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(54) **COMBINATIONS OF RHAMNOLIPIDS AND ENZYMES FOR IMPROVED CLEANING**

KOMBINATIONEN AUS RHAMNOLIPIDEN UND ENZYMEN FÜR VERBESSERTE REINIGUNG

COMBINAISONS DE RHAMNOLIPIDES ET D'ENZYMES POUR NETTOYAGE AMÉLIORÉ

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Description

TECHNICAL FIELD

[0001] This invention relates to cleaning compositions comprising mono-rhamnolipids in combination with enzymes.

BACKGROUND

[0002] Rhamnolipids are a class of glycolipid. They are constructed of rhamnose combined with beta-hydroxy fatty acids. Rhamnose is a sugar. Fatty acids are ubiquitous in animals and plants. The carboxyl end of the fatty acid end is connected to the rhamnose. Rhamnolipids are compounds of only three common elements; carbon, hydrogen, and oxygen. They are a crystalline acid. Rhamnolipids may be produced by strains of the bacteria *Pseudomonas aeruginosa.* There are two major groups of rhamnolipids; mono-rhamnolipids and di-rhamnolipids.

[0003] Mono-rhamnolipids have a single rhamnose sugar ring. A typical mono-rhamnolipid produced by *P. aeruginosa* is L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate ($RhaC_{10}C_{10}$). It may be referred to as Rha-C_{10}-C_{10}, with a formula of $C_{26}H_{48}O_9$. Mono-rhamnolipids have a single rhamnose sugar ring. The IUPAC Name is 3-[3-[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxydecanoyloxy]decanoic acid.

[0004] Di-rhamnolipids have two rhamnose sugar rings. A typical di-rhamnolipid is L-rhamnosyl-L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate ($Rha2C_{10}C_{10}$). It may be referred to as Rha-Rha-C_{10}-C_{10}, with a formula of $C_{32}H_{58}O_{13}$. The IUPAC name is 3-[3-[4,5-dihydroxy-6-methyl-3-(3,4,5-trihydroxy-6-methyloxan-2-yl)oxyoxan-2-yl]oxydecanoyloxy]decanoic acid.

[0005] In practice a variety of other minor components with different alkyl chain length combinations, depending upon carbon source and bacterial strain, exist in combination with the above more common rhamnolipids. The ratio of mono-rhamnolipid and di-rhamnolipid may be controlled by the production method. Some bacteria only produce mono-rhamnolipid, see US5767090: Example 1, some enzymes can convert mono-rhamnolipid to di-rhamnolipid.

[0006] In various publications mono-rhamnolipids have the notation Rha-, which may be abbreviated as Rh or RL2. Similarly di-rhamnolipids have the notation Rha-Rha or Rh-Rh- or RL1. For historical reasons "rhamnolipid 2" is a mono-rhamnolipid and "rhamnolipid 1" is a di-rhamnolipid. This leads to some ambiguity in the usage or "RL1" and "RL2" in the literature. Throughout this patent specification, we use the terms mono- and di-rhamnolipid in order to avoid this possible confusion. However, if abbreviations are used R1 is mono-rhamnolipid and R2 is di-rhamnolipid. For more information on the confusion of terminology in the prior art see the introduction to US 4814272.

[0007] The following rhamnolipids have been detected as produced by the following bacteria: (C12:1, C14:1 indicates fatty acyl chains with double bonds).

[0008] Rhamnolipids produced by *P. aeruginosa* (mono-rhamnolipids):

Rha-C_8-C_{10}, Rha-C_{10}-C_8, Rha-C_{10}-C_{10}, Rha-O_{10}-O_{12}, Rha-C_{10}-$C_{12:1}$, Rha-C_{12}-C_{10}, Rha-$C_{12:1}$-C_{10}

[0009] Rhamnolipids produced by P. *aeruginosa* (di-rhamnolipids):

Rha-Rha-C_8-C_{10}, Rha-Rha-C_8-$C_{12:1}$, Rha-Rha-C_{10}-C_8, Rha-Rha-C_{10}-C_{10}, Rha-Rha-C_{10}-$C_{12:1}$, Rha-Rha-C_{10}-C_{12}, Rha-Rha-C_{12}-C_{10}, Rha-Rha-$C_{12:1}$-C_{12}, Rha-Rha-C_{10}-$C_{14:1}$

[0010] Rhamnolipids produced by *P. aeruginosa* (unidentified as either mono- or di-rhamnolipids):

C_8-C_8, C_8-C_{10}, C_{10}-C_8, C_8-$C_{12:1}$, $C_{12:1}$-C_8, C_{10}-C_{10}, C_{12}-C_{10}, $C_{12:1}$-C_{10}, C_{12}-C_{12}, $C_{12:1}$-C_{12}, C_{14}-C_{10}, $C_{14:1}$-C_{10}, C_{14}-C_{14}.

