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(54) Improvements relating to detergent analysis

(57) One problem with the laboratory use of grass stains is that the results show poor reproducibility. The present invention provides a swatch of fabric having an area of from 0.25 m^2 to $4x10^{-6}m^2$, said swatch being stained in one or more patches over at least 0.1% of its surface with debris comprising chloroplast material wherein at least 80%wt of the chloroplast material present in a patch is from a single species of green plant other than tea or spinach. Preferably, the remaining 20%wt of the debris is from no more than five, preferably three, preferably one other species. In a referred embodiment the stain is derived from a single species. By ensuring that a small and known number of species of green plant are used reproducible "grass stains" can be obtained that have a consistent level of difficulty as regards their removal. A second aspect of the invention subsists in a method for preparing a swatch which comprises the step of contacting a fabric substrate in sheet form having an area greater than $4x10^{-6}m^2$ (greater than $4mm^2$) with chloroplast-containing green plant material wherein at least 80% of the debris is from a single species of green plant other than tea or spinach. A third aspect of the invention provides a method for determining the effectiveness of laundry compositions which includes that step of treating swatches according to the present invention with the laundry composition in a particularly preferred embodiment of the invention the laundry composition comprises at least one enzyme.

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Description

Technical Field

[0001] The present invention is concerned with improvements relating to detergent analysis and in particular to improvements relating to the preparation of so-called "swatches" for use in such analysis.

Background of the Invention:

[0002] In the art of laundry detergent formulation a "swatch" is a piece of generally flexible material such as a fabric that has a stain applied thereto. The material can be, for example, a fabrics made of cotton, polyester or various mixtures of natural and synthetic fibres. The swatch can be non-woven (e.g. filter paper or nitrocellulose) or even a piece of a hard material, for example, ceramic. The stain may include blood, milk, ink, grass, tea, wine, spinach, gravy, chocolate, egg, cheese, clay, pigment, oil, or mixtures of these components.

[0003] Swatches are commonly used in the evaluation of laundry detergent compositions. In the traditional "model wash" analysis, as they are a small part of the wash-load, the bulk of the load being made up of "ballast", which comprises various sheets of cloth. One or more swatches (also known as "monitors") and a quantity of "ballast", are washed under known conditions so that the degree of stain removal can be determined.

[0004] United States Patent 7122334 discloses a more modern technique for analysis of detergent compositions. In this, a "smaller swatch" - a piece of the swatch that has been cut or otherwise removed from the swatch of material either before or after fixing the stain to the swatch is placed into the well of a 24, 48 or 96 well micro-titer plate. The "smaller swatch" can also be made by applying a stain to a small piece of material. Typically these "smaller swatches" are around 5mm in diameter. In the context of the present invention "swatch" includes both larger and smaller swatches.

[0005] Swatches having stains of known "strength" on various types of material are commercially available (from EMPA, St. Gallen, Switzerland; WFK-Testgewebe GmbH, Krefeld Germany; or Center for Test Materials, Vlaardingen, The Netherlands) and/or can be made by the practitioner (as described for example in Morris and Prato, Textile Research Journal 52(4):280 286 (1982)).

[0006] It has been suggested, in US 7122334 to use tea (*Camellia sinensis*) and spinach (*Spinacia oleracea)* as a source for stains.

[0007] Mechanically-produced plant stains are the more common source for such stains is generally referred to as "grass". "Grass" is a very general term and in common parlance is often used to refer to monocotyledonous green plants of the family Poaceae (also known as the *Gramineae*). As will be apparent, spinach and tea stains on clothing and other fabric articles are less likely to occur through outdoor activity than "grass stains", and more likely to result from spillage of food or drink whereas "grass stains" are more of a problem on sports/outdoor clothing and/or children's clothing.

[0008] One of the main problems with the laboratory use of grass stains is that the results show poor reproducibility. It is therefore difficult to optimise laundry detergent compositions for the effective and efficient removal of grass stains. Much effort has been given to exactly how the grass stain should be formed on the swatch and various complicated devices have been proposed to solve this problem.

Brief Description of the Invention

[0009] We have determined that improved "grass stain" swatches can be prepared if the majority of the green plant material present is mostly or all from a single species of green plant.

[0010] Accordingly the present invention provides a swatch of fabric having an area of from 0.25 m^2 to $4x10^{-6}m^2$, said swatch being stained in one or more patches over at least 0.1% of its surface with debris comprising chloroplast material wherein at least 80%wt of the chloroplast material present in a patch is from a single species of green plant other than tea or spinach.

[0011] Preferably the fabric is cellulosic, polyester or a mixture thereof.

[0012] Preferably, the remaining 20%wt of the debris is from no more than five, preferably three, preferably one other species. In a preferred embodiment the stain is derived from a single species.

[0013] By ensuring that a small and known number of species of green plant are used reproducible "grass stains" can be obtained that have a consistent level of difficulty as regards their removal.

[0014] Preferably the debris is one or more species of the genera:

Agropyron, Agrostis, Axonopus, Bothriochloa, Bouteloua,
Fescue, Chamaemelum, Dactyloctenium, Chrysopogon,
Deschampsia, Desmodium, Dichanthium, Cynodon, Lolium,
Trifolium, Belliss, Taraxacum, Hilaria, Hydrocotyle,

Pennisetum, Leptinella, Koeleria, Ischaemum, Eremochloa, Eragrostis, Elymus, Microlaena, Plantago, Paspalum, Eurhynchium, Dichondra, Digitaria, Brachythecium, Polytrias, Pratia, Puccinellia, Ranunculus, Senecio, Distichlis, Holcus, Stenotaphrum, Urochloa, Zoysia, Trisetum and/or Poa.

