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(54) CLEANING PREPARATION FOR SURFACES AS FLOORS, GLASS WALLS, OBJECTS AND THE LIKE, AND METHOD FOR CLEANING SUCH SURFACES

(57) A cleaning preparation for surfaces as floors, walls, glass walls, objects and the like, comprising at least an enzyme provided for degrading organic substances which can be anchored to the surface to be cleaned and/or to settlings present on the surface to be cleaned, and at least an immobilizing agent for immobilizing and maintaining the activity of said enzyme over time following its anchoring to the surface and/or to settlings present on such surface, so as to extend the degradation activity of the organic substances.

A method for cleaning surfaces such as floors, walls, glass walls, objects and the like is also proposed.

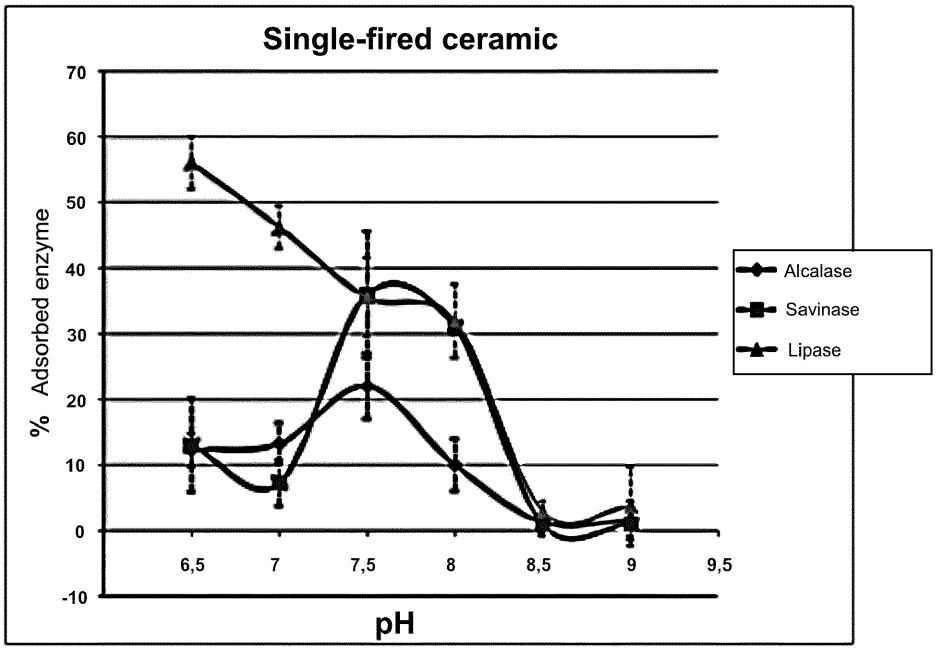

FIG. 1

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Description

TECHNICAL FIELD OF THE INVENTION

[0001] The present invention relates to a cleaning preparation for surfaces such as floors, walls, windows, objects and the like, and to a method for cleaning such surfaces.

PRIOR ART

[0002] As is known, the activities for cleaning surfaces such as floors, walls, windows, objects and the like and/or the layering that may possibly be present on such surfaces in general, provide for the use of cleaning products and/or disinfectants of various types, which are distributed over the surface to be cleaned and/or on the layers present on such a surface to remove both organic (fats, proteins, sugars, etc.) and inorganic substances that generally make up the accumulations of dirt, as well as to eliminate any bacterial contamination formed on the surface itself (and which typically proliferates on the above accumulations of dirt).

[0003] However, the standard detergent and disinfectant products carry out their action mostly at the time of the application, i.e. they remove dirt and bacteria already present in a short time.

[0004] The cleaning systems applied today provide, in most cases, at least three daily applications of cleaning preparations with a high content of surfactants and/or molecules having a detergent and/or disinfectant effect.

[0005] This frequency of application is due to the fact that, subsequently to the cleaning action, the organic and/or inorganic substances mainly forming the dirt and the consequent growth of pathogens harmful to health from the external environment begin to contaminate the same surfaces again.

[0006] These surfaces are therefore passive to such an accumulation, with the result of the deterioration of the environmental health conditions.

[0007] Therefore, it becomes necessary again to proceed with the cleaning operations at the end of the check of potentially contaminant agents several times during the day.

[0008] As a reference for the definition of cleanliness of an area and/or a surface, a maximum of 10 cfu/cm^2 (colony forming units per square centimeter) is considered in the established practice.

[0009] Above this level, the surface is considered dirty, while below this level the surface is considered clean. Moreover, in order to obtain an effective cleaning it is necessary to carry out a rinsing step which allows removing the residues of dirt and bacteria, but it also inevitably causes the removal of the detergents themselves.

[0010] In fact, in most cases, in order to carry out their dirt removal action, the cleaning agents and disinfectants normally used require the rinsing step, since their action is simply to make the substances forming the dirt water-soluble, and do not show any chemical-physical feature that may degrade (i.e. break) the molecules forming the dirt.

[0011] Furthermore, normally it is absolutely necessary to remove the substances contained in the detergents and/or disinfectants, since residues of such substances on the surfaces and/or layers present on such surfaces impart a non-clean appearance to the same surfaces. Therefore, it is clear that in order to ensure a constant and prolonged cleaning of the surfaces of interest, it is necessary to periodically (even several times per day) apply the products on the surfaces and then rinse them, thereby resulting in a high consumption of detergents/disinfectants as well as of water.

OBJECTS OF INVENTION

[0012] The technical task of the present invention therefore is to improve the prior art.

[0013] Within this technical task, an object of the present invention is to solve the drawbacks described above by providing a cleaning preparation which allows reducing the number of operations required to obtain the optimum cleaning of the surface of interest.

[0014] Another object of the present invention is to provide a cleaning preparation which allows reducing the consumption of materials, in particular water and chemical substances, at least having the same results obtained on the surfaces concerned.

[0015] A further object of the invention is to provide a cleaning preparation which ensures high reliability of operation over time on the surfaces on which it is applied and/or on the layers present on such surfaces.

[0016] Yet another object of the present invention is to provide a cleaning preparation which is cost-effective and safely applied.

[0017] A further object of the present invention is to provide a cleaning preparation which is easily obtainable starting from elements and materials commonly commercially available.

[0018] Last but not least, another object of the present invention is to also develop a method for cleaning surfaces such as floors, walls, windows, objects and the like which allows reducing the number of manual and/or mechanical interventions, for the temporary removal of dirt and of the resulting proliferation of agents potentially harmful to health.

[0019] This task and these objects are achieved by a cleaning preparation according to the appended claim 1, and by a cleaning method according to the appended claim 10.

[0020] The cleaning preparation according to the invention comprises at least one enzyme responsible for the degradation of organic substances which can be anchored to the surface to be cleaned and/or to the layers present on the surface to be cleaned, and at least one immobilizing agent for immobilizing and maintaining the activity of said enzyme over the time subsequent to its anchoring to the surface and/or to the layers present on said surface, so as to prolong the degradation activity of the organic substances.

[0021] Such a degradation is able to reduce the contaminating substances directly on the surface and/or on the layers present on that surface.

[0022] Furthermore, the cleaning method according to the present invention comprises a step of applying, on the surface to be cleaned or on layers present on such a surface to be cleaned, a cleaning preparation or a solution comprising at least one enzyme responsible for the degradation of organic substances.

[0023] The process also comprises a step of applying, on the surface to be cleaned or on the layers present on such a surface, at least one immobilizing agent for immobilizing and maintaining the activity of said enzyme over the time subsequent to its anchoring to the surface and/or to layers present on such a surface, so as to prolong the degradation activity of the organic substances.

[0024] The cleaning preparation is applied, according to the invention, through materials consisting of polymers of a different nature.

[0025] In one non-limiting embodiment, such polymers may be polyamides, polyethylene, polypropylene, polylactates, polymers with a high degree of bio-decomposition, etc. Subsequently, for a period of time that can reach 42 days, unlike the current cleaning methods, the manual cleaning operations can be avoided or significantly reduced anyway, due to the ability of the cleaning preparation to perform its activity of degradation of dirt which accumulates over the time subsequent to the application and/or cleaning step.

[0026] It is possible to provide for such manual operations to be varied and/or simplified without by this reducing the effective efficiency of the preparation and of the cleaning method in the degradation of the organic and/or inorganic substances mainly making up the dirt and the consequent reduction in the growth of pathogens harmful to health.

[0027] The cleaning method according to the invention can include, in a non-exclusive manner, a cleaning operation with the cleaning preparation object of the invention, through the standard techniques.

[0028] Following the application of such a cleaning preparation on surfaces such as floors, walls, windows, objects and the like, and/or on the layers present on such surfaces, the enzyme and/or enzymes contained in the preparation remain anchored to the surface to be cleaned and/or to the layers present on said treated surface for an extended period of time (5 hours to 42 days).

[0029] The degradation efficacy of the preparation against the organic and/or inorganic substances mainly forming the dirt is maintained over time, thus allowing a reduction in the accumulation of such substances due to the environment and to the persons who spend time in or contaminate such surfaces.

[0030] The reduction in the accumulation of dirt resulting from sources external to the surface and/or to the layers present on such a treated surface consequently allows a control of the growth of pathogens harmful to health.

[0031] In fact, it is known that the formulations containing surfactants may have a negative effect on the catalytic activity in solution, so as to deeply inhibit the immobilization on surfaces.

[0032] Therefore, one of the advantages of the invention consists in the stabilization of the enzyme anchoring to the surface to be cleaned and/or to the stratified substances present thereon, and the enzyme efficacy is maintained for more than 7 days.

[0033] The immobilization efficacy and the maintenance of the enzymatic activity for such a long period of time in an environment exposed to contamination is a peculiarity guaranteed by the preparation and by the method of the invention.

[0034] Another peculiarity of the invention consists in the fact that the preparation described above is able to prevent the harmful phenomena arising from the accumulation of the substances making up the dirt, thus having a prolonged efficacy over time.

[0035] Subsequently to the application step, described above, of the above cleaning preparation, reduced cleaning operations are provided for a period of at least 6 h, or at least 12 h, or at least 72 h, or at least 96 h, or at least 120 h, up to, in some cases, 42 days. Subsequently to the step of application of the cleaning preparation, it is possible to non-exclusively provide a mechanical cleaning operation, which may provide for the use of slightly damp supports, with the purpose of removing surface contaminants introduced from the outside in an excessive and unpredictable manner.

[0036] The features of the invention will be better understood by any man skilled in the art from the following description and from the appended drawings, given by way of a non-limiting example, in which:

figure 1 is a chart summarizing the immobilization of three enzymes (Alcalase, Savinase, Lipolase) on single-fired tiles;

figure 2 is a chart summarizing the immobilization of the same three enzymes on linoleum tiles;

figure 3 is a chart summarizing the immobilization of the same three enzymes on PVC tiles;
figure 4 is a chart summarizing the amount of dirt removed from single-fired tiles using the cleaning preparation according to the invention, and according to different operating methods;
figure 5 is a chart summarizing the amount of dirt removed from linoleum tiles using the cleaning preparation according to the invention, and according to different operating methods;
figure 6 is a chart summarizing the amount of dirt removed from PVC tiles using the cleaning preparation according to the invention, and according to different operating methods;
figure 7 is a graph of the time course of the microbial load on single-fired tiles previously treated with standard dirt and then with the cleaning preparation according to the invention, and according to different operating methods;
figure 8 is a graph of the time course of the microbial load on linoleum tiles previously treated with standard dirt and then with the cleaning preparation according to the invention, and according to different operating methods;
figure 9 is a graph of the time course of the microbial load on PVC tiles previously treated with standard dirt and then with the cleaning preparation according to the invention, and according to different operating methods;
figure 10 is a representative graph of a test performed in a healthcare facility and which shows the time course of the total bacterial count with and without treatment with the preparation according to the invention;
figure 11 is a representative graph of a test performed on a toilet seat of a healthcare facility which shows the time course of the total bacterial count in the first 72 hours after treatment with the preparation according to the invention;
figure 12 is a representative graph of a test performed on a sink of the bathroom of a hospital room of a healthcare facility, which shows the time course of the total bacterial count in the first 72 hours after treatment with the preparation according to the invention;
figure 13 is a representative graph of a test performed on a toilet seat of a healthcare facility which shows the time course of the total bacterial count in the first 96 hours after treatment with the preparation according to the invention;
figure 14 is a representative graph of a test performed on a sink of the bathroom of a hospital room of a healthcare facility, which shows the time course of the total bacterial count in the first 72 hours after treatment with the preparation according to the invention.

[0037] The cleaning preparation according to the invention is particularly, but not exclusively, intended for use on surfaces to be cleaned such as floors or walls (for example made of PVC, linoleum or tile), windows, objects and the like (although not excluding the use, still falling within the scope of protection claimed herein, for cleaning other elements, depending on specific requirements).

[0038] According to the invention, the cleaning preparation comprises at least one enzyme which can be stably anchored to the surface to be cleaned and/or to the layers present on said surface, and which is responsible for the degradation of organic substances, such as fats, proteins and/or sugars, and inorganic substances such as, by way of non-limiting example, carbonates salt powders.

[0039] As better clarified hereinafter, in the first place the enzyme may thus stably reduce or eliminate the accumulation of dirt present on the surface and/or on the layers present on such a surface at the time of the application of the preparation (and precisely consisting of the organic and/or inorganic substances mentioned above), since it allows the degradation thereof and promotes the solubility thereof, thereby allowing the removal thereof with a possible subsequent rinsing and/or slight mechanical action.

[0040] In addition, since the enzyme remains stably anchored to the surface and/or to the layers present on such a surface over time, it is able to exert its degradation activity even at a later time, for a more or less prolonged period (even several days) subsequent to the time of application of the preparation, and without the contribution of manual and/or mechanical operations by means of systems external to the same surface and/or to the layers present on such a surface for the whole period indicated above, thereby achieving the object set.

[0041] The degradation activity of the enzyme towards the substances that make up the dirt over time also allows controlling the development of pathogens harmful to health, and the consequent reduction of their amount compared to the normal proliferation which occurs as a result of the accumulation of such substances after the conventional cleaning operation.

[0042] According to another aspect of the invention, and precisely to allow the action of the enzyme even after the application of the preparation, the latter comprises one or more substances which make the immobilization of the enzyme itself effective, which will be defined as immobilizing agents hereinafter. Such agents allow immobilizing the enzyme without altering the efficacy (activity) over time, in order, precisely, to prolong the activity of degradation of organic substances, subsequent to its anchoring to the surface and/or layers present on said surface.

[0043] This solution allows avoiding the danger of loss of efficacy of the enzyme that could otherwise occur, for example, due to exposure to atmospheric agents or in any case to the external environment or through the mechanical rubbing to which the surfaces treated are subjected as a result of the normal use thereof.

[0044] The immobilizing agents which maximize the efficacy (activity) of the enzyme in removing dirt include, or consist of, pH regulators and/or surfactants.

[0045] The surfactants present in the preparation may be ionic and/or anionic and/or amphoteric and/or cationic and/or a combination thereof.

[0046] Preferably but not exclusively, they may be ionic and/or cationic surfactants.

[0047] Due to the components present in the preparation, and in particular due to the pH regulators, the enzyme is thus also able to withstand changes in pH, temperature, etc. over time, which might otherwise cause the deactivation thereof.

[0048] The concentrations of the enzymes and/or the other preparation components are such as to allow a degree of immobilization of more than 1% of the enzyme itself.

[0049] In particular, according to one embodiment of considerable practical interest, mentioned for illustrative purposes and not limitative of the application of the invention, the immobilizing agents include nonionic surfactants and/or cationic surfactants, or a combination thereof in a percentage not higher than 5% w/w of the preparation object of the invention.

[0050] In particular, according to a further solution of practical interest, mentioned for illustrative purposes and not limitative of the application of the invention, the enzyme immobilization may occur through the application of a further preparation or solution (containing no enzymes) comprising at least one immobilizing agent, with the aim, in fact, to immobilize the surfactants on the surface to be treated and/or on the layers present thereon by absorption and/or adsorption.

[0051] The surfactants allow the enzyme and/or enzymes, through even weak chemical interactions, to be effectively immobilized, even in the presence of layers of substances present on the surfaces themselves.

[0052] As better described hereinafter, the presence of such surfactants does not alter the catalytic properties of the enzyme and/or enzymes.

[0053] As said, the enzyme can be stably anchored to the surface and/or to the layers present on said surface by absorption or by adsorption.

[0054] In the first case, as is known, the enzyme penetrates and spreads to the inner layers of the surface and/or layers present on such a surface to be cleaned.

[0055] In the second case, the enzymes form a chemical bond or establish a chemical-physical interaction, through Van der Waals forces, limited to the outer layers of the surface and/or layers present on such a surface.

[0056] The anchoring of the enzyme occurs in a pH range of the cleaning preparation comprised between 6 and 8.5 and preferably between 6.5 and 8.5, and even more preferably between 7 and 8.

[0057] These pH ranges allow maximizing the immobilization yield of the enzyme itself, determining the maximum enzymatic activity of degradation of the organic and/or inorganic substances over time.

[0058] It should be noted that however, the possibility of obtaining the anchoring and immobilization of the enzyme according to other methods is not excluded and falls within the scope of protection claimed herein. The anchoring methods described above have the feature, not obvious, to maintain the enzymatic activity over time (days) at sufficient levels and such as to degrade the component substances of dirt, resulting in controlling the growth of harmful organisms, and to be resistant to chemical contamination phenomena and/or mechanical actions exerted on the surface and/or the layers present on such a surface.

[0059] The non-obvious particularity of the invention is the maintenance of the enzymatic activity over time, even in the presence of secondary components such as surfactants.

[0060] In fact, it is known that surfactants may be a means of immobilization of the enzymes themselves, but at the same time they inhibit their activity both immediately and over time.

[0061] Preferably, but not exclusively, the enzyme is selected from a protease, a lipase and an amylase, in order to be able to effectively degrade the organic substances such as proteins, fats and sugars, respectively, coming from sources external to the surface and/or to the layers present on such a surface to be cleaned.

[0062] In a particular but non-limiting embodiment of the invention, the enzymes may be sibtilisin and/or cellulase and/or lipase.

[0063] The concentrations of these enzymes and/or of the other additives in the preparation object of the invention is such as to obtain an immobilization yield greater than 10% on the surfaces and/or layers present on such surfaces.

[0064] Suitably, the surfactants present in the preparation allow increasing the wettability of the surface and/or the layers present on such a surface intended to be treated with the preparation.

[0065] Consequently, an optimal application of the preparation on the surface and/or on the layers present on such a surface is ensured, ensuring that each of its portions is reached by the enzymes responsible, according to the methods described above, for removing the accumulations of dirt.

[0066] Beyond the features of the cleaning method carried out with the preparation according to the invention, a method which also - as said - is a specific object of the present invention, in general the method of using the cleaning preparation is therefore clear from what has been described: in order to clean a surface and/or the layers present on such a surface such as a floor, a wall, a window, objects or the like, the preparation described above is applied on the surface and/or on the layers themselves so as to allow, in the first place, the enzyme contained therein to carry out its degradation activity on the organic substances, on the accumulations of dirt already present.

[0067] Thereafter, due to the enzyme anchoring on the surface and/or layers present on said surface, which withstands the rinsing step, the preparation is able to carry out a residual degradation activity which can last over time even for several days.

[0068] The cleaning preparation is adapted to carry out the degradation function even after its application and without the need for subsequent manual and/or mechanical operations for a time of at least 1 day, preferably for at least 3 days, more preferably for at least 42 days.

[0069] Furthermore, the preparation is adapted to control and reduce the proliferation of pathogens harmful to health on the surface to be cleaned and/or on the layers present on such a surface by at least 20%, preferably at least 30%, even more preferably at least 80%.

[0070] Such degradation activity allows preventing the formation of new accumulations of dirt, also due to the immobilization efficiency ensured by the pH regulator components and by the surfactants, so as to impart greater resistance to the enzyme against external environmental factors, which could otherwise cause its deactivation.

[0071] The absence of accumulations of organic substances, in addition, means absence of the element essential for the proliferation of pathogens harmful to health, and therefore, over time, the surface and/or the layers present on such a surface are also protected from such pathogens.

[0072] The preparation according to the invention thus ensures a prolonged cleaning over time without the need for continuous washing actions, resulting in an evident saving of product, in addition to that of water needed for rinsing (and possibly for the dilution of the preparation itself).

[0073] As seen, the anchoring of the enzyme to the surface and/or to the layers present on such a surface is obtained without resorting to specific devices, such as polymeric films or coatings, thereby ensuring greater structural simplicity and a reduced number of necessary components, thus allowing a useful containment of the overall cost.

[0074] The results of some application, but non-limiting, examples are shown below and further clarify the fundamental aspects of the present invention.

[0075] The application examples described hereinafter relate to the treatment of floor surfaces, and specifically relate to three types of floors, i.e. single-fired, linoleum and PVC.

[0076] A first aspect of the application examples described relates to the determination of optimal pH values, for each type of floor, in terms of enzyme immobilization. In particular, the amount of enzyme immobilized in mg/mL and in percentage was evaluated for each protein (enzyme) and each type of floor, and each pH value (in the range of 6.5 to 9).

[0077] The enzymes used are, in particular, Lipolase®, Alcalase® and Savinase®.

[0078] The values obtained are shown in the following tables along with standard deviation, while the graphs in figures 1, 2 ,3 summarize the percentage amount of immobilized enzyme as a function of pH for each surface.

[0079] The following tables A, B, C refer to tests on single-fired tiles with an amount of enzyme used of 1 mg/mL, with pH between 6.5 and 9 and a duration of immobilization of 3h.

Table A: Alcalase®

pH	[Immobilized enzyme] (mg/ml) $\pm\sigma$	% Immobilized e.
6.5	0.123 $\pm$ 0.025	12.3 $\pm$ 2.5
7	0.133 $\pm$ 0.031	13.3 $\pm$ 3.1
7.5	0.220 $\pm$ 0.050	22 $\pm$ 5
8	0.100 $\pm$ 0.040	10 $\pm$ 4
8.5	0.013 $\pm$ 0.015	1.3 $\pm$ 1.5
9	0.016 $\pm$ 0.029	1.6 $\pm$ 2.9

Table B: Savinase®

pH	[Immobilized enzyme] (mg/ml) $\pm\sigma$	% Immobilized e.
6.5	0.130 $\pm$ 0.071	13 $\pm$ 7.1
7	0.073 $\pm$ 0.035	7.3 $\pm$ 3.5
7.5	0.360 $\pm$ 0.096	36 $\pm$ 9.6
8	0.31 $\pm$ 0.012	31 $\pm$ 1.2
8.5	0.01 $\pm$ 0.017	1 $\pm$ 1.7

(continued)

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
9	0.01 ± 0.017	1 ± 1.7

Table C: Lipolase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.560 ± 0.040	56 ± 4
7	0.462 ± 0.032	46.2 ± 3.5
7.5	0.356 ± 0.006	36 ± 0.6
8	0.320 ± 0.056	32 ± 5.6
8.5	0.030 ± 0.015	3 ± 1.5
9	0.037 ± 0.006	3.7 ± 6

[0080] By analyzing tables A, B, C, and the graph in figure 1, it is clear that at the more basic pH (8.5-9) none of the three enzymes shows a significant adsorption.

[0081] Moreover, two different trends may be seen between lipase and protease, as well as a very similar trend for Alcalase® and Savinase®. In particular, Alcalase® shows a maximum absorption corresponding to about 20% of the total amount of enzyme placed in contact with the surface, while Savinase® is almost 40%. On the other hand, Lipolase® shows a contrary trend. It also shows no adsorption at basic pH, but the maximum immobilization is seen at pH 6 with nearly 60% of the total enzyme immobilized on the tiles.