[0011] Rhamnolipids produced by *P. chlororaphis* (mono-rhamnolipids only):

Rha-C_{10}-C_8, Rha-C_{10}-C_{10}, Rha-C_{12}-C_{10}, Rha-$C_{12:1}$-C_{10}, Rha-C_{12}-C_{12}, Rha-$C_{12:1}$-C_{12}, Rha-C_{14}-C_{10}, Rha-$C_{14:1}$-C_{10}.

[0012] Rhamnolipids produced by *Burkholdera pseudomallei* (di-rhamnolipids only):

Rha-Rha-C_{14}-C_{14}.

[0013] Rhamnolipids produced by *Burkholdera (Pseudomonas) plantarii* (di-rhamnolipids only):

Rha-Rha-C_{14}-C_{14}.

[0014] Because rhamnolipids are produced in various chemical formulas, each with a different HLB, it is known that rhamnolipids can be produced or mixed to have a range of foaming properties. Rhamnolipids are an anionic surfactant with both hydrophilic end and a lipophilic end. When their concentration increases to a certain level it is known that the rhamnolipids join together inside a liquid in a micelle.

[0015] It has been suggested that rhamnolipids with two shorter fatty acids are more active in reducing surface tension and as an emulsifier. Those rare rhamnolipids with a single fatty acid chain are not as effective.

[0016] The bacterium *Pseudomonas aeruginosa* is found naturally in soils, in water, and on plants. Metabolically, *P. aeruginosa* is chemoheterotrophic, generally aerobic, utilizing a wide range of organic compounds for sources of carbon and nitrogen.

[0017] There are over 100 strains of *P. aeruginosa* on file at the American Type Culture Collection (ATCC). There are also a number of strains that are only available to manufacturers of commercial Rhamnolipids. Additionally there are probably thousands of strains isolated by various research institutions around the world. Some work has gone into typing them into groups. Each strain has different characteristics including how much rhamnolipid is produced, which types of rhamnolipids are produced, what it metabolizes, and conditions in which it grows. Only a small percentage of the strains have been extensively studied.

[0018] Through evaluation and selection, strains of *P. aeruginosa* can be isolated to produce rhamnolipids at higher concentrations and more efficiently. Strains can also be selected to produce less byproduct and to metabolize different feedstock or pollutants. This production is greatly affected by the environment in which the bacterium is grown.

[0019] Various documents have proposed to use Rhamnolipids in detergent compositions. US 2004/0152613 A1 (Ecover), also EP1445302, describe compositions including those having mixtures of Rhamnolipid surfactants and synthetic conventional surfactants. In Example 5, the surfactant system was tested by adding it to a conventional laundry base powder comprising zeolite builder, but no enzymes.

[0020] US 5417879 A1 (Unilever) suggests a mixed micellar Glycolipid and lamellar surfactant composition, that can be either glycolipid, or not. Compositions are proposed for use at 0.5 to 50 g/l. Examples using Rhamnolipid did not use any enzyme. In column 12 lines 24 to 25, it is mentioned as possible to combine the biosurfactants with an undisclosed amount of enzyme. To arrive at a combination of enzyme with Rhamnolipid it is necessary to make several selections from this document.

[0021] US2006106120 describes a mixture of micro-organism, biosurfactant and a plastic degrading enzyme for the bioremediation of man-made materials. The biosurfactant may be a rhamnolipid (para 62). The enzyme may be a lipase (para 64). No preference is given for any components of the rhamnolipid. Neither rhamnolipids nor lipases are exemplified and rhamnolipids are not specifically claimed.

[0022] US2004072713A (Unilever) discloses an article for use in an enzymatic fabric cleaning process, said article containing one or more types of harmless micro-organisms capable of excreting enzymes useful in said fabric cleaning process. It is especially useful if, in addition to enzymes, the micro-organisms are also capable of producing other chemical entities that contribute to the cleaning process, e.g. biosurfactants, for example lipopolysaccharides as described in EP924221. These biosurfactants are not Rhamnolipids. The levels of biosurfactants generated were very low indeed and certainly would not have exceeded 0.5g/l. The micro-organisms are said to be capable of producing and secreting useful laundry enzymes such as Oxidoreductases, Carbohydrases, Proteases, Lipases, Transferases and Glycosidases.

