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(54) Title **Method for elimination of microorganisms**

(57) Abstract

The invention relates to a water inlet or outlet duct 10 with adjustable length for use on fish farms 20 and a fish farm 20 with such a duct. Furthermore, the invention concerns the installation on such a duct on a fish farm. The duct 10 5 includes an upper duct connection ring 16 secured water port 14 on the fish farming structure. A lower ring 13 forms a port at the end of the adjustable length water flow duct 10. The adjustable length water flow duct 10 allows the distance between the upper duct connection ring 16 and the lower ring 13 be varied. At least three external lifting wires 26 are secured on an external portion of the fish 10 farming water inlet or outlet duct 10. The at least three external lifting wires 26 are provided to pull the adjustable length water flow duct between an extended position and a retracted position. The at least three external lifting wires 26 are adapted to be secured to at least one wire pulling device on the fish farm.

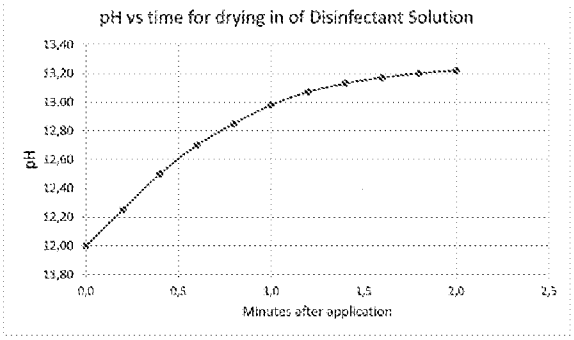

FIGURE 1

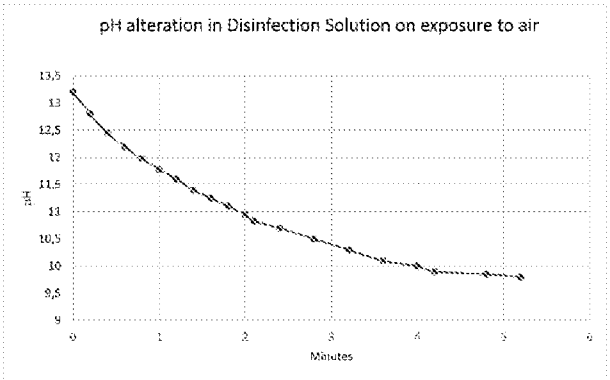

FIGURE 2

TITLE

Method for elimination of microorganisms

FIELD OF INVENTION

The present disclosure relates to a method for elimination of microorganisms on a substrate surface. More specifically, the disclosure relates to a method for elimination of microorganisms on a substrate surface as defined in the introductory parts of the independent claims.

BACKGROUND OF THE INVENTION

Removal of infective agents like bacteria, virus and fungi from surfaces like skin, benches, tables, handles, rails, armrests, car interior and similar, is important in hygienic control regimes. For these purposes, a variety of biocide agents has been introduced covering all aspects from intensive cleaning of hospital surgery units to daily disinfection of hand surfaces. For many purposes where human skin or mucous membranes are not involved, acting agents like chlorine- and other halogen-based compounds, quaternary ammonia compounds, strong detergents and various organic solvents are often used. For purposes like rinsing of human skin and more sensitive materials that should not be damaged, decolorized or in any other way damaged, less aggressive chemical substances or compositions are desirable. Among such disinfectant solutions frequently used are ordinary hand-soap, mildly acting detergent solutions, various alcohols or alcohol mixtures like ethanol, propan-2 ol, or a mixture of these. Also, low concentration hypochlorite solutions or other halogen-based solutions as well as low concentrations of various quaternary ammonia compounds and hydrogen peroxide are used.

Practical use of current biocides is often hampered by various disadvantages. Although ordinary hand-soap and detergent solutions are efficient, they can act toxic is swallowed, are irritant upon contact with eyes or mucous membranes, and require the presence of water and towels that often render it less convenient for the users' purposes. Likewise, solutions of alcohols are efficient, and although ready-made and more convenient than soap and water it is hampered by being highly flammable, toxic, irritant to eyes and mucous membranes, and is dehydrating skin and certain synthetic materials like «Skai» that is frequently used in car

interior. Alcohols and other organic solvents may also affect the quality of materials used in furniture, and related applications. Other biocide compositions like hydrogen peroxide and those containing halogen-based compounds may be irritant and toxic, even in low concentrations. Furthermore, they may cause discoloration and may also leave an unpleasant smell.

Applications using traditional soaps with an alkaline pH are also widely known. However, such soaps typically created a pH in the range 9.5-10.5 when mixed with water, and have their biocidal effect through the detergent fatty acids. Furthermore, soap-water solutions are not acting biocidal by drying in on the surfaces applied to, but is rather rinsed off with water.

The important factors desirable for disinfection methods are 1) efficient elimination of unwanted microorganisms, 2) ready-made solution that can conveniently be used at the site with no use of other items, 3) not causing damage or altering the surfaces it is used on, 4) non-irritant or non-toxic, and 5) environmentally friendly.

The inventor have noticed that such properties can be obtained by application of solutions of alkaline salts, preferably inorganic salts that are environmentally friendly, and when applied according to a distinct, but simple procedure.

Thus, there is still a need in the art to search for new disinfectant solutions and methods for removal of unwanted microorganisms from surfaces.

It may be seen as another object of the invention to wholly or partly overcome the disadvantages and drawbacks of the disinfective principles known from the prior art, particularly for applying a disinfectant solution for the described purpose that is user friendly, non-toxic, non-irritant, not flammable, and environmental friendly.

