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(54) Title: METHOD FOR CONTROLLING MALODORS, IN PARTICULAR IN DISH WASHING MACHINES, USING BACTERIAL SPORES CAPABLE OF INHIBITING OR PREVENTING THE PRODUCTION OF MALODOR

(57) Abstract: The present invention generally relates to a method for degrading malodors with regard to the treatment of hard surfaces, and more particularly relates to the degradation of malodors in the context of dish washing, by contacting said surfaces with bacterial spores of at least one species of Bacillus.

Method for controlling malodors, in particular in dish washing machines, using bacterial spores capable of inhibiting or preventing the production of malodor

The present invention generally relates to a method for degrading malodors preferably with regard to the treatment of hard surfaces, and more particularly relates to the degradation of malodors in the context of dish washing.

Malodor is a growing problem, particularly in dish washers, with the changed habits of lower temperature washing and the tendency to avoid disinfectants for ecological reasons.

An important consumer requirement therefore consists in the elimination or at least diminution of malodors (i.e. off-odors) or undesired odors. Off-odors derive from specific olfactorily active compounds that are also referred to as "malodorants." Malodorants are foul-smelling compounds having so-called kakosmophoric groups, e.g. amine derivatives and sulfur derivatives. The presence of such off-odors generally results in a negative effect on human comfort, and for that reason the consumer makes an effort to extinguish these odors. Often, however, the off-odors are not extinguished but merely masked. It is usual to use for this purpose products that contain volatile, usually pleasant-smelling substances, and that even in small quantities can mask foul odors.

These solutions, however, are not completely effective as they are short-term. There is a need in the art for new solutions for controlling the problem of malodor.

It is therefore the object of the present invention to provide the consumer with a further capability for bringing about an inhibition, degradation or prevention of malodors.

The present invention provides a method of inhibiting or preventing the production of malodor comprising contacting tableware or a dish washing machine with bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the

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registration reference PTA-7543, registration office ATCC,
registration reference SD-6992, registration office ATCC,
registration reference NRRL B-50607, registration office ARS,
registration reference NRRL B-50606, registration office ARS,

registration reference NRRL B-50887, registration office ARS,
registration reference PTA-7549, registration office ATCC,
registration reference NRRL B-50017, registration office ARS,
and its mixtures.

Above mentioned strains were deposited by Novozymes Biologicals, Inc. 5400 Corporate Circle, Salem, VA 24513, USA, at the American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, Virginia 20108, USA, or at the Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Agricultural Research Service (ARS), U.S. Department of Agriculture, 1815 North University Street, Peoria, Illinois 61604, USA, under terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

The contacting can occur before, during, or after a cleaning process. Tableware including cutlery preferably is contacted with the bacterial spores during the washing process; dish washing machines may alternatively or additionally be contacted with the bacterial spores in between two cleaning processes.

Combinations of bacterial spores of one or more of such strains may also be used, such as, blends of two or more strains, three or more strains, four or more strains, five or more strains, etc.

The present invention also provides the use of said bacterial spores for inhibiting malodor in cleaning machines and cleaning processes. The present invention also provides compositions for use in inhibiting malodor in cleaning machines and cleaning processes.

The methods and compositions of the present invention may be used to treat an existing odor problem and/or as a preventative treatment to prevent a potential odor problem. The present invention may be used, for example, to inhibit malodor in dish washing machines/processes and other cleaning machines/processes.

Malodor may be generated from a number of sources, mostly microbial and in particular bacterial sources (including compounds derived or produced therefrom). Different surfaces or surfaces in different environments cause different habitats for microorganisms in household and household appliances. Important factors of influence on the microorganism habitat are the types of surface, such as textile, hard surfaces of plastic or ceramic, and sponges, the amounts of humidity, the sources of nutrients and their availabilities, growth conditions, and environmental sources of microbial contamination. While in most household surfaces waterborne germs, mainly gram negative bacteria, play a role, the population of microorganisms at dish-washing sites differ from

the population at laundry washing sites: The latter, such as the interior of automatic laundry washing machines, tend to comprise microorganisms originating from the source "man", such as gram positive bacteria, whereas the former, such as the interior of automatic dish washing machines, normally lack microorganisms from this source, because human contact to clothing normally is more intense by far than human contact to tableware or cutlery. Microorganisms populations at dish-washing sites normally tend to comprise gram negative proteobacteria, such as for example Alphaproteobacteria. The current invention is capable to inhibit or prevent the production of malodor of such sources, so that preferred embodiments of the invention relate to inhibiting or preventing the production of malodor caused by gram negative proteobacteria and/or substances derived therefrom.

The methods and compositions may also be applied directly to any tableware including cutlery cleaned in the cleaning machine or cleaning process, such as, to a dish cleaned in the machine. The tableware may be treated before cleaning, during the cleaning process, after the cleaning processes and any combination thereof.

The treating may include contacting the odor-generating organism(s) or odor-generating compound(s) present in the cleaning machine or cleaning process with the bacterial spores. Such contacting may include contacting a surface of a machine with the bacterial spores and/or contacting a process water or cleaning composition used in the cleaning machine with the bacterial spores.

Contacting means contacting the odor-causing organism and/or odor causing compound with the bacterial spores.

The ability to prepare spores and vegetative cells is considered routine in the art. See Tzeng, Y. M., Y. K. Rao, et al. (2008). "Effect of cultivation conditions on spore production from *Bacillus amyloliquefaciens* B128 and its antagonism to *Botrytis elliptica*." *Journal of Applied Microbiology* 104(5): 1275-1282.

Compositions of the invention comprise bacterial spores as described herein. The bacterial spores should be present in effective amounts. The terms "effective amount", "effective concentration" or "effective dosage" are defined herein as the amount, concentration or dosage of odor-controlling bacterial spores that can inhibit the malodor caused by the odor causing organism or substances derived therefrom on articles, articles subjected to a cleaning machine or cleaning process, and/or cleaning machines. The actual effective dosage in absolute numbers depends on factors including: the odor causing organisms(s) in question; whether the aim is prevention or reduction of malodor; other ingredients present in the composition, and also the articles and/or cleaning machine in question.

In an embodiment an effective dosage of the bacterial spores of the strains as described herein would be introduced to an automatic dishwashing agent at a final concentration of 1×10^2 - 1×10^9 CFU/g of automatic dishwashing agent, with a preferred range of 1×10^3 - 1×10^5 CFU/g of automatic dishwashing agent.

In another embodiment an effective dosage of the bacterial spores of the strains as described herein would be introduced to a manual dishwashing agent at a final concentration of 1×10^1 - 1×10^9 CFU/g of the manual dishwashing agent, with a preferred range of 1×10^2 - 1×10^5 CFU/g of the manual dishwashing agent.

Effective amounts can be determined by one skilled in the art using routine assays.

The bacterial spores of the invention can be used in combination with or as an ingredient of a washing product, such as manual or automatic dishwashing agents in particular, including but not limited to aerosols, powders, solids, creams, etc., for use, e.g., in dish washers, dish washing processes and/or articles treated in dishwashers or dish washing processes, such as, tableware.

An aspect of the present invention also includes cleaning compositions or compositions for use in dishwashers or dish washing processes which comprise bacterial spores described herein and a carrier. The composition may be in the form of a solid, semi-solid, gel, liquid, aerosol, emulsion, and/or powder. Most preferably, the bacterial spores are of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the registration reference SD-6991, registration office ATCC, registration reference PTA-7543, registration office ATCC, registration reference SD-6992, registration office ATCC, registration reference NRRL B-50607, registration office ARS, registration reference NRRL B-50606, registration office ARS, registration reference NRRL B-50887, registration office ARS, registration reference PTA-7549, registration office ATCC, registration reference NRRL B-50017, registration office ARS, and its mixtures

The most preferred strain according to the invention is PTA-7543.

The compositions may in particular embodiments comprise blends of bacterial spores of two or more strains, including at least two, at least three, at least four, and at least five of the bacterial spores described herein.