[0023] The ability of microbes to co-generate enzyme and biosurfactants is disclosed in "Lipase and biosurfactant production for utilisation in bioremediation of vegetable oils and hydrocarbon". Martins VG et al (2008) Quimica Nova No 31 vol 8, 1942-1947. and in "Isolation and characterisation of a lipid degrading bacterium and its application to lipid containing wastewater treatment". Matsumiya Y. et al (2007) Journal of Bioscience and Bioengineering No 103, Vol 4, 325-330.

[0024] US2006080785A (Nero) describes carpet cleaning by applying a cleaning composition having biosurfactants and enzymes to the carpet; and bonnet cleaning the material. The enzymes derived from Sea Kelp are not further specified. Rhamnolipids of unknown composition are mentioned but not exemplified

SUMMARY OF THE INVENTION

[0025] According to the present invention there is provided a detergent composition with a novel ratio of mono to di rhamnolipids in combination with lipase. The amount of mono-rhamnolipid present is more than the amount of di-rhamnolipid present (if any). Preferably, at least 80 wt%, more preferably at least 90 wt% or even 100 wt% of the rhamnolipid in the composition is mono-Rhamnolipid.

[0026] The lipase is preferably derived from either fungal or bacterial sources. By bacterial sources, we include expression from other microbes, such as yeast, of genes that have been cloned from bacteria.

[0027] The rhamnolipid is preferably present in an amount of from 0.5 to 40 wt%. The lipase is preferably present in an amount of from 0.0001 to 5 wt%.

[0028] The detergent composition is preferably unbuilt. That is zeolite, phosphate or silicate builders are absent.

[0029] The detergent composition is preferably a liquid detergent composition and if citric acid builder is present, it is limited to a maximum level of 2 wt%. The composition is especially useful as a laundry detergent and may be used with advantage for washing in water with a low water hardness of less than 15°F. A process whereby the laundry and the composition are washed in presoftened water is particularly advantageously used with the compositions of the invention.

[0030] The compositions are used to remove fatty soils from laundry, especially from cotton cloths. Removal of soils from cotton is of increasing concern because many of the sophisticated soil removal and soil release technologies included in modern laundry detergents work best on polyester cloths. Accordingly, it is advantageous to combine the detergent system of the present invention with a polyester soil release polymer.

[0031] Using mono-rhamnolipids and lipase in a detergent composition according to the invention leads to enhanced cleaning benefits and possibly synergies with synthetic anionic surfactants, for example C12-14 alkyl benzene sulphonate synthetic anionic surfactant. This surfactant is commonly employed in laundry detergent compositions and is typically used with a nonionic surfactant, such as the ethoxylated nonionic surfactant used in US5417879. For environmental reasons it is desirable to eliminate this nonionic surfactant from the composition. The rhamnolipids with mono to di rhamnolipid ratio claimed provide a suitable substitute for the nonionic surfactant component, especially when used to remove fatty soils and particularly when used to remove soils from cotton cloth. The compositions are suited to low wash temperatures and fast wash times, which support energy and time savings.

[0032] A preferred fatty soil is beef fat.

DETAILED DESCRIPTION OF THE INVENTION

[0033] A large proportion of biosurfactants are generated by the action of bacteria on renewable feedstocks and are increasingly becoming more and more viable options as sustainable replacements of current synthetic surfactants. Within the current portfolio of biosurfactants that are currently commercialised, Rhamnolipids, formed by the degradation of oils and fats by Pseudomonas Aeg, show poor cleaning benefits when used at concentrations of components generated by the bacterial breakdown process. However, when the mono and di rhamnolipid components of the expressed rhamnolipids are extracted and the mono-rhamnolipid is used with lipase superior cleaning results. Moreover, by producing blends and mixing with synthetic anionic surfactants further enhancement in detergency may be achieved.

[0034] The detergent composition may comprise other ingredients commonly found in laundry liquids. Especially polyester substantive soil release polymers, hydrotropes, opacifiers, colorants, perfumes, other enzymes, other surfactants, microcapsules of ingredients such as perfume or care additives, softeners, polymers for anti redeposition of soil, bleach, bleach activators and bleach catalysts, antioxidants, pH control agents and buffers, thickeners, external structurants for rheology modification, visual cues, either with or without functional ingredients embedded therein and other ingredients known to those skilled in the art. The composition is preferably a liquid and is advantageously packaged in either a multidose bottle or in a unit dose soluble pouch.