[0082] The immobilization is decreasing with an increasing pH, with about 35% of adsorption at a pH of 7.5, at the relative maximum of the protease.

[0083] It may be concluded that, with regard to the single-fired tiles, the optimum working pH with the enzymatic mixture (Alcalase®, Savinase® and Lipolase®) is 7.5, which corresponds to the maximum adsorption of the two proteases and to a good level of immobilization of Lipolase®.

[0084] The following tables D, E, F refer to tests on linoleum with an amount of enzyme used of 1 mg/mL, with pH between 6.5 and 9 and a duration of immobilization of 3h.

Table D: Alcalase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.160 ± 0.053	16 ± 5.3
7	0.026 ± 0.046	2.6 ± 4.6
7.5	0.006 ± 0.011	6 ± 11
8	0.110 ± 0.036	11 ± 3.6
8.5	0.003 ± 0.050	3 ± 5
9	0.040 ± 0.040	4 + 4

Table E: Savinase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.220 ± 0.046	22 ± 4.6
7	0.001 ± 0.017	2.6 ± 4.6
7.5	0 ± 0	6 ± 11
8	0.430 ± 0.190	43 ± 19
8.5	0.070 ± 0.070	7 ± 7

(continued)

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
9	0.110 ± 0.075	11 ± 7.5

Table F: Lipolase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.220 ± 0.046	22 ± 4.6
7	0.24 ± 0.045	24 ± 4.5
7.5	0.066 ± 0.041	6.6 ± 4.1
8	0.050 ± 0.053	5 ± 5.3
8.5	0.010 ± 0.017	1 ± 1.7
9	0.036 ± 0.047	3.6 ± 4.7

[0085] The remarks made for the single-firing also substantially apply to linoleum.

[0086] At alkaline pH, the immobilization percentages of all the three industrial enzyme formulations are very limited and the typical trend of the two proteases (with optimum adsorption at almost neutral pH) and Lipolase® (which instead shows a good immobilization at more acidic pH values) is also noted in this case.

[0087] The data obtained also allow making some additional evaluations.

[0088] The waxed surface of linoleum (like PVC) limits the amount of enzymes adsorbed on the tiles in absolute terms.

[0089] In fact, the values obtained are lower than those obtained for the single-firing: it suffices to consider Lipolase®, for which the percentage of immobilization drops from 55% (for single-firing) to a little over 20% (for linoleum).

[0090] Single-fired surfaces possess a good level of porosity and facilitate the enzyme adsorption even in the inner interstices of the tile, increasing the available surface.

[0091] Both Savinase® and Alcalase® show a limited but significant level of non-specific adsorption at the so-called "extreme" pH of our considered range (6.5 and 9), associated with a visible decrease in the opacity of the floor.

[0092] The following tables G, H, I refer to tests on PVC with an amount of enzyme used of 1 mg/mL, with pH between 6.5 and 9 and a duration of immobilization of 3h.

Table G: Alcalase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.093 ± 0.055	9.3 ± 5.5
7	0.120 ± 0.037	12 ± 3.7
7.5	0.01 ± 0.017	1 ± 1.7
8	0.140 ± 0.040	14 + 4
8.5	0.063 ± 0.025	6.3 ± 2.5
9	0.040 ± 0.036	4 ± 3.6

Table H: Savinase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.093 ± 0.055	9.3 ± 5.5
7	0.193 ± 0.035	19.3 ± 3.5
7.5	0.253 ± 0.057	25.3 ± 5.7
8	0.160 ± 0.144	16 ± 14.4
8.5	0.173 ± 0.015	17.3 ± 1.5

(continued)

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
9	0.123 ± 0.060	12.3 ± 6.0

Table I: Lipolase®

pH	[Immobilized enzyme] (mg/ml) ± σ	% Immobilized e.
6.5	0.147 ± 0.006	14.7 ± 0.6
7	0.177 ± 0.012	17.7 ± 1.2
7.5	0.080 ± 0.017	8 ± 1.7
8	0.030 ± 0.052	3 ± 5.2
8.5	0.053 ± 0.061	5.3 ± 6.1
9	0.047 ± 0.015	4.7 ± 1.5

[0093] PVC is the surface that shows the most homogeneous trend, as a function of pH, as regards the enzyme immobilization.

[0094] In order to evaluate the actual activity of the immobilized enzymes on the surface, spectrophotometric evaluations were also carried out directly on the affected areas and not in solution.

[0095] In particular, two experiments were developed, both accompanied by quantitative data that prove the enzymatic activity.

[0096] The first set of tests had a dual purpose:

- checking the reliability of the Bradford assay used to evaluate the immobilization;
- evaluating the residual activity of the adsorbed enzymes.

[0097] Therefore, for each assay, the two single-fired tiles showing greater and lesser enzyme adsorption according to Bradford's quantification were used.

[0098] Each reaction mixture (final volume of 2 mL) was incubated for 3 hours, and then the absorbance values related to the generation of enzyme-catalyzed reaction products were determined spectrophotometrically.

[0099] More in detail, two single-fired tiles were used since the data given above show that this is the floor which ensures greater experimental reproducibility.

[0100] The spectrophotometric measurements given hereinafter unequivocally show that enzymes are not only immobilized but also active:

LIPASE ASSAY (410 nm)

- Control: 0.0041
- Sample: 0.955

PROTEASE ASSAY (440 nm)

- Control: 0.0047
- Sample: 0.150

[0101] The second set of tests was conducted in duplicate and was aimed to confirm the activity of Lipolase® despite the small percentage of immobilization at pH 8 compared to both Savinase® and Alcalase®.

[0102] In order to verify that the reduced adsorption was compensated by the high intrinsic activity of the enzyme, two tiles for each type were used on all the three floors, using the same preparation solution without enzyme as negative control.

[0103] The absorbance values at 410 nm confirm the hypotheses explained (it should be noted that the samples were diluted 1: 2, while the controls were not):

Controls (1:1):

- single firing: 0.774
- linoleum: 0.728
- PVC: 0.9335

Samples (1:2) :

- single firing: 0.919
- linoleum: 0.9155
- PVC: 0.8375

[0104] The experimental evidences also comprise the "standard dirt" removal test for each type of material.

[0105] Three tiles (nine, in reality, since each analysis was repeated in triplicate) were used for each type of floor on which the enzymes were first immobilized and then the "standard dirt" was applied.

[0106] As regards the immobilization, each of the three tiles for each type was treated as follows:

a tile used as "sample" on which the cleaning preparation according to the invention was immobilized;
a tile used as "control A" treated in a manner similar to the previous sample but without enzymatic mixture;
a tile used as "control B" treated in a manner similar to the previous samples in the presence of H_2O alone. In this way, it was possible to compare the effects of subsequent washing of the surfaces made using only water.

[0107] In order to quantitatively determine the dirt removed with the washings, the individual tiles were weighed (post-immobilization) before and after the application of the dirt so as to estimate the amount of "standard dirt" applied on each tile, as from the following table J (where "M" indicates single-fired, "L" indicates linoleum and "P" indicates PVC).

Table J (dirt applied)

Dirt applied (in mg)									
	Samples			Controls A			Controls B		
	M	L	P	M	L	P	M	L	P
1°	80	60	95	74	68	72	92	60	55
2°	106	82	70	82	70	97	82	64	86
3°	99	73	88	101	63	69	91	83	100

[0108] Successive washings were then carried out, 24 hours apart from one another, washing the floors using 5 mL of H_2O and stirring gently for 90 seconds, in order to simulate the cleaning operations.

[0109] Aliquots of 4 mL of each sample were then taken.

[0110] Each washing water was subjected to turbidimetric assay (600 nm), and the absorbance values obtained were converted to mass of dirt removed using calibration lines referred to the standard dirt.

[0111] Tables K, L, M shown hereinafter and the graphs in figures 4, 5, 6 show the trend of the dirt removal (in mg) from the surfaces with immobilized enzymes after 4 consecutive days of washes, with respect to both types of controls.

Table K: dirt removed (single-firing)

Dirt removed in mg (single-firing)			
	Samples (Tris-HCl pH8, "Basic detergent" (3%) and enzyme mixture)	Controls A (Tris-HCl pH 8, "Basic detergent" (3%))	Controls B (Tris-HCl pH 8)
Days			
1	(5.18 ± 0.35)	(2.53 ± 0.45)	(2.00 ± 0.92)
2	(4.80 ± 0.57)	(2.80 ± 0.22)	(1.97 ± 0.34)
3	(6.12 ± 0.74)	(4.33 ± 0.45)	(2.43 ± 0.50)

(continued)

Dirt removed in mg (single-firing)			
	Samples (Tris-HCl pH8, "Basic detergent" (3%) and enzyme mixture)	Controls A (Tris-HCl pH 8, "Basic detergent" (3%))	Controls B (Tris-HCl pH 8)
Days			
4	(6.03 ± 1.48)	(3.30 ± 0.92)	(3.48 ± 0.79)

Table L: dirt removed (Linoleum)

Dirt removed in mg (Linoleum)			
	Samples (Tris-HCl pH8, "Basic detergent" (3%) and enzyme mixture)	Controls A (Tris-HCl pH 8, "Basic detergent" (3%))	Controls B (Tris-HCl pH 8)
Days			
1	(4.57 ± 0.49)	(3.63 ± 0.39)	(2.00 ± 1.06)
2	(5.10 ± 0.22)	(4.20 ± 0.28)	(2.83 ± 0.17)
3	(5.17 ± 0.77)	(5.13 ± 0.26)	(3.50 ± 0.36)
4	(5.33 ± 0.74)	(4.70 ± 1.07)	(4.03 ± 0.81)

Table M: dirt removed (PVC)

Dirt removed in mg (Linoleum)			
	Samples (Tris-HCl pH8, "Basic detergent" (3%) and enzyme mixture)	Controls A (Tris-HCl pH 8, "Basic detergent" (3%))	Controls B (Tris-HCl pH 8)
Days			
1	(11.13 ± 1.28)	(5.23 ± 0.90)	(3.33 ± 0.52)
2	(4.60 ± 1.22)	(4.10 ± 0.51)	(3.80 ± 0.78)
3	(5.93 ± 1.25)	(4.37 ± 0.54)	(3.17 ± 0.33)
4	(7.00 ± 0.22)	(4.87 ± 0.48)	(3.20 ± 0.45)

[0112] Despite the apparent diversity of the individual graphs shown in figures 4, 5, 6, the data obtained allow highlighting some aspects.

[0113] In the first place, it is seen that for each floor, in absolute terms, the amount of "standard dirt" removed with each washing is always higher for the tiles treated with the enzyme mixture, even net of the reported standard deviations.

[0114] This trend confirms that the adsorbed enzymes facilitate and improve the removal of a set of lipid and protein compounds from the surfaces.

[0115] In addition, by comparing the values corresponding to the two controls, it is seen that the presence of the so-called "basic detergent" (without enzyme mixture) has a very positive effect compared to treatment with water and buffer alone.

[0116] It can therefore be concluded that the so-called "basic detergent" alone allows better removal of "standard dirt", but the addition of the enzyme mixture further implements the capacity of cleaning of the surfaces. The catalytic action of the enzymes, in fact, facilitates the solubility of the lipid and protein compounds, increasing the efficiency of washing.

[0117] This involves a better removal of dirt net of a same treatment.

[0118] Having verified that the presence of immobilizing enzymes increases the amount of "standard dirt" removed by acting in combination with the action of the "basic detergent", it was subsequently examined whether the increased removal of dirt is also associated with a decrease in the total bacterial load found on the treated surfaces.

[0119] Three tiles of each type of material were therefore treated in the manner described above (a "sample" with

enzyme mixture and two "controls A, B"), and subsequently soiled with a fresh preparation of "standard dirt".

[0120] The washes were carried out once a week for 42 days, and aliquots of 100 μl of the washing waters were transferred on Petri dishes containing agar LB medium (Tryptone 10 g/L, Yeast extract 5 g/L, NaCl 10 g/L, Agar 15 g/L).

[0121] In particular, three different serial dilutions of each sample were analyzed for each determination of the bacterial load.

