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(54) **A composition for the environmentally-friendly washing of clothes**

(57) The present invention relates to an environmentally friendly washing product comprising a mixture of a surface-tension lowering composition and a bacterial composition, and that effectively cleans clothes and fabrics, minimises damage thereto and reduces dam-

age to washing machines. It further relates to a receptacle suitable for storing and dispensing said washing product.

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Description**FIELD OF THE INVENTION**

[0001] The present invention relates to a composition comprising a mixture of bacteria and non-ionic surfactant for use in washing clothes. It further relates to a receptacle suitable for storing and dispensing said composition.

BACKGROUND

[0002] Environmental concerns have an influence on governmental policy and of purchasing decisions made by the consumer. Most developed countries have introduced laws and regulations in order to control the composition of domestic and industrial waste. Laundering products such as washing powders or liquids have statutory limits placed on the amount of phosphates and other environmentally-polluting compounds that can be present. Therefore, in order to comply with regulations as down in legislating countries and to meet demand from consumers in non-legislating countries, there is a need for environmentally-friendly washing products.

[0003] Since limits on phosphate levels have been set, problems have arisen that appear to be linked the rise in phosphate-free products for washing clothes which operate at temperatures of around 40°C. Washing machines suffer from a build-up of internal greasy deposits which cause unpleasant odours and rotting door gaskets, water hoses and can block pumping systems. Therefore, there is a need for an environmentally-friendly products for washing clothes which can effectively dissolve grease and oil, so preventing damage to washing machines.

[0004] Washing machines operating in hard water areas suffer from calcium (or limescale) deposition in piping and in particular on the heating element. Solutions to the problem of limescale deposition already exist, however, they generally rely on the addition of an extra product to the wash cycle. Thus, there is a reliance on the operator to undertake additional expenditure and inconvenience to overcome limescale deposition. There is a need for a method of preventing limescale deposition in washing machines which obviates the need for the addition of an extra product to the wash cycle.

[0005] There is a demand from consumers for environmentally-friendly cleaning products that are equally as effective in cleaning clothes as the old style-products. Not only should they remove stains, but they should not damage clothing fibres, particularly natural fibres. They should not cause colours to fade and they should leave clothes feeling soft. Thus, there is a need for a washing product that fulfils these requirements.

[0006] European Patent application 0 245 560 discloses a cleaning composition comprising abrasive particles, viable micro-organisms and a detergent. The application make no mention of the suitability of the composition for cleaning clothes.

[0007] European Patent application 0 569, 140 describes a liquid composition for opening drains. The application make no mention of the suitability of the composition for cleaning clothes.

US patent number 6,162,635 discloses novel Bacillus strains and their use in various application including laundry detergents. There is no mention therein of biodegradability or environmental toxicity, of the composition or of the role of the composition in the protection against damage to clothes and/or washing machines.

AIMS OF THE INVENTION

[0008] The aim of the invention is to provide an environmentally-friendly washing product that effectively cleans clothes and fabrics, minimises damage thereto and reduces damage to the washing machine.

DETAILED DESCRIPTION OF THE INVENTION

[0009] The invention relates to the finding that a mixture of a surface-tension-lowering composition and a bacterial composition is an effective cleaning agent in the washing of clothes and fabrics - both referred herein as "clothes". It relates to the further finding that the waste water produced from washing using said mixture contains lower than acceptable levels of pollutants (pH, toxic metal ions, anionic surfactants, non-dissolved oils and fats), in compliance with regulations, such as, for example, those according to Belgian law. It further relates to the finding that the mixture is almost completely biodegradable.

[0010] The inventors have found that a mixture according to the invention is not toxic to humans, animals or plants. The micro-organisms therein are not genetically modified and have been tested for the presence of salmonella and other non-toxic bacteria. Thus they are non-pathogenic and belong to group 1 of the EU and WHO directive. They cannot cause disease or deficiencies in humans. They are present in a sporulated form and when they are used in a suitable environment, they start to geminate and to form cells. The testing methods are according to standard procedures.

[0011] Thus, the mixture of the present invention is an effective, environmentally-friendly cleaning product.

[0012] By environmentally friendly, as used herein, means that its use has less detrimental impact on the environment as compared to ordinary products. The likelihood of detrimental effect may be judged according to methods known in the art. It may be judged according to standard tests and criteria as set out by legislation, for example, of that in Belgium. Examples of tests which may be performed on washing products to judge suitability for use in terms of environmental impact are provided in the Examples section. They include tests of the waste water after a wash for pH, levels of colourant and amount of undissolved fat and grease, and tests of the washing product for metal ions, anionic surfactants, phosphates and biodegradability.

[0013] One embodiment of the present invention is a mixture of a surface-tension lowering composition and a general bacterial composition and a fats and oils bacterial composition. The inventors have surprisingly found that combination of the surface-tension lowering composition and the bacteria is synergistic, and provides enhanced cleaning compared with using just two of a surface-tension lowering composition, a general bacterial composition or a fats and oils bacterial composition.

[0014] The surface-tension lowering composition comprises a non-ionic surfactant. Numerous nonionic surfactants are within the scope of the present invention. Such surfactants include, but are not limited to, alkyl aryl polyether alcohols having degrees of ethoxylation from 1.5 to 120, including but not limited to, alkyl phenol ethoxylates having an alkyl chain length of between about 6 and 18 carbons, such as nonylphenol ethoxylates, octylphenol ethoxylates and dodecylphenol ethoxylates; alkyl polyether alcohols having degrees of ethoxylation from 1.5 to 120, including but not limited to, linear polyether alcohols having an alkyl chain length from between about 4 and 22 carbons, mixed linear alcohol ethoxylates, secondary alcohol ethoxylates having an alkyl chain length from between about 6 and 22 carbons, branched alkyl alcohol ethoxylates having between about 8 and 22 carbons, such as tridecylalcohol ethoxylates, trimethylnonyl ethoxylates, and isodecyl alcohol ethoxylates, isotridecyl alcohol ethoxylates; nonionic amides such as alkanolamides, including but not limited to, 1:1 diethanolamides, monoethanol amides, monoisopropanolamides, 2:1 alkanolamides and modifications thereof, ethoxylated alkanolamides, and bisamides; nonionic esters, including but not limited to, alcohol, glycerol, and glycol esters, polyethylene glycol (PEG) esters such as diethylene glycol monostearates, glycerol monostearate, PEG laurates, PEG dilaurates, PEG monooleates, and PEG dioleates, where PEG has a molecular weight ranging between about 100 and 1000; ethoxylated acids and oils, including derivatives of castor oil, oleic acid, linoleic acid, myristic acid, lauric acid, and stearic acid, among others, where the organic acids have from between about 6 to 20 carbons having linear and branched chain structures, and degrees of ethoxylation from 1.5 to 200; sorbitan esters and ethoxylated sorbitol esters, including but not limited to sorbitan monolaurate, ethoxylated sorbitan monolaurate, sorbitan monooleate, ethoxylated sorbitan monooleate, sorbitan trioleate and ethoxylated sorbitan trioleate, where the polyhydric alcohols and sugars have a degree of ester substitution of between about 1 and 4, and degrees of ethoxylation from between about 0 to 200; polysorbates, including but not limited to polysorbate 20, polysorbate 30, polysorbate 40, polysorbate 50, polysorbate 60, polysorbate 70, polysorbate 80; alkyl polyglucoside surfactants having between about 1 and 10 saccharide units and alkyl substitution from between about 0.5 and 2.5; ethoxylated mercaptans having an alkyl chain length from between about 6 and 18 carbons and a degree of ethoxylation from between about 4 and 20; low foaming surfactants, including ethylene oxide/propylene oxide (EO/PO) block copolymers such as the Pluronic® and Tetronic® products available from BASF Corporation, Parsippany, N.J., linear alcohol EO/PO, branched alcohol EO/PO, aryl alkaryl EO/PO, and linear alcohol EO with a chlorine cap; ethylene oxide/propylene oxide copolymers, including both block and random copolymers, having a molecular weight from between about 1000 and 25 000 and cloud point from 10 °C to greater than 100 °C; and amine ethoxylates having a degree of ethoxylation from 1.5 to 75 and alkyl groups having from between about 4 to 22 carbons. The composition of the present invention does not encompass use of amine oxides as the nonionic surfactants.

[0015] In one embodiment of the invention, the surface-tension lowering composition comprises polysorbate in a concentration between the ranges of 5 and 60 %, 5 and 55%, 5 and 50%, 5 and 45%, 5 and 40%, 5 and 35%, 5 and 30%, 5 and 25%, 5 and 20%, 5 and 15%, 5 and 10%, 10 and 60 %, 10 and 55%, 10 and 50%, 10 and 45%, 10 and 40%, 10 and 35%, 10 and 30%, 10 and 25%, 10 and 20%, 10 and 15%, 15 and 60 %, 15 and 55%, 15 and 50%, 15 and 45%, 15 and 40%, 15 and 35%, 15 and 30%, 15 and 25%, 15 and 20%, 20 and 60 %, 20 and 55%, 20 and 50%, 20 and 45%, 20 and 40%, 20 and 35%, 20 and 30%, 20 and 25%, 25 and 60 %, 25 and 55%, 25 and 50%, 25 and 45%, 25 and 40%, 25 and 35%, 25 and 30%, 30 and 60 %, 30 and 55%, 30 and 50%, 20 and 45%, 30 and 40%, 30 and 35%, 35 and 60 %, 35 and 55%, 35 and 50%, 35 and 45%, 35 and 40%, 40 and 60 %, 40 and 55%, 40 and 50%, 40 and 45%, 50 and 60 %, 50 and 55%, or 55 and 60 %.

[0016] In another embodiment of the invention, the surface-tension lowering composition comprises PEG in a concentration between the ranges of 5 and 60 %, 5 and 55%, 5 and 50%, 5 and 45%, 5 and 40%, 5 and 35%, 5 and 30%, 5 and 25%, 5 and 20%, 5 and 15%, 5 and 10%, 10 and 60 %, 10 and 55%, 10 and 50%, 10 and 45%, 10 and 40%, 10 and 35%, 10 and 30%, 10 and 25%, 10 and 20%, 10 and 15%, 15 and 60 %, 15 and 55%, 15 and 50%, 15 and 45%, 15 and 40%, 15 and 35%, 15 and 30%, 15 and 25%, 15 and 20%, 20 and 60 %, 20 and 55%, 20 and 50%, 20 and 45%, 20 and 40%, 20 and 35%, 20 and 30%, 20 and 25%, 25 and 60 %, 25 and 55%, 25 and 50%, 25 and 45%, 25 and 40%, 25 and 35%, 25 and 30%, 30 and 60 %, 30 and 55%, 30 and 50%, 20 and 45%, 30 and 40%, 30 and 35%, 35 and 60 %, 35 and 55%, 35 and 50%, 35 and 45%, 35 and 40%, 40 and 60 %, 40 and 55%, 40 and 50%, 40 and 45%, 50 and 60 %, 50 and 55%, or 55 and 60 %.

35%, 35 and 60 %, 35 and 55%, 35 and 50%, 35 and 45%, 35 and 40%, 40 and 60 %, 40 and 55%, 40 and 50%, 40 and 45%, 50 and 60 %, 50 and 55%, or 55 and 60 %. The solubility of PEG may be enhanced by the addition of alcohol to a sufficient quantity to allow PEG to dissolve in the composition. Said alcohol in the surface-tension lowering composition may range, for example, between 1 to 5%, 1 to 10%, or 1 to 20%.

[0017] In another embodiment of the invention, the surface-tension lowering composition comprises polysorbate and PEG in the ranges disclosed above, optionally combined with citrus extract and colour agent. The citrus extract may be present in quantities of <1%, <2%, <3% or <4%. The colour agent may be present in quantities of <1%, <2%, <3% or <4%.