SUMMARY OF THE INVENTION

It is an object of the present disclosure to mitigate, alleviate or eliminate one or more of the above-identified deficiencies and disadvantages in the prior art and solve at least the above mentioned problem.

By the present invention it has surprisingly been found that applying an aqueous solution of preferentially inorganic salts, that are able to generate a pH in the range 11-12.5, to various surfaces like human skin, furniture, rails, benches and tables, car interior, handles; allowing said aqueous salt solution to dry in thereby creating a concomitant rise in pH, and thereafter turning non-irritant as the pH is lowered to <10 by contact with air.

Thus, a first aspect of the invention provides a method for elimination of microorganisms on a substrate surface comprising the following steps: i) applying to the substrate surface an aqueous salt solution of alkaline salts having a pH in the range of 11 to 12.5, ii) allowing the aqueous salt solution to dry in on the substrate surface, and optionally iii) exposing the substrate surface to air after the aqueous salt solution has dried in.

Thus, according to some embodiments, said alkaline salts are selected from the group consisting of metasilicate, orthosilicate, pyrosilicate, phosphate, hydroxide, carbonate, borate and any combinations thereof. Preferably, the metasilicates, orthosilicates, pyrosilicates, phosphates, or carbonates are present in concentrations of 0.5 – 25 mmol/L.

According to some embodiments, the cations of the alkaline salts are selected from the group consisting of sodium and potassium.

According to some embodiments, the aqueous salt solution further comprises sodium chloride to provide an osmolarity of 308 ± 50 mosmol/L.

According to some embodiments, the aqueous salt solution comprises Na_2SiO_3, Na_3PO_4 and NaCl. In a preferred embodiment, the aqueous salt solution comprises Na_2SiO_3 in a concentration ranging from about 5 to 15 mmol/L, Na_3PO_4 in a concentration ranging from about 1 to 2 mmol/L and NaCl in a concentration ranging from about 0.05 to 0.15 mol/L. In a particularly preferred embodiment, the aqueous salt solution consists of 8 mmol/L Na_2SiO_3, 1.5 mmol/L Na_3PO_4 and 0.1 mol/L NaCl. The aqueous salt solution is non-irritant to human skin.

According to some embodiments, the elimination of microorganisms are determined by a reduction in the microorganism count by at least 2 log, preferably at least 3 log, and most preferably at least 4 log as measured by European Standard EN 16615.

According to some embodiments, said microorganisms are selected from the group consisting of bacteria, fungi, and viruses.

The present disclosure will become apparent from the detailed description given below. The detailed description and specific examples disclose preferred embodiments of the disclosure by way of illustration only. Those skilled in the art understand from guidance in the detailed description that changes and modifications may be made within the scope of the disclosure.

Hence, it is to be understood that the herein disclosed disclosure is not limited to the particular component parts of the device described or steps of the methods described since such device and method may vary. It is also to be understood that the terminology used herein is for purpose of describing particular embodiments only, and is not intended to be limiting. It should be noted that, as used in the specification and the appended claim, the articles "a", "an", "the", and "said" are intended to mean that there are one or more of the elements unless the context explicitly dictates otherwise. Thus, for example, reference to "a unit" or "the unit" may include several devices, and the like. Furthermore, the words "comprising", "including", "containing" and similar wordings does not exclude other elements or steps.

In this context the term aqueous salt solution, disinfection solution and disinfectant solution are used interchangeably.

BRIEF DESCRIPTIONS OF THE DRAWINGS

The above objects, as well as additional objects, features and advantages of the present disclosure will be more fully appreciated by reference to the following illustrative and non-limiting detailed description of example embodiments of the present disclosure, when taken in conjunction with the accompanying drawings.

Figure 1 shows pH vs time for drying in the disinfectant solution on a surface.

Figure 2 shows pH alteration in disinfectant solution on exposure to air.

Figure 3 shows reduction in CFU after application of disinfectant solution on human skin.

DETAILED DESCRIPTION

The present disclosure will now be described with reference to the accompanying drawings, in which preferred example embodiments of the disclosure are shown. The disclosure may, however, be embodied in other forms and should not be construed as limited to the herein disclosed embodiments. The disclosed embodiments are provided to fully convey the scope of the disclosure to the skilled person.

The invention relates to a method for removal of unwanted microorganisms from surfaces, and in particular surfaces that are sensitive to more aggressively acting chemicals, applying an aqueous salt solution. Furthermore, the invention seeks to avoid the use of disinfectant chemicals that produce unpleasant, irritant or even toxic gases. Surfaces particularly useful for disinfection according to the invention are, but not limited to, human or animal skin, skin based products like leather, synthetic materials like car interior materials commonly known as «Skai», surfaces in areas for human or veterinary medical and/or dental procedures, areas and machinery for food production, and restaurants and other publically visited areas like public transport vehicles, meeting rooms or exhibition facilities. The method may also be useful for surface disinfection of fruits, vegetables and berries.

In comparison, methods applying commonly used disinfectants are hampered by various disadvantages like creating irritant reactions to skin and mucous membranes, toxic effects to animals and humans, damaging surface materials, and generating irritant or even toxic vapours and gases.

Thus, the object of the current invention is to providing a method that lacks most of the disadvantages related to application of disinfectant media in the prior art, and still be able to remove unwanted microorganisms from various surfaces.