[0015] Preferably the debris is from a Dicotyledone.

[0016] Preferably the debris is from one or more of the species:

Agrostis canina, Agrostis capillaris, Agrostis clavata, Agrostis curtisii, Agrostis gigantea, Agrostis castellana, Agrostis mertensii, Agrostis scabra, Agrostis stolonifera, Agrostis tenuis, Agrostis palustris, Agrostis vineale, Festuca rubra, Lolium perenne, Trifolium repens, Bellis perennis, Taraxacum officinale, Eurhynchium praelongum, Brachythecium rutabulum, Ranunculus repens, Holcus lanatus, Senecio jacobaea and Plantago major, Poa trivialis, Poa. pratensis and Poa. Annua.

[0017] Preferably, the debris is from one or more of the order *Ranunculales* or the family *Asteraceae*.

[0018] Many of these plants are more familiar under their common names: *Agrostis* are the bent grasses (*Agrostis stolonifera* - "*creeping bent*" is commonly used on golf-course greens), and are widely used on lawns. The *Fescue* genus are tufted grasses (used on bowling greens and for fodder), *Lolium* are the ryegrasses, *Poa* includes European meadowgrass, bluegrass and tussock, while *Holcus* are also grasses. However many of the preferred plants are not grasses. For example, *Trifolium* includes the clovers, *Bellis* includes the daisy, *Taraxacum* includes the dandelion, *Ranunculus* includes the buttercup. *Eurhynchium* is a moss genus as are the *Brachytheciaceae*.

[0019] Particularly preferred are stains which comprise one or more of plants selected from the genus *Lolium,* preferably *Lolium perenne* (a perennial ryegrass). *Festuca*, preferably Festuca *pratensi* (a meadow fescue). *Trifolium,* preferably *Trifolium pratense* (a red clover). Other suitable stains may comprise *Agrostis* (bent grass), *trifolium repens* (white clover) and/or *Taraxacum officinale* (dandelion).

[0020] A swatch may comprise two or more stains which are different cultivars or varieties of the same species. Thus, where the swatch comprises a stain derived from *trifolium repens* (white clover) separate stains derived from the *Aber Dai* cultivar and the *Aber Ace* cultivar may be present.

[0021] It is preferred that at least one non-grass species is present and particularly preferred that this is selected from the non-vascular plants (Bryophytes), from non-angiosperm vascular plants or from the angiosperm families *Asteraceae, Rubiaceae* or *Fabaceae*. Swatches can be prepared wherein the debris derived from true grasses (Poaceae) are less than 50%wt, preferably less than 25%wt of the material present. Swatches may be prepared in which none of the material present is derived from grasses.

[0022] More than one species-specific stain can be present on each swatch. Preferably there are 1-30 stained patches on a larger swatch, each covering at least 0.1% of the area of the swatch. 4-10 patches are particularly preferred.

[0023] A smaller swatch may be uniformly stained or cut from a larger swatch.

[0024] A second aspect of the present invention subsists in a method for preparing a swatch which comprises the step of contacting a fabric substrate in sheet form having an area greater than $4x10^{-6}m^2$ (greater than $4mm^2$) with chloroplast-containing green plant material wherein at least 80% of the debris is from a single species of green plant other than tea or spinach.

[0025] In this second aspect of the invention the preferred genera and species are as noted above and the process may be repeated to give a swatch bearing a plurality of stains.

[0026] A third aspect of the present invention provides a method for determining the effectiveness of laundry compositions which includes that step of treating swatches according to the present invention with the laundry composition. In a particularly preferred embodiment of the invention the laundry composition comprises at least one enzyme.

[0027] As chloroplasts contain DNA, it is relatively easy to determine the number and type of genera and species represented on a particular swatch.

Detailed Description of the Invention

Enzymes

[0028] As noted above, in a particularly preferred embodiment of the invention the laundry composition being tested

comprises at least one enzyme. Especially contemplated enzymes include proteases, alpha-amylases, cellulases, lipases, peroxidases/oxidases, pectate lyases, and mannanases, or mixtures thereof.

[0029] Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically modified or protein engineered mutants are included. The protease may be a serine protease or a metallo protease, preferably an alkaline microbial protease or a trypsin-like protease. Examples of alkaline proteases are subtilisins, especially those derived from *Bacillus,* e.g., subtilisin Novo, subtilisin Carlsberg, subtilisin 309, subtilisin 147 and subtilisin 168 (described in WO 89/06279). Examples of trypsin-like proteases are trypsin (e.g. of porcine or bovine origin) and the *Fusarium* protease described in WO 89/06270 and WO 94/25583.

[0030] Examples of useful proteases are the variants described in WO 92/19729, WO 98/20115, WO 98/20116, and WO 98/34946, especially the variants with substitutions in one or more of the following positions: 27, 36, 57, 76, 87, 97, 101, 104, 120, 123, 167, 170, 194, 206, 218, 222, 224, 235 and 274. Preferred commercially available protease enzymes include Alcalase™, Savinase™, Primase™, Duralase™, Dyrazym™, Esperase™, Everlase™, Polarzyme™, and Kannase™, (Novozymes A/S), Maxatase™, Maxacal™, Maxapem™, Properase™, Purafect™, Purafect OxP™, FN2™, and FN3™ (Genencor International Inc.).

[0031] Suitable lipases include those of bacterial or fungal 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).

[0032] 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 and WO 97/07202.

[0033] Preferred commercially available lipase enzymes include Lