrients on the surface and in biofilm [Priest (1977) Bacteriol. Rev. 41(3): 711-53]. This reduction exerts an inhibitory action on the food availability to other microorganisms via nutrient depletion and the general concept of competitive exclusion [Ragione et al. (2003) Vet. Microbiol. 94(3): 245-56].

**[0046]** Treating a surface with a surface treatment composition containing a probiotic component can affect the concentration of unwanted and potentially harmful microorganisms.

**[0047]** Such an effect is further optimized by the selection of a mixture the aforementioned four *Bacillus spp.,* i.e. *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens.* As these *Bacillus spp.* have a different rate in germination, therefore also a different concentration distribution over time, a constant presence of the probiotic component on the surface to be treated is assured. Said selection will result in an even more pronounced moisture depletion as well as an optimized diversity of extracellular enzymes.

**[0048]** It has now unexpectedly been found that the addition of a prebiotic component to such a composition and the use thereof in e.g. a cleaning product affects the

concentration of pathogens on the treated surface even further. The observed effect is twofold. Firstly, the prebiotic component accelerates in time the activity of the probiotic component.

**[0049]** Secondly, the combination of a probiotic component and a prebiotic component reduces even further the concentration of pathogens on the treated surface, resulting in a lower concentration of pathogens on the treated surface in comparison with a similar treatment for such a surface without the prebiotic component.

**[0050]** The synbiotic surface treatment composition can be used to purify a surface from organic dirt, which may be in the form of a biofilm matrix, as well as to lower the number of other microorganisms such bacteria, viruses, yeasts and fungi. It has been found that the combination of prebiotics with probiotics strengthen the purifying effect of the composition, the prebiotic component thereby seemingly supporting and strengthening the effect of the probiotics on biofilm removal and lowering other micro-organisms.

**[0051]** According to embodiments of the invention, the spores of said probiotic component and said at least one prebiotic component are at least partly encapsulated in microcapsules.

**[0052]** For the purpose of the invention, microcapsules are particles containing a prebiotic and/or a probiotic. Such capsules are described in US 20120076864. Advantageously, the composition can be encapsulated in such microcapsules to provide the composition with longer stability/shelf life. Furthermore, after a "free" first part of the probiotic and prebiotic components of the composition, which part was not encapsulated in microcapsules, has already exerted its effect, said microcapsules may provide for a delayed release of a second part of the probiotic and prebiotic components on the surface or in biofilm. As a result thereof, a persistent activity of the composition for up to 6 weeks can be obtained, provided that sufficient friction is present.

**[0053]** If the preparation is thereby applied to a surface that suffers from friction (such as human or animal activity (walking, touching) or mechanical activity (interaction with machines or vehicles and the like)), the presence of the microcapsules is an established way of ensuring that the preparation exerts a lasting action.

**[0054]** Consequently, it is advantageous to include such microcapsules in the composition for the treatment of a surface where frequent treatment of the surface is not possible.

**[0055]** Typically, the microcapsules are composed of a brittle shell comprising a liquid containing the spores. Said shell breaks open due to friction and releases the contents of the capsule. The microcapsules are typically 1 to 300 μm in diameter.

**[0056]** According to embodiments of the invention, the shell is composed of a polymer layer, such as a polymer of gelatin, polyurethane, polyolefins, polyamides, polyesters, polysaccharides, silicone resins, epoxy resins, chitosan and aminoplastic resins such as a melamine formaldehyde resin.

**[0057]** According to embodiments of the invention, said probiotic and prebiotic components may be present together or separately in a microcapsule. However, said microcapsules may also be provided containing individual bacterial strains.

**[0058]** According to embodiments of the invention, the liquid in the capsules is typically non-aqueous, and more specifically is immiscible with water (e.g. an organic oil, a silicone oil, a fluorocarbon, or mixtures thereof).

**[0059]** The weight ratio of the shell to the liquid contained therein is typically 1:500 to 1:5000. The outer layer of this shell can contain functional reactive groups for chemical binding.

**[0060]** According to embodiments of the invention, up to 10 wt%, 20 wt%, 30 wt%, 50 wt%, or even 75 wt% of the prebiotic and/or probiotic component may be encapsulated in the microcapsules. Microcapsules may be dispersed in a liquid or viscous composition.

**[0061]** According to embodiments of the invention, the composition is processed into cleaning products (detergents), or products for spraying, for (ultrasound) fogging, for painting or for water dosing. Such products are typically in liquid form.