[0122] The Petri dishes were finally incubated for 5 days at 30 °C and the results are listed in tables N, O, P, Q, R, S shown hereinafter (in all the above tables, "M" indicates single-fired, "L" indicates linoleum and "P" indicates PVC).

[0123] The data shown only refer to bacteria-formed colonies since they are the main microorganisms that determine the total bacterial load.

Table N: bacterial counts at 7 days from the application of "standard dirt"

Bacterial counts (7 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^0	0	1	2	5	0	0	1	3	0
10^{-1}	4	1	1	1	0	2	0	0	0
10^{-2}	1	0	0	0	0	0	0	0	0

[0124] After the first assay (first week), it was decided to use further dilutions for determination of the bacterial load.

[0125] Since no microorganisms were observed in the dilution 10^{-2}, it was decided to eliminate it and replace it with 10^1, in which an aliquot of 1.5 mL of wash water was centrifuged and resuspended in 150 μL of sterile H_2O.

Table O: bacterial counts at 14 days from the application of "standard dirt"

Bacterial counts (14 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^1	12	1	2	0	4	5	0	296	31
10^0	14	0	1	0	0	1	0	5	2
10^{-1}	1	0	0	0	0	1	0	1	1

Table P: bacterial counts at 21 days from the application of "standard dirt"

Bacterial counts (21 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^1	22	0	2	23	25	114	8	6	0
10^0	2	0	1	7	2	10	1	6	0
10^{-1}	0	0	1	0	1	6	3	0	0

Table Q: bacterial counts at 28 days from the application of "standard dirt"

Bacterial counts (28 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^1	5	13	7	1	4	4	5	20	4
10^0	2	0	6	2	1	0	0	1	0

(continued)

Bacterial counts (28 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^{-1}	2	1	0	1	2	0	0	1	0

Table R: bacterial counts at 36 days from the application of "standard dirt"

Bacterial counts (36 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^{1}	9	0	0	2	6	18	2	188	10
10^{0}	0	5	3	2	2	64	1	57	3
10^{-1}	1	0	1	3	0	0	0	13	1

Table S: bacterial counts at 44 days from the application of "standard dirt"

Bacterial counts (44 days from the application of "standard dirt")									
	Samples			Controls A			Controls B		
Dilutions	M	L	P	M	L	P	M	L	P
10^{1}	10	4	2	4	4	0	1	2	18
10^{0}	3	3	2	5	5	0	0	1	0
10^{-1}	1	0	0	1	0	0	0	0	0

[0126] The washing water volume transferred on each Petri dish being known (100 μL), it is easily possible to derive the value of colony forming units (cfu) per milliliter of washing water from the number of colonies observed in each plate.

[0127] The graphs in figures 7, 8, 9 show, for each type of surface, the trend of cfu/mL as a function of time, both of the "samples" treated with the enzyme mixture, and of "controls A" (in the presence of "basic detergent") and "controls B" (without "basic detergent").

[0128] The maximums of the number of cfu/mL which are observed in the three graphs are due to random contamination of surfaces.

[0129] The values obtained prove the ease of pollution of the surfaces by microorganisms present in the air and this ease seems to be limited by the removal of standard dirt (with the consequent reduction of the total microbial load).

[0130] In all three types of surfaces, in fact, the most irregular trends are those relating to "controls A and B", while the microbial loads observed in the washing water taken from the floors treated with the enzyme mixture are tendentially constant.

[0131] These data confirm the efficacy of the enzymatic preparations in helping to prevent the microbial growth on surfaces as a result of random contamination.

[0132] In the case of the two waxed floors, in fact, for the whole duration of the experiment (6 weeks), the cfu/mL found on the surfaces containing enzymes never exceeded 13 units, remaining at an average of 3-4 cfu/mL.

[0133] These values are extremely low if compared with those of "controls A and B", which reach about 100 cfu/mL (control A - PVC), and even higher in the case of control B referred to linoleum.

[0134] It is important to note a further aspect. On both waxed floors (linoleum and PVC) after about 30-35 days of exposure to "standard dirt", the trend of the cfu/mL values of the samples compared to that of the controls radically diverges. The samples on which the lipase and protease mixture had been immobilized show a reduction of the total microbial load, while the two "controls A and B" (especially PVC) show an increasing trend. If this data were confirmed, it could demonstrate an effect of reduction of the total amount of bacterial load on the tiles in the long run, data substantially correlated with the progressive dirt removal with continued washings.

[0135] The remarks regarding linoleum and PVC are definitely different from those relating to the single-firing. In this

latter case, in fact, the trend of the microbial load in the tiles treated with enzymes does not differ significantly from the trends observed in the control tiles.

[0136] Probably, the porous and not homogeneous structure of this type of floor favors an increase in the total bacterial load, in addition to a lower protective effect by the enzymes. Even in this case, however, a decreasing trend of the microbial load may be seen in the tiles containing the Lipolase® Alcalase® and Savinase® mixture, reflecting the possible effect of reduction of the total microbial load values in the long run.

[0137] As mentioned above, the object of the present invention is also a method for cleaning surfaces such as floors, walls, windows, objects and the like.

[0138] In general, the method comprises at least a step of applying, on the surface to be cleaned or on layers present on such a surface to be cleaned, a solution comprising at least one enzyme responsible for the degradation of organic substances.

[0139] The method further comprises a step of applying, on such a surface to be cleaned or on the layers present thereon, at least a further preparation or solution comprising an immobilizing agent for immobilizing and maintaining the activity of the enzyme over the time subsequent to its anchoring to the surface and/or to layers present on such a surface, so as to prolong the degradation activity of the organic substances.

[0140] In one embodiment of the invention, the step of applying a further preparation or solution comprising an immobilizing agent is carried out after the step of applying the cleaning preparation or solution comprising at least one enzyme.

[0141] In another embodiment of the invention, to be considered preferred, the step of applying a further preparation or solution comprising an immobilizing agent is carried out at the same time as the step of applying the cleaning preparation or solution comprising at least one enzyme.

[0142] In the latter case, and in a preferred embodiment of the method, what is applied is precisely the cleaning preparation according to the invention described above, comprising at least one enzyme and at least one immobilizing agent.

[0143] The method also comprises at least one step of applying, on the surface to be cleaned or on the layers present on such a surface to be cleaned, a second cleaning preparation, the latter without enzymes.

[0144] In particular, such a second cleaning preparation is of the type with low content of surfactants.

[0145] Even more in particular, and in a specific embodiment of the method, such a second cleaning preparation without surfactants can be of the type already known and available on the market.

[0146] The step of applying the second cleaning preparation (without surfactant) is subsequent to the step of applying the solution comprising at least one enzyme, i.e., in a preferred embodiment, the cleaning preparation according to the invention as described above.

[0147] In some other preferred embodiments of the invention, and according to an interesting aspect of the same, the method provides an alternation of steps of application of the cleaning preparation according to the invention and of application of the second cleaning preparation (without enzymes).

[0148] More in detail, according to this aspect of the invention, the method may comprise, in some embodiments thereof, the alternating application of the two preparations (the one according to the invention and the second preparation without enzymes) at alternating days and/or weeks and/or months.

[0149] In other preferred embodiments of the invention, and according to another interesting aspect of the same, the method provides a specific sequence of application steps of the cleaning preparation according to the invention, characterized by different modes of application in terms of frequency over time, as will become clearer hereinafter.

[0150] With the features of the present invention, and as will become clearer hereinafter, the application of the second cleaning preparation can be significantly reduced in terms of amount and/or frequency of application.

[0151] By way of non-limiting example, two different embodiments - or application examples - of the cleaning method according to the present invention are described in detail hereinafter.

[0152] A specific embodiment relates to the light cleaning of small surfaces.

[0153] According to this embodiment, the method provides a step of application of the cleaning preparation (of the invention) on the surfaces concerned using a cloth. Said cloth may be made of materials consisting of polymers of a different nature, such as polyamides, polyethylene, polypropylene, polylactates, polymers with a high bio-decomposition degree, etc.

[0154] The peculiarity of this application is that no mechanical pressure of the cloth on the surface in question and/or the dirt is required.

[0155] In fact, the purpose of such application/distribution of the cleaning preparation according to the invention is to make the surface treated by the enzyme uniform. In the context of hospital or health-care facilities, the conventional cleaning procedures provide for more than three daily operations to maintain the degree of dirt, or of bacterial contamination (expressed in cfu/cm^2) to a value of less than 10.

[0156] According to the present embodiment of the invention, instead, the cleaning preparation containing at least one enzyme is applied at most once a day for the first week.

[0157] In the next second week, however, the method according to the invention provides for the application of the

second cleaning preparation (without enzymes and with a low content of surfactants), for normal light cleaning operations, also at most once a day.

[0158] In the third week, the method again provides for the application of the cleaning preparation according to the invention, again - at most - once a day.

[0159] This application scheme (also defined as "system initiation") is then repeated, according to the invention, every other month, since the enzyme anchored on the surfaces allows the effective degradation of the dirt from the outside environment.

[0160] The alternation of the application of the above "initiation", described above, allows a further reduction of the cleaning operations and/or of the products used for the control of contaminants.

[0161] The experimental data provided hereinafter - see in particular TEST 3 - show how the method according to the invention allows control of the bacterial load (and of the consequent effects harmful to the health of the areas, also as regards the unpleasant odors) reducing by at least 10%, preferably 20%, preferably 30% the times and the cleaning operations.

[0162] In fact, compared to the traditional methods which involve applications of detergent products with a high concentration of surfactants, the method according to the invention provides only one daily application of the product, also with a low content of detergent surfactants.

[0163] The other embodiment of the method of which, as said, a detailed description is given by way of example only, relates to the cleaning of larger surfaces, such as floors, walls, rooms and bathroom fixtures with a high contamination of dirt and/or pathogens harmful to health.

[0164] The first step of the method, according to this further embodiment, consists in applying the cleaning preparation according to the invention (containing the enzyme to be immobilized) at most once a day for the first 7 days.

[0165] This operational step is also compatible with a possible, but not necessary, light cleaning operation carried out with a traditional cleaning preparation low in surfactants and/or molecules with a detergent and/or disinfectant effect.

[0166] In the second week, the method provides a step of application of the preparation according to the invention for a maximum period of 3 days, with the same procedure described for the previous embodiment.

[0167] In the third week, the method provides a further step of application of the preparation according to the invention for a maximum period of 2 days, with the same procedure.

[0168] This last step is further repeated weekly for a maximum period of three months.

[0169] The so-called "initiation" step described above is thus completed.

[0170] In essence, therefore, in this embodiment of the method the application of the cleaning preparation is carried out according to a sequence of steps; each of these steps is characterized by a respective number of applications per week.

[0171] The number of weekly applications is therefore progressively decreasing during at least the first three weeks from the beginning of the method or treatment.

[0172] At this point, the cleaning operations intended to control the environmental dirt and/or the proliferation of pathogens may be carried out in a maximum number of two times daily, preferably once daily, preferably once every two days.

[0173] In a particular embodiment, relating to the cleaning of bathroom fixtures and/or areas commonly defined as bathrooms or rooms before bathrooms, the method provides the application of the cleaning preparation according to the invention at most once a week. Subsequently, the cleaning operations intended to control the environmental dirt and/or the proliferation of pathogens can be carried out at most twice daily, preferably once daily, preferably once every two days, by the use of cleaning preparations low in surfactants and/or molecules with a detergent and/or disinfectant effect (such as, but not exclusively, the second cleaning preparation described above).

[0174] In another specific embodiment of the cleaning method of the invention, relating to high-contact surfaces in environments having a so-called non-negligible risk (such as, for example, floors of hospitals, nursing homes, sanitary areas, etc.), the step of applying the cleaning preparation according to the invention is carried out once every two days.

[0175] Thereafter, the normal cleaning operations intended to control the environmental dirt and/or the proliferation of pathogens may be reduced to up to at most three times a day, preferably twice a day, preferably once a day, using cleaning preparations with a low content of surfactants and/or molecules with a detergent and/or disinfectant effect.