Thus, the first aspect of the invention is directed to a method for elimination of microorganisms on a substrate surface comprising the following steps: i) applying to the substrate surface an aqueous salt solution of alkaline salts having a pH in the range of the 11 to 12.5; ii) allowing the aqueous salt solution to dry in on the substrate surface; and optionally iii) exposing the substrate surface to air after the aqueous salt solution has dried in.

It is evident that the method according to the invention is most efficient when the aqueous salt solution is allowed to dry in. In particular, this may be a precaution for efficient elimination of more biocide resistant Gram+ bacteria like those of the Staphylococcus strain. Thus, as part of the invention is a particular use of the aqueous salt solution by allowing it to dry in after application to surfaces.

The aqueous salt solution applied is preferably based on inorganic salts commonly occurring in the nature, able to generate a pH in the range 11-12.5. Particularly useful inorganic salts are metasilicates, orthosilicates, pyrosilicates, phosphate, hydroxide, carbonate, borate and any combinations thereof. Preferably, metasilicates, orthosilicates, pyrosilicates and phosphate combined with hydroxide ions to arrive at the desired pH may be useful. It should however, be understood that also any other salts with similar properties are useful. Thus, also quaternary organic amino salts can be applied. In principle, any cationic counter ions can be used for the above-mentioned salts, but sodium and potassium ions are in particular useful. Thus, examples of salts that can be used in the solution are, but not limited to, Na_2SiO_3, K_2SiO_3, Na_4SiO_4, K_4SiO_4, $Na_6Si_2O_7$, $K_6SiO_2O_7$, Na_3PO_4, K_3PO_4, NaOH, KOH, Na_2CO_3, K_2CO_3. Preferably, metasilicates, orthosilicates, pyrosilicates and phosphate combined with hydroxide ions to arrive at the desired pH may be useful.

In order to create a disinfection method with limited irritant consequences, the present invention takes advantage of the property that the aqueous salt solution applied is gradually neutralised when exposed to certain surfaces or to air, and when the above mentioned salts in the solution are preferably used in concentrations of 0.5 – 25 mmol/L, although the invention is not limited to application of aqueous salt solution with such concentrations.

In preferred embodiments, the aqueous salt solution comprises Na_2SiO_3, Na_3PO_4 and NaCl. In a preferred embodiment, the aqueous salt solution comprises Na_2SiO_3 in a concentration ranging from about 5 to 15 mmol/L, Na_3PO_4 in a concentration ranging from about 1 to 2 mmol/L and NaCl in a concentration ranging from about 0.05 to 0.15 mol/L. In a particularly preferred embodiment, the aqueous salt solution consists of 8 mmol/L Na_2SiO_3, 1.5 mmol/L Na_3PO_4 and 0.1 mol/L NaCl.

A further aspect of proving a method for disinfection with a minimum irritant action is to apply an aqueous salt solution where a further inorganic salt, preferentially sodium chloride, is added to provide an isotonic solution targeting 308 mosmol/L or an almost isotonic solution of

308 ± 50 mosmol/L. Application of such salt compositions prove to be almost non-irritant, even in contact with mucous membranes. Thus, in a preferred embodiment the present invention relates to an aqueous salt solution further comprising sodium chloride to provide an isotonic salt solution, i.e. to provide an osmolarity of 308 + 50 mosmol/L.

The method of the invention prove to have very favourable properties. **Figure 1** shows the development of pH after distribution to a surface. The version of disinfectant solution used in this case has the composition of 8 mmol/L Na_2SiO_3, 1.5 mmol/L Na_3PO_4 and 0.1 mol/L NaCl. As the aqueous salt solution dries in, the pH rise to about 13 when the least measurable volume is left (about 10%), and probably rise even more just before the aqueous salt solution dries in.

A long-lasting exposure of human skin to such levels of pH would for most circumstances be regarded harmful. However, a patch-test where the disinfection method is used on human skin proves that the procedure surprisingly could be classified «non irritant».

Figure 2 shows how pH of the aqueous salt solution develops over time. Starting with a version of the aqueous salt solution concentrated 10 times the composition described above, i.e. 8 mmol/L Na_2SiO_3 , 1.5 mmol/L Na_3PO_4 and 0.1 mol/L NaCl, this mimics the situation when the disinfectant solution is dried in to 10% of the original volume, and the pH when newly mixed could be measured to 13.1. When 5 mL of this concentrated aqueous salt solution is left in a petridish to generate a large surface exposed to air, pH in the solution surprisingly drops rapidly and after about 20 minutes flattens out at about pH 9.5, a pH-level considered harmless for most circumstances.

Although not wishing to be bound by any theory, it is believed that the entire effect of applying the aqueous salt solution is that it gives a temporary rise in pH sufficient to kill most microorganisms, followed by a subsequent drop in pH rendering the disinfectant solution at a harmless pH-level after short time.

The invention relates to the method of applying an aqueous salt solution for removal of microorganisms. For application to various surfaces like human skin, furniture, rails, handles, the maximum disinfectant properties of the method is obtained when allowed to dry in on the surfaces applied to. This procedure creates a certain rise in pH, efficiently eliminating microorganisms, and thereafter surprisingly turning non-irritant as the pH lowers to <10 by contact with air and/or surfaces like skin. Thus, a test on human skin applying the aqueous salt

solution at a pad to 20 volunteers rendered no skin damage or irritant reactions even after days covered by a plaster. Thus, the aqueous salt solution provided is non-irritant to human skin.