The compositions of the present invention may in an embodiment have a pH in the range of 5-10 and may further include water and/or one or more preservatives. For preservation of compositions comprising bacterial spores of *Bacillus amyloliquefaciens*, for example, the following preservatives can be useful: chloromethylisothiazolinone/methylisothiazolinone (CMIT/MIT) (Kathon or others); MIT (Neolone or others); 1,2-benzisothiazolin-3-one (BIT) (if allowed in personal care); CMIT/MIT + EDTA; CMIT/MIT + Biodegradable Chelator; MIT + EDTA; MIT + Biodegradable Chelator; BIT + EDTA; BIT + Biodegradable Chelator; Bronopol; 2-Phenoxyethanol; 2-Phenoxyethanol + Biodegradable Chelator; Potassium sorbate (used at low pH); Sodium benzoate (used at low pH); Salt; Glycerol; Propylene Glycol; Essential Oils; Dichlorobenzyl alcohol; Triclosan; Parabens; and 1-Phenoxy-2-propanol and 2-Phenoxy-1-propanol. In an embodiment, the preservative is 2-Phenoxyethanol; 2-Phenoxyethanol + Biodegradable Chelator; Potassium Sorbate (used at low pH); Sodium Benzoate (used at low pH); Salt; Glycerol; Propylene Glycol; or one of more Essential Oils - e.g., white mustard seed, tea tree, rosewood, or some citrus oils. In another embodiment, the preservative is 2-Phenoxyethanol; 2-Phenoxyethanol + Biodegradable Chelator; or Glycerol. Accordingly, an embodiment of the present invention is directed to a composition comprising bacterial spores as described herein and a preservative selected from the group consisting of chloromethylisothiazolinone/ methylisothiazolinone (CMIT/MIT) (Kathon or others); MIT (Neolone or others); 1,2-benzisothiazolin-3-one (BIT) (if allowed in personal care); CMIT/MIT + EDTA; CMIT/MIT + Biodegradable Chelator; MIT + EDTA; MIT + Biodegradable Chelator; BIT + EDTA; BIT + Biodegradable Chelator; Bronopol; 2-Phenoxyethanol; 2-Phenoxyethanol + Biodegradable Chelator; Potassium sorbate (used at low pH); Sodium benzoate (used at low pH); Salt; Glycerol; Propylene Glycol; Essential Oils; Dichlorobenzyl alcohol; Triclosan; Parabens; and 1-Phenoxy-2-propanol and 2-Phenoxy-1-propanol. In an embodiment, the preservative is 2-Phenoxyethanol; 2-Phenoxyethanol + Biodegradable Chelator; Potassium Sorbate (used at low pH); Sodium Benzoate (used at low pH); Salt; Glycerol; Propylene Glycol; or one of more Essential Oils - e.g., white mustard seed, tea tree, rosewood, or some citrus oils, 2-Phenoxyethanol; 2-Phenoxyethanol + Biodegradable Chelator; or Glycerol, and wherein the composition is a liquid, solid or gel composition.

In one preferred aspect, the invention provides a composition adapted for application to the interior of a dish washing machine. A composition of the invention may be in solid or liquid form. The composition may be a concentrate to be diluted, rehydrated and/or dissolved in a solvent, including water, before use. The composition may also be a ready-to-use (in-use) composition. The composition may furthermore be an active cleaning base ingredient to be incorporated into other cleaning or washing compositions.

In one embodiment, the composition is adapted for delivery to a dish washing machine to prevent fouling by bacterial species capable of causing malodor. In another embodiment, the composition is further adapted for delivery to a dish washing machine by applications which include, but are not

limited to, solid, semi-solid, gel, liquid, aerosol, emulsion, and/or powder applications alone and/or in combination with liquid, solid, semisolid, aerosol, emulsion, and/or gel detergents, alone and/or in combination with any other dish washing machine additive.

Preferred embodiments of the present invention encompass dish washing agents comprising bacterial spores described herein.

The dish washing agents according to the present invention, which can be present as powdered or tableted solids, homogeneous solutions, or suspensions, can in principle contain, in addition to the bacterial spores used according to the present invention, all known ingredients that are usual in such agents. The agents according to the present invention can contain, in particular, builder substances, surface-active surfactants, peroxygen compounds, water-miscible organic solvents, enzymes, sequestering agents, electrolytes, pH regulators, and further adjuvants such as silver corrosion inhibitors, foam regulators, additional peroxygen activators, and coloring agents and fragrances. Agents according to the present invention provided for use in automatic dishwashing methods are preferably so-called "3 in 1" products that combine the conventional cleaner, rinse-agent, and regenerating-salt agents in one agent.

Suitable in principle as water-soluble builder components, in particular in the low-alkalinity cleaning agents, are all builders usually used in automatic dishwashing agents, for example polymeric alkali phosphates, which can be present in the form of their alkaline, neutral, or acid sodium or potassium salts. Examples thereof are tetrasodium diphosphate, disodium dihydrogendiphosphate, pentasodium triphosphate, so-called sodium hexametaphosphate, and the corresponding potassium salts, or mixtures of sodium and potassium salts. Their quantities can be in the range of up to approximately 35 wt %, based on the entire agent; preferably, however, the agents according to the present invention are free of such phosphates. Further possible water-soluble builder components are, for example, organic polymers or natural or synthetic origin, chiefly polycarboxylates, which act as co-builders especially in hard-water regions. Possibilities are, for example, polyacrylic acids and copolymers of maleic acid anhydride and acrylic acid, as well as the sodium salts of said polymeric acids. Commercially usual products are, for example, Sokalan® CP 5 and PA 30 of the BASF company. Among the polymers of natural origin usable as co-builders are, for example, oxidized starch as known, for example, from International Patent Application WO 94/05762, and polyamino acids such as polyglutamic acid or polyaspartic acid. Further possible builder components are naturally occurring hydroxycarboxylic acids such as, for example, mono-, dihydroxysuccinic acid, α -hydroxypropionic acid, and gluconic acid. Among the preferred builder components are the salts of citric acid, in particular sodium citrate. Possibilities as sodium citrate are anhydrous trisodium citrate and, by preference, trisodium citrate dihydrate. Trisodium citrate dihydrate can be used as a finely or coarsely crystalline powder. Depending on the pH ultimately

established in the agents according to the present invention, the acids corresponding to the aforesaid co-builder salts can also be present.