[0176] In a further embodiment of the cleaning method according to the invention, referred to bathrooms areas with public access (to be understood as provided for access not only to the internal staff users of the facility, but also to people external to the specific use of the facility itself, such as relatives, visitors in hospitals, etc.), the method provides a step of applying the cleaning preparation according to the invention at most once a day.

[0177] Several tests were conducted in the study and testing steps of the enzymatic mixture contained in the cleaning preparation according to the invention; these tests were carried out in real areas and in comparison with standard systems.

[0178] The tests were conducted on areas and surfaces of health-care facilities, i.e. patient rooms, toilets, fixtures, sinks regularly used.

TEST 1

[0179] The sample data shown in the graph in figure 10 refer to the hygienic conditions before (columns indicated with P) and after (columns indicated with D) the cleaning, with the enzyme mixture, respectively, of an area of the floor immediately below the toilet of a health facility.

[0180] The data show an average 79% reduction of cfu.

TEST 2

[0181] In other experimental conditions (field tests), the following data relating to the total bacterial count were obtained.

[0182] In particular, the data - shown in the graphs in figures 11, 12, 13, 14 - refer to the actual behavior of the preparation according to the present invention in a health-care facility, in the first 72 and 96 hours, respectively, after treatment with the preparation according to the invention (first application and ordinary condition).

[0183] It is noted that, in each of the graphs in figures 11, 12, 13, 14:

- the column indicated with C1 represents the total bacterial count (TBC) at 7:00 am, before cleaning;
- the column indicated with C2 represents the total bacterial count (TBC) at 9:00 am, after cleaning;
- the column indicated with C3 represents the total bacterial count (TBC) at 2:00 pm;
- the column indicated with C4 represents the total bacterial count (TBC) at 6:00 pm.

[0184] More in detail, figure 11 is a representative graph of a test performed on a toilet seat of a healthcare facility which shows the time course of the total bacterial count (TBC) in the first 72 hours (3 days) after treatment with the preparation according to the invention.

[0185] On the other hand, figure 12 is a representative graph of a test performed on a sink of the bathroom of a hospital room of the same facility, showing the time course of the total bacterial count (TBC) in the first 72 hours after treatment.

[0186] Figure 13 is a representative graph of a test performed on a toilet seat of the facility, showing the time course of the total bacterial count (TBC) in the first 96 hours after treatment.

[0187] Finally, figure 14 is a representative graph of a test performed on a sink of the bathroom of a hospital room of the facility, showing the time course of the total bacterial count (TBC) in the first 96 hours after treatment.

TEST 3

[0188] A further important and significant test was carried out through direct experiments in different hospitals and health-care facilities.

[0189] In particular, more than 4,000 measurements were carried out with microbiological plates and bioluminescence tests at ISO/IEC17025 accredited laboratories (Laboratory Research & Development Area of the supplier Laboratori di Microbiologia Ospedaliera). In this context, an analysis and evaluation was carried out of the conditions for the implementation of the test method in different application contexts, different surfaces, protocols and methodologies already in place (e.g. existing biofilm layers).

[0190] The associated check methods are microbiological checks, bioluminescence checks, colorimetric presence checks, operators accuracy checks with UV methodology. Once again, the object of the test is the verification of the total bacterial count (TBC) on the surfaces concerned at a given moment in time after the beginning of the treatment.

[0191] To this end, it may be noted that it is established experience - also confirmed by preliminary tests in the different health-care settings tested - that a good sanitization procedure allows, in the checks carried out within half an hour from the intervention (in low recontamination conditions), having TBC values of less than 5-10 cfu/cm^2.

[0192] This test focuses in particular on measurements taken 4-7 hours after the sanitization of the morning (at about 2:00-3:00 pm) to evaluate the efficacy of containment of the regrowth of the bacterial load.

[0193] In the following tables T, U, each reported value is the average result of two determinations carried out on the floor in the central areas of the rooms of the health-care or hospital facility in question.

[0194] In a first part of this test, the sample was taken in a health-care facility of Ferrara (Italy) at 2:00 pm with preliminary wet sweeping, 35 days after the beginning of the treatment according to the method according to the invention described above.

[0195] Each 24 cm^2 RODAC plate used was photographed after incubation and reading.

Table T: Testing in health-care facility of Ferrara

Nursing department							
Evaluated microorganisms		U. of	Room 12	Room 15	Room 16	Room 17	Average value
1	Total bacterial load	ufc × cmq	1.19	8.33	1.21	8.33	4.77
2	Staphylococcus aureus	ufc × cmq	0.13	0.02	0.40	0.00	0.14
3	Pseudomonas aeruginosa	ufc × cmq	0.00	0.00	0.00	0.00	0.00
4	Aspergillus niger/Candida albicans	ufc × cmq	0.00	0.06	0.02	0.00	0.02
5	Escherichia coli	ufc × cmq	0.00	0.00	0.00	0.00	0.00

[0196] In a second part of the test, the sample was taken in a hospital of Bologna (Italy) at 3:00 pm with preliminary wet sweeping, 65 days after the beginning of the treatment (method according to the invention).

[0197] Each 24 cm^2 RODAC plate used was photographed after incubation and reading.

Table U: Tests at a hospital in Bologna

Medicine recovery ward, 4th floor							
Evaluated microorganisms		U. of	Rec. room 1	Rec. room 2	Rec. room 3	Waiting room	Average value
1	Total bacterial load	ufc × cmq	3.15	5.48	2.08	5.69	4.10
2	Staphylococcus aureus	ufc × cmq	0.00	0.00	0.00	0.00	0.00
3	Pseudomonas aeruginosa	ufc × cmq	0.00	0.00	0.00	0.00	0.01
4	Aspergillus niger/Candida albicans	ufc × cmq	0.08	0.04	0.04	0.00	0.04
5	Escherichia coli	ufc × cmq	0.08	0.04	0.04	0.00	0.04

[0198] In control samples, originated with a standard cleaning procedure with sampling at 3:00 pm (according to the prior art), a 40 to 80% range of off-scale values (higher than the possibility of counting on the 24 cm^2 RODAC plates) was found.

[0199] In contrast, in the data shown in tables T and U, originated by applying the method according to the present invention, the total bacterial count is always well below the threshold value of 10 cfu/cm^2.

[0200] In conclusion, the preparation described above, due to the features and efficacy also described in the embodiment examples illustrated, therefore allows, through the method according to the invention also described above, reducing the normal cleaning operations, as a result of the fact that dirt that has accumulated after the cleaning operation is continually degraded over time by the enzyme anchored to the surface and/or to the layers present on such a surface. The continuous degradation of the dirt from sources external to the surface and/or to the layers present on such a surface also causes the control and reduction of pathogens harmful to health that may proliferate due to the accumulation of fats, proteins, sugars that mainly constitute the composition thereof.

[0201] The continuous degradation over time allows a reduction (in terms of time or number) of the routine (manual and mechanical) cleaning operations equal to 10%, instead by 30%, preferably by 50%.

[0202] Therefore, it is possible to identify in the present innovation the ability to reduce cleaning operations, through the application of the preparation, which allows the degradation over time and without any external contribution, neither chemical nor mechanical, of the organic and/or inorganic substances that normally contaminate the surfaces.

[0203] The invention thus conceived is subject to numerous modifications and variants, all falling within the scope of the inventive concept; moreover, all details can be replaced by other technically equivalent elements.

[0204] In the exemplary embodiments, single features described with reference to specific examples may actually be interchanged with other different features, existing in other exemplary embodiments.

[0205] In the practice, the materials used may be any, according to the requirements and to the prior art.

Claims

1. Cleaning preparation for surfaces as floors, walls, glass walls, objects and the like, **characterised in that it** comprises at least an enzyme provided for degrading organic substances which can be anchored to the surface to be cleaned and/or to settlings present on the surface to be cleaned, and
at least an immobilizing agent for immobilizing and maintaining the activity of said enzyme over time following its anchoring to the surface and/or to settlings present on such surface, so as to extend the degradation activity of the organic substances.

2. Cleaning preparation according to claim 1, wherein said enzyme is preferably selected from a protease, a lipase, a lyase and amylase, for respectively degrading organic substances such as proteins, fats, celluloses and sugars, coming from sources external to the surface and/or to settlings present on such surface to be cleaned.

3. Cleaning preparation according to claim 1 or 2, wherein said immobilizing agent comprises at least a pH-regulating agent and/or surfactants, adapted to obtain rising of the wettability of the surface to be cleaned and/or of settlings present on such surface, said pH regulating agent being adapted to keep pH value between 6.5 and 8.5.

4. Cleaning preparation according to one of the preceding claims, wherein said enzyme is firmly anchored to the surface and/or settlings present on such surface by absorption and/or adsorption.

5. Cleaning preparation according to one of the preceding claims, adapted to carry out the degradation function also following its application and without any need of subsequent manual and/or mechanical operations, for a time period of at least 1 day, preferably for at least 3 days, even more preferably for at least 42 days.

6. Method for cleaning surfaces such as floors, walls, glass walls, objects and the like, **characterised in that it** comprises a step of applying, on the surface to be cleaned or on settlings present on such surface to be cleaned, a cleaning preparation or a solution comprising at least an enzyme provided for degrading organic substances.

7. Method according to claim 6, comprising a step of applying, on said surface to be cleaned or on settlings present on such surface, at least a further immobilizing agent preparation or solution for immobilizing and maintaining the activity of said enzyme over time following its anchoring to the surface and/or settlings present on such surface, so as to extend the degradation activity of the organic substances.

8. Method according to claim 7, wherein said step of applying said further preparation or solution comprising at least an immobilizing agent is carried out subsequently to or at the same time of said step of applying said cleaning preparation or said solution.

9. Method according to claim 8, wherein said cleaning preparation comprises said immobilizing agent in form of a solution.

10. Method according to one of claims 6-9, wherein said enzyme is preferably selected from among a protease, a lipase, a lyase and an amylase, for respectively degrading organic substances such as proteins, fats, celluloses and sugars, coming from sources external to the surface and/or settlings present on such surface to be cleaned.

11. Method according to one of claims 7-10, wherein said immobilizing agent comprises at least a pH regulating agent and/or ionic and/or cationic surfactants adapted to obtain rising of the wettability of the surface to be cleaned and/or

settlings present on such surface, said pH regulating agent being adapted to maintain pH value between 6.5 and 8.5.

12. Method according to one of claims 6-11, wherein said enzyme is firmly anchored to the surface and/or settlings present on such surface by absorption and/or adsorption.

13. Method according to one of claims 6-12, comprising at least a step of applying a second cleaning preparation having a low surfactant content subsequently to said step of applying said cleaning preparation comprising at least an enzyme.

14. Method according to claim 13, wherein said steps of applying said cleaning preparation comprising at least an enzyme and applying said second cleaning preparation are carried out in an alternating manner over time, i.e. every other day and/or week and/or month.

15. Method according to one of claims 6-14, comprising a time sequence of said steps of applying said cleaning preparation comprising at least an enzyme, each of said steps being **characterised by** a respective number of week applications, said number of week applications being progressively decreasing during at least the first three weeks since the beginning of the process.

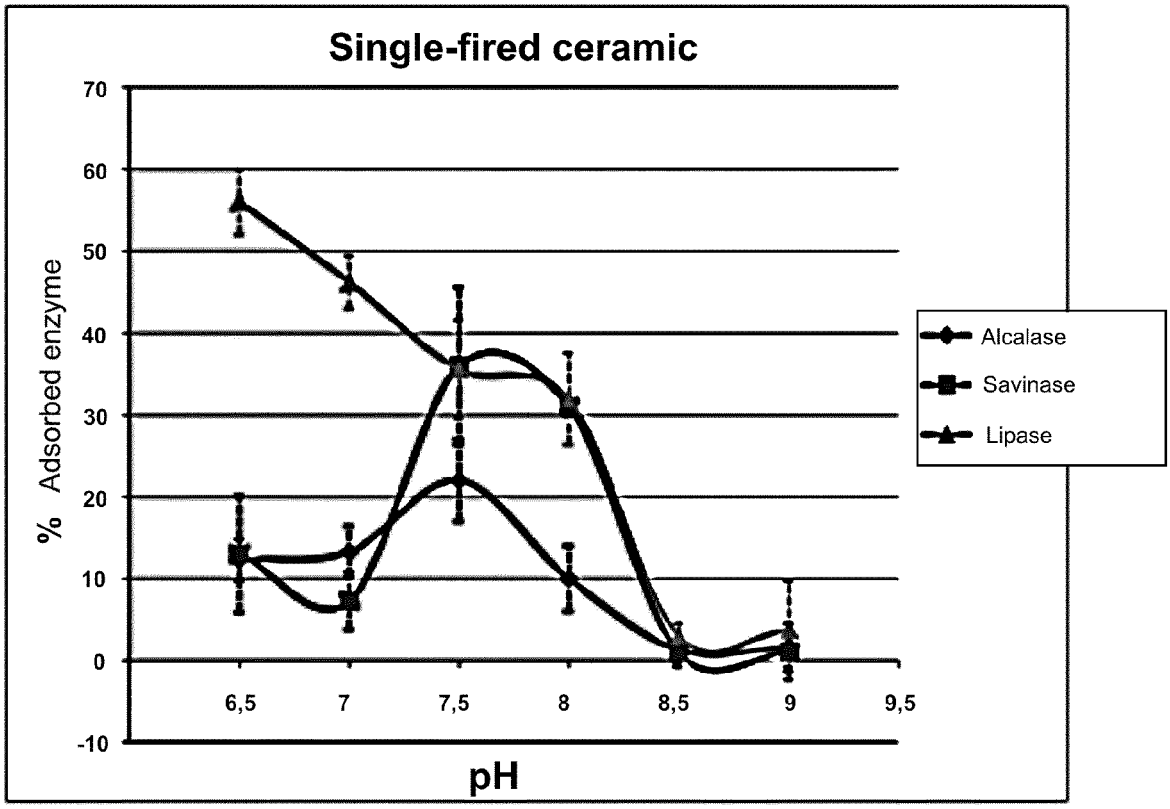
Single-fired ceramic
% Adsorbed enzyme
70
60
50
40
30
20
10
0
-10
6,5
7
7,5
8
8,5
9
9,5
pH
Alcalase
Savinase
Lipase

FIG. 1

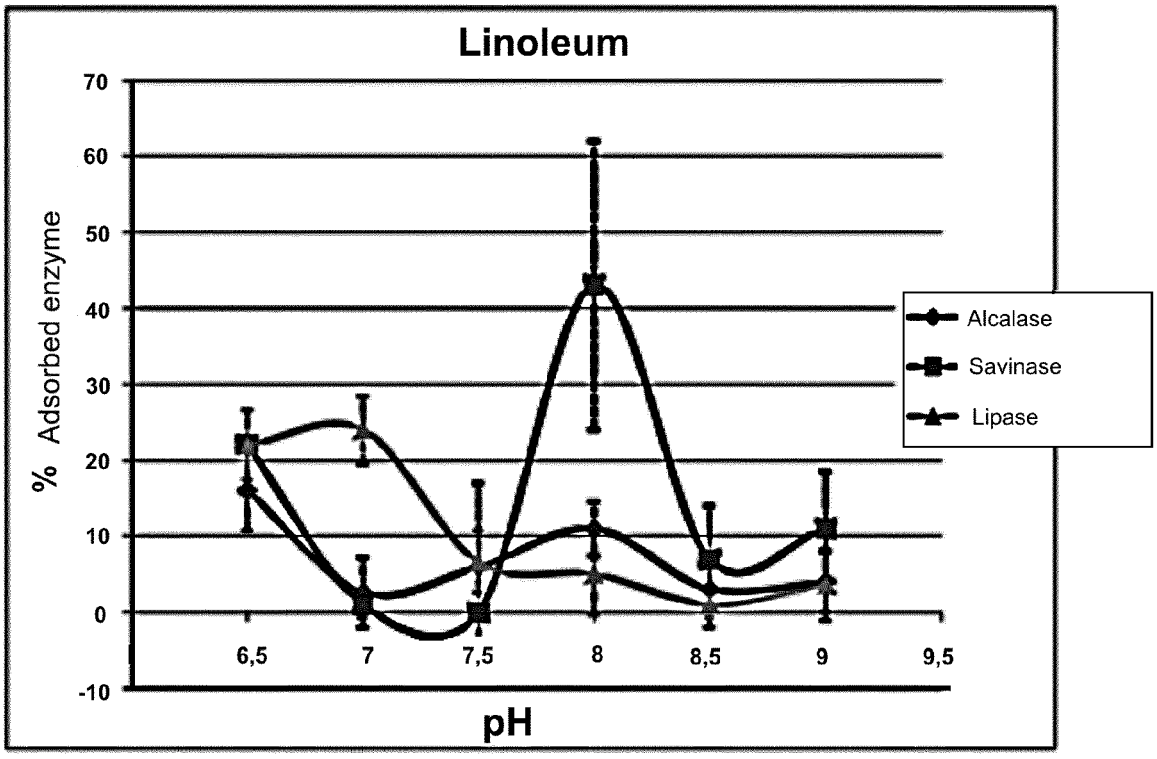
Linoleum
% Adsorbed enzyme
70
60
50
40
30
20
10
0
-10
6,5
7
7,5
8
8,5
9
9,5
pH
Alcalase
Savinase
Lipase

FIG. 2

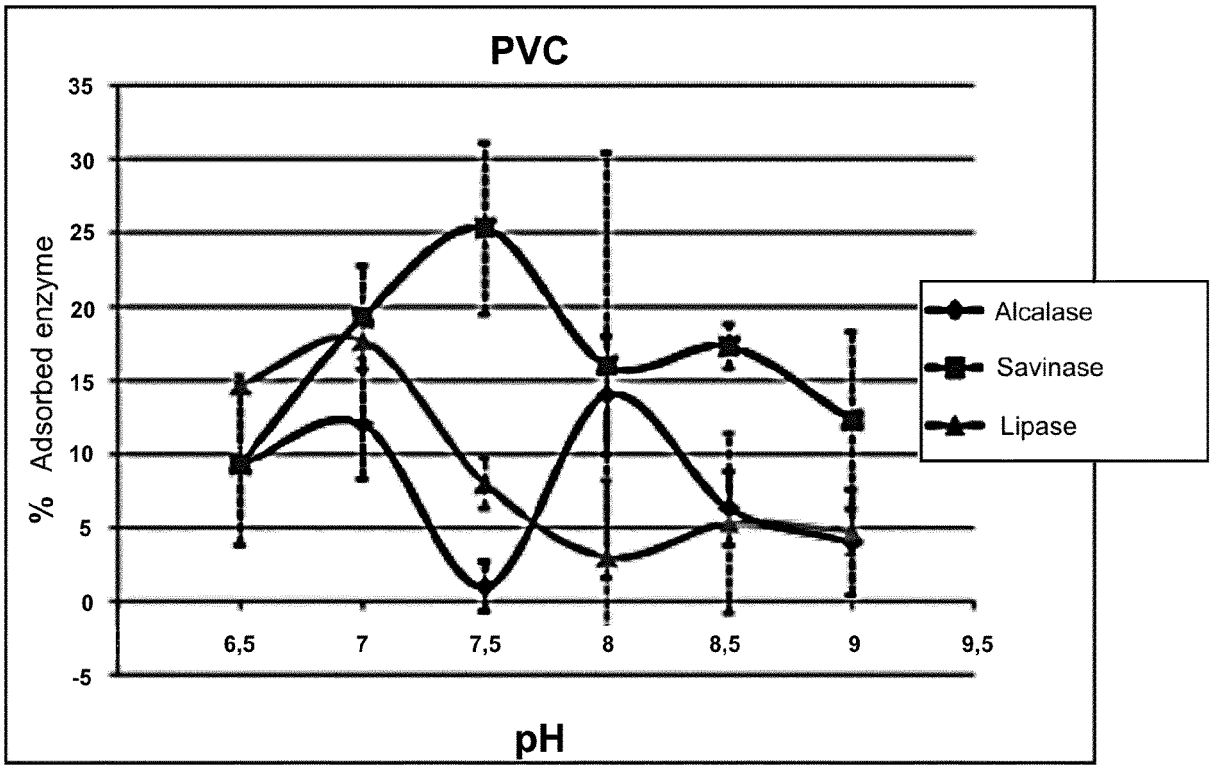
PVC
% Adsorbed enzyme
35
30
25
20
15
10
5
0
-5
6,5
7
7,5
8
8,5
9
9,5
pH
Alcalase
Savinase
Lipase

FIG. 3

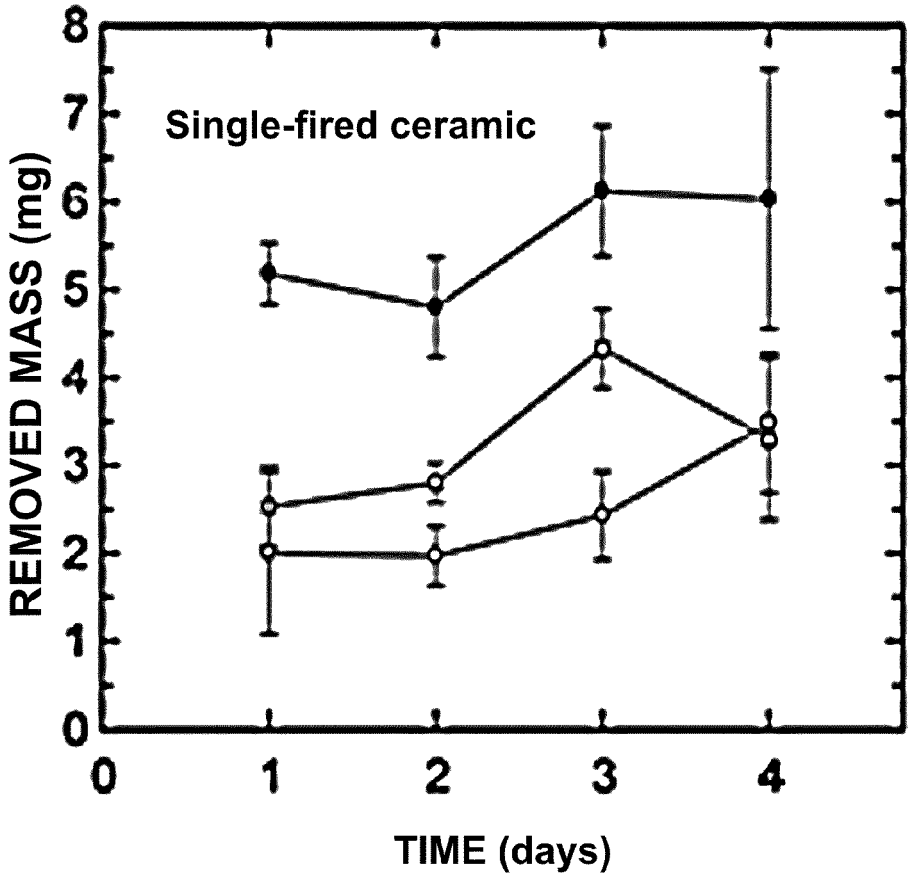
Single-fired ceramic
REMOVED MASS (mg)
0
1
2
3
4
5
6
7
8
0
1
2
3
4
TIME (days)

FIG. 4

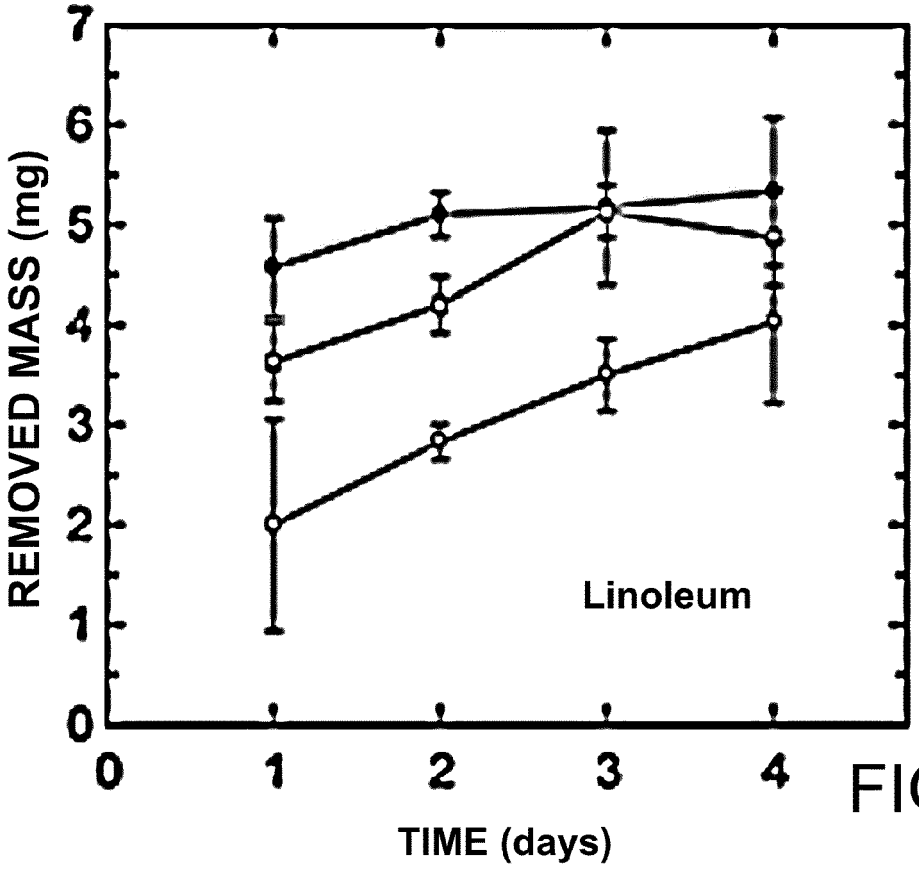
REMOVED MASS (mg)
0
1
2
3
4
5
6
7
0
1
2
3
4
TIME (days)
Linoleum

FIG. 5

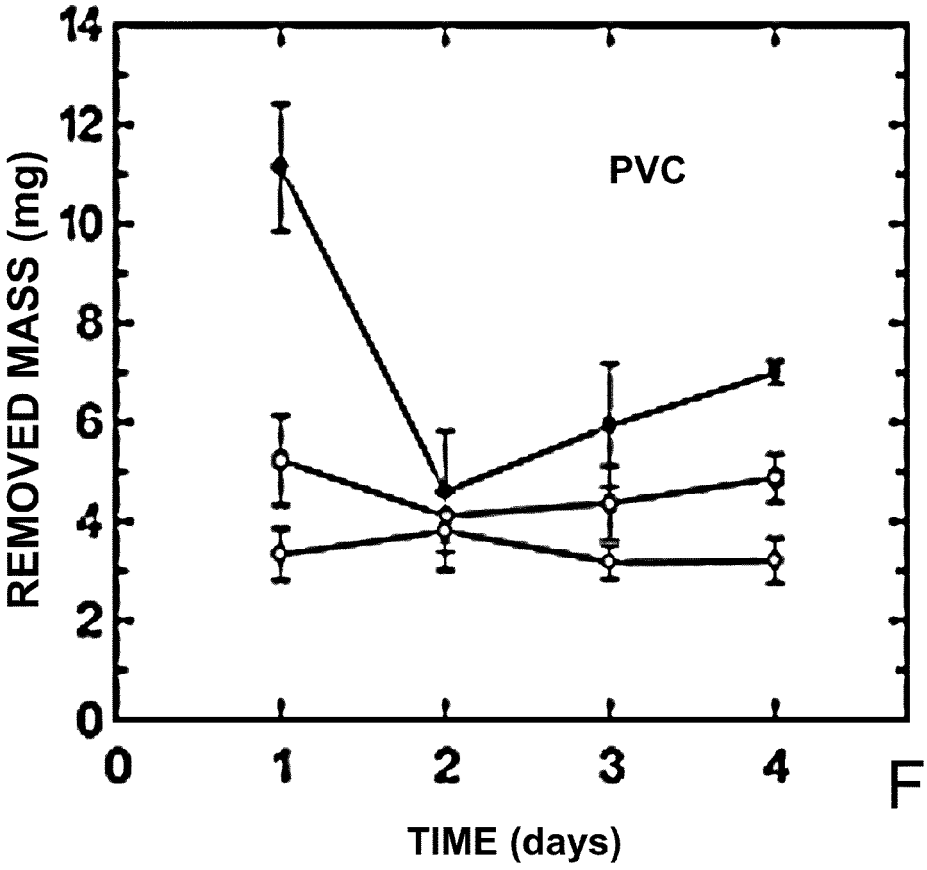
REMOVED MASS (mg)
0
2
4
6
8
10
12
14
0
1
2
3
4
TIME (days)
PVC

FIG. 6

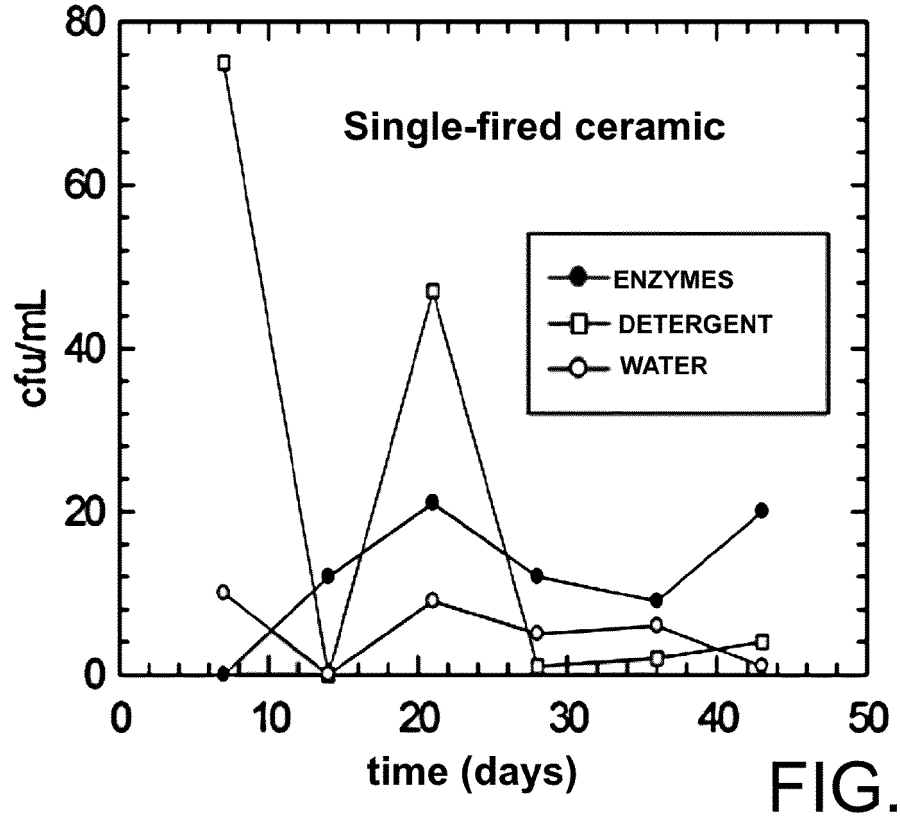
Single-fired ceramic
ENZYMES
DETERGENT
WATER
cfu/mL
time (days)
0
20
40
60
80
0
10
20
30
40
50

FIG. 7

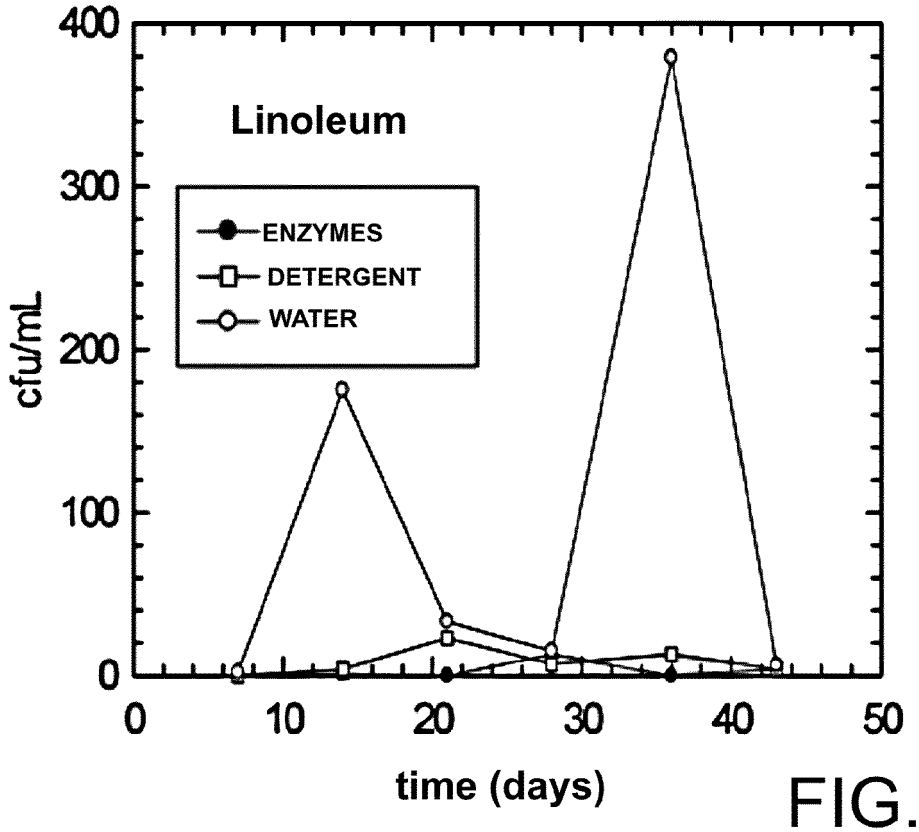
Linoleum
ENZYMES
DETERGENT
WATER
cfu/mL
time (days)
0
100
200
300
400
0
10
20
30
40
50

FIG. 8

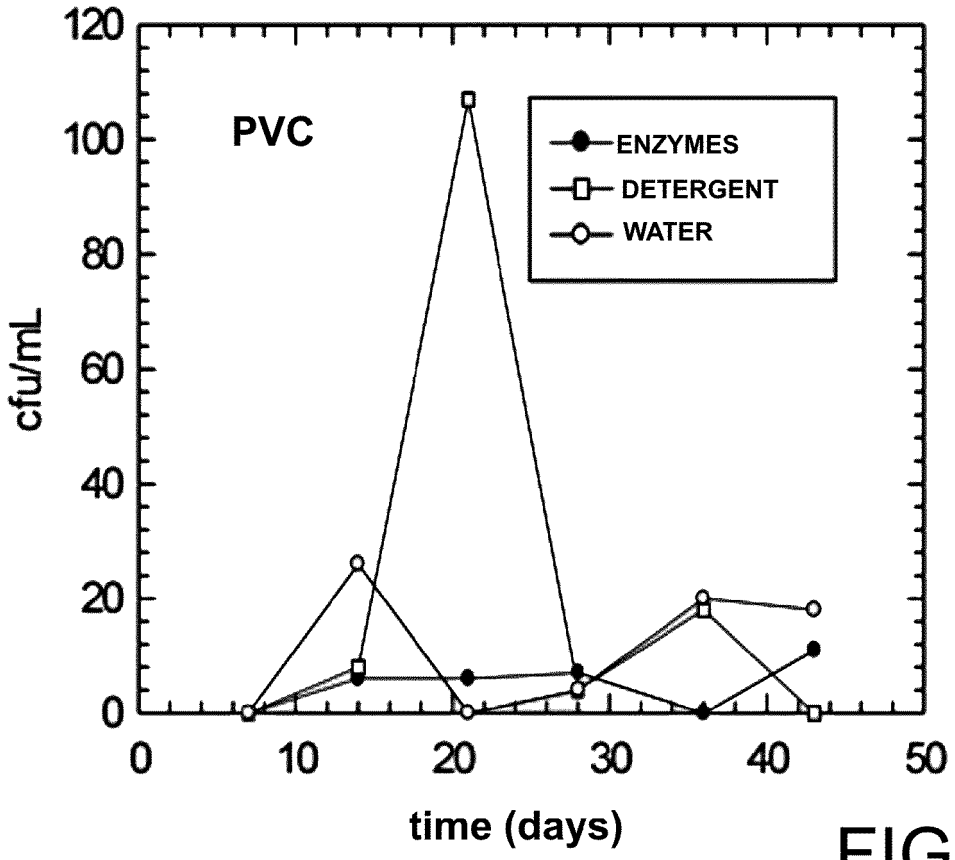
PVC
ENZYMES
DETERGENT
WATER
cfu/mL
time (days)
0
20
40
60
80
100
120
0
10
20
30
40
50