The method according to the invention applying the aqueous salt solution efficiently eliminates microorganisms such as bacteria, fungi, and viruses. Examples of viruses are all enveloped viruses thus including, but not limited to, adenovirus, norovirus, rotavirus and coronavirus. Furthermore, the method efficiently eliminates bacteria from strains like Escherichia, Staphylococcus, Pseudomonas, Salmonella, and Vibrio by $\geq$ log 5. Furthermore, the method efficiently eliminates fungi such as fungi from the strain Candida by log $\geq$ 5. When considering the general meaning of the term «removal of unwanted microorganisms» it is not evident that any complete removal is obtained. Elimination of microorganisms will rather be considered as a reduction in microorganism count by at least 2 log, preferably at least 3 log, and most preferably a least 4 log as measured by the European Standard EN 16615. Thus, in preferred embodiments, the elimination of microorganisms are determined by a reduction in the microorganism count by at least 2 log, preferably at least 3 log, and most preferably at least 4 log as measured by European Standard EN 16615.

It is evident that the method according to the invention is most efficient when the aqueous salt solution is allowed to dry in. In particular, this may be a precaution for efficient elimination of more biocide resistant Gram+ bacteria like those of the Staphylococcus strain. Thus, as part of the invention is a particular use of the aqueous salt solution by allowing it to dry in after application to surfaces.

The concept of «drying in» shall generally mean that in the context of this invention a visual inspection of a surface where the method is applied to, is not rendering any visible liquid. However, common understanding of the term «dry» does not necessarily mean «water free» since it is not likely that water can be completely removed from a surface whereto the method according to the invention is applied, because the remaining salt layer of the treated surface will necessarily be exposed to air that for all practical purposes will contain a certain level of water vapour. «Drying in» will for most purposes occur within two minutes after application of the aqueous salt solution to a surface. However, it is evident that the time for drying in will depend on many factors like amount of liquid applied to a given surface area, the temperature, air moisture, ventilation a.s.o.

Certain applications tested did not include a step of drying in of the aqueous salt solution, demonstrating that after prolonged exposure of the microorganisms in an aqueous environment the aqueous salt solution even so had significant effect on the microorganism counts. However, application of the aqueous salt solution to a surface will not allow any long standing exposure to an excess of this solution, and the effect of the aqueous salt solution must be potentiated through letting it dry in to rise the pH.

The concept of «application to a surface» is generally meaning that the aqueous salt solution is evenly distributed on the desired part of a surface where microorganisms should be removed, so that the entire surface is covered. The application of the aqueous salt solution may be performed by any convenient means like spraying, clothing, swabbing, dipping etc.

The invention will now be further illustrated with reference to the following non-limiting examples.

EXAMPLES

Example 1

An alkaline aqueous salt solution was made by adding to water Na_2SiO_3 to arrive at a concentration of 8 mmol/L, Na_3PO_4 to arrive at a concentration of 1.5 mmol/L and 0.1 mol/L NaCl. This aqueous salt solution had a pH of 12.0 $\pm$ 0.1.

Unless otherwise remarked, this version of the aqueous salt solution was used in the further examples.

Example 2

A test demonstrating the effect of drying in of the aqueous salt solution for maximum effect on certain bacterial species, was performed. The standard protocol for antiseptics EN 16615 «4-Field Test» was applied for this experiment. The bacteria species tested were

1. Stapholycoccus aureus ATTC 25923
2. Escherichia coli ATTC 25922
3. Candida africana

The bacteria were cultivated in LB medium overnight at 37°C while shaking. 0-samples and stock solutions were seeded for each experiment.

1. For each bacterial species tested, two parallel squares of 5X5cm^2 were marked on a surface of stainless steel. Each square was inoculated with 50µl, approx. 1 x 10^9 CFU and allowed to dry for about 1h.

2. Thereafter one square was sprayed with sufficient disinfectant solution to allow it stay liquid for at least 15 minutes, whereas the other square was sprayed with less disinfectant solution rendering this square dry after about 5 min.

3. Shortly thereafter, and then after 1, 5, 10, and 15 min, samples were absorbed from each square by means of a cotton swab. Two cotton swabs and a tube with bovine serum albumin, BSA, were used per sampling. Firstly, a cotton swab saturated with BSA-solution was rubbed over a square and then placed in 10ml BSA solution by cutting off the stick end. Furthermore, a dry cotton stick was rubbed over the same square and its tip was cut off and placed in the same tube as above. BSA neutralised the further effect of the aqueous salt solution. The tube was shaken for 15 seconds. 100 µl of this suspension was cultivated on blood agar plates and incubated at 37°C over night before counting the colonies.
The results are shown in table1 below:

Table 1

	CFU* applied	**CFU in liquified square after 15 min**	**CFU in dry square**
Staphylococcus aureus	3 x 10^{10}	overgrowth	2.2 x 10^5
Escherichia coli	2 x 10^9	15 x 10^3	30 x 10^3
Candida africana	3 x 10^9	30 x 10^3	4 x 10^3

* Colony Forming Units

To pass the test according to the standard EN 16615 «4-Field Test», at least a 5 log reduction of bacteria (S.aureus and E.coli) or at least a 4 log reduction of fungi (Candida) should be obtained. As can be seen from the table, this requirement is fulfilled. However, it can be seen that for a more biocide resistant G+ bacteria like S.aureus, a full effect is most likely obtained after the aqueous salt solution is allowed to dry in.