Suitable oxygen-based bleaching agents are principally alkali perborate mono- or tetrahydrate and/or alkali percarbonate, sodium being the preferred alkali metal. The use of sodium percarbonate has advantages especially in cleaning agents for tableware, since it has particularly favorable effects on corrosion properties on glassware. The oxygen-based bleaching agent is therefore by preference an alkali percarbonate, in particular sodium percarbonate. Additionally or, in particular, alternatively, known peroxy-carboxylic acids, for example dodecanediperic acid or phthalimidopercarboxylic acids, which if applicable can be substituted on the aromatic portion, can be contained. The addition of small quantities of known bleaching-agent stabilizers such as, for example, phosphonates, borates or metaborates, and metasilicates, as well as magnesium salts such as magnesium sulfate, may furthermore be expedient. Because cleaning agents are usually utilized in an air atmosphere, the agents according to the present invention can also be free of bleaching agents, since the bleach catalysts according to formula (I) already exhibit an effect in the presence of atmospheric oxygen.

Transition-metal salts or complexes known as bleach-activating active substances, and/or conventional bleach activators, i.e. compounds that, under perhydrolysis conditions, yield optionally substituted perbenzoic acid and/or peroxy-carboxylic acids having 1 to 10 carbon atoms, in particular 2 to 4 carbon atoms, can be used; the presence of peroxygen-based bleaching agent is then necessary, however. The usual bleach activators cited above, which carry O- and/or N-acyl groups having the aforesaid number of carbon atoms, and/or which carry optionally substituted benzoyl groups, are suitable. Multiply acylated alkylenediamines, in particular tetraacetylenediamine (TAED), acylated glycolurils, in particular tetraacetyl glycoluril (TAGU), acylated triazine derivatives, in particular 1,5-diacetyl-2,4-dioxohexahydro-1,3,5-triazine (DADHT), acylated phenylsulfonates, in particular nonanoyl- or isononanoyloxybenzenesulfonate, acylated polyvalent alcohols, in particular triacetin, ethylene glycol diacetate, 2,5-diacetoxy-2,5-dihydrofuran, as well as acetylated sorbitol and mannitol, and acylated sugar derivatives, in particular pentaacetyl glucose (PAG), pentaacetyl fructose, tetraacetyl xylose, and octaacetyl lactose, as well as acetylated, optionally N-alkylated glucamine and gluconolactone, are preferred. Combinations of conventional bleach activators can also be used.

Automatic dishwashing agents according to the present invention preferably contain the usual alkali carriers such as, for example, alkali silicates, alkali carbonates, and/or alkali hydrogencarbonates. Included among the alkali carriers usually used are carbonates, hydrogencarbonates, and alkali silicates having a $\text{SiO}_2/\text{M}_2\text{O}$ molar ratio ($\text{M}=\text{alkali atom}$) from 1.5:1 to 2.5:1. Alkali silicates can be contained in quantities of up to 30 wt % based on the entire agent. It is preferable to dispense entirely with the use of the highly alkaline metasilicates as alkali carriers. The alkali carrier system

preferably used in the agents according to the present invention is a mixture of carbonate and hydrogencarbonate, by preference sodium carbonate and hydrogencarbonate, which is contained in a quantity of up to 60 wt %, by preference 10 wt % to 40 wt %. The ratio of carbonate used to hydrogencarbonate used varies depending on the pH that is ultimately desired, although an excess of sodium hydrogencarbonate is usually used, so that the weight ratio between hydrogencarbonate and carbonate is generally 1:1 to 15:1.

In a further preferred embodiment of agents according to the present invention, 20 wt % to 40 wt % water-soluble organic builders, in particular alkali citrate, 5 wt % to 15 wt % alkali carbonate, and 20 wt % to 40 wt % alkali disilicate are contained.