[0018] The general bacterial composition comprises at least 3 strains of bacteria that are not genetically modified, non-pathogenic and belong to group 1 of the EU and WHO directive, and which produce multiple enzymes such as, for example, lipases to digest fat, proteases to degrade proteins, amylases to degrade starch, enzymes to enhance the degradation of organic compounds in the wastewater, and enzymes suitable for removing stains and odours.

[0019] In one embodiment of the present invention, the general bacterial composition comprises different groups from the *Bacillus* family, *Enterobacter* family and *Clostridium* family in the sporulated form. The general bacterial composition may also comprise plant extracts and liquid medium to ensure the bacteria stay alive in the sporulated form. The inventors have surprisingly found that three different strains of bacteria present in a composition in combination with a liquid medium allows the bacteria to stay alive and active, whereas in a composition comprising two different bacterial strains, said bacterial strains do not remain active.

[0020] The fats and oils bacterial composition comprises fats and oils-digesting bacteria. The purpose the fats and oils bacterial composition is to enhance the solubilisation of grease, fats and oils and to make said substances bioavailable. These bacteria should be not toxic to humans, animals or plants, not be genetically modified, non-pathogenic and belong to group 1 of the EU and WHO directive. They should not cause disease or deficiencies in humans. They should be present in a sporulated form and when they are used in a suitable environment, they should start to germinate and to form cells. Methods for evaluating fats and oils bacteria are available in the art. Examples of suitable fats and oils bacteria include, but are not limited to *Bacillus subtilis*, *Bacillus polymyxa* and *Bacillus licheniformis*.

[0021] In one embodiment of the present invention, the general bacterial composition and the oils and fats bacterial composition are stored premixed as a first composition, and the surface-tension lowering composition is stored as a second composition. Prior to or during use, the first and second compositions are mixed.

[0022] In one embodiment of the present invention, the mixture comprises:

- a volume of said first composition, and
- a volume of said second composition such that the concentration of total bacteria in the mixture is in the range of 10^7 to 10^{11} , 10^7 to 10^{10} , 10^7 to 10^9 , 10^8 to 10^{11} , 10^9 to 10^{11} , 10^7 to 10^{10} , 10^8 to 10^{10} , 10^9 to 10^{10} , 10^8 to 10^9 , or 10^9 cells per millilitre.

[0023] Alternatively, in one embodiment of the present invention, the mixture comprises:

- 5 to 15 %, 5 to 14%, 5 to 13%, 5 to 12%, 5 to 11%, 5 to 10%, 6 to 13%, 6 to 12%, 6 to 11%, 6 to 10%, 7 to 15%, 7 to 14%, 7 to 13%, 7 to 12%, 7 to 11%, 7 to 10%, 8 to 15%, 8 to 14%, 8 to 13%, 8 to 12%, 8 to 11%, 8 to 10%, 9 to 15%, 9 to 14%, 9 to 13%, 9 to 12%, 9 to 11%, 9 to 10%, 10 to 15%, 10 to 14%, 10 to 13%, 10 to 12%, 10 to 11%, or 10%, v/v general bacterial composition,
- 5 to 15%, 5 to 14%, 5 to 13%, 5 to 12%, 5 to 11%, 5 to 10%, 6 to 13%, 6 to 12%, 6 to 11%, 6 to 10%, 7 to 15%, 7 to 14%, 7 to 13%, 7 to 12%, 7 to 11%, 7 to 10%, 8 to 15%, 8 to 14%, 8 to 13%, 8 to 12%, 8 to 11%, 8 to 10%, 9 to 15%, 9 to 14%, 9 to 13%, 9 to 12%, 9 to 11%, 9 to 10%, 10 to 15%, 10 to 14%, 10 to 13%, 10 to 12%, 10 to 11%, or 10%, v/v oils and fats bacterial composition,
- the remaining volume being surface-tension lowering composition.

[0024] In another embodiment of the invention, the concentration of bacteria in the mixture is 10^7 to 10^{11} , 10^7 to 10^{10} , 10^7 to 10^9 , 10^8 to 10^{11} , 10^9 to 10^{11} , 10^7 to 10^{10} , 10^8 to 10^{10} , 10^9 to 10^{10} , 10^8 to 10^9 , or 10^9 cells per millilitre.

[0025] In another embodiment of the present invention the pH of the mixture is in the range 6.5 to 9.0, 6.5 to 8.9, 6.5 to 8.8, 6.5 to 8.7, 6.5 to 8.6, 6.5 to 8.5, 6.5 to 8.4, 6.5 to 8.3, 6.5 to 8.2, 6.5 to 8.1, 6.5 to 8.0, 6.5 to 7.9, 6.5 to 7.8, 6.5 to 7.7, 6.5 to 7.6, 6.5 to 7.5, 6.5 to 7.4, 6.5 to 7.3, 6.5 to 7.2, 6.5 to 7.1, 6.5 to 7.0, 6.5 to 6.9, 6.5 to 6.8, 6.5 to 6.7, 6.5 to 6.6, 6.6 to 9.0, 6.6 to 8.9, 6.6 to 8.8, 6.6 to 8.7, 6.6 to 8.6, 6.6 to 8.5, 6.6 to 8.4, 6.6 to 8.3, 6.6 to 8.2, 6.6 to 8.1, 6.6 to 8.0, 6.6 to 7.9, 6.6 to 7.8, 6.6 to 7.7, 6.6 to 7.6, 6.6 to 7.5, 6.6 to 7.4, 6.6 to 7.3, 6.6 to 7.2, 6.6 to 7.1, 6.6 to 7.0, 6.6 to 6.9, 6.6 to 6.8, 6.6 to 6.7, 6.7 to 9.0, 6.7 to 8.9, 6.7 to 8.8, 6.7 to 8.7, 6.7 to 8.6, 6.7 to 8.5, 6.7 to 8.4, 6.7 to 8.3, 6.7 to 8.2, 6.7 to 8.1, 6.7 to 8.0, 6.7 to 7.9, 6.7 to 7.8, 6.7 to 7.7, 6.7 to 7.6, 6.7 to 7.5, 6.7 to 7.4, 6.7 to 7.3, 6.7 to 7.2, 6.7 to 7.1, 6.7 to 7.0, 6.7 to 6.9, 6.7 to 6.8, 6.8 to 9.0, 6.8 to 8.9, 6.8 to 8.8, 6.8 to 8.7, 6.8 to 8.6, 6.8 to 8.5, 6.8 to 8.4, 6.8 to 8.3, 6.8 to 8.2, 6.8 to 8.1, 6.8 to 8.0, 6.8 to 7.9, 6.8 to 7.8, 6.8 to 7.7, 6.8 to 7.6, 6.8 to 7.5, 6.8 to 7.4, 6.8 to 7.3, 6.8

to 7.2, 6.8 to 7.1, 6.8 to 7.0, 6.8 to 6.9, 6.9 to 9.0, 6.9 to 8.9, 6.9 to 8.8, 6.9 to 8.7, 6.9 to 8.6, 6.9 to 8.5, 6.9 to 8.4, 6.9 to 8.3, 6.9 to 8.2, 6.9 to 8.1, 6.9 to 8.0, 6.9 to 7.9, 6.9 to 7.8, 6.9 to 7.7, 6.9 to 7.6, 6.9 to 7.5, 6.9 to 7.4, 6.9 to 7.3, 6.9 to 7.2, 6.9 to 7.1, 6.9 to 7.0, 7.0 to 9.0, 7.0 to 8.9, 7.0 to 8.8, 7.0 to 8.7, 7.0 to 8.6, 7.0 to 8.5, 7.0 to 8.4, 7.0 to 8.3, 7.0 to 8.2, 7.0 to 8.1, 7.0 to 8.0, 7.0 to 7.9, 7.0 to 7.8, 7.0 to 7.7, 7.0 to 7.6, 7.0 to 7.5, 7.0 to 7.4, 7.0 to 7.3, 7.0 to 7.2, 7.0 to 7.1, 7.1 to 9.0, 7.1 to 8.9, 7.1 to 8.8, 7.1 to 8.7, 7.1 to 8.6, 7.1 to 8.5, 7.1 to 8.4, 7.1 to 8.3, 7.1 to 8.2, 7.1 to 8.1, 7.1 to 8.0, 7.1 to 7.9, 7.1 to 7.8, 7.1 to 7.7, 7.1 to 7.6, 7.1 to 7.5, 7.1 to 7.4, 7.1 to 7.3, 7.1 to 7.2, 7.2 to 9.0, 7.2 to 8.9, 7.2 to 8.8, 7.2 to 8.7, 7.2 to 8.6, 7.2 to 8.5, 7.2 to 8.4, 7.2 to 8.3, 7.2 to 8.2, 7.2 to 8.1, 7.2 to 8.0, 7.2 to 7.9, 7.2 to 7.8, 7.2 to 7.7, 7.2 to 7.6, 7.2 to 7.5, 7.2 to 7.4, 7.2 to 7.3, 7.3 to 9.0, 7.3 to 8.9, 7.3 to 8.8, 7.3 to 8.7, 7.3 to 8.6, 7.3 to 8.5, 7.3 to 8.4, 7.3 to 8.3, 7.3 to 8.2, 7.3 to 8.1, 7.3 to 8.0, 7.3 to 7.9, 7.3 to 7.8, 7.3 to 7.7, 7.3 to 7.6, 7.3 to 7.5, 7.3 to 7.4, 7.4 to 9.0, 7.4 to 8.9, 7.4 to 8.8, 7.4 to 8.7, 7.4 to 8.6, 7.4 to 8.5, 7.4 to 8.4, 7.4 to 8.3, 7.4 to 8.2, 7.4 to 8.1, 7.4 to 8.0, 7.4 to 7.9, 7.4 to 7.8, 7.4 to 7.7, 7.4 to 7.6, 7.4 to 7.5, 7.5 to 9.0, 7.5 to 8.9, 7.5 to 8.8, 7.5 to 8.7, 7.5 to 8.6, 7.5 to 8.5, 7.5 to 8.4, 7.5 to 8.3, 7.5 to 8.2, 7.5 to 8.1, 7.5 to 8.0, 7.5 to 7.9, 7.5 to 7.8, 7.5 to 7.7, 7.5 to 7.6, 7.6 to 9.0, 7.6 to 8.9, 7.6 to 8.8, 7.6 to 8.7, 7.6 to 8.6, 7.6 to 8.5, 7.6 to 8.4, 7.6 to 8.3, 7.6 to 8.2, 7.6 to 8.1, 7.6 to 8.0, 7.6 to 7.9, 7.6 to 7.8, 7.6 to 7.7, 7.7 to 9.0, 7.7 to 8.9, 7.7 to 8.8, 7.7 to 8.7, 7.7 to 8.6, 7.7 to 8.5, 7.7 to 8.4, 7.7 to 8.3, 7.7 to 8.2, 7.7 to 8.1, 7.7 to 8.0, 7.7 to 7.9, 7.7 to 7.8, 7.8 to 9.0, 7.8 to 8.9, 7.8 to 8.8, 7.8 to 8.7, 7.8 to 8.6, 7.8 to 8.5, 7.8 to 8.4, 7.8 to 8.3, 7.8 to 8.2, 7.8 to 8.1, 7.8 to 8.0, 7.8 to 7.9, 7.9 to 9.0, 7.9 to 8.9, 7.9 to 8.8, 7.9 to 8.7, 7.9 to 8.6, 7.9 to 8.5, 7.9 to 8.4, 7.9 to 8.3, 7.9 to 8.2, 7.9 to 8.1, 7.9 to 8.0, 7.9 to 9.0, 7.9 to 8.9, 7.9 to 8.8, 7.9 to 8.7, 7.9 to 8.6, 7.9 to 8.5, 7.9 to 8.4, 7.9 to 8.3, 7.9 to 8.2, 7.9 to 8.1, 7.9 to 8.0, 8.0 to 9.0, 8.0 to 8.9, 8.0 to 8.8, 8.0 to 8.7, 8.0 to 8.6, 8.0 to 8.5, 8.0 to 8.4, 8.0 to 8.3, 8.0 to 8.2, 8.0 to 8.1, 8.1 to 9.0, 8.1 to 8.9, 8.1 to 8.8, 8.1 to 8.7, 8.1 to 8.6, 8.1 to 8.5, 8.1 to 8.4, 8.1 to 8.3, 8.1 to 8.2, 8.2 to 9.0, 8.2 to 8.9, 8.2 to 8.8, 8.2 to 8.7, 8.2 to 8.6, 8.2 to 8.5, 8.2 to 8.4, 8.2 to 8.3, 8.3 to 9.0, 8.3 to 8.9, 8.3 to 8.8, 8.3 to 8.7, 8.3 to 8.6, 8.3 to 8.5, 8.3 to 8.4, 8.4 to 9.0, 8.4 to 8.9, 8.4 to 8.8, 8.4 to 8.7, 8.4 to 8.6, 8.4 to 8.5, 8.5 to 9.0, 8.5 to 8.9, 8.5 to 8.8, 8.5 to 8.7, 8.5 to 8.6, 8.6 to 9.0, 8.6 to 8.9, 8.6 to 8.8, 8.6 to 8.7, 8.7 to 9.0, 8.7 to 8.9, 8.7 to 8.8, 8.8 to 9.0, 8.8 to 8.9, or 8.9 to 9.0.