**[0062]** Furthermore, depending on the type of treatment and surface type in question, the composition may be used as a component in or formulated as: a detergent, a cleaning product, a liquid, an emulsion, a cream, an ointment, a lotion, a gel, an oil, a solution, a trigger spray, an aerosol spray, a powder, or a semi-solid formulation.

**[0063]** Advantageously, said composition can be processed in a product for fogging, preferably ultrasound fogging, and combined with a fogging device. An ultrasound fogging device refers herein to a device that uses high-frequency sound vibrations to produce an extra fine water mist that is then expelled to add moisture to the room. It has been found that such an application allows surface treatment of surfaces that cannot be reached by conventional cleaning techniques or that cannot be treated by means of water dosing.

**[0064]** Surface treatment compositions for treatment by (ultrasound) fogging and methods therefore, may be particularly interesting for the treatment of HVAC installation surfaces, especially the surface of the inner sides of ducts. It was found that the installation of an ultrasound fogging device in the duct of an air-conditioning installation, said device fogging a surface treatment composition according to the invention had a beneficial influence on the presence of harmful microorganisms.

**[0065]** In preferred embodiments, water droplets have an average diameter ranging from 0.5 to 10 μm. It was found that under these conditions, the composition could be maximally distributed into an available space.

**[0066]** While the invention has been described hereinabove with reference to specific embodiments, this is done to illustrate and not to limit the invention, the scope of which is defined by the accompanying claims.

**Experimental Results**

**[0067]** The invention will be now described in more details with reference to the following examples, whose purpose is merely illustrative and not intended to limit the scope of the invention.

Galacto-oligosaccharides: Ergomax GOS
Fructo-oligosaccharides: Supersmart Fructo
Inulin: Beneo Orafti Inulin
Germination speeds as well as cfu count were performed by use of ISO 20391-1:2018.

**Example 1 (E1) - Composition of a synbiotic preparation**

**[0068]** The surface treatment composition has the following components, per gram of the preparation:

- $2 \times 10^9$ spores of each of the following species: *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens;*
- 50 mg of inulin;
- 25 mg of galacto-oligosaccharides;
- 15 mg of fructo-oligosaccharides;
- 5 mg of microcapsules.

**Comparative Example 2 (CE2) - Composition of a probiotic preparation**

**[0069]** The surface treatment composition has the following components, per gram of the preparation:

- $2 \times 10^9$ spores of each of the following species: *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens;*
- 5 mg of microcapsules.

**Example 3 (E3) - Effect of a prebiotic component**

**[0070]** A sample of Example 1 (E1) and a sample of Comparative Example 2 (CE2) were each diluted in water at a 1:100 dilution ratio and then allowed to germinate. Figure 1 shows the germination speed of both samples.

**[0071]** From this figure, it can be concluded that germination of the probiotic component occurs more rapidly in a synbiotic formulation. In other words, including a prebiotic component in a product containing a probiotic component results in an increased presence of the probiotic component in the product when evaluated at shorter periods of time.

**Example 4 (E4) - Comparative Examples 5-6 (CE5-CE6) - Cleaning product**

**[0072]** An all-purpose cleaning product was defined, having one of the following compositions, as summarized in Table 1:

Table 1 - compositions cleaning product

| **Component** | E4 | CE5 | CE6 |
|---|---|---|---|
| Water | 91,8 | 91,8 | 91,8 |
| Sodium Laureth Sulphate | 5,5 | 5,5 | 5,5 |
| Probiotic Mixture | 1 | 1 | 0 |
| Phenoxyethanol | 0,45 | 0,45 | 0,45 |
| Inulin | 0,5 | 0 | 0 |
| Galacto-oligosaccharide | 0,25 | 0 | 0 |
| Fructo-oligosaccharide | 0,15 | 0 | 0 |
| CI 42051 | 0,05 | 0,05 | 0,05 |
| Fragrance | 0,3 | 0,3 | 0,3 |
| Ethanol | 0 | 0 | 0 |
| **Total weight (g)** | 100 | 99,1 | 98,1 |

wherein said components of said cleaning product are expressed in weight and wherein said Probiotic Mixture is an aqueous mixture of *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens.* Each component of said probiotic mixture is present in a concentration of $2 \times 10^9$ cfu per gram of the total composition, meaning that each component of said mixture has a number of spores which is 25% with reference to the total number of spores.