The agents according to the present invention can also, if applicable, have surfactants added to them, in particular low-foaming nonionic surfactants that provide better detachment of grease-containing stains and serve as a wetting agent and, if applicable, as a granulating adjuvant in the context of manufacture of the cleaning agents. Their quantity can be up to 10 wt %, in particular up to 5 wt %, and is preferably in the range from 0.5 wt % to 3 wt %. Extremely low-foaming compounds are usually used, especially in cleaning agents for use in automatic dishwashing processes. These compounds include, by preference, C12-C18 alkylpolyethylene glycol-polypropylene glycol ethers having respectively up to 8 mol of ethylene oxide and propylene oxide units in the molecule. It is also possible, however, to use other known low-foaming nonionic surfactants such as, for example, C12-C18 alkylpolyethylene glycol-polybutylene glycol ethers having respectively up to 8 mol of ethylene oxide and butylene oxide units in the molecule, end-capped alkylpolyalkylene glycol mixed ethers, and the foaming but environmentally attractive C8-C14 alkyl polyglucosides having a degree of polymerization from approximately 1 to 4 (e.g. APG® 225 and APG® 600), and/or C12-C14 alkylpolyethylene glycols having 3 to 8 ethylene oxide units in the molecule. Likewise suitable are surfactants from the family of the glucamides such as, for example, alkyl-N-methylglucamides, in which the alkyl part preferably derives from a fatty alcohol having a carbon chain length of C6 to C14. It is in some cases advantageous if the above-described surfactants are used as mixtures, for example the combination of alkyl polyglycoside with fatty alcohol ethoxylates, or glucamide with alkyl polyglycosides.

Preferred silver corrosion protection agents are organic disulfides, divalent phenols, trivalent phenols, optionally substituted benzotriazole, salts and/or complexes of manganese, titanium, zirconium, hafnium, vanadium, cobalt, or cerium, in which the aforesaid metals are present in one of the oxidation states II, III, IV, V, or VI.

In addition, the agents according to the present invention can contain enzymes such as proteases, amylases, pullulases, cutinases, and lipases, for example proteases such as BLAP®, Optimase®, Opticlean®, Maxacal®, Maxapem®, Esperase®, and/or Savinase®, amylases such as

Termamyl®, Amylase-LT®, Maxamyl®, and/or Duramyl®, lipases such as Lipolase®, Lipomax®, Lumafast®, and/or Lipozym®. The enzymes that are used if applicable can be adsorbed onto carrier substances and/or embedded into encasing substances in order to protect them from premature inactivation. They are contained in the cleaning agents according to the present invention in quantities by preference not above 2 wt %, in particular from 0.1 wt % to 0.7 wt %.

If the cleaning agents foam excessively upon use, they can also have added to them up to 6 wt %, by preference approximately 0.5 wt % to 4 wt %, of a foam-suppressing compound, by preference from the group of the silicone oils, mixtures of silicone oil and hydrophobized silicic acid, paraffins, paraffin-alcohol combinations, hydrophobized silicic acid, the bis-fatty acid amides, and other known defoamers obtainable commercially. Further optional ingredients in the agents according to the present invention are, for example, perfume oils.

Among the organic solvents usable in the agents according to the present invention, especially when the latter are present in liquid or paste form, are alcohols having 1 to 4 carbon atoms, in particular methanol, ethanol, isopropanol, and tert.-butanol, diols having 2 to 4 carbon atoms, in particular ethylene glycol and propylene glycol, as well as mixtures thereof, and the ethers derivable from the aforesaid compound classes. Water-miscible solvents of this kind are present in the cleaning agents according to the present invention at preferably no more than 20 wt %, in particular from 1 wt % to 15 wt %.

In order to establish a desired pH that does not of itself result from the mixture of the other components, the agents according to the present invention can contain system-compatible and environmentally compatible acids, for example citric acid, acetic acid, tartaric acid, malic acid, lactic acid, glycolic acid, succinic acid, glutaric acid, and/or adipic acid, but also mineral acids, in particular sulfuric acid, or alkali hydrogensulfates or bases, in particular ammonium hydroxides or alkali hydroxides pH regulators of this kind are contained in the agents according to the present invention by preference at no more than 10 wt %, in particular from 0.5 wt % to 6 wt %.

Manufacture of the solid agents according to the present invention presents no difficulties and can in principle be effected in known fashion, for example by spray drying or granulation, the peroxygen compound and bleach catalyst optionally being separately added later. If applicable, the bleach catalyst is mixed with further raw materials and/or compounds, and the mixture is then compressed into tablets or phases thereof.

Cleaning agents according to the present invention in the form of aqueous solutions or those containing other usual solvents are manufactured particularly advantageously by simply mixing the ingredients, which can be placed, in substance or as a solution, into an automatic mixer.

The agents according to the present invention are present by preference as powdered, granular, or tableted preparations that can be manufactured in known fashion, for example by mixing, granulating, roller compacting, and/or by spray drying of the thermally insensitive components and mixing in the more-sensitive components, included among which are, in particular, enzymes, bleaching agents, and the bleach catalyst.