ENZYMES

[0035] Suitable lipases for use in the compositions of the invention include those of bacterial, fungal or yeast origin. Chemically modified or protein engineered mutants are included. Examples of useful lipases include lipases from *Humicola* (synonym *Thermomyces*), e.g. from *H. lanuginosa (T. lanuginosus)* as described in EP 258 068 and EP 305 216 or from *H. insolens* as described in WO 96/13580, a *Pseudomonas* lipase, e.g. from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), P. *cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens, Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), a *Bacillus* lipase, e.g. from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

[0036] Other examples are lipase variants such as those described in WO 92/05249, WO 94/01541, EP 407 225, EP 260 105, WO 95/35381, WO 96/00292, WO 95/30744, WO 94/25578, WO 95/14783, WO 95/22615, WO 97/04079, WO 97/07202, US2008004186, US2006205628, US5869438, US6017866, US2002110854, US6939702, US2009221034, US200802425, US2004053360, US2005281912, US2006075518, US2005059130, US20041542180, US2003199069, WO98106215 and WO08088489.

[0037] Further examples of suitable lipases are described and referenced but not limited to those in Juardo et al J Surfact Deterg (2007) 10: 61-70, Horchani et al J Molecular Catalysis: Enzymatic 56 (2009) 237-245, Aloulou et al Biochimica et Biophysica acta 1771 (2007) 1446-1456, Mogensen et al Biochemistry (2005) 44: 1719-1730, Nicanuzia dos Prazeres et al Brazilian J of Microbiology (2006) 37: 505-509, Fernandez-Lorente et al Biotechnology and Bioengineering, 97: vol2 242-250, Gilbert (1993) Enzyme Microb. Technol. Vol 15 634-645, Bora and Kalita (2008) J of Chemical Technology and Biotechnology 83: 688-693, Liu et al (2009) Biochemical Engineering Journal 46: 265-270, Thirunavukarasu et al (2008) Process Biochemistry 43: 701-706, Saisubramanian et al (2008) Appl Biochem Biotechnol 150:

139-156, Joshi and Vinay (2007) Res J Biotech vol2 no2 50-56. Yeast lipases such as those from Candida sp and Cryptococcus sp are suitable.

[0038] Preferred commercially available lipase enzymes include Lipolase™ and Lipolase Ultra™, Lipex™, Novozym 525L (Novozymes A/S).

[0039] The composition may comprise a cutinase. classified in EC 3.1.1.74. The cutinase used according to the invention may be of any origin. Preferably, cutinases are of microbial origin, in particular of bacterial, of fungal or of yeast origin.

[0040] Cutinases (Esterases) are enzymes that are able to degrade cutin. In a preferred embodiment, the cutinase is derived from a strain of *Aspergillus,* in particular *Aspergillus oryzae,* a strain of *Alternaria,* in particular *Alternaria brassiciola,* a strain of *Fusarium,* in particular *Fusarium solani, Fusarium solani pisi, Fusarium roseum culmorum,* or *Fusarium roseum sambucium,* a strain of *Helminthosporum,* in particular *Helminthosporum sativum,* a strain of *Humicola,* in particular *Humicola insolens,* a strain of *Pseudomonas,* in particular *Pseudomonas mendocina,* or *Pseudomonas putida,* a strain of *Rhizoctonia,* in particular *Rhizoctonia solani,* a strain of *Streptomyces,* in particular *Streptomyces scabies,* or a strain of *Ulocladium,* in particular *Ulocladium consortiale.* In a most preferred embodiment the cutinase is derived from a strain of *Humicola insolens,* in particular the strain *Humicola insolens* DSM 1800. *Humicola insolens* cutinase is described in WO 96/13580.The cutinase may be a variant, such as one of the variants disclosed in WO 00/34450 and WO 01/92502, which are hereby incorporated by reference. Preferred cutinase variants include variants listed in Example 2 of WO 01/92502, which is hereby specifically incorporated by reference.

[0041] Other suitable esterases are those described in US2002012959, WO09085743, WO09002480, US2002137177, US2003024009, US2010151542, US2003032161, US2002007518 and US2007167344. This also includes the Transferase enzyme class.

[0042] Preferred commercial cutinases include NOVOZYM™ 51032 (available from Novozymes A/S, Denmark).