**Example 5 (E7) - Purification of a surface**

**[0073]** Cleaning products according to the composition E4 and CE5 were used to clean stone tile floors of toilets of a confined experimental space under controlled conditions. The stone tile floors were suffering from severe organic pollution and biofilm, mainly in the concrete joints between the tiles. A picture of a stone tile floor before the treatment is shown in Fig. 2A.

**[0074]** A first of such floors was cleaned with an all-purpose cleaning product having a composition according to CE5. A second of said stone tile floors was cleaned with an all-purpose cleaning product having a composition according to E4, being a synbiotic composition.

**[0075]** Said first and second floor were then cleaned on a daily basis for a period of three weeks, with said cleaning product on the basis of CE5 and E4 respectively.

**[0076]** Fig 2B shows the result after three weeks of daily cleaning with the (probiotic) cleaning product having a composition according to CE5. The probiotic cleaning product was capable of removing superficial biofilm on the tiles surface and partly in the joints.

**[0077]** Fig. 2C shows the result after three weeks of daily cleaning with the (synbiotic) E4 cleaning product. The synbiotic cleaning product was capable of removing much more organic dirt and biofilm deep into the joints between the tiles, making them bright again at most plac-

es, indicating that the addition of a prebiotic component to a probiotic cleaning product has an unexpected invigorative effect.

**Example 6 (E8) - Purification of a surface**

**[0078]** All-purpose cleaning products according to the composition E4 (synbiotic composition), CE5 (probiotic composition), and CE6 (forming a diluted detergent) were applied at a daily basis to a floor in a confined experimental space under controlled conditions for a total duration of 10 days. Microbiological samples were taken from the surface of the floor in order to determine the presence of the hospital bacteria Staphylococcus aureus. Such samples were taken after 1, 6, 24, 48, 72, 120 and 240 hours. As of 24 hours, the samples were taken just before product application so as to measure the effect of the previous 24 hours of application.

**[0079]** The results in Figure 3 show the following:

- the application of the diluted detergent ("control" -cleaning product based on composition CE6) does not seem to affect the level of *S. aureus* CFU's.
- Applying the probiotic cleaning ("probiotic" - cleaning product based on composition CE5) results in a drop of S. *aureus* CFU's as of 24 hours in order to reach a minimum level (i.e. 430 cfu/$m^2$) after 240 hours.
- Applying the synbiotic cleaning product ("synbiotic" - based on composition E4) leads to a faster decrease of *S. aureus* CFU's resulting in a lower level (i.e. 220 cfu/$m^2$) after 240 hours.

**Example 7 (E9) - Ultrasound fogging of synbiotic preparation**

**[0080]** The synbiotic preparation as described in E1 was diluted tenfold in water containing 0,1 wt% of Sodium Laureth Sulphate. The dilution was subsequently introduced in an ultrasound fogging device. A Bionaire® BUL9500B-U Warm and Cool Mist Ultrasonic Humidifier device, a commercially available air humidifier, was therefore used.

**[0081]** A fog with particles having a diameter being predominantly between 0.8$\mu$m and 10$\mu$m was generated.

**[0082]** The device was placed in one corner of a 120$m^3$ room and sprayed every 15 minutes for 20 seconds, with an output of 1ml of synbiotic solution per minute. Nutrient agar plates were placed on a table at the other side of the room, with open covers. Every 15 minutes, after each fogging, one nutrient agar plate was closed and incubated. A probiotic count was as such determined at starting point (Fig. 4A) and after 15 (Fig. 4B), 30 (Fig. 4C) and 60 (Fig. 4D) minutes.

**[0083]** As illustrated by Figs. 4A-4D, a high probiotic count was measured already after 30 minutes, indicating that ultrasound fogging is a very effective distribution of probiotics through the air.

**Claims**

1. A method for treating surfaces with a surface treatment composition, said composition comprising spores of non-pathogenic species of the genus *Bacillus* and a prebiotic component,

   wherein said non-pathogenic species of the genus *Bacillus* comprises a mixture of *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* and *Bacillus amyloliquefaciens,* each of said four *Bacillus* species having a number of spores which is between 10% and 70%, with reference to the total number of spores of said four *Bacillus spp,,* and
   wherein said prebiotic component comprises at least one of a fructo-oligosaccharide, a galacto-oligosaccharide and/or inulin,
   wherein the surface is wood, paper, leather, rubber or textile; wherein the surface is an abiotic material, or wherein the surface is a mixture of said aforementioned materials;
   said method comprising the steps of cleaning, spraying, ultrasound fogging, painting, or water dosing said surfaces with said composition.

2. The method according to claim 1, wherein said mixture of said non-pathogenic species is present in a combined concentration of $1 \times 10^6$ to $1 \times 10^{10}$ cfu per gram composition.

3. The method according to any one of previous claims, wherein said prebiotic component is present in a concentration of 100 mg to 100 g per kg composition.

4. The method according to any one of previous claims, wherein said spores of said non-pathogenic species and said prebiotic component are at least partly encapsulated in microcapsules.

5. The method according to claim 4, wherein said microcapsule comprises a brittle shell and is furthermore filled with a non-aqueous solution.

6. The method according to claim 4 or 5, wherein the diameter of the microcapsules is 1 to 300 $\mu$m.

7. The method according to any one of previous claims, wherein said composition is formulated as a detergent, cleaning product, liquid, emulsion, gel, oil, solution, trigger spray, aerosol spray, powder, or semi-solid formulation.

**Patentansprüche**

1. Verfahren zur Behandlung von Oberflächen mit einer Oberflächenbehandlungszusammensetzung,

wobei die Zusammensetzung Sporen von nicht-pathogenen Spezies der Gattung *Bacillus* und eine präbiotische Komponente umfasst,

wobei die nicht-pathogene Spezies der Gattung *Bacillus* eine Mischung aus *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* und *Bacillus amyloliquefaciens* umfasst, wobei jede der vier *Bacillus-Spezies* eine Sporenzahl aufweist, die bezogen auf die Gesamtzahl der Sporen der vier Bacillus spp. zwischen 10 % und 70 % liegt, und
wobei die präbiotische Komponente mindestens eines aus einem Fructo-Oligosaccharid, einem Galacto-Oligosaccharid und/oder Inulin umfasst,
wobei es sich bei der Oberfläche um Holz, Papier, Leder, Gummi oder Textil handelt; wobei es sich bei der Oberfläche um ein abiotisches Material handelt oder wobei es sich bei der Oberfläche um eine Mischung der vorgenannten Materialien handelt;
wobei das Verfahren die Schritte des Reinigens, Besprühens, Ultraschallbenebelns, Anstreichens oder der Wasserdosierung der Oberflächen mit der Zusammensetzung umfasst.

**2.** Verfahren nach Anspruch 1, wobei die Mischung der nicht-pathogenen Spezies in einer kombinierten Konzentration von $1 \times 10^6$ bis $1 \times 10^{10}$ KBE pro Gramm Zusammensetzung vorhanden ist.

**3.** Verfahren nach einem der vorhergehenden Ansprüche, wobei die präbiotische Komponente in einer Konzentration von 100 mg bis 100 g pro kg Zusammensetzung vorhanden ist.

**4.** Verfahren nach einem der vorhergehenden Ansprüche, wobei die Sporen der nicht-pathogenen Spezies und die präbiotische Komponente zumindest teilweise in Mikrokapseln eingekapselt sind.

**5.** Verfahren nach Anspruch 4, wobei die Mikrokapsel eine spröde Hülle umfasst und außerdem mit einer nichtwässrigen Lösung gefüllt ist.

**6.** Verfahren nach Anspruch 4 oder 5, wobei der Durchmesser der Mikrokapseln 1 bis 300 μm beträgt.

**7.** Verfahren nach einem der vorhergehenden Ansprüche, wobei die Zusammensetzung als Waschmittel, Reinigungsprodukt, Flüssigkeit, Emulsion, Gel, Öl, Lösung, Triggerspray, Aerosolspray, Pulver oder halbfeste Formulierung formuliert ist.