For the manufacture of cleaning agents according to the present invention in tablet form, it is preferable to proceed in such a way that all the constituents, or all the constituents provided for use together in one phase of the tablet, are mixed together in a mixer, and the mixture, or the mixtures in succession, are compressed by means of conventional tablet presses, for example eccentric presses or rotary tablet presses, at compression pressures in the range from 200 10^5 Pa to 1500 10^5 Pa. Break-resistant tablets that are nevertheless sufficiently rapidly soluble under the utilization conditions, having flexural strength values normally above 150 N, are thereby obtained without difficulty. A tablet manufactured in this fashion preferably has a weight from 15 g to 40 g, in particular from 20 g to 30 g. It can be round, for example with a diameter from 35 mm to 40 mm, or can have any other desired shape, for example rectangular with optionally rounded edges.

Agents according to the present invention in the form of non-dusting, shelf-stable, pourable powders and/or granulates having high bulk densities in the range from 800 to 1000 g/l can be manufactured by mixing the builder components with at least a portion of liquid mixture components in a first partial step of the method, with an increase in the bulk density of this premixture, and then, if desired after intervening drying, combining the further constituents of the agent, among them the bacterial spores, with the premixture thus obtained.

Agents according to the present invention for cleaning tableware can be used in both household and commercial dishwashers. They are added manually or by means of suitable metering devices. The utilization concentration in the cleaning bath is as a rule approximately 1 to 8 g/l, by preference 2 to 5 g/l.

An automatic washing cycle is generally supplemented and completed by several intermediate rinse cycles with clean water following the cleaning cycle, and a rinse cycle with an ordinary rinse agent. When agents according to the present invention are used, tableware that is completely clean and unobjectionable in hygienic terms is obtained after drying.

Examples

Example 1: Odor Mitigation in hard surface cleaning

15 % egg yolk in water was used as artificial soil. Various amounts of spore suspension to be tested were added to 10 ml of such artificial soil, so that the total amount of spores given in table 1 was achieved. Sponges designed for hard surface cleaning were repeatedly immersed in the contaminated soil and wrung out. The sponges were put in Erlenmeyer flasks for 2 days at room temperature. Persons trained in olfaction smelled at the opening of the flask after 1 day, after 2 days and after 3 days and rated the intensity of the malodor on a scale of 0 to 3 (0 being no odor, 1 = weak odor, 2 = moderate odor, and 3 = strong odor). The results of malodor scores after 2 days are presented in Table 1.

Table 1: Malodor scores

Control (without spores)	With spores PTA-7543				
	1x10E7 cfu/ml	1x10E5 cfu/ml	1x10E3 cfu/ml	1x10E2 cfu/ml	1x10E1 cfu/ml
3.5	2.5	2.2	2.6	2.3	2.1

Example 2: Odor Mitigation in hard surface cleaning

The procedure of example 1 was repeated, with the exception that egg yolk was replaced by a standardized artificial soil (IKW) developed for automatic dishwashing performance tests. The results of malodor scores after 2 days are presented in Table 2.

Table 2: Malodor scores

Control (without spores)	With spores PTA-7543	
	1x10E7 cfu/ml	1x10E5 cfu/ml
2.8	1.6	1.3

Print Out (Original in Electronic Form)
 (This sheet is not part of and does not count as a sheet of the international application)

0-1	Form PCT/RO/134 Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.251e MT/FOP 20141031/0.20.5.20
0-2	International Application No.	
0-3	Applicant's or agent's file reference	PT033564 PCT
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	1
1-2	line	26
1-3	Identification of deposit	
1-3-1	Name of depository institution	ATCC American Type Culture Collection (ATCC)
1-3-2	Address of depository institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
1-3-3	Date of deposit	14 May 2015 (14.05.2015)
1-3-4	Accession Number	ATCC SD-6991
1-4	Additional Indications	Authorization Novozymes + Depository confirmation
1-5	Designated States for Which Indications are Made	All designations
2	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
2-1	page	1
2-2	line	27
2-3	Identification of deposit	
2-3-1	Name of depository institution	ATCC American Type Culture Collection (ATCC)
2-3-2	Address of depository institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
2-3-3	Date of deposit	20 April 2006 (20.04.2006)
2-3-4	Accession Number	ATCC PTA-7543
2-4	Additional Indications	Authorization Novozymes + Depository confirmation
2-5	Designated States for Which Indications are Made	All designations

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 (This sheet is not part of and does not count as a sheet of the international application)