[0043] The composition may also comprise phospholipase classified as EC 3.1.1.4 and/or EC 3.1.1.32. As used herein, the term phospholipase is an enzyme which has activity towards phospholipids. Phospholipids, such as lecithin or phosphatidylcholine, consist of glycerol esterified with two fatty acids in an outer (sn-1) and the middle (sn-2) positions and esterified with phosphoric acid in the third position; the phosphoric acid, in turn, may be esterified to an amino-alcohol. Phospholipases are enzymes which participate in the hydrolysis of phospholipids. Several types of phospholipase activity can be distinguished, including phospholipases A_1 and A_2 which hydrolyze one fatty acyl group (in the sn-1 and sn-2 position, respectively) to form lysophospholipid; and lysophospholipase (or phospholipase B) which can hydrolyze the remaining fatty acyl group in lysophospholipid. Phospholipase C and phospholipase D (phosphodiesterases) release diacyl glycerol or phosphatidic acid respectively.

[0044] The term phospholipase includes enzymes with phospholipase activity, e.g., phospholipase A (A_1 or A_2), phospholipase B activity, phospholipase C activity or phospholipase D activity. The term "phospholipase A" used herein in connection with an enzyme of the invention is intended to cover an enzyme with Phospholipase A_1 and/or Phospholipase A_2 activity. The phospholipase activity may be provided by enzymes having other activities as well, such as, e.g., a lipase with phospholipase activity. The phospholipase activity may, e.g., be from a lipase with phospholipase side activity. In other embodiments of the invention the phospholipase enzyme activity is provided by an enzyme having essentially only phospholipase activity and wherein the phospholipase enzyme activity is not a side activity.

[0045] The phospholipase may be of any origin, e.g., of animal origin (such as, e.g., mammalian), e.g. from pancreas (e.g., bovine or porcine pancreas), or snake venom or bee venom. Preferably the phospholipase may be of microbial origin, e.g., from filamentous fungi, yeast or bacteria, such as the genus or species *Aspergillus,* e.g., *A. niger, Dictyostelium,* e.g., *D. discoideum; Mucor,* e.g. *M. javanicus, M. mucedo, M. subtilissimus; Neurospora,* e.g. *N. crassa; Rhizomucor,* e.g., *R. pusillus; Rhizopus,* e.g. *R. arrhizus, R. japonicus, R. stolonifer, Sclerotinia,* e.g., *S. libertiana; Trichophyton,* e.g. *T. rubrum; Whetzelinia,* e.g., *W. sclerotiorum; Bacillus,* e.g., *B. megaterium, B. subtilis; Citrobacter,* e.g., *C. freundii; Enterobacter,* e.g., *E. aerogenes, E. cloacae Edwardsiella, E. tarda; Erwinia,* e.g., *E. herbicola; Escherichia,* e.g., *E. coli; Klebsiella,* e.g., *K. pneumoniae; Proteus,* e.g., *P. vulgaris; Providencia,* e.g., *P. stuartii; Salmonella,* e.g. *S. typhimurium; Serratia,* e.g., *S. liquefasciens, S. marcescens; Shigella,* e.g., *S. flexneri; Streptomyces,* e.g., *S. violeceoruber, Yersinia,* e.g., *Y. enterocolitica.* Thus, the phospholipase may be fungal, e.g., from the class *Pyrenomycetes,* such as the genus *Fusarium,* such as a strain of *F. culmorum, F. heterosporum, F. solani,* or a strain of *F. oxysporum.* The phospholipase may also be from a filamentous fungus strain within the genus *Aspergillus,* such as a strain of *Aspergillus awamori, Aspergillus foetidus, Aspergillus japonicus, Aspergillus niger* or *Aspergillus oryzae.*

[0046] Preferred phospholipases are derived from a strain of *Humicola,* especially *Humicola lanuginosa.* The phospholipase may be a variant, such as one of the variants disclosed in WO 00/32758, which are hereby incorporated by reference. Preferred phospholipase variants include variants listed in Example 5 of WO 00/32758, which is hereby specifically incorporated by reference. In another preferred embodiment the phospholipase is one described in WO 04/111216, especially the variants listed in the table in Example 1.

[0047] In another preferred embodiment the phospholipase is derived from a strain of *Fusarium,* especially *Fusarium oxysporum.* The phospholipase may be the one concerned in WO 98/026057 displayed in SEQ ID NO: 2 derived from

Fusarium oxysporum DSM 2672, or variants thereof.

[0048] In a preferred embodiment of the invention the phospholipase is a phospholipase A_1 (EC. 3.1.1.32). In another preferred embodiment of the invention the phospholipase is a phospholipase A_2 (EC.3.1.1.4.).

[0049] Examples of commercial phospholipases include LECITASE™ and LECITASE™ ULTRA, YIELSMAX, or LIPOPAN F (available from Novozymes A/S, Denmark).

[0050] The composition may further comprise other enzymes enhancing the detergency of the composition such as softening agents, an amylase (e.g. Fungamyl(R) from Novo Nordisk A/S, Denmark), a lipase (e.g. Novocor(R) AD from Novo Nordisk A/S, Denmark), a cellulase (e.g. Celluzyme(R), Carezyme(R), and/or Celluclast(R), all from Novo Nordisk A/S, Denmark), a xylanase (e.g. Biofeed(R) PLUS or Shearzyme(TM) from Novo Nordisk A/S, Denmark), a beta-glucanase (e.g. Viscozyme(R) or Ultraflo(TM) from Novo Nordisk A/S, Denmark), a pectinase (e.g. Pectinex(TM) Ultra from Novo Nordisk A/S, Denmark), a peroxidase (e.g. Guardzyme(TM) from Novo Nordisk A/S, Denmark), a laccase (e.g. obtained from Myceliophthora or Polyporus), an enhancing agent for the peroxidase/laccase (e.g. PPT or methylsyringic acid methylsyringate or derivatives thereof) and/or a buffer (e.g. citric acid).

[0051] The invention will be further described with reference to the following non-limiting examples.

EXAMPLES

Example 1

[0052] Various Lipase and Rhamnolipid compositions were examined to determine their ability to remove a coloured beef stain.

[0053] Wash solutions were prepared by dispersing lipase at a concentration of 4mg protein per litre together with detergent surfactant at the required concentration in phosphate buffered saline (PBS) adjusted to pH 8 and 12° FH water hardness. 10 mls of the wash solution were mixed in 25 ml plastic vials at 37 °C with agitation at 200 rpm in an orbital incubator for 30 minutes. Swatches (approximately 1 cm^2) of cotton cloth stained with Sudan Red coloured Beef fat were then added and the vials returned to the shaking incubator. Swatches were removed at timed intervals, rinsed in cold water and dried at 37 °C. The residual colour was monitored using a Macbeth Colour Eye, and compared with untreated stained cloths.

[0054] Bacterial enzyme is "Lipomax", a bacterially derived Lipase variant M21 L of the lipase of *Pseudomonas alcaligenes* as described in WO 94/25578 to Gist-Brocades (M.M.M.J. Cox, H.B.M. Lenting, L.J.S.M. Mulleners and J.M. van der Laan).

[0055] Fungal enzyme is "Lipolase", derived from *Humicola languginosa* as described in EP 0 258 068 and available from NovoZymes A/S.

[0056] Details of the surfactants were as follows:

RL = Rhamnolipid: a biosurfactant of bacterial origin. Commercially available from Jeneil as RBR425 (25%AM). The composition of this material was analysed and is given in Table 3 below.

[0057] In addition to the commercially supplied Rhamnolipid, two further samples were made up by separating it into R1 and R2 rich fractions. These fractions were also used in combination with the lipases. The cleaning data for 1 hour and 4 hours is given in Tables 1 and 2.

Table 1 - 1 hour

Rhamnolipid	No Enzyme	Bacterial enzyme	Fungal enzyme
0.5 g/l RL	1.15	8.88	1.04
0.5 g/l R1	9.85	11.31	12.25
0.5 g/l R2	0.80	8.87	1.05

Table 2 - 4 hours

Rhamnolip	No Enzyme	Bacterial enzyme	Fungal enzyme
0.5 g/l RL	1.18	10.68	1.89
0.5 g/l R1	14.52	12.43	14.19

(continued)

Rhamnolip	No Enzyme	Bacterial enzyme	Fungal enzyme
0.5 g/l R2	1.14	11.42	2.85

Table 3 - Analysis of JBR425 via HPLC/MS

Rhamnolipid Congeners	m/z	%
Di - C10-C8	621	1.6
Di - C8 - C10	621	1.3
Di - C10-C10	649	67.4
Di - C10-C12:1	675	0.78
Di - C12:1-C10	675	0.016
Di - C10-C12	677	3.18
Di - C12-C10	677	1.12
Mono - C10-C8	475	0.63
Mono - C8-C10	475	0.47
Mono C10-C10	503	21.6
Mono - C10-C12:1	529	0.69
Mono -C12:1-C10	529	0.014
Mono C10-C12	531	1.12
Mono -C12-C10	531	0.023

[0058] For our analysis of this Rhamnolipid in Table 3, a known amount of JBR425 was acidified to pH 3 using 12M HCl and placed in a refrigerator overnight. The supernatant was then extracted three times using a 2:1 mixture of Chloroform and Ethanol. The solvent was then removed by rotary evaporation and the isolated rhamnolipid mixture was then re-dissolved in methanol.

[0059] The process of separating and characterising the mixture was carried out using an HPLC connected to an Ion Trap Electrospray ionisation Mass Spectrometer. The mode of ionisation was in negative mode with a scanning range of 50-1200Da. The column used to separate was a Phenomenex luna C18 250 x 4.6mm 5 μm column. The mobile phase: water (mobile phase A) and acetonitrile (mobile phase B) were used to separate via a gradient of 60:40 (A:B) changing to 30:70 (A:B) over 30 minutes. The system was then held for 5 minutes before returning to the start conditions all at a flow rate of 0.5ml/min. The injection volume was 10 μl.

Claims

1. A detergent composition comprising rhamnolipids and lipase, wherein the weight percent of the rhamnolipid made up by mono-rhamnolipids is more than 50 wt%.

2. A composition according to claim 1 wherein the weight percent of the rhamnolipid made up by mono-rhamnolipids is more than 80 wt%.

3. A composition according to claim 1 or 2 wherein the weight percent of the rhamnolipid made up by mono-rhamnolipids is more than 90 wt%.

4. A composition according to any preceding claim in which 100 wt% of the rhamnolipid is mono-rhamnolipid.

5. A composition according to any preceding claim in which the rhamnolipid is present in an amount of from 0.5 to 40 wt%

6. A composition according to any preceding claim in which the lipase is present in an amount of from 0.0001 to 5 wt%

7. A composition according to any preceding claim further comprising at least 5 wt% of synthetic anionic surfactant.

8. A composition as claimed in any preceding claim in which the synthetic anionic surfactant comprises C12-14 linear alkyl benzene sulphonate.

9. A composition according to any preceding claim, which has less than 2% detergent builder

10. A composition according to any preceding claim that has more synthetic anionic surfactant than rhamnolipid.

11. A composition according to any preceding claim that is a laundry liquid comprising from 10 to 40% total surfactant.

12. A laundry liquid according to claim 11 comprising less than or equal to 2% citric acid.

13. A laundry detergent composition according to any preceding claim, further comprising a polyester substantive soil release polymer.

14. Use of a composition according to any of claims 1 to 13 for washing in water with a low water hardness of less than 15°F.

15. Use of a composition according to any one of claims 1 to 13 to remove fatty soils from laundry.

16. Use according to claim 15 wherein the fatty soils are removed from cotton cloth.

17. Use according to claim 15 or 16 wherein the fatty soil is beef fat.

18. A process for cleaning a substrate comprising the steps of immersing the substrate in water, adding a composition according to claims 1 to 13 to the water to form a wash liquor and washing the substrate, **characterised in that** the wash cycle time is less than 60 minutes, preferably less than 30 minutes and the water temperature is less than 35 °C.

Patentansprüche

1. Waschmittelzusammensetzung, umfassend Rhamnolipide und Lipase, wobei der Gewichtsprozentsatz des Rhamnolipids, der durch Mono-Rhamnolipide gebildet wird, mehr als 50 Gew.-% beträgt.

2. Zusammensetzung nach Anspruch 1, wobei der Gewichtsprozentsatz des Rhamnolipids, der durch Mono-Rhamnolipide gebildet wird, mehr als 80 Gew.-% beträgt.

3. Zusammensetzung nach Anspruch 1 oder 2, wobei der Gewichtsprozentsatz des Rhamnolipids, der durch Mono-Rhamnolipide gebildet wird, mehr als 90 Gew.-% beträgt.

4. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, wobei 100 Gew.-% des Rhamnolipids Mono-Rhamnolipid ist.

5. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, wobei das Rhamnolipid in einer Menge von 0,5 bis 40 Gew.-% vorliegt.

6. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, wobei die Lipase in einer Menge von 0,0001 bis 5 Gew.-% vorliegt.

7. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, die ferner mindestens 5 Gew.-% synthetisches anionisches Tensid umfasst.

8. Zusammensetzung wie in irgendeinem vorhergehenden Anspruch beansprucht, wobei das synthetische anionische Tensid lineares C_{12-14}-Alkylbenzolsulfonat umfasst.

9. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, die weniger als 2 Gew.-% Waschmittelgerüstsubstanz umfasst.

10. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, die mehr synthetisches anionisches Tensid als Rhamnolipid aufweist.