FIG. 9

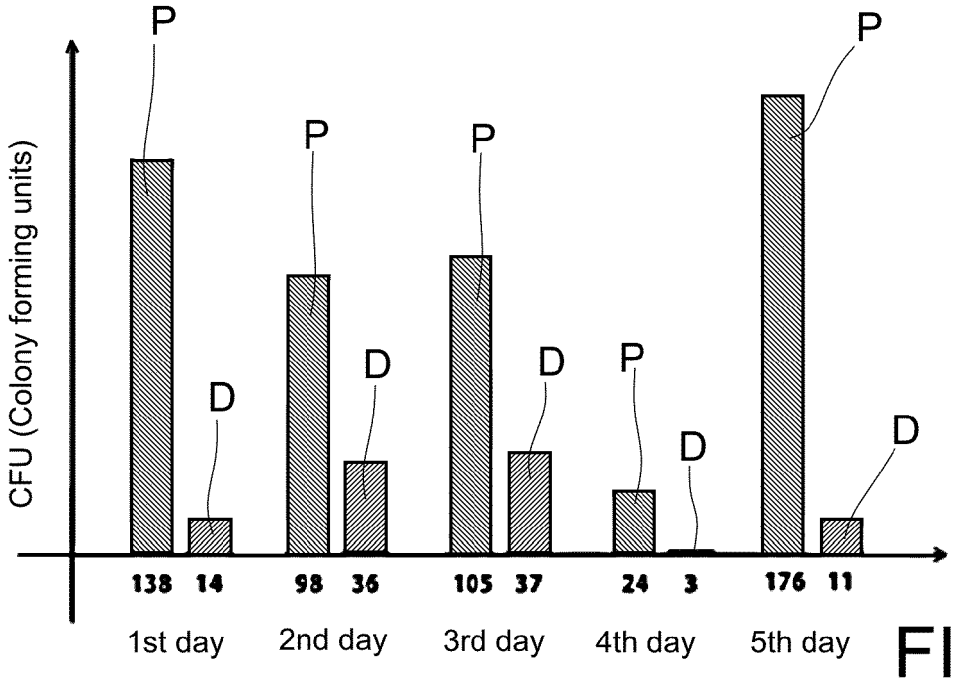
CFU (Colony forming units)
P
D
138
14
98
36
105
37
24
3
176
11
1st day
2nd day
3rd day
4th day
5th day

FIG. 10

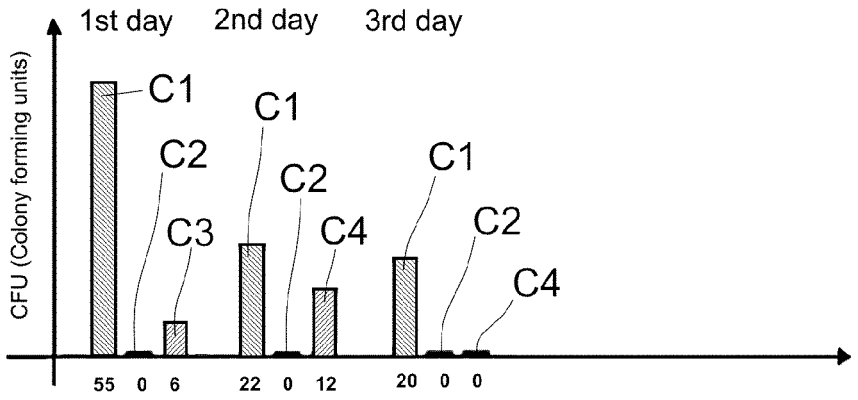

FIG. 11

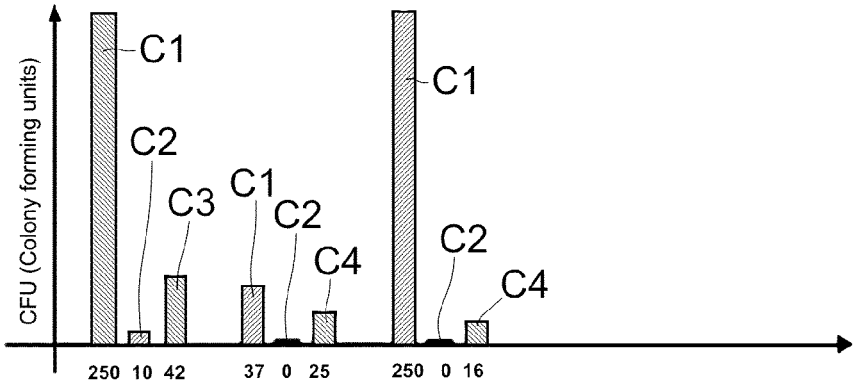

FIG. 12

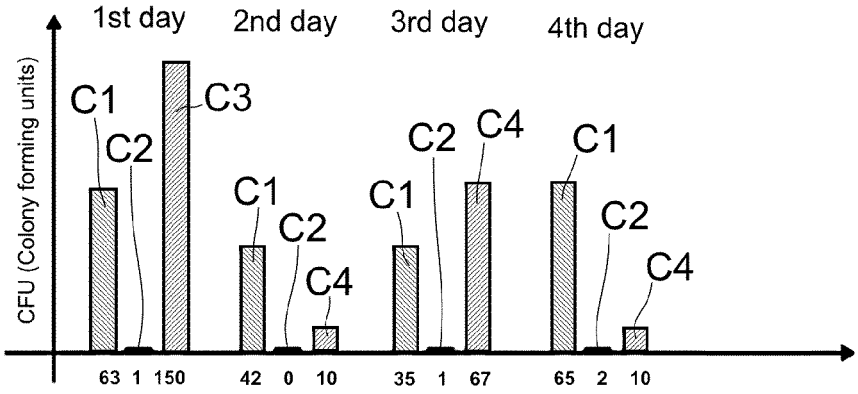

FIG. 13

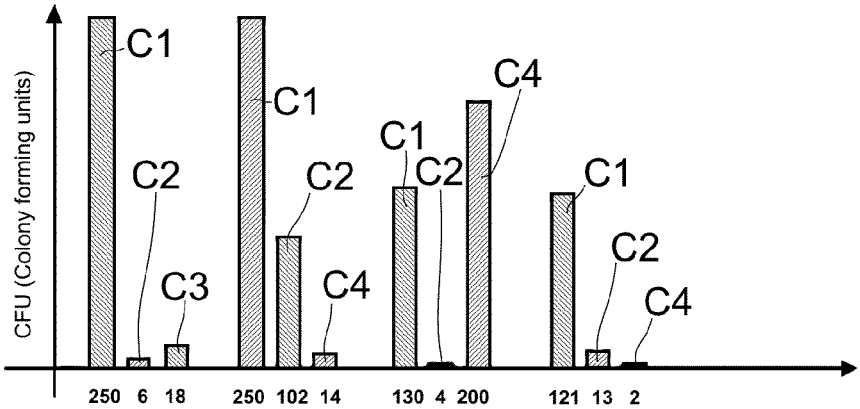

FIG. 14

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PARTIAL EUROPEAN SEARCH REPORT

under Rule 62a and/or 63 of the European Patent Convention. This report shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number
EP 16 16 3756

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	EP 0 476 915 A2 (CLOROX CO [US]) 25 March 1992 (1992-03-25) * page 12, lines 24-36 *	1-15	INV. C11D3/386
X	WO 98/39403 A1 (PROCTER & GAMBLE [US]; HERBOTS IVAN MAURICE ALFONS JA [BE]; MOESE ROSA) 11 September 1998 (1998-09-11) * example 20 *	1-15	
X	DATABASE WPI Week 201435 Thomson Scientific, London, GB; AN 2014-J72421 XP002752472, & CN 103 666 780 A (QINGDAO DAOHE BIOLOGICAL TECHNOLOGY CO) 26 March 2014 (2014-03-26) * abstract *	1-15	
X	US 2013/137623 A1 (SIEGERT PETRA [DE] ET AL) 30 May 2013 (2013-05-30) * paragraph [0079]; examples *	1-15	TECHNICAL FIELDS SEARCHED (IPC) C11D

-/--

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC so that only a partial search (R.62a, 63) has been carried out.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

see sheet C

Place of search	Date of completion of the search	Examiner
Munich	17 October 2016	Saunders, Thomas

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04E07)

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 16 16 3756

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (IPC)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
X	WO 02/33035 A1 (3840158 CANADA INC [CA]; TEASDALE STEVE [CA]; LAFRANCE CORINNE [CA]) 25 April 2002 (2002-04-25) * pages 1, 3; claims 7, 8 *	1-15	
X	WO 03/018734 A1 (TREYCO SUPPLY INC [US]; MANNING JAMES T JR [US]) 6 March 2003 (2003-03-06) * page 1; example XIII *	1-15	
X	WO 2014/006424 A1 (XEROS LTD [GB]) 9 January 2014 (2014-01-09) * example 2 *	1-15	
X	WO 2014/067933 A1 (LECTA GMBH C [DE]) 8 May 2014 (2014-05-08) * example 4 *	1-15	TECHNICAL FIELDS SEARCHED (IPC)
X	US 2012/276617 A1 (JIA HONGFEI [US] ET AL) 1 November 2012 (2012-11-01) * example 4 *	1-15	
X	WO 2004/015074 A2 (PROCTER & GAMBLE [US]) 19 February 2004 (2004-02-19) * page 1, paragraph 2; claim 9; example 2 *	1-15	

EPO FORM 1503 03.82 (P04C10)

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INCOMPLETE SEARCH
SHEET C

Application Number
EP 16 16 3756

Claim(s) searched incompletely:
1-15

Reason for the limitation of the search:

The initial phase of the search revealed a very large number of documents relevant to the issue of novelty. The scope of the present claims includes any composition comprising an enzyme in combination with a substance which is not pH-neutral and/or in combination with a cationic, anionic or amphoteric surfactant. The number of patents relating to such compositions is well in excess of 5000.

So many documents were retrieved that it is impossible to determine which parts of claims 1-15 may be said to define subject-matter for which protection might legitimately be sought.

For these reasons, a meaningful search of the whole claimed subject-matter of claims 1-15 could not be carried out. The extent of the search was consequently limited.

The search of claims 1-15 was restricted to the use of enzyme-containing compositions for cleaning hard surfaces such as floors, walls and glass, wherein the compositions explicitly comprise an enzyme immobilising or stabilising agent and/or comprise a pH regulating agent and/or a surfactant.

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 16 16 3756

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The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

17-10-2016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE 69126079 D1	19-06-1997
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		US 2002082184 A1	27-06-2002
		WO 0233035 A1	25-04-2002
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		HK 1206777 A1	15-01-2016
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		WO 2014006424 A1	09-01-2014
WO 2014067933 A1	08-05-2014	NONE	

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17-10-2016

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2012276617 A1	01-11-2012	JP 5964648 B2	03-08-2016
		JP 2012233186 A	29-11-2012
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		US 2004029242 A1	12-02-2004
		WO 2004015074 A2	19-02-2004

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82