**Revendications**

**1.** Procédé de traitement de surfaces avec une composition de traitement de surface, ladite composition comprenant des spores d'espèces non pathogènes du genre *Bacillus* et un composant prébiotique,

dans lequel lesdites espèces non pathogènes du genre *Bacillus* comprennent un mélange de *Bacillus subtilis, Bacillus licheniformis, Bacillus megaterium* et *Bacillus amyloliquefaciens,* chacune desdites quatre espèces de *Bacillus* ayant un nombre de spores compris entre 10 % et 70 %, par rapport au nombre total de spores desdites quatre espèces de *Bacillus,* et
dans lequel ledit composant prébiotique comprend au moins l'un d'un fructo-oligosaccharide, d'un galacto-oligosaccharide et/ou d'inuline,
dans lequel la surface est en bois, papier, cuir, caoutchouc ou textile ; dans lequel la surface est un matériau abiotique, ou dans lequel la surface est un mélange desdits matériaux précédemment mentionnés ;
ledit procédé comprenant les étapes de nettoyage, pulvérisation, nébulisation par ultrasons, peinture ou dosage en eau desdites surfaces avec ladite composition.

**2.** Procédé selon la revendication 1, dans lequel ledit mélange desdites espèces non pathogènes est présent à une concentration combinée de $1 \times 10^6$ à $1 \times 10^{10}$ cfu par gramme de composition.

**3.** Procédé selon l'une quelconque des revendications précédentes, dans lequel ledit composant prébiotique est présent à une concentration de 100 mg à 100 g par kg de composition.

**4.** Procédé selon l'une quelconque des revendications précédentes, dans lequel lesdites spores desdites espèces non pathogènes et ledit composant prébiotique sont au moins partiellement encapsulés dans des microcapsules.

**5.** Procédé selon la revendication 4, dans lequel ladite microcapsule comprend une coque friable et est en outre remplie d'une solution non aqueuse.

**6.** Procédé selon les revendications 4 ou 5, dans lequel le diamètre des microcapsules est de 1 à 300 μm.

**7.** Procédé selon l'une quelconque des revendications précédentes, dans lequel ladite composition est formulée comme un détergent, un produit de nettoyage, un liquide, une émulsion, un gel, une huile, une solution, un spray à gâchette, un spray aérosol, une poudre ou une formulation semi-solide.

Fig. 1

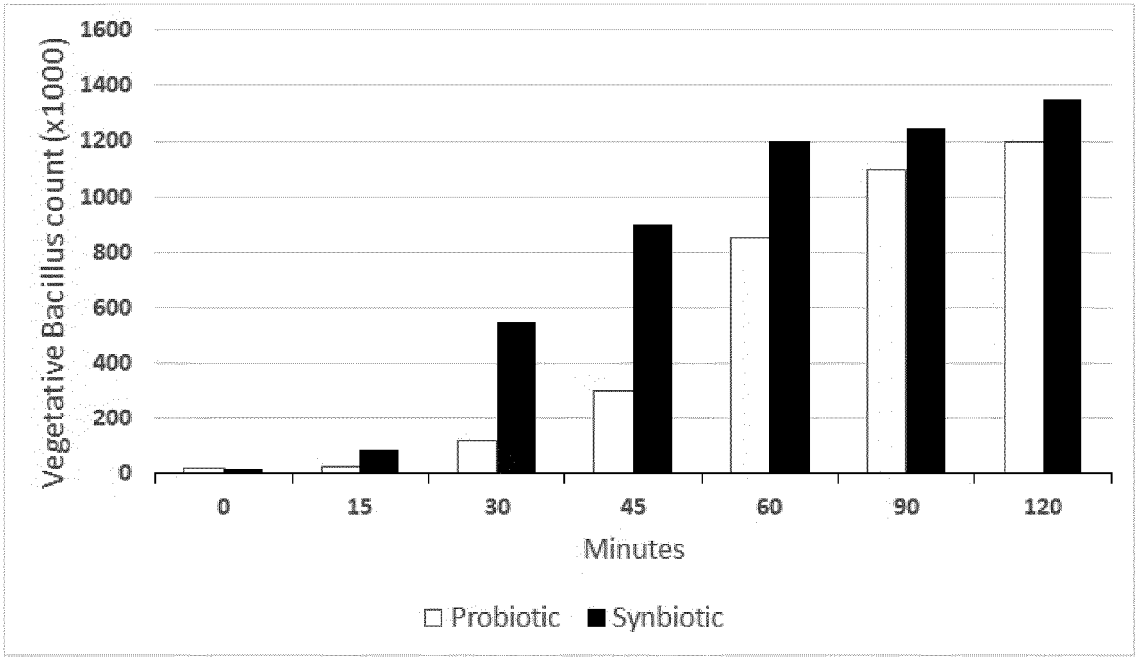

Vegetative Bacillus count (x1000)
1600
1400
1200
1000
800
600
400
200
0
0
15
30
45
60
90
120
Minutes
Probiotic
Synbiotic