11. Zusammensetzung nach irgendeinem vorhergehenden Anspruch, die eine Waschflüssigkeit darstellt, die insgesamt 10 bis 40% Gesamttensid umfasst.

12. Waschflüssigkeit nach Anspruch 11, umfassend weniger als oder gleich 2% Citronensäure.

13. Waschmittelzusammensetzung nach irgendeinem vorhergehenden Anspruch, die des Weiteren ein für Polyester substantives schmutzabweisendes Polymer umfasst.

14. Verwendung einer Zusammensetzung nach irgendeinem der Ansprüche 1 bis 13 zum Waschen in Wasser mit einer geringen Wasserhärte von weniger als 15°F.

15. Verwendung einer Zusammensetzung nach irgendeinem der Ansprüche 1 bis 13 zur Entfernung von fettigem Schmutz von Wäsche.

16. Verwendung nach Anspruch 15, wobei der fettige Schmutz von Baumwollgewebe entfernt wird.

17. Verwendung nach Anspruch 15 oder 16, wobei der fettige Schmutz Rinderfett ist.

18. Verfahren zum Reinigen eines Substrats, umfassend die Schritte des Eintauchens des Substrats in Wasser, der Zugabe einer Zusammensetzung nach den Ansprüchen 1 bis 13 zum Wasser, um eine Waschlauge zu erzeugen, und des Waschens des Substrats, **dadurch gekennzeichnet, dass** die Waschzykluszeit weniger als 60 Minuten beträgt, vorzugsweise weniger als 30 Minuten, und die Wassertemperatur niedriger als 35°C ist.

Revendications

1. Composition de détergent comprenant des rhamnolipides et une lipase, dans laquelle le pourcentage en poids du rhamnolipide constitué de mono-rhamnolipides est supérieur à 50 % en poids.

2. Composition selon la revendication 1, dans laquelle le pourcentage en poids du rhamnolipide constitué de mono-rhamnolipides est supérieur à 80 % en poids.

3. Composition selon la revendication 1 ou 2, dans laquelle le pourcentage en poids du rhamnolipide constitué de mono-rhamnolipides est supérieur à 90 % en poids.

4. Composition selon l'une quelconque des revendications précédentes, dans laquelle 100 % en poids du rhamnolipide sont constitués de mono-rhamnolipide.

5. Composition selon l'une quelconque des revendications précédentes, dans laquelle le rhamnolipide est présent dans une quantité de 0,5 à 40 % en poids.

6. Composition selon l'une quelconque des revendications précédentes, dans laquelle la lipase est présente dans une quantité de 0,0001 à 5 % en poids.

7. Composition selon l'une quelconque des revendications précédentes comprenant de plus au moins 5 % en poids de tensioactif anionique synthétique.

8. Composition selon l'une quelconque des revendications précédentes, dans laquelle le tensioactif anionique synthétique comprend un benzènesulfonate d'alkyle linéaire en C12-14.

9. Composition selon l'une quelconque des revendications précédentes, laquelle présente moins de 2 % d'adjuvant de détergent.

10. Composition selon l'une quelconque des revendications précédentes qui présente plus de tensioactif anionique synthétique que de rhamnolipide.

11. Composition selon l'une quelconque des revendications précédentes qui est un liquide pour le linge comprenant de 10 à 40 % de tensioactif total.

12. Liquide pour le linge selon la revendication 11 comprenant une quantité inférieure ou égale à 2 % d'acide citrique.

13. Composition de détergent pour le linge selon l'une quelconque des revendications précédentes, comprenant de plus un polymère de polyester de libération substantive de salissure.

14. Utilisation d'une composition selon l'une quelconque des revendications 1 à 13 pour un lavage dans de l'eau avec une faible dureté d'eau inférieure à 15°F.

15. Utilisation d'une composition selon l'une quelconque des revendications 1 à 13 pour éliminer des salissures grasses du linge.

16. Utilisation selon la revendication 15, dans laquelle les salissures grasses sont éliminées d'un tissu en coton.

17. Utilisation selon la revendication 15 ou 16, dans laquelle la salissure grasse est de la graisse de boeuf.

18. Procédé de nettoyage d'un substrat comprenant les étapes d'immersion du substrat dans de l'eau, d'addition d'une composition selon les revendications 1 à 13 à l'eau pour former une liqueur de lavage et de lavage du substrat, **caractérisé en ce que** la durée de cycle de lavage est inférieure à 60 minutes, de préférence inférieure à 30 minutes et la température de l'eau est inférieure à 35°C.

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