3	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
3-1	page	1
3-2	line	28
3-3	Identification of deposit	
3-3-1	Name of depositary institution	ATCC American Type Culture Collection (ATCC)
3-3-2	Address of depositary institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
3-3-3	Date of deposit	14 May 2015 (14.05.2015)
3-3-4	Accession Number	ATCC SD-6992
3-4	Additional Indications	Authorization Novozymes + Depository confirmation
3-5	Designated States for Which Indications are Made	All designations
4	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
4-1	page	1
4-2	line	29
4-3	Identification of deposit	
4-3-1	Name of depositary institution	NRRL Agricultural Research Service Culture Collection (NRRL)
4-3-2	Address of depositary institution	1815 North University Street, Peoria, Illinois 61604, United States of America
4-3-3	Date of deposit	30 November 2011 (30.11.2011)
4-3-4	Accession Number	NRRL B-50607
4-4	Additional Indications	Authorization Novozymes + Depository confirmation
4-5	Designated States for Which Indications are Made	All designations
5	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
5-1	page	1
5-2	line	30
5-3	Identification of deposit	
5-3-1	Name of depositary institution	NRRL Agricultural Research Service Culture Collection (NRRL)
5-3-2	Address of depositary institution	1815 North University Street, Peoria, Illinois 61604, United States of America
5-3-3	Date of deposit	30 November 2011 (30.11.2011)
5-3-4	Accession Number	NRRL B-50606
5-4	Additional Indications	Authorization Novozymes + Depository confirmation
5-5	Designated States for Which Indications are Made	All designations

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6	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
6-1	page	2
6-2	line	1
6-3	Identification of deposit	
6-3-1	Name of depositary institution	NRRL Agricultural Research Service Culture Collection (NRRL)
6-3-2	Address of depositary institution	1815 North University Street, Peoria, Illinois 61604, United States of America
6-3-3	Date of deposit	12 December 2013 (12.12.2013)
6-3-4	Accession Number	NRRL B-50887
6-4	Additional Indications	Authorization Novozymes + Depository confirmation
6-5	Designated States for Which Indications are Made	All designations
7	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
7-1	page	1
7-2	line	2
7-3	Identification of deposit	
7-3-1	Name of depositary institution	ATCC American Type Culture Collection (ATCC)
7-3-2	Address of depositary institution	10801 University Boulevard, Manassas, Virginia 20110-2209, United States of America
7-3-3	Date of deposit	20 April 2006 (20.04.2006)
7-3-4	Accession Number	ATCC PTA-7549
7-4	Additional Indications	Authorization Novozymes + Depository confirmation
7-5	Designated States for Which Indications are Made	All designations
8	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
8-1	page	2
8-2	line	3
8-3	Identification of deposit	
8-3-1	Name of depositary institution	NRRL Agricultural Research Service Culture Collection (NRRL)
8-3-2	Address of depositary institution	1815 North University Street, Peoria, Illinois 61604, United States of America
8-3-3	Date of deposit	14 March 2007 (14.03.2007)
8-3-4	Accession Number	NRRL B-50017
8-4	Additional Indications	Authorization Novozymes + Depository confirmation
8-5	Designated States for Which Indications are Made	All designations

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0-4	This form was received with the international application: (yes or no)	yes
0-4-1	Authorized officer	De Jong-de Koster, Marion

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0-5	This form was received by the international Bureau on:	
0-5-1	Authorized officer	

Claims

1. A method of inhibiting or preventing the production of malodor comprising contacting tableware or a dish washing machine with bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the registration reference SD-6991, registration office ATCC, registration reference PTA-7543, registration office ATCC, registration reference SD-6992, registration office ATCC, registration reference NRRL B-50607, registration office ARS, registration reference NRRL B-50606, registration office ARS, registration reference NRRL B-50887, registration office ARS, registration reference PTA-7549, registration office ATCC, registration reference NRRL B-50017, registration office ARS, and its mixtures.
2. The method according to claim 1, wherein the method comprises contacting tableware or a dish washing machine with bacterial spores of the *Bacillus amyloliquefaciens* strain PTA-7543.
3. The method according to claim 1 or 2, wherein the method comprises contacting tableware or a dish washing machine with combinations of bacterial spores of one or more of the strains of claim 1, in particular blends of two or more strains, three or more strains, four or more strains or five or more strains.
4. The method according to any of claims 1 to 3, wherein the malodor is caused by gram negative proteobacteria and/or substances derived therefrom.
5. A composition for inhibiting or preventing the production of malodor in a dish washing machine comprising bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the registration reference SD-6991, registration office ATCC, registration reference PTA-7543, registration office ATCC, registration reference SD-6992, registration office ATCC, registration reference NRRL B-50607, registration office ARS, registration reference NRRL B-50606, registration office ARS, registration reference NRRL B-50887, registration office ARS,