Fig. 2A

Fig. 2B

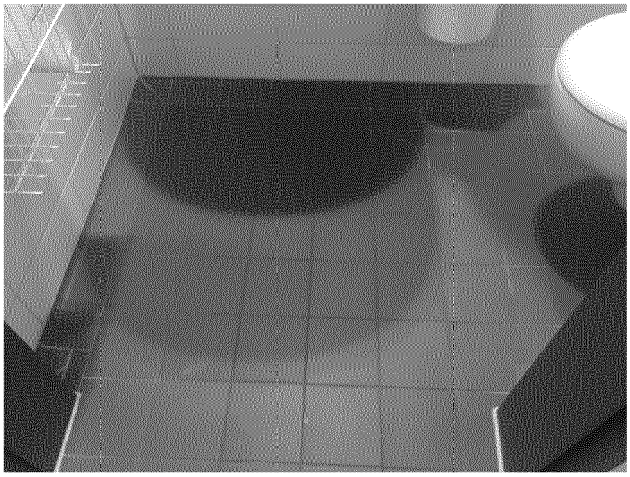


Fig. 2C

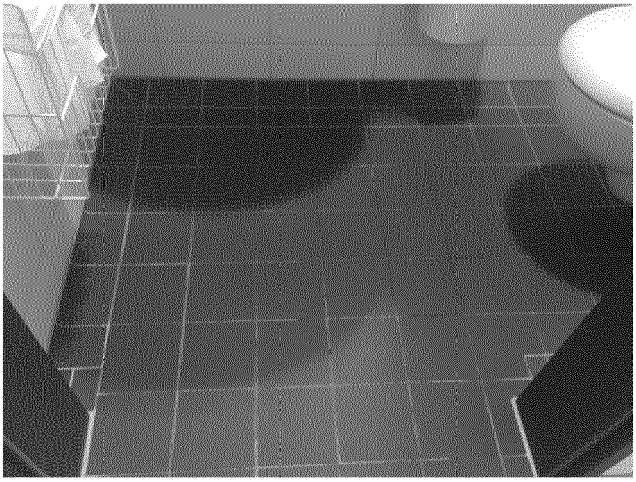


Fig. 3

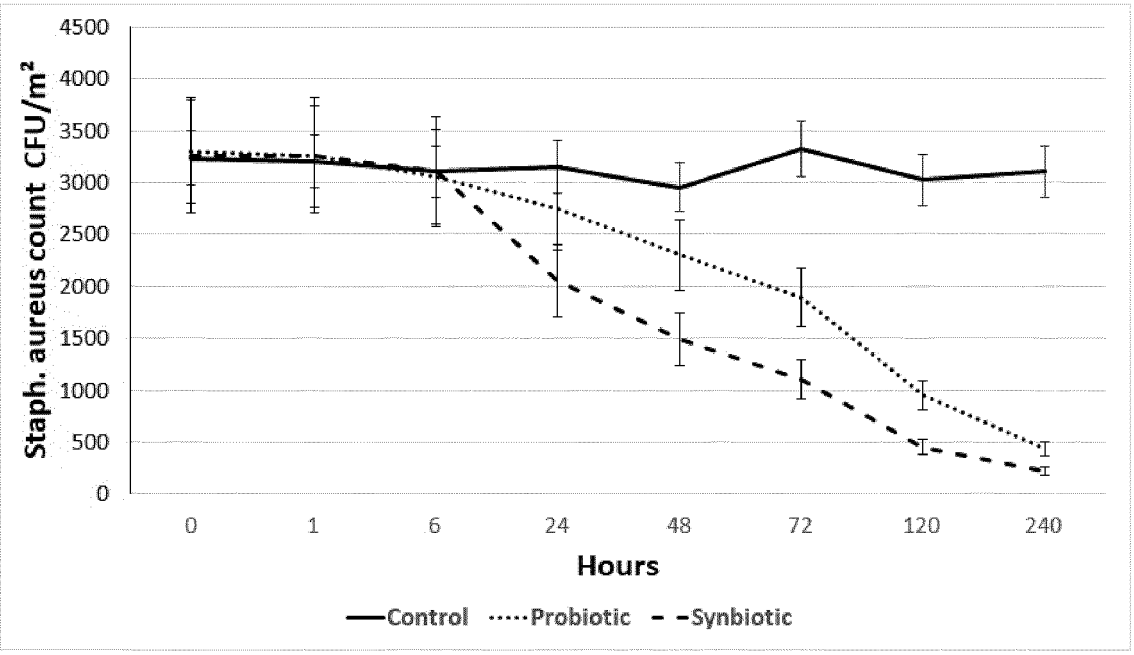

4500
4000
3500
3000
2500
2000
1500
1000
500
0
Staph. aureus count CFU/m²
0
1
6
24
48
72
120
240
Hours
Control
Probiotic
Synbiotic