[0026] The pH of the composition may be reached by adjusting the pH of the first composition and of the second composition such that the pH of the mixture is in the range according to the invention.

[0027] Another embodiment of the present invention is the mixture of the present invention for use in washing clothes. The purpose of washing may be, for example, to remove soiling, remove odours, and/or to soften clothes. Said washing may be performed by non-agitated soaking, by agitated washing or any other washing method known in the art. Agitated washing includes, but is not limited to, washing by hand, washing using ultrasound, washing using a mechanised form of agitation, including a washing machine.

[0028] A washing machine of the present invention comprises a washing compartment to hold clothes and water, a means of agitation, a means of heating and a means of draining waste water. A washing machine includes, but is not limited to, domestic automatic washing machines, domestic semi-automatic washing machines, industrial washing machines, and twin tub washing machines.

[0029] The concentration of the mixture of the present invention may adjusted by dilution with a suitable diluent. Concentration may be adjusted, for example, such that it is compatible with the capacity of the washing compartment of the washing machine and/or the capacity of the detergent dispensing-drawer of said machine, if present.

[0030] According to the invention, additives may be combined with the mixture of the invention to increase or decrease the viscosity. Viscosity may be adjusted, for example, to suit the means of administering the washing product e.g. as a liquid in a dispensing-drawer, or as a liquid in a "wash-ball".

[0031] The volume of the mixture of the present invention may adjusted by the user depending on the quantity of washing and the degree of soiling and/or odour.

[0032] The mixture of the present invention may be administered directly onto stains of clothes.

[0033] Another embodiment of the present invention is the mixture of the present invention for use in the environmentally-friendly washing of clothes.

[0034] Another embodiment of the present invention is the mixture of the present invention for use in a washing machine for the washing of clothes, and for the prevention of grease and limescale deposition within said machine.

[0035] Another embodiment of the invention is a product suitable for the washing of clothes, comprising two or more separate, liquid holding means, called herein a receptacle. According to one aspect of the invention, one said receptacle contains the first composition and another said receptacle contains the second composition. The receptacle may take the form of, but is not limited to, a resealable or non-resealable bottle, a resealable or non-resealable sachet, a resealable or non-resealable carton, a resealable or non-resealable vial, a degradable sachet for introduction into the washing water without unsealing said sachet. The receptacle may take the form of a liquid holding means that comprises more than one separate compartments. Alternatively, the receptacle may take the form of a liquid holding means that comprises one compartment. The receptacle may comprise a means for measuring an appropriate amount of respective composition. The receptacle may hold sufficient volume of the respective composition for a single use or several uses.

[0036] Another embodiment of the invention is a product suitable for the washing of clothes, comprising a solid

formulation of the first and second compositions according to the invention. The solid formulation may comprise separate solid formulations for the first and second compositions. Alternatively, a single solid formulation may comprise solid formulations of the first and second compositions, as an heterologous solid. A solid formulation of the first and second compositions is made according to methods known in the art.

5 **[0037]** The first and second compositions may be introduced into the washing water in various ways. The first and second compositions may be mixed prior to introduction into the wash water. For example, the first and second compositions stored in separate receptacles may be poured into a suitable container, and the mixture so made introduced therefrom into the washing water. In another example, the liquid holding means may contain two separate compartments each holding respective compositions, and prior to introduction into wash water, the compositions are allowed to mix
10 in the liquid holding means. Alternatively, the liquid holding means may contain two separate compartments each holding respective compositions; the respective compositions may be dispensed therefrom by a single action such as, for example, pouring, squeezing, pumping, or opening a valve, which allows both compositions to flow from the liquid holding means at the same time. The rate of flow of the respective compositions from the liquid holding means may be adjusted to suit the proportions of respective compositions required in the final mix. In another aspect of the invention,
15 the first and second compositions may be mixed in the wash water. For example, the first composition is introduced into the wash water, followed by the second, or vice versa, or both at the same time. Alternatively, where a solid formulation is used, the first and second compositions may be introduced separately and mixed in the wash water. Alternatively, a single solid formulation, comprising solid formulations of the first and second compositions, as an heterologous solid may be introduced in on addition and mixed in the wash water.

20 **[0038]** Figure 7 depicts a non-limiting example of a receptacle according to the invention. The receptacle [1] is bottle shaped, having a base [2] and an open top [3] and comprises an inner compartment [4] which is suitable for holding a composition of the present invention, and an outer compartment [5] which is suitable for holding separately another composition of the present invention. The bottle is orientated upright in storage by means of the base [2], such that the fluids in the respective compartments do not mix during storage. The compartments are connected at the open top
25 of the bottle [3]. The open top may contain a means of supporting the walls of the inner and outer compartments, and/or of the inner bottle. Examples of these means include the one or more connections between the outer wall of the bottle and the wall of the inner compartment. Figure 7 depicts a supporting means in profile wherein the walls of the bottle and inner compartment are clipped together; an enlargement of the clipping means [15] is shown in Figure 8 and further disclosures thereto are below.

30 **[0039]** According to one aspect of the invention, the bottle optionally comprise a circular lid [6], set at an angle to the rim of the top [3] and which has edges [7] preventing the flow of mixture from the top, except through the spout [8]. The lid may be fixed at the angled position so rendering the bottle permanently open, or it may be hinged [9] such that the lid may open and close, so respectively exposing and concealing the spout upon use and storage.

35 **[0040]** According to one aspect of the invention, aspect of the invention, the detachable cap [10] attaches to the upper portion of the bottle by means of a rim on the cap which is able of receive a rim on the top portion of the bottle, one rim having one or more protrusions, and the other rim having one or more grooves to receive said protrusions. In one example, according to Figure 8, which is an enlargement of Figure 7, a protrusion [20] on the cap [10] is located on the circumference of the rim of the cap; the groove [21] on the top of the bottle is located along the outer circumference of the rim thereof. Upon attaching the cap [10] to the bottle, the protrusion [20] of the cap [10] seats in the groove [21]
40 of the bottle, so reversibly locking the cap to the bottle. The cap and bottle are constructed from materials known in the art which have suitable tensile and elastic properties for this purpose. Suitable materials therefor are known in the art, and methods of testing suitable material therefor are known in the art. The detachable cap may be used to seal and/or measure the volume of mixture. The cap may be unmarked, or it may have indicators of volume, either with [11] or without numerals.

45 **[0041]** According to another aspect of the invention, the detachable cap [10] attaches to the upper portion of the bottle by means of a rim on the cap which is able of receive a rim on the top portion of the bottle, both rims having one or more protrusions. The protrusion on cap is located on the inner circumference of the rim thereof; the protrusion on the top of the bottle is located along the outer circumference of the rim thereof. Upon attaching the cap to the bottle, both protrusions are forced past each other, so reversibly locking the cap to the bottle. The cap and bottle are constructed from materials known in the art which have suitable tensile and elastic properties for this purpose. Suitable materials therefor are known in the art, and methods of testing suitable material therefor are known in the art. The detachable cap may be unmarked, or it may have indicators of volume, either with [11] or without numerals.
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[0042] The bottle may be constructed using materials, methods and designs known in the art. Examples of designs of the bottle include a single moulded design, and a design wherein the inner compartments walls are formed by means of joints to the base and top of the bottle. Alternatively, the bottle may be constructed according to Figure 7, wherein an inner bottle [12] is inserted into an outer bottle [13]. One compartment [4] is formed by the inner wall of the inner bottle, and the other compartment [5] is formed by the space between the inner wall of the outer bottle and the outer wall of the inner bottle. The base of both are seated by means of one or more locating means [14]. The locating means
55

may locate and lock the inner bottle, such that the inner bottle is prevented from falling out. Alternatively, the locating means may locate the inner bottle, and the inner bottle is prevented from falling out by other means, such as, for example, connections between the tops of the respective bottles.

[0043] According to an aspect of the invention, the tops of both bottles are connected and supported by means of a clip [15] as depicted in Figure 8, which is an enlargement of Figure 7; the clip [15] and the bottles [18] and [19] make use of protrusions [16] and [17] to reversibly lock the clip to the bottles. Figure 8 illustrates a protrusion-protrusion locking attachment which is already described for attaching the cap, however, the invention is not limited to just one type of attachment; the clip may attach to the bottles by means of protrusion-groove which is already described herein, or a mixture of protrusion-groove and protrusion-protrusion. The strength of the attachment achieved by the clip can be varied according to the properties of materials used in the clip and bottles, the size of the protrusion(s) and/or the diameters of the bottles, the clip, its rims. A plan view of the clipping means would reveal one or more connections between the inner and outer clipped compartments, connected such that compositions contained in the respective bottles are able to flow substantially unrestricted upon pouring. According to another aspect of the invention, the tops of both bottles are supported by means of one or more permanent connections between the tops of the inner and outer bottles; connected such that compositions contained in the respective bottles are able to flow substantially unrestricted upon pouring.

[0044] According to one aspect of the invention, the protrusions and grooves as disclosed herein may encompass the circumference of the respective rims. Alternatively, they may intermittently encompass the respective rims. Alternatively, they may be present at specific points on the circumference of the respective rims.

[0045] A three dimensional representation of the receptacle according to Figures 7 and 8 is depicted in Figure 9.

[0046] One embodiment of the present invention is a composition comprising a mixture of a surface-tension lowering composition and a bacterial composition.

[0047] Another embodiment of the present invention is a composition as defined above wherein said surface-tension lowering composition comprises:

- 5 to 30% polysorbate,
- 5 to 30% polyethylene glycol,
- sufficient alcohol to allow the polyethylene glycol to dissolve, and
- the remainder being demineralised water.

[0048] Another embodiment of the present invention is a composition as defined above wherein said bacterial composition comprises one or more *Bacillus* bacteria, one or more *Enterobacter* bacteria, and one or more *Clostridium* bacteria, and one or more fats and oils-digesting bacteria.

[0049] Another embodiment of the present invention is a composition as defined above wherein the concentration of bacteria in the mixture is in the range 10^7 to 10^{10} cells per millilitre.

[0050] Another embodiment of the present invention is a composition as defined above wherein said surface-tension lowering composition and said bacterial composition are stored separately, and are mixed prior to or during use.

[0051] Another embodiment of the present invention is a composition as defined above comprising a volume of said surface-tension lowering composition and a volume of said bacterial composition, said volumes determined such that the concentration of bacteria in the mixture is 10^7 to 10^{10} cells/ml.

[0052] Another embodiment of the present invention is a composition as defined above for use in washing clothes.

[0053] Another embodiment of the present invention is a composition as defined above for use in environmentally-friendly washing of clothes.

[0054] Another embodiment of the present invention is a composition as defined above for use in environmentally-friendly washing of clothes in a washing machine, to reduce damage due to grease deposition and/or lime-scale deposition in said machine.

[0055] Another embodiment of the present invention is a method for removing odours from clothes comprising the use of a composition as defined above.

[0056] Another embodiment of the present invention is a method for washing clothes comprising the use of a composition as defined above.

[0057] Another embodiment of the present invention is a method for reducing damage to washing machines comprising the use of a composition as defined above during a wash cycle.

[0058] Another embodiment of the present invention is a method as defined above, wherein said method is environmentally-friendly.

[0059] Another embodiment of the present invention is a product suitable for washing clothes comprising composition as defined above.

[0060] Another embodiment of the present invention is a product as defined above, wherein said surface-tension lowering composition and said bacterial composition are provided in separate receptacles.

[0061] Another embodiment of the present invention is a product as defined above, wherein said composition reduces damage to washing machines used during a wash cycle.

[0062] Another embodiment of the present invention is a receptacle, comprising two compartments suitable for dispensing two liquids simultaneously therefrom, comprising:

- a) a first bottle having a size of opening, base and internal cavity suitable for accepting a second bottle, and a locating means therefor in the base,
- b) a second bottle having a reciprocal locating means in the base for the first bottle,
- c) one or more clips, suitable for attachment of the wall of the first bottle to the wall of the second bottle, such that the flow of liquid from both compartments is substantially unrestricted upon dispensing,

such that the first compartment is formed in the space between the outer wall of the second bottle and the inner wall of the first bottle, and the second compartment is formed in the space between the inner wall of the second bottle.

[0063] Another embodiment of the present invention is a receptacle as defined above wherein said clip comprises two or more rims, wherein

- a) the shape of one rim matches the circumference of the top of the first bottle and contacts the inner or outer surface of the top of the first bottle, and optionally another rim contacts the other surface,
- b) the shape of one rim matches the circumference of the top of the second bottle and contacts the inner or outer surface of the top of the second bottle, and optionally another rim contacts the other surface,
- c) the rims of a) and b) are connected such that the flow of liquid from both compartments is substantially unrestricted upon dispensing therefrom.

[0064] Another embodiment of the present invention is a receptacle as defined above wherein the connection means according to step c) connects said rims across top edges of said rims and across the opening of the first compartment.

[0065] Another embodiment of the present invention is a receptacle according to any as defined above, further comprising a detachable cap.

[0066] Another embodiment of the present invention is a receptacle as defined above wherein the detachable cap attaches to the upper portion of the bottle, said receptacle comprising:

- a rim on the circumference of the cap, the inner surface of which substantially contacts the circumference of outer surface of the top of the first bottle,
- one or more protrusions located on the rim of the inner surface of the cap,
- one or more grooves located on the outer surface of the top of the first bottle,
- said protrusion(s) and groove(s) located such that upon attaching the cap to the first bottle, the protrusion(s) seats in the groove(s), so reversibly locking the cap to the bottle.

[0067] Another embodiment of the present invention is a product as defined above, wherein said surface-tension lowering composition and said bacterial composition are provided in a receptacle having separate compartments.

[0068] Another embodiment of the present invention is a product as defined above, wherein said surface-tension lowering composition and said bacterial composition are provided in a receptacle having separate compartments as defined above.

FIGURES

[0069]

- Figure 1. Set-up of the experimental system of Example 1.
- Figure 2. Evolution of the cumulative O₂-consumption of the inoculum
- Figure 3 Evolution of the O₂-consumption of the reference item
- Figure 4. Evolution of the O₂-consumption of the test item Bio Blue
- Figure 5. Evolution of the net O₂-consumption of the reference and the test item (averages)
- Figure 6. Evolution of the average biodegradation percentages for cellulose and Bio Blue.
- Figure 7. An illustration of a receptacle according to the invention.
- Figure 8. An expanded view of a supporting means according to the invention.
- Figure 9. A three-dimensional illustration of a receptacle according to the invention.

EXAMPLESExample 1 - test for aqueous aerobic biodegradation of the mixture of the invention

[0070] The aqueous biodegradation test determines the biodegradation of test items under laboratory conditions by a consortium of bacteria from different sources. The test material is brought into a chemically defined (mineral) liquid medium, essentially free of other organic carbon sources, and spiked with micro-organisms.

[0071] During the aerobic biodegradation of organic materials in an aqueous medium, oxygen is consumed and carbon is converted to gaseous, mineral carbon (under the form of carbon dioxide, CO₂). Part of the organic material is assimilated for cell growth. NaOH pellets trap the CO₂ released and the induced pressure-drop is directly related to the consumed oxygen and hence to the biodegradation of the test item.

[0072] The amount of biodegradation is expressed as the ratio of the BOD (corrected for the blank) and the Chemical Oxygen Demand (COD) of the used Test item.

[0073] The test is performed according to the international standard:

[0074] OECD Guidelines for the testing of chemicals ft 301 C: "Modified MITJ test".

ISO 9408 Water Quality - Evaluation in an aqueous medium of the "ultimate" aerobic biodegradability of organic compounds -Method by determining the oxygen demand in a closed respirometer.

1. Materials And Methods**[0075]**

Test item: "Bio Blue" - a mixture according to the invention

Physical form: liquid

Color: light orange

Storage conditions: room temperature in the dark

COD: 82.8 g/l on a liquid basis (determined by the inventors)

Batch number: Unknown

Production date: Unknown

Reference item: Cellulose

Nr: Cellulose (Batch C-105)

Purity: Native cellulose powder for thin layer chromatography (Avicel)

Expiration Date: May2006

Physical form: Powder

Color: White

Storage conditions: Room temperature in the dark

ThOD: 1190 mg/g

Brand: Merck-Eurolab N0 1-02331-0500

2. Preparation of the inoculum

[0076] The source of test organisms is a mixture of ten samples taken from activated-sewage sludge treatment plants (Brecht, Zele, Lokeren, Gent, Belgomilk), soil (Landegem, 2 samples) and/or compost extracts (Pilot scale composting test at OWS (Gent), 3 samples). Prior to use, the mixture of sludges and extracts was aerated for 1 day in order to reduce the background activity.

Sufficient volume of the mixed liquor was sampled and sieved over 0.2 mm sieve, homogenised for 2 minutes at full speed in a Waring blender. It was then filtered over a 45 micron filter. The filtrate is further used as an inoculum.

3. Preparation of Control, Reference and Test Reactors

[0077] At the start of the experiment, each bottle was filled with 240 ml of mineral medium. The procedure for preparing 1 liter of mineral medium was as follows:

- add 1 ml each of the ferric chloride, magnesium sulfate and calcium chloride stock solution;
- add 10 ml of the phosphate buffer stock solution;
- add demineralised water to a volume of 1000 ml.

[0078] In total 3 liters were prepared. This mixture was aerated with CO₂-free air for 1 hour, to make the solution

oxygen saturated.

After the first aeration period, the reference and test items were added directly to the mineral medium in the reactors.

[0079] The reference item was added to a concentration of approximately 120 mg/l by the addition 30 mg of reference item, and test item was also added to a concentration of approximately 120 mg/l by the addition of 5 ml of a prepared stock solution (1/25 dilution) for the test item.

[0080] Finally 5 ml of activated sludge inoculum was added to each bottle to obtain a total of 250 ml in each reactor.

[0081] The vessels were aerated for another 15 minutes in the incubator after mixing inoculum, sample (test or reference item) and mineral medium before closing and initiating the incubation period for biodegradation.

4. Experimental procedure

[0082] For the set-up of the experimental system, see Figure 1.

[0083] After filling, the reactors they were put on the inductive stirrer and closed after 15 minutes of acclimation in order to reach the temperature of the incubation room. NaOH pellets were added to the rubber carrier and the OXI-TOP-OC heads were connected. All heads were started using the OXI-TOP-OC- 100. A magnetic rod was added to keep the reference items, test items and the growing biomass in suspension throughout the test.

[0084] During the test, the NaOH pellets absorbed the CO₂ produced. The reactors were incubated at a constant temperature (within at least ± 1 °C) at 25 °C, in the dark for a period of 28 days. See Figure 1

5. Analytical Methods

Weight Determination

[0085] During the test, two balances were used. A Sartorius AC 210 5 with internal calibration (max. 200 g; d = 0.1 mg) for the determination of dry and volatile matter and for the weight of test and reference items. A Shimadzu Libror EB-3200 D dual range (max. 3120 g; d = 0.1 g and max. 600 g; d = 0.01 g) was used for the weight of inoculum and mineral medium.

Dry Matter or Total Solids

[0086] The dry matter was determined by drying at 105°C until constant weight was reached and weighing, as described in "SOP on determination of dry matter (DM) or total solids (IS), Edition 9". The dry matter is given in percent on wet Weight.

Volatile Solids - Ash

[0087] The volatile solids and ash content was determined by heating the dried sample at 550°C for a few hours and weighing, as described in "SOP on determination of volatile solids (VS) and ash, Edition 9." The results are given in percent on dry matter.

COD

[0088] Most types of Organic Matter are completely destroyed by a in boiling mixture of chromic and sulphuric acid. A sample is refluxed with known amounts of potassium dichromate and sulphuric acid, and the excess dichromate is titrated with ferrous ammonium sulphate. The amount of Oxidizable Organic Matter, measured as oxygen equivalent, is proportional to the potassium dichromate consumed. The COD was determined as described in "SOP on determination of COD, Edition 3".

TSS

[0089] The total suspended solids were determined using centrifugation. A well-known sample of the sludge (50-ml) was brought in a tube and centrifuged for 15 minutes at 4000rpm. After decanting the supernatant, the pellet was dried (105°C overnight) as described in "SOP on determination of suspended solids (TSS) and volatile suspended solids (VSS), Edition 3". The results are given in g/l.

pH

[0090] The pH was determined as described in "SOP on use of the CONSORT C83 1 for pH measurements, Edition

4".

6. Analyses Of Inoculum And Test Substances

[0091] The source of test organisms was a mixture of ten samples taken from activated-sewage sludge treatment plants, soil and compost extracts. The different sources are listed in Table 1.

Table 1.

Type	Place	Pretreatment
Municipal & Industrial Sewage	Zeile	None
Municipal & Industrial Sewage	Lokeren	None
Industrial sewage	Brecht	None
Milk Wastewater	Belgomilk	None
Municipal Sewage	Gent-Ossemeersen	None
Soil (sandy)	Landegem	Extract [#]
Forest	Landegem	Extract [#]
Old Compost	Gent - Lab OWS	Extract [#]
Young Compost	Gent - Lab OWS	Extract [#]
Soil	Lokeren	Extract [#]

[#]Extract. Dilution of 1 part of sample/4 parts of water.

[0092] Prior to use, the mixture aerated for 24 hours in order to reduce the background activity. A sufficient volume of the mixed liquor was sampled and homogenised for 2 minutes at full speed in a Waring blender. It was then filtered over a 45 micron filter.

[0093] The inoculum had a pH of 7.68.

[0094] Analysis indicates the total solids of the inoculum used were as follows:

TSS: 2.76 g/l

VSS: 1.38 g/l

A total 56 mg/l of suspended solids and a total of 28 mg/l of volatile suspended solids were obtained (5 ml inoculum/ 250 ml test medium) in the test medium

[0095] The test items were analysed for COD (see Table 2).

Table 2.

COD value of the test item (stock solution) and ThOD of cellulose. Average of 2 replicates of a 1/25 dilution of the test item		
Test substance	COD (mg/l) Avg	COD (mg/l) Std
Bio Blue (1/25)	3312	0
Bio Blue	82800	0
Cellulose	1190	0

7. Test Conditions and Set-Up

[0096] A set of 9 identical reactor vessels with a total volume of 0.5 l each was used. The total volume of the test solution was 0.250 liters. The experimental set-up is given in Table 3.

The reference item, cellulose was added directly to the reactor. The test item was added as a 1/25 dilution with demineralised water.

Table 3.

test set-up				
Reactor number	Reactor type	Innoculum (ml/reactor)	Cellulose (mg/reactor)	Test item [#] (g/reactor)
1	Blank	5	/	/
2	Blank	5	/	/
3	Blank	5	/	/
4	Cellulose	5	31.6	/
5	Cellulose	5	30.1	/
6	Cellulose	5	32.1	/
7	Test item	5	/	4.98
8	Test item	5	/	4.98
9	Test item	5	/	4.98

Test item= 1/25 dilution of Bio Blue

8. Net and cumulative O₂ consumption during biodegradation

[0097] The evolution of the cumulative O₂-consumption of the inoculum (blank) is represented in Figure 2. The total O₂-consumption after 28 days of testing was 21.2 ± 6.2 mg O₂/l medium.

[0098] The background activity, as indicated by the blank reactors holding only mineral medium and inoculum, was lower than 60 mg O₂/l at the end of the test, indicating that the test was valid

[0099] Figure 3 shows the evolution of the O₂-consumption of the reference item (cellulose). The final O₂-consumption for the reference item was 162.3 ± 8.5 mg O₂/l. The final net O₂ - consumption for the reference item was 141.2 ± 8.5 mg O₂/l.

[0100] Figure 4 shows the evolution of the O₂-consumption of the test item Bio Blue. The final O₂ - consumption after 28 days was 90.6 ± 6.7 mg O₂/l. The final net O₂ -consumption after 28 days was 69.5 ± 6.7 mg O₂/l.

[0101] The evolution of the net O₂-consumption of the reference and the test item (average) is represented in Figure 5.

9. Analysis at the end of the test

[0102] At the end of the 28 day test period, the pH of each reactor was measured. As can be seen in Table 4, almost no difference was found between the reactors. The presence of oxidised nitrogen was also checked by means of nitrogen strips. The results are given in Table 4. As no difference was found between the nitrate concentrations and no nitrite was present, the nitrification was similar in all reactors and did not lead to differences in O₂ consumption. The net oxygen consumption in the test reactors was therefore related only to the biodegradation of test item. As the total nitrogen of the dilution (1/25) of the test item was less than 10 mg/l, it had no influence on the results as only 5 ml was added per vessel or less than 0.05 mg of nitrogen.

Table 4.

pH, Nitrite presence, Nitrate presence and amount of Nitrate after 28 days.				
Reactor number	pH	Nitrite present?	Nitrate	
			Present?	mg NO ₃ /l
1	7.44	Negative	Positive	±10
2	7.47	Negative	Positive	±10
3	7.46	Negative	Positive	±10
4	7.50	Negative	Positive	±10
5	7.50	Negative	Positive	±10
6	7.53	Negative	Positive	±10

Table 4. (continued)

pH, Nitrite presence, Nitrate presence and amount of Nitrate after 28 days.				
Reactor number	pH	Nitrite present?	Nitrate	
			Present?	mg NO ₃ /l
7	7.53	Negative	Positive	±10
8	7.55	Negative	Positive	±10
9	7.54	Negative	Positive	±10

10. Percentage biodegradation based on COD

[0103] Figure 6 shows the evolution of the average percentage of biodegradation for cellulose and the Test item Bio Blue. In Table 6 the results for the biodegradation are given based on COD, and in Table 7, the results for the biodegradation after 28 days are given, based on COD.

Table 6.

Results of the biodegradation based on the net Oxygen consumption.				
Test item	COD (g/g reference) (g/l Test item)	Test item (mg COD/l test medium)	Net O ₂ (mg/l test medium)	Biodegradation (%)
Cellulose	1.190	150.4	137.8	91.7
Cellulose	1.190	143.2	134.8	94.1
Cellulose	1.190	152.8	150.8	98.7
Test item	82.8	66.2	74.6	112.7
Test item	82.8	66.2	71.8	108.4
Test item	82.8	66.2	61.9	93.5

Table 7.

Biodegradation percentage after 28 days (averages)					
Test item	COD (g/g reference) (g/l Test item)	Test item (mg COD/l test medium)	Net O ₂ (mg/l test medium)	Biodegradation (%)	
				AVG	STD
Cellulose	1.190	148.8	141.2	94.8	3.6
Test item	82.8	66.2	69.5	104.9	10.1

[0104] The biodegradation percentage above 100% can be explained by a synergistic effect, sometimes called priming. A priming effect occurs if the inoculum in the test reactor consumes more O₂ than the inoculum in the control reactors. This results in a net O₂ consumption that is not exclusively due to the test item and, in case of readily degradable products, results in a biodegradation percentage of more than 100%.

11. Evaluation And Discussion

[0105] During 28 days the biodegradation of Bio Blue was tested together with cellulose as reference item with a ten sources inoculum in an aquatic, aerobic biodegradation test. The test was executed according to the standard procedure IOS 9408, which is almost identical to the OECD guideline #301 C.

[0106] The reference item started to degrade after 1-2 days and had reached 70 % within 10 days. The final percentage degradation was 94.8 ± 3.6 %.

[0107] For the test item an adaptation was not necessary as 40 % of the biodegradation had already taken within the first two days. After 10 days, 90 % biodegradation had been reached. At day 20, a plateau was reached. The final result obtained was 104.9 ± 10.1 % for Bio Blue.

[0108] Both biodegradation percentages are based on oxygen consumption only.

[0109] According to the OECD guideline (# 301 C) the test is valid if the percentage biodegradation of the reference reaches more than 65% at the end of the test. In this case it was found that after 28 days, more than 90 % of BioBlue had biodegraded, therefore, BioBlue is well within the limits as set out by the guidelines.

[0110] As test item Bio Blue showed a degradation of 104.9 ± 10.1 %, it can be considered as completely degradable.

Example 2 - analysis of the content of the waste water after washing with the mixture of the invention

1. Introduction

[0111] According to the regulations for the discharge of household waste water, waste water must satisfy a number of parameters before it can be discharged. There is a difference between (1) discharge stipulations for discharging household water in the normal surface waters or in the artificial discharge methods for rain and (2) discharge stipulations for discharging household water in the public sewerage.

[0112] Given that the stipulations for discharging household water in the normal surface waters or the artificial discharge means of discharging rainwater have the most extensive and strict standards, the test product (washing water) has been analysed in conformity with these parameters.

2. Collecting the test product

[0113] The washing water was been produced by the sponsor. A total of approximately 20 l of washing water was produced by the sponsor. The washing water was collected on 27/05/02 by OWS N.V. by Bioblue Global N.V.

[0114] The washing water was a mixture of 2 machine washings of standard household linen: one washing with only a main washing at 40 °C and one washing with only a main washing at 60 °C. Each wash contained 100 ml Bio Blue - the mixture of the invention - which contained 15 ml general Bacteria and 15 ml grease and oil bacteria. Only the washing water of both washings has been collected (and not the rinsing water).

3. Procedure

[0115] The washing water was subjected to a number of analyses and tests. The following analyses were carried out on the washing water directly:

- pH
- Presence of anionic surfactants via the methylene blue method
- Visual observation of the presence of oils, fats or other floating matters

[0116] Wastewater may enter the normal surface water after via overflow from a septic tank. Therefore, a simulation test of a septic tank was also carried out in the laboratory.

[0117] After the simulation test, the above-mentioned fluid (that normally floods out of the septic tank) was analysed according to the following parameters:

- Biological use of oxygen
- Precipitated substances
- Substances that stay in suspension
- Non-polar hydrocarbons extractable with carbon tetrachloride

4. Results - analysis of the waste water

[0118] Table 8 gives an overview of the analysis results carried out on the waste water directly.

Table 8.

Overview of the analyses results carried out on the waste water	
Parameter	Result
pH	6.85
Methylene blue method	Discolouring after 2 days

Table 8. (continued)

Overview of the analyses results carried out on the waste water	
Parameter	Result
Visual observations of oils, fats and other floating substances	No visual floating layers

4. Results analysis of the septic tank water waste water

[0119] A simulation test on a septic tank was carried out in the laboratory. The washing water (31) was mixed with 11 anaerobic sludge (RWZI Zele). 11 anaerobic sludge was mixed with 31 phosphate buffer, as a control. 4 reactors were set up in total: 2 experimental control reactors and 2 test reactors (with washing water).

[0120] The reactors were incubated at 37° for 3 days. The reactors were mixed manually on a daily basis.

[0121] After 3 days, after settling for 1 hour, the above-mentioned fluid was removed for further analysis.

[0122] Table 9 gives an overview of the results of the analysis.

Table 9.

An overview of the analyses carried out on the above-mentioned fluid after simulation test in the septic tank				
Parameter	Result#			Vlarem II standard
	Control reactors	Test reactors	Net after allowance of blank	
Biological Use Of Oxygen	202 mg/l	343 mg/l	141 mg/l	Max 50 mg/l
Sediment	1.50 ml/l	1.75 ml/l	0.25 ml/l	Max 0.5 ml/l
Floating substances	240 mg/l	248 mg/l	8 mg/l	Max 60 mg/l
Non-polar hydrocarbons	0.13 mg/l	0.22 mg/l	0.09 mg/l	Max 3 mg/l

The results are based on an average of 2 analyses (1 analysis per reactor)

5. Conclusions

[0123] The result indicate that washing water using Bio Blue satisfies stringent standards (e.g. the Vlarem II standard, Section 4.2.7.2 of Vlarem II) for discharging household water in the public sewerage system. Therefore, in normal usage, BioBlue would be considered environmentally friendly

[0124] Most of the parameters indicate that the washing water is within the limits set by certain standards for discharging household water in normal surface water or in artificial discharge methods for rainwater.

Example 3 - analysis of the contents of the mixture of the invention

1. Introduction

[0125] According to the Belgian Order in Council of 25 October 1988 'regarding the degree of biological degradability of certain surfactants in detergents' (BS, 10 November 1988) it is forbidden to add detergent, bring it onto the market and to use it whenever the quantity of biologically degradable surfactants present therein is not indicated.

[0126] The washing product was analysed for presence of two of the four groups of surfactants:

- Anionic surfactants and
- Non-ionic surfactants

[0127] Cationic ampholytic surfactants were not analysed because they are present in insignificant amounts in detergents for household use.

[0128] According to the Flemish Environmental Law VLAREM II for the discharge of household waste water in normal surface water or in artificial discharge methods of rainwater (subsection 4.2.7.1) 'the discharged waste water may comprise no substances belonging to the families and groups of substances mentioned in Annex 2C of VLAREM I, nor any other substance with a percentage that directly or indirectly could damage human health or be toxic for flora or fauna.'

[0129] In order to see if the washing product satisfied the above-mentioned article, the presence and concentration of the following elements was analysed: antimony, arsenic, lead, nickel, zinc, copper, mercury, cobalt, selenium, tin, vanadium, barium, cadmium, chromium, thallium, molybdenum and titanium.

2. Components of the substance under investigation

[0130] Tested for the presence of surfactants and dangerous substances was a mix according to the invention (called herein 'Bio Blue')

[0131] Physical properties: light orange-coloured suspension.