Example 3

The aqueous salt solution was tested for its effect on bacteria on human skin, according to the same test principle as described in Example 2. A square of 5X5cm^2 was drawn on the palm of the inventor, and the square area was inoculated with 50µl suspension of Staphylococcus

aureus. After the bacterial culture had dried in, the aqueous salt solution was sprayed on to the square, and samples were taken by a cotton swab immediately after application and the subsequently after 1, 2, 3, 4 and 5 min. The palm was considered dry after about 2-3 minutes. The samples were further handled and cultivated as in Example 2.

The CFU of the stock solution applied was 3×10^{10}. After 5 min at the palm, when completely dried in, the CFU was 9×10^{5}, thus rendering a CFU reduction close to 5 log.

The reduction in CFU after application of disinfection solution is set forth in Figure 3.

Example 4

Test on effect on bacteria in unclean water was performed according to the method /criteria given in NS-EN ISO 6222-1 and NMKL 125 -4. Water from a slightly polluted creek was mixed 1:1 with the aqueous salt solution of the invention, incubated at 20°C for five minutes and subsequently tested for colony-forming bacteria after application to blood agar plates and incubation at 37°C overnight. The test showed that >99% of unspecified, cultivable, colony-forming, bacterial species were eliminated. The test shows that the aqueous salt solution has significant anti-microbial properties when allowed to be exposed to bacteria over time, and without applying the drying in procedure that is considered necessary on short time exposure.

Example 5

Test on effect on bacteria on a laminated laboratory bench plate surfaces was performed by applying a thin layer of a liquid suspension of the bacterium Escherichia coli to the actual surface, and allowed to dry. Distinct segments areas of the polluted bench surface was then treated by 1) No washing; 2) Washing with tap water; and 3) Washing by application of the aqueous salt solution that was allowed to dry in. The respective surface areas were thereafter tested in to parallels with petrifilm for colony forming bacteria as shown in Table 2 below:

Table 2

Test petrifilm	Colonies	Effect according to Standard
Unwashed surface	>300	Not acceptable
Surface washed with tap water	127 (average)	Not acceptable
Surface treated with aqueous salt solution	1	Very good effect

Example 6

Aqueous salt solution and a control liquid (0.9% NaCl) were added to bacterial cultures to 20% v/v. The bacterial cultures contained $2.5 – 5.7 \times 10^{6}$ bacteria/mL. The cultures were left at 20°C for 3, 6 and 24 hours and subsequently tested for colony forming bacteria on agar plates. The control culture added only 0.9% NaCl increased in bacterial numbers, as expected. The other

samples added aqueous salt solution demonstrated a marked reduction of living bacteria as shown in table 3 below.

Table 3

Bacterium sp.	Log reduction in colonies after addition of aqueous salt solution		
	3 hours	6 hours	24 hours
Escherichia coli	>5	>5	>5
Pseudomonoas aeruginosa	>5	>5	>5
Salmonella sp.	>5	>5	>5
Staphylococcus aureus	5	>5	5.3
MRSA	3.4	>5	>5
Vibrio parahaemolyticus	>5	>5	>5

There was consequently a significant, and for practical purposes, a full elimination of the bacteria tested after treatment of the cultures with aqueous salt solution also without drying in on a substrate, when the exposure time was sufficiently long.

Example 7

Antiviral effect of the aqueous salt solution was tested.

Firstly, any cytotoxic effect of the disinfectant solution under the experimental conditions to be used was determined on the eukaryotic cells: Human epithelial type 2 (Hep-2) cells; Human Rhabdomyosarcoma cells (RD); RAW 264.7 macrophage (ATCC). Dilutions 10^{-1} to 10^{-8} of the disinfective solution in culture medium with 3.0 g/L BSA plus 3,0 ml/l erythrocytes were incubated in ice-cold water for 30 min and then 100 µL of each dilution were inoculated onto monolayers of Hep-2, RD and RAW cells in the wells of culture plates. Any microscopic changes in the cells after 5-days incubation were recorded.

No cytotoxic effect was observed on Hep-2, RD and RAW cells in all dilutions of the Disinfective Solution under the described conditions thus rendering the experiments with the Disinfective Solutions effects on virus possible.

The anti-viral test was performed according to 14476:2013+A2:2019: "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase2/Step 1)".

The viruses tested were:
Adenovirus type 5 (ATCC VR-5); and Murine Norovirus (Strain S99 Berlin)

The cells used for viral infection were:
Human epithelial type 2 (Hep-2) cells; Human Rhabdomyosarcoma cells (RD);

Principle of the test: A 97% dilution of the aqueous salt Solution was added to a test suspension of titrated viruses in bovine serum albumin solutions of 3.0 g/L plus 3,0 ml/l erythrocytes (dirty conditions). The mixtures were maintained at 20°C for 3 min. At the end of contact time, an aliquot was taken and the virucidal activity was suppressed by dilutions in ice-cold maintenance medium. The dilutions were then inoculated onto cell monolayers in 96-well culture plates for the titration of the remaining viruses. The titers of the viruses expressed in TCID50 values, after 5-days incubation, were determined and expressed in log scale. Reduction of the viruses' infectivity was calculated from the differences of the log virus titers before (control) and after treatment with the product. According to the EN 14476 standard a product has antiviral activity when the reduction of the virus is at least 4 log.

The viruses were propagated in the appropriate cell culture system to produce a high titer: Hep-2 monolayers for adenovirus titration, and RAW cell monolayers for M.norovirus titration. Each virus was tested in decimal dilutions 10^{-3} up to 10^{-10}. Each dilution was inoculated 10x in wells of 96-well culture plates with the appropriate cell monolayer. The infected cells were incubated at 37°C in a 5% CO_2 atmosphere for 5 days. The Tissue Culture Infectious Dose (TCID50) i.e. the infection dose of a virus suspension inducing a Cytopathic Effect (CPE) in 50% of cell culture units was estimated by the end-point Spearman-Karber method:

$$\text{Log TCID50} = L - d(S - 0.5),$$

where L is the highest virus concentration used, d is the log difference of dilutions, S is the sum of % affected (CPE) at each dilution.

By using of the Spearman-Karber formula on the aforementioned CPE results, the calculated TCID50 of the Adenovirus, and the Norovirus strains were $10^{-7.9}$ and $10^{-7.5}$ respectively. Taking into account the standard error of the above calculation, the titers of the three strains used in the tests were

Initial titer of Adenovirus type 5	Log TCID50/0.1mL = 7.9±0.163
Initial titer of Murine norovirus	Log TCID50/0.1mL = 7.5±0.141

Formaldehyde 0.7% (w/v) was included as reference for test validation. Cytotoxicity test as well as antiviral activity determination was performed on RD cells using serial dilutions of up to 10^{-8} of the aforementioned formaldehyde test solution. Contact times were 30 min and 60 min.

The antiviral activity of the product against the adenovirus, and murine norovirus strains was determined for 3 min at 20±1 °C in 3.0 g/L (dirty conditions). Immediately at the end of contact time, a 1/10 dilution was made in ice-cold cell maintenance medium and 30 min later, subsequent serial dilutions (step 1:10) were inoculated onto cell culture monolayers. After incubation, the titer of each virus was calculated, and the reduction of the virus infectivity was determined from the log differences of virus titers before and after treatment with the product.

A sufficient antiviral activity of the aqueous salt solution would require a reduction of at least 4 log of the viruses tested. The test demonstrated: a 5.2 log reduction of the Adenovirus type

5 (ATCC VR-5), a ≥6.0 log reduction of the Murine Norovirus (Strain S99 Berlin). The disinfectant solution thus demonstrated antiviral activity according to EN14476 against the viruses tested. According to the EN 14476 standard, products that have antiviral activity against the adenovirus and the murine norovirus are considered active against all enveloped viruses further also including, but not limited to, rotavirus and coronavirus. This effect is thus taking place also without the "drying-in-step» which renders it likely that when drying in is performed, the viral elimination effect will be even more abundant than shown in this example.

Example 8

A test on assessment of skin tolerance of the aqueous salt solution was performed. After a single application of aqueous salt solution followed by 48 hours occlusive patch on 20 human volunteers was conducted in accordance with all international ethical standards and regulations, and in particular the COLIPA Guidelines edited on 1997 for the “Product Test Guidelines for the Assessment of Human Skin Compatibility”.

Patch test is the assessment of skin tolerance of a product that normally will be used for skin applications, and involves a single application during 48 hour occlusive patch test. Negative controls are used to facilitate evaluation. Treatment sites are assessed before the first application of the test material (baseline), after treatment at 30 minutes, upon patch removal and at 24 and 48 hours after patch removal. The patch is applied on the skin for 48 hours. Skin reactions are scored throughout the test by the same experienced assessor, a dermatologist, who made the baseline assessment and under the same lighting source, following a pre-defined scoring scale. The quantification of the skin irritation is given through a numeric scale (erythema, oedema, dryness/desquamation, vesicles). The average irritant score of the product tested is calculated from the average of the reactions obtained for each volunteer, allowing to rank the product from "non irritant to very irritant" as shown in Table 4 below.

Table 4

Average irritation index	Classification
0 - 0.08	Non irritant
0.08 - 0.16	Very slightly irritant
0.16 - 0.56	Slightly irritant
0.56 - 1	Moderately irritant
1 - 1.16	Irritant
> 1.16	Very irritant

Of the 20 subjects studied, 10 were considered having «normal skin», whereas 10 were considered having «sensitive skin». Testing of the aqueous salt solution resulted in an average irritant score of the product at 0.03. The conclusion thus was that the method of the current invention can be considered as «Non irritant» regarding its primary skin tolerance.

Example 9

The potential effect of application of the aqueous salt solution to a variety of materials was investigated in order to identify potential damages, discolouration or in other ways other unacceptable alterations when applied the Disinfectant Solution was applied to:

Car interior (Skai)
Natural leather
Coloured leather
Coloured wool
Coloured cotton
Coloured acryl
Coloured viscose
Coloured polyester

Aqueous salt solution was sprayed on distinct areas of each test-material in doses of 2 x 1 mL, and allowed to dry in. A control solution with 0.9% NaCl was applied next to the aqueous salt solution. The test-materials were then left at room temperature. Visual inspections of the materials were performed daily to look for any changes to colour or texture.
After two weeks, none of the materials tested showed any sign of alteration thus indicating the procedure is safe to use on a variety of surfaces and materials.

CLAIMS

1. A method for elimination of microorganisms on a substrate surface comprising the following steps: i) applying to the substrate surface an aqueous salt solution of alkaline salts having a pH in the range of 11 to 12.5, ii) allowing said aqueous salt solution to dry in on the substrate surface, and optionally iii) exposing the substrate surface to air after the aqueous salt solution has dried in.

2. The method according to claim 1, wherein said alkaline salts are selected from the group consisting of metasilicate, orthosilicate, pyrosilicate, phosphate, hydroxide, carbonate, borate and any combinations thereof.

3. The method according to claim 1, wherein the cations of the alkaline salts are selected from the group consisting of sodium and potassium.

4. The method according to any of the preceding claims, wherein the aqueous salt solution further comprises sodium chloride to provide an osmolarity of 308 $\pm$ 50 mosmol/L.

5. The method according to any of the preceding claims, wherein the aqueous salt solution comprises Na_2SiO_3, Na_3PO_4 and NaCl.

6. The method according to claim 6, wherein the aqueous salt solution consists of 8 mmol/L Na_2SiO_3, 1.5 mmol/L Na_3PO_4 and 0.1 mol/L NaCl.

7. The method according to claim 1, wherein the elimination of microorganisms are determined by a reduction in the microorganism count by at least 2 log, preferably at least 3 log, and most preferably at least 4 log as measured by European Standard EN 16615.

8. The method according to claim 1, wherein said microorganisms are selected from the group consisting of bacteria, fungi, and viruses.

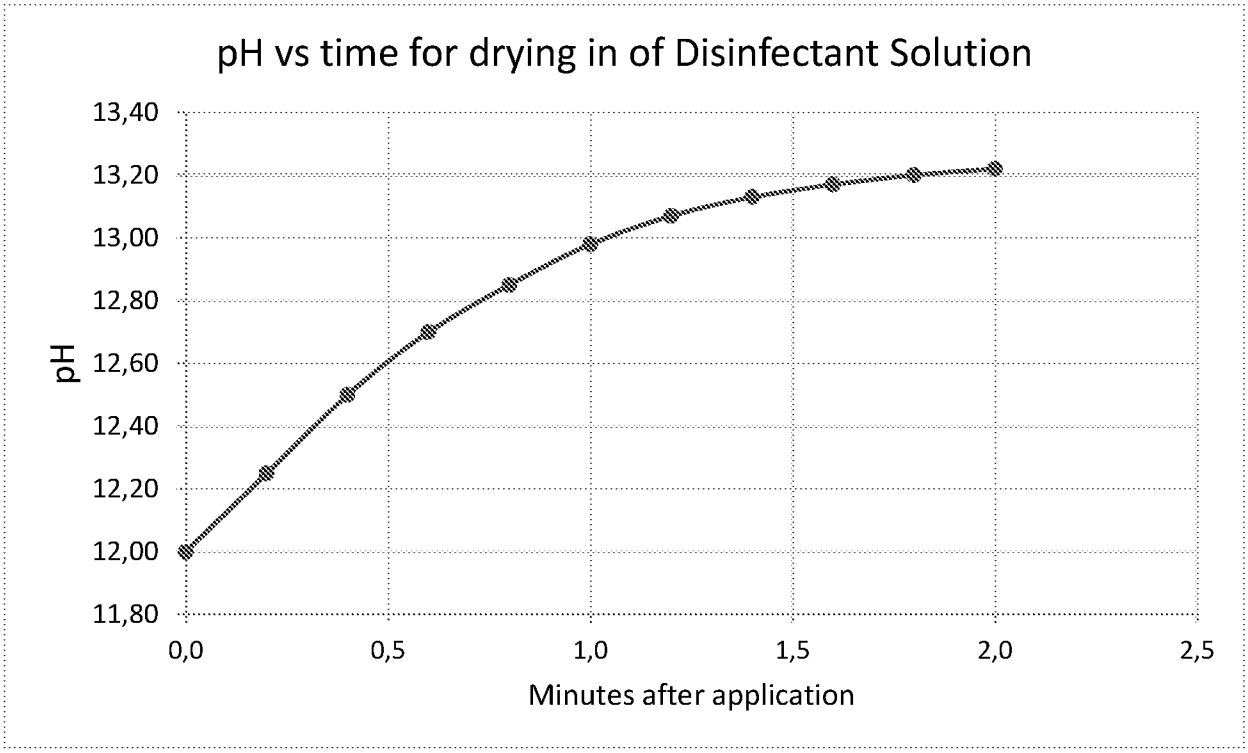

FIGURE 1

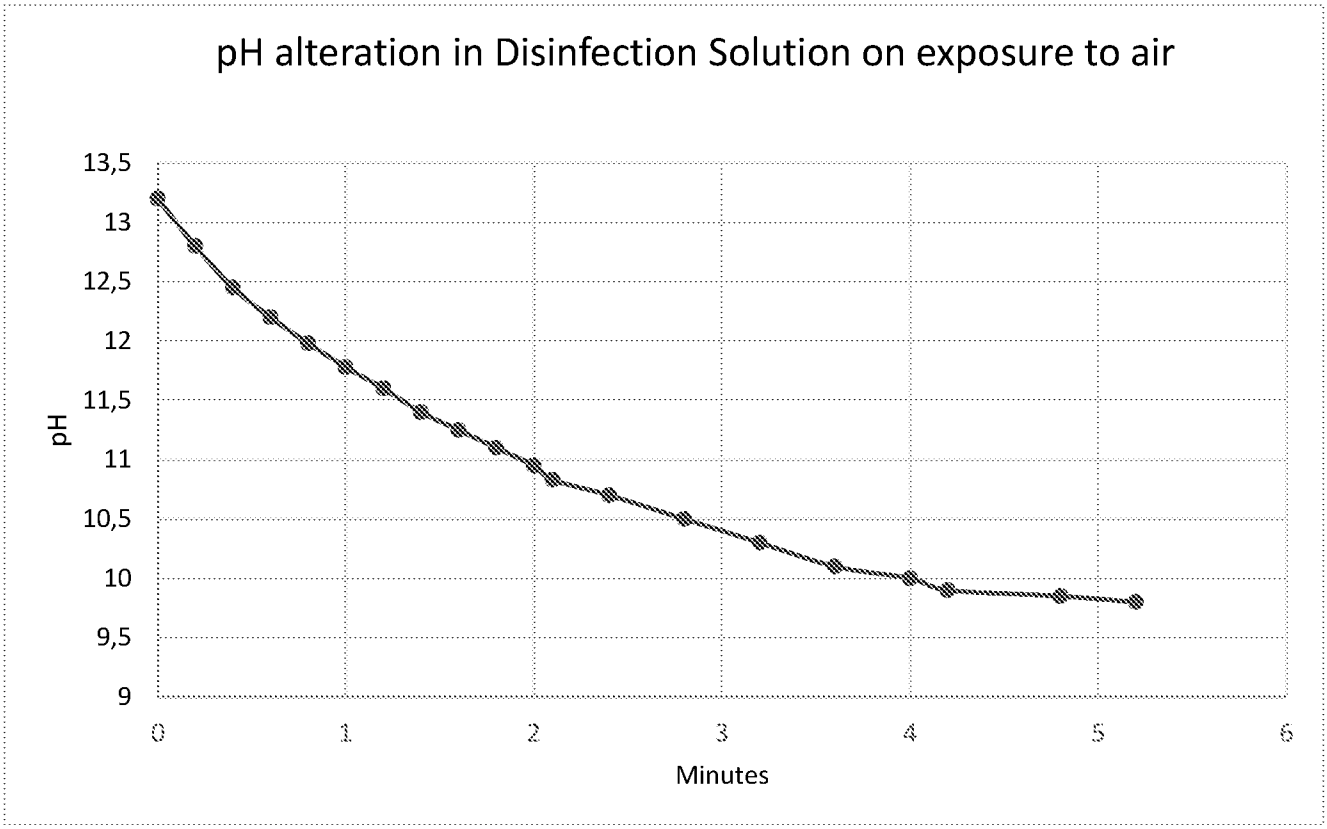

FIGURE 2

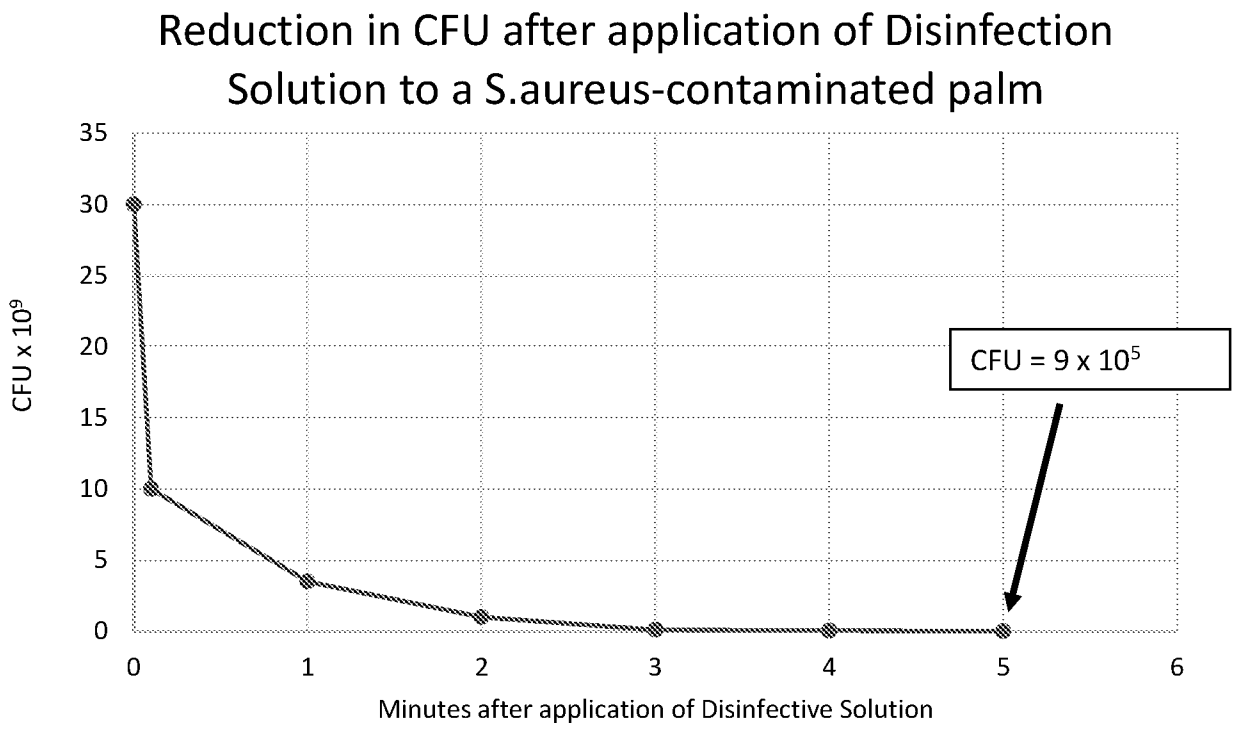
Reduction in CFU after application of Disinfection Solution to a S.aureus-contaminated palm
CFU x 10^9
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
CFU = 9 x 10^5
Minutes after application of Disinfective Solution

FIGURE 3