Fig. 4

**Fig 4A**

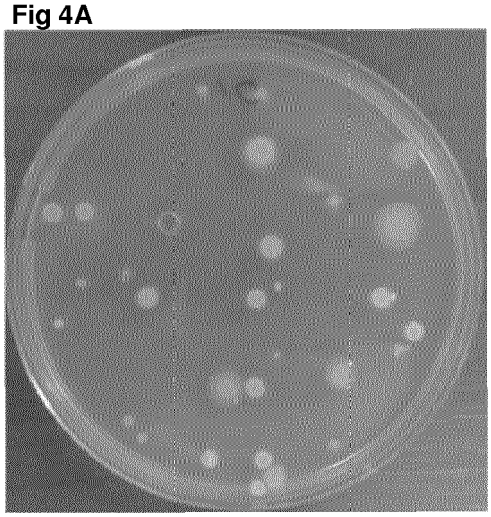


**Fig. 4B**

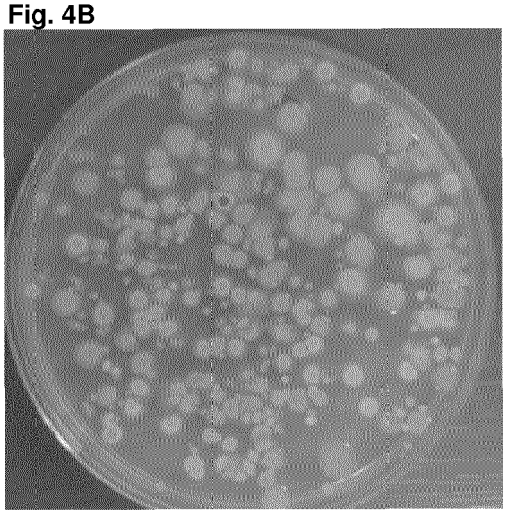


**Fig 4C**

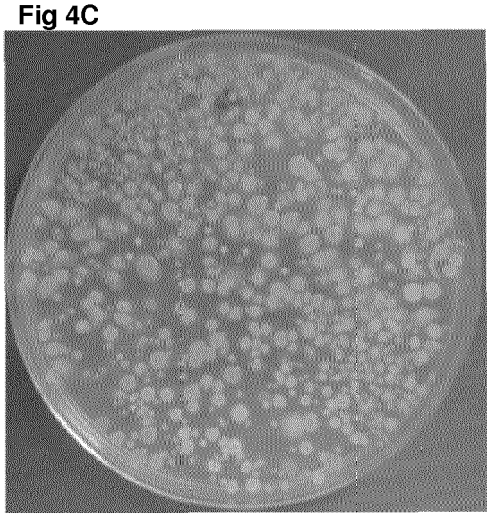
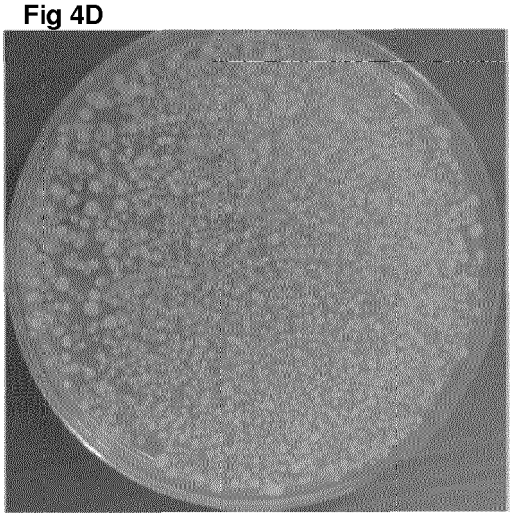


**Fig 4D**



## REFERENCES CITED IN THE DESCRIPTION

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