- registration reference PTA-7549, registration office ATCC,
registration reference NRRL B-50017, registration office ARS,
and its mixtures.
6. The composition according to claim 5 for use as a cleaning composition or in manual or automatic dishwashing agents which further comprises a carrier.
7. An automatic dishwashing agent comprising bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the
registration reference SD-6991, registration office ATCC,
registration reference PTA-7543, registration office ATCC,
registration reference SD-6992, registration office ATCC,
registration reference NRRL B-50607, registration office ARS,
registration reference NRRL B-50606, registration office ARS,
registration reference NRRL B-50887, registration office ARS,
registration reference PTA-7549, registration office ATCC,
registration reference NRRL B-50017, registration office ARS,
and its mixtures at a concentration of 1×10^2 - 1×10^9 CFU/g of automatic dishwashing agent,
with a preferred range of 1×10^3 - 1×10^5 CFU/g of automatic dishwashing agent.
8. A manual dishwashing agent comprising bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the
registration reference SD-6991, registration office ATCC,
registration reference PTA-7543, registration office ATCC,
registration reference SD-6992, registration office ATCC,
registration reference NRRL B-50607, registration office ARS,
registration reference NRRL B-50606, registration office ARS,
registration reference NRRL B-50887, registration office ARS,
registration reference PTA-7549, registration office ATCC,
registration reference NRRL B-50017, registration office ARS,
and its mixtures at a concentration of 1×10^1 - 1×10^9 CFU/g of the manual dishwashing agent,
with a preferred range of 1×10^2 - 1×10^5 CFU/g of the manual dishwashing agent.

9. Use of bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the registration reference SD-6991, registration office ATCC, registration reference PTA-7543, registration office ATCC, registration reference SD-6992, registration office ATCC, registration reference NRRL B-50607, registration office ARS, registration reference NRRL B-50606, registration office ARS, registration reference NRRL B-50887, registration office ARS, registration reference PTA-7549, registration office ATCC, registration reference NRRL B-50017, registration office ARS, and its mixtures for inhibiting malodor in cleaning machines and cleaning processes.

10. Use of bacterial spores of at least one species of *Bacillus*, in particular bacterial spores of *Bacillus amyloliquefaciens* and/or *Bacillus atrophaeus*, especially bacterial spores of bacilli selected from the group consisting of *Bacillus* strains with the registration reference SD-6991, registration office ATCC, registration reference PTA-7543, registration office ATCC, registration reference SD-6992, registration office ATCC, registration reference NRRL B-50607, registration office ARS, registration reference NRRL B-50606, registration office ARS, registration reference NRRL B-50887, registration office ARS, registration reference PTA-7549, registration office ATCC, registration reference NRRL B-50017, registration office ARS, and its mixtures in automatic or manual dishwashing agents for inhibiting or preventing the production of malodor.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/055629

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12R1/07 C11D3/38 C11D3/00
 ADD.
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 C12R C11D C12N A61L D06F

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/207699 A1 (MCHATTON SARAH C [US] ET AL) 16 August 2012 (2012-08-16) paragraphs [0005], [0006], [0009], [0010] page 2, paragraphs 0011,0012 page 3, paragraph 0018 - page 4, paragraph 0025 example 5 ----- -/--	1-10

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search 1 June 2017	Date of mailing of the international search report 09/06/2017
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Gault, Nathalie
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/055629

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/274673 A1 (KANG YAOWEI [US] ET AL) 10 November 2011 (2011-11-10) abstract claims 1-3,9 page 1, paragraphs 0003,0011,0012 page 2, paragraph 0028-0029 page 3, paragraphs 0040,0055 page 4, paragraphs 0057,0071-0072 page 5, paragraph 0085-0086 page 6, paragraph 0088 -----	1-10
X	EP 2 759 590 A1 (POLLET SA [BE]) 30 July 2014 (2014-07-30) claims 1,2,15 page 5, paragraphs 0030, 0037 page 3, paragraph 0008 page 2, paragraph 0002 -----	1-10
X	WO 00/63338 A1 (SPARTAN CHEMICAL COMPANY INC [US]) 26 October 2000 (2000-10-26) page 1, paragraph 3; claim 1 page 2, last paragraph - page 3, paragraph 1 page 9, last paragraph - page 10, paragraph 1 page 11, paragraph 2 -----	1-10
X	Julius Hoesch GmbH: "Bacterial spores for odour control/ prevention in aqueous systems or cleaner formulations", 1 June 2013 (2013-06-01), XP55376815, Retrieved from the Internet: URL: http://www.julius-hoesch.de/Aufstellung_Bakterien_ENGLISCH.pdf [retrieved on 2017-05-30] the whole document -----	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/EP2017/055629

Patent document cited in search report	Publication date	Patent family member(s)	Publication date																																																
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