Composition: 10ml 'Bacteria Mix' and 10ml 'Fats and Oils Bacteria' per 100ml Bio Blue

[0132] Composition of 'General Bacteria Mix'

- different groups from the Bacillus family, Enterobacter family and Clostridium family
- a safety/preserving bottom, that ensure that the micro-organisms stay alive
- plant extracts
- a quantity of demineralised water

[0133] Composition of 'Fats and Oils Bacteria': unknown

3. Examination into the presence of surfactants

[0134] The test product was analysed for presence of anionic surfactants and non-ionic surfactants.

[0135] The determination of anionic surfactants is based on the fact that the cationic pigment methylene blue forms a blue complex with the anionic surfactants which may be extracted with chloroform. To obtain the best results, a first extraction was performed with an alkaline solution, followed by the extraction of the acidic methylene blue. The absorbance of the separated organic phases were measured using the maximum extension coefficient (650 nm) of the complex.

[0136] The determination of non-ionic surfactants is based on the fact that cobalt thiocyanate forms a coloured complex with the non-ionic surfactants which may be extracted with methanol. The absorbance of the separated organic phases were measured using the maximum extension coefficient (630 nm) of the complex.

4. Examination into the presence of dangerous substances

[0137] The test product was analysed for the presence of the most important metals listed in Annex 2C of Vlare II.

[0138] The metals (with the exception of mercury) were determined via ICP-AES (inductively coupled plasma atom emission spectrometry) (OVAM Compendium, 2//B.1, 1994). The metals were released by means of a semi-open microwave oven treatment with HCl and HNO₃ (3/1).

[0139] Mercury was determined via fluorescent spectroscopy after reduction (OVAM Compendium, 2//B.3, 1991). Mercury was released from the test product made means of a closed microwave oven treatment with HNO₃.

5. Results - The presence surfactants

[0140] The washing product 'Bio Blue' was analysed for the presence of surfactants. The following values were found after averaging of 2 analyses:

- Anionic surfactants: 7.1 mg/l
- Non-ionic surfactants: 36350 mg/l

[0141] Clearly there is a very small quantity of anionic surfactants, and a majority of non-ionic surfactants.

6. Results - The presence of dangerous substances

[0142] In conformity with Art 4.2.7.1.1. it is not permitted for there to be a concentration of any substances (including a number of metals) present in discharged water that may directly or indirectly be dangerous for man, flora or fauna. In this respect, the waste water (which possibly contains remains of the washing product 'Bio Blue') must undergo a number of ecotoxic tests (given that this concerns waste water, they are primarily aquatic toxicity tests). No ecotoxic tests are mentioned in the legislation.

[0143] In order to make a more precise evaluation of the possible presence of dangerous metals, an estimation is

made of the concentration of each metal in household waste water, resulting from the use of Bio Blue as a washing product. The following dilution factors were taken into account:

- Dilution of 200 (100 ml Bio Blue/20 l washing water)
- Dilution of 10 (estimated production of household waste water: 200 l per household/day - estimated production of washing water 20 l per household/day)

The global diluting factor of Bio Blue in household waste water is therefore, 2000.

[0144] The concentration of each metal in household waste water as a result of using Bio Blue was compared with on the one hand, the basic environmental quality standards for surface water and on the other hand, the environmental quality standards for surface water, designed for the production of drinking water, as laid out in Vlarem II. Note that no account is taken of the dilution that appears by mixing waste water with surface water ('worst case scenario').

[0145] On the basis of the results laid out in Table 10 it seems that the metals present in household waste water as a result of using Bio Blue as a washing product, were lower than the basis environmental quality standards for surface water and were also lower than the environmental quality standards for surface water designed for the production of drinking water. The washing water itself already satisfies the basis environmental quality standards for surface water and environmental quality standards for surface water designed for the production of drinking water.

Table 10 -

an overview of the presence and concentration of a number of metals in the test product 'Bio Blue'.

Parameter	Bio Blue ¹ (µg/l)	Bio Blue (2000x dilution) (µg/l)	Surface water	
			Basic quality ² (µg/l)	Drinking water ³ (µg/l)
Antimony	84	0.042	N.L.	N.L.
Arsenic	171	0.085	≤30	<100
Lead	<20	<0.10	≤50	<50
Nickel	255	0.127	≤50	<50
Zinc	499	0.249	≤200	<5000
Copper	121	0.060	≤50	<1000
Mercury	<0.1	<0.0001	<0.5	<1
Cobalt	<10	<0.005	N.L.	N.L.
Selenium	467	0.234	<10	<10
Tin	272	0.136	N.L.	N.L.
Vanadium	<10	<0.005	N.L.	N.L.
Barium	19	0.010	<1000	<1000
Cadmium	4.6	0.002	<1	<5
Chromium	<10	<0.005	≤50	<50
Thallium	<40	<0.02	N.L.	N.L.
Molybdenum	<10	<0.005	N.L.	N.L.
Titanium	<10	<0.005	N.L.	N.L.

¹ Results are an average of 2 test

² Basis of environmental quality standards for surface water

³ Environment quality standards for surface water, designed for production of drinking water

N.L. - No defined limit

7. Conclusions

[0146] The detergent Bio Blue contains few anion-active surface-active compounds (7.1 mg/l). If the dilution factor during normal use of 2000 is taken into account, the concentration of anionic surfactants present in the effluent drops

to a maximum of 3.6µg/l. The basic environment quality standard for surface water is less than 100 µg/l anionic detergents (annex 2.3.1 of Vlare II), therefore Bio Blue easily falls within the acceptable guidelines.

[0147] There is an obvious presence of non-ionic surface compounds in the detergent Bio Blue (36350 mg/l). In accordance with the Royal Decree of October 25, 1988 (with regard to the degree of biological biodegradability of certain surface-active compounds in detergents) it should be proved that the surface-active compounds are biodegradable. Example 1 demonstrates that Bio Blue completely biodegrades.

Example 4 - Examples of compositions which may be formulated according to the invention

[0148]

Table 11 -

Examples of formulations according to the invention					
Polysorbate % (w/v)	PEG % (w/v)	General bacterial composition % (v/v)	Oils and fats bacterial composition % (v/v)	Total concn bacteria, cells/ml	Other additives
5	5	10	10	10 ⁸	Alcohol Plant extracts Liquid medium Citrus extract Colour agent Natural preserving agents
10	5	10	10	10 ⁸	Ditto
5	10	10	10	10 ⁸	Ditto
5	5	15	10	10 ⁸	Ditto
5	5	10	15	10 ⁸	Ditto
5	5	10	10	10 ⁹	Ditto
10	10	10	10	10 ⁸	Ditto
10	5	15	10	10 ⁸	Ditto
10	5	10	15	10 ⁸	Ditto
10	5	10	10	10 ⁹	Ditto
10	10	15	10	10 ⁸	Ditto
10	10	15	15	10 ⁸	Ditto
10	10	15	10	10 ⁹	Ditto
10	10	15	15	10 ⁹	Ditto
20	5	10	10	10 ⁸	Ditto
5	20	10	10	10 ⁸	Ditto
30	5	10	10	10 ⁸	Ditto
5	30	10	10	10 ⁸	Ditto
40	5	10	10	10 ⁸	Ditto
5	40	10	10	10 ⁸	Ditto

Claims

1. A composition comprising a mixture of a surface-tension lowering composition and a bacterial composition.

2. A composition according to claim 1 wherein said surface-tension lowering composition comprises:

- 5 to 30% polysorbate,
- 5 to 30% polyethylene glycol,
- sufficient alcohol to allow the polyethylene glycol to dissolve, and
- the remainder being demineralised water.

3. A composition according to claims 1 and 2 wherein said bacterial composition comprises one or more *Bacillus* bacteria, one or more *Enterobacter* bacteria, and one or more *Clostridium* bacteria, and one or more fats and oils-digesting bacteria.

4. A composition according to claims 1 and 3 wherein the concentration of bacteria in the mixture is in the range 10^7 to 10^{10} cells per millilitre.

5. A composition according to claims 1 to 4, wherein said surface-tension lowering composition and said bacterial composition are stored separately, and are mixed prior to or during use.

6. A composition according to claims 1 to 5 comprising a volume of said surface-tension lowering composition and a volume of said bacterial composition, said volumes determined such that the concentration of bacteria in the mixture is 10^7 to 10^{10} cells/ml.

7. A composition according to claims 1 to 6 for use in washing clothes.

8. A composition according to claims 1 to 6 for use in environmentally-friendly washing of clothes.

9. A composition according to claims 1 to 6 for use in environmentally-friendly washing of clothes in a washing machine, to reduce damage due to grease deposition and/or lime-scale deposition in said machine.

10. A method for removing odours from clothes comprising the use of a composition according to claims 1 to 6.

11. A method for washing clothes comprising the use of a composition according to claims 1 to 6.

12. A method for reducing damage to washing machines comprising the use of a composition according to claims 1 to 6 during a wash cycle.

13. A method according to claims 10 to 12, wherein said method is environmentally-friendly.

14. A product suitable for washing clothes comprising composition according to claims 1 to 9.

15. A product according to claim 14, wherein said surface-tension lowering composition and said bacterial composition are provided in separate receptacles.

16. A product according to claims 14 and 15, wherein said composition reduces damage to washing machines used during a wash cycle.

17. A receptacle, comprising two compartments suitable for dispensing two liquids simultaneously therefrom, comprising:

- a) a first bottle having a size of opening, base and internal cavity suitable for accepting a second bottle, and a locating means therefor in the base,
- b) a second bottle having a reciprocal locating means in the base for the first bottle,
- c) one or more clips, suitable for attachment of the wall of the first bottle to the wall of the second bottle, such that the flow of liquid from both compartments is substantially unrestricted upon dispensing,

such that the first compartment is formed in the space between the outer wall of the second bottle and the inner wall of the first bottle, and the second compartment is formed in the space between the inner wall of the second bottle.

18. A receptacle according to claim 17 wherein said clip comprises two or more rims, wherein

- a) the shape of one rim matches the circumference of the top of the first bottle and contacts the inner or outer surface of the top of the first bottle, and optionally another rim contacts the other surface,
- b) the shape of one rim matches the circumference of the top of the second bottle and contacts the inner or outer surface of the top of the second bottle, and optionally another rim contacts the other surface,
- c) the rims of a) and b) are connected such that the flow of liquid from both compartments is substantially unrestricted upon dispensing therefrom.

19. A receptacle according to claim 18 wherein the connection means according to step c) connects said rims across top edges of said rims and across the opening of the first compartment.

20. A receptacle according to any of claims 17 to 19, further comprising a detachable cap.

21. A receptacle according to claim 20 wherein the detachable cap attaches to the upper portion of the bottle, said receptacle comprising:

- a rim on the circumference of the cap, the inner surface of which substantially contacts the circumference of outer surface of the top of the first bottle,
- one or more protrusions located on the rim of the inner surface of the cap,
- one or more grooves located on the outer surface of the top of the first bottle,
- said protrusion(s) and groove(s) located such that upon attaching the cap to the first bottle, the protrusion(s) seats in the groove(s), so reversibly locking the cap to the bottle.

22. A product according to claim 15, wherein said surface-tension lowering composition and said bacterial composition are provided in a receptacle having separate compartments.

23. A product according to claim 15, wherein said surface-tension lowering composition and said bacterial composition are provided in a receptacle having separate compartments according to any of claims 17 to 21.

FIGURE 1

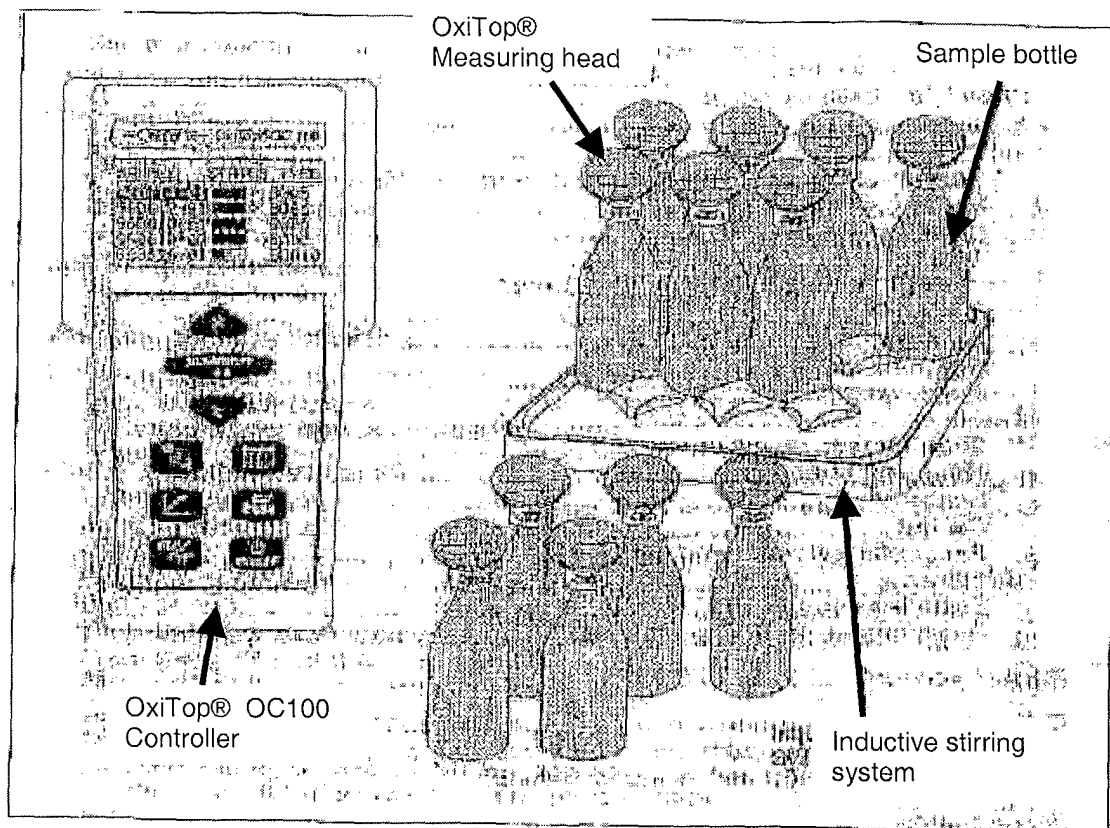


FIGURE 2

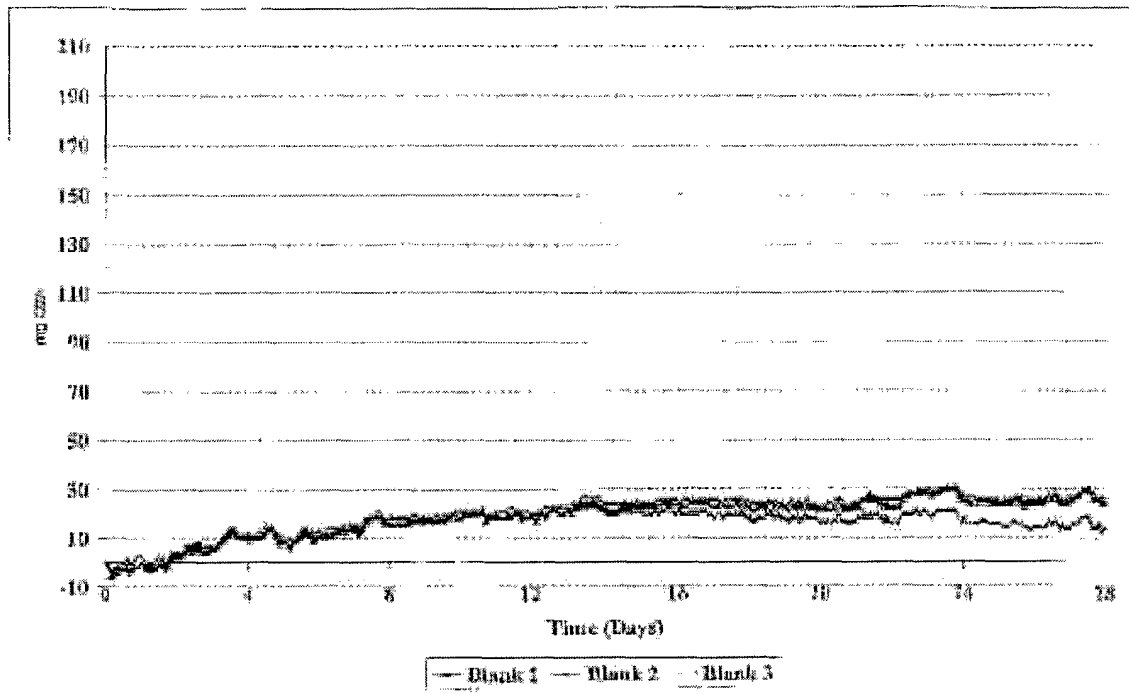


FIGURE 3

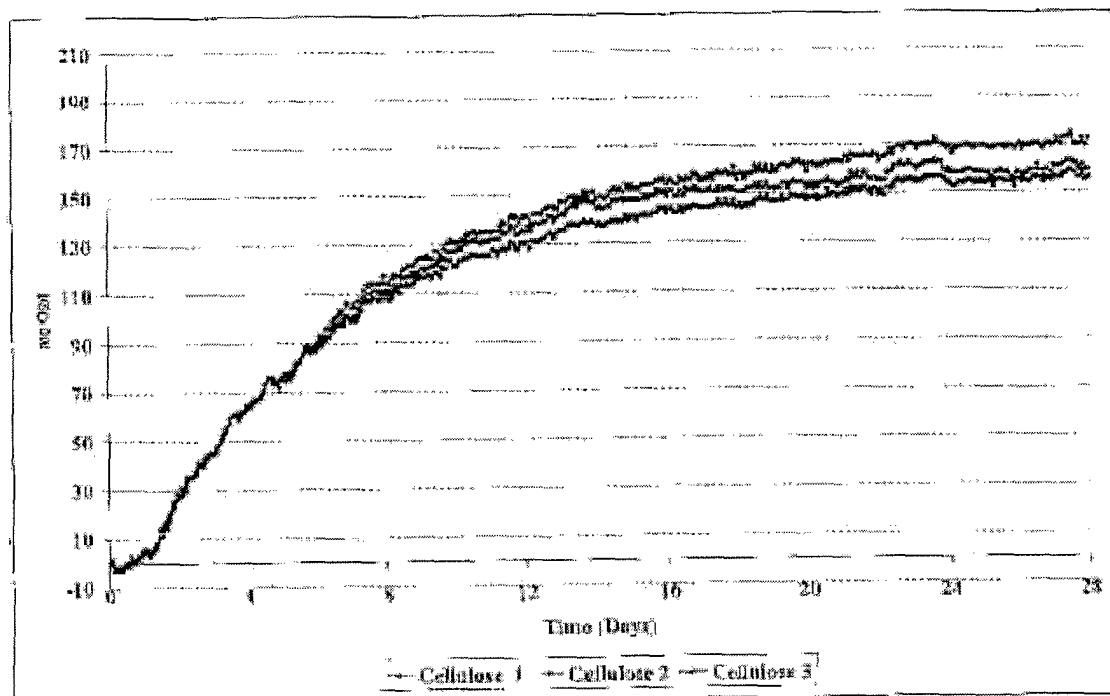


FIGURE 4

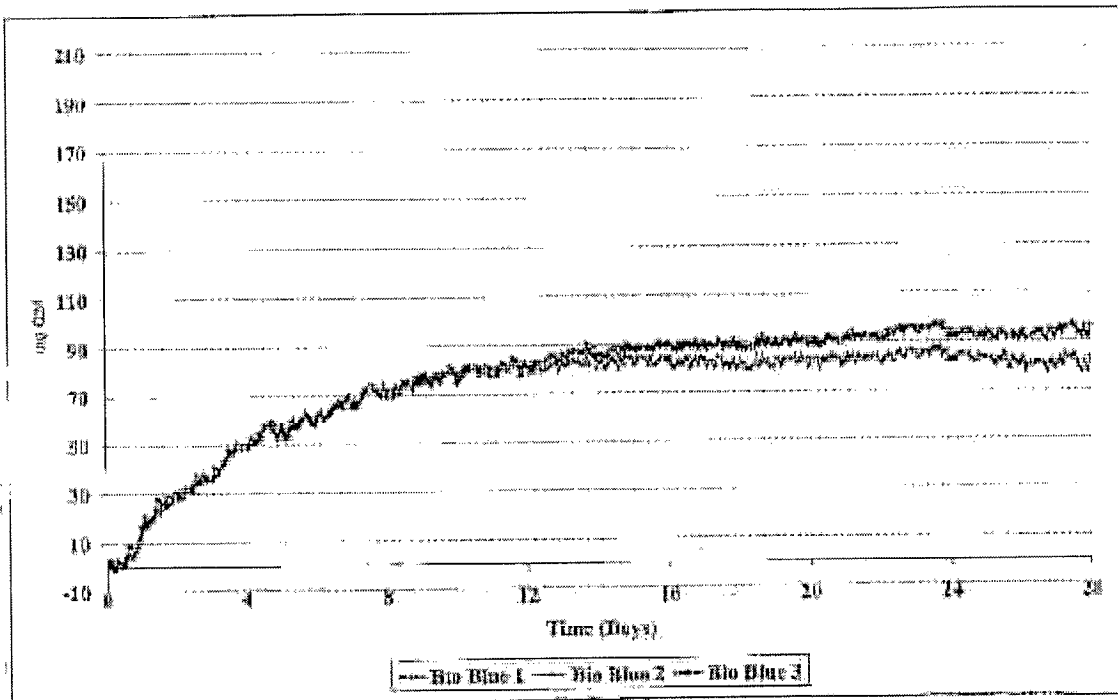


FIGURE 5

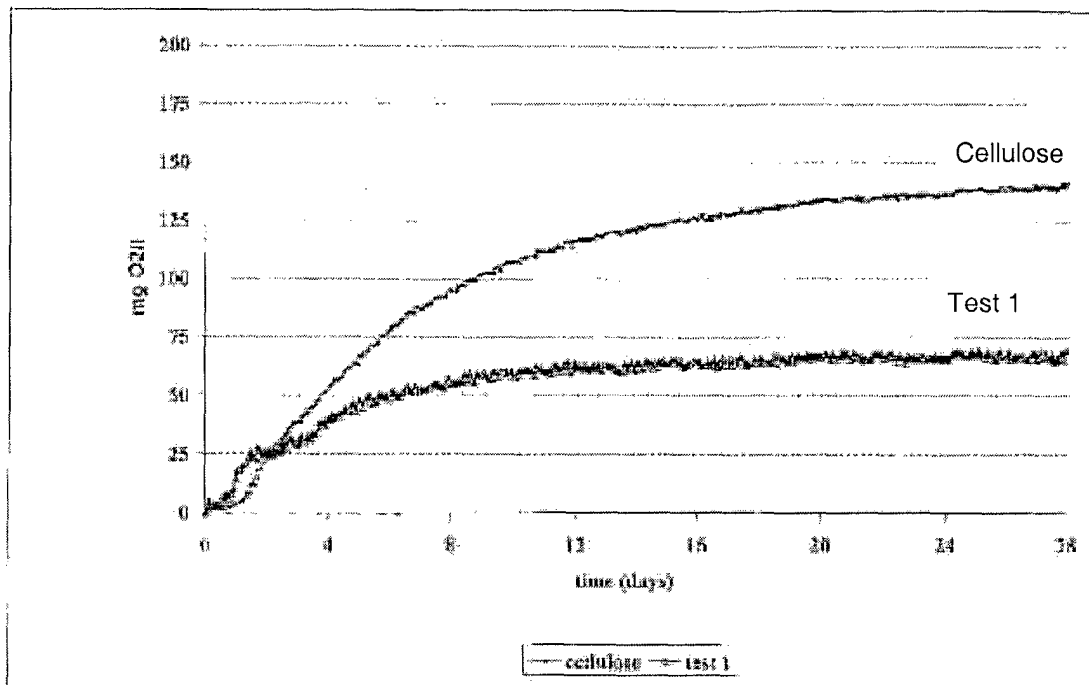


FIGURE 6

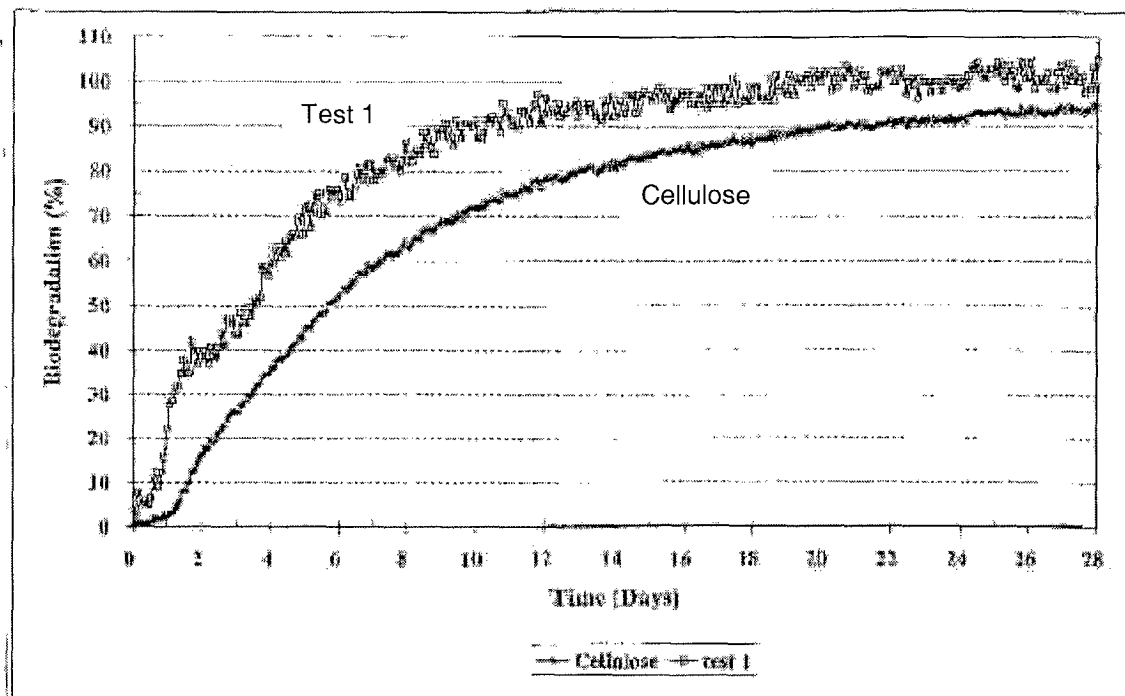


FIGURE 7

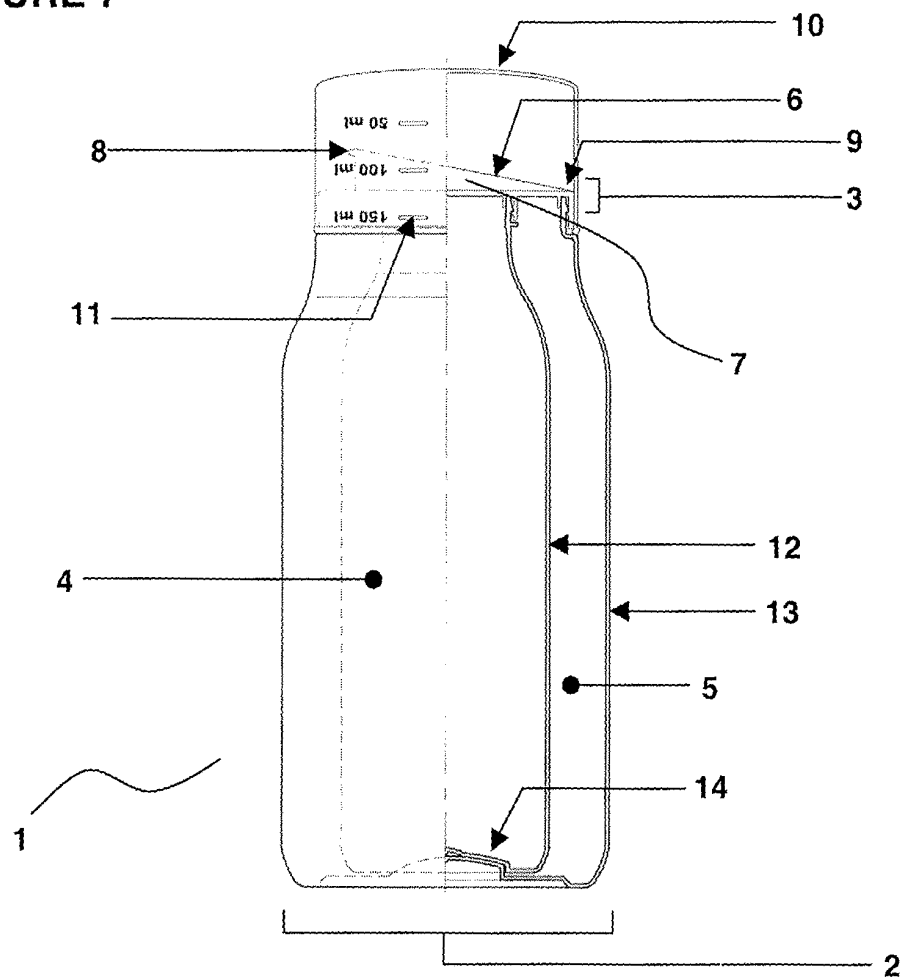


FIGURE 8

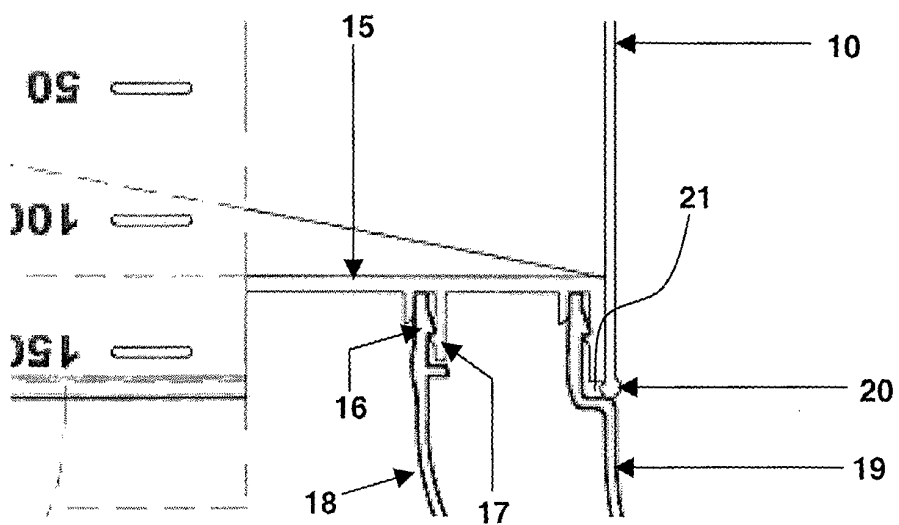
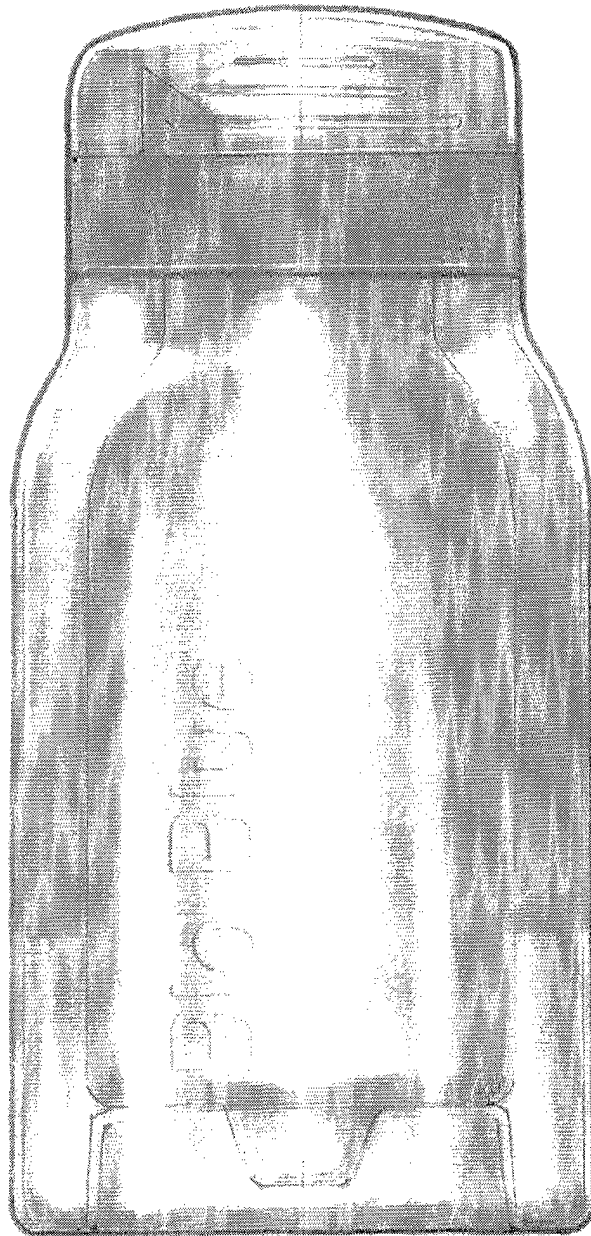


FIGURE 9





European Patent
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EUROPEAN SEARCH REPORT

Application Number
EP 03 07 5439

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,X	EP 0 569 140 A (SYBRON CHEMICALS) 10 November 1993 (1993-11-10) * claims 1-3,6-10 * * page 5, line 38 - line 49 * * page 2, line 32 - line 37 *	1,4-6	C11D3/38 C11D17/04 C11D1/66
D,X	US 6 162 635 A (LAWLER DAVID ET AL) 19 December 2000 (2000-12-19) * column 1, line 60 - line 66 * * column 2, line 12 - line 16 * * abstract *	1,5,7-9, 11,13,14	
X	WO 02 33035 A (CANADA INC (CA)) 25 April 2002 (2002-04-25) * claims 1,2,6-9 * * table * * page 2, paragraph 2 * * page 3, last paragraph - page 4, paragraph 1 *	1,4-6	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C11D
The present search report has been drawn up for all claims			
Place of search		Date of completion of the search	Examiner
The Hague		24 June 2003	Neys, P.
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 (P4/C01)



European Patent
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Application Number
EP 03 07 5439

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

Claims 1-16, 22, 23.



European Patent
Office

LACK OF UNITY OF INVENTION
SHEET B

Application Number
EP 03 07 5439

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. claims: 1-16,22,23

A composition comprising a mixture of a surface-tension lowering composition and a bacterial composition. Methods for respectively removing odours, washing clothes and reducing damage to washing machines by using the said composition. Products comprising the said composition.

2. claims: 17-21

A receptacle, comprising two compartments suitable for dispensing two liquids simultaneously therefrom, comprising:
(a) a first bottle; (b) a second bottle located in the first bottle ; (c) one or more clips for attaching the wall of the first bottle to that of the second bottle.

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 07 5439

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
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24-06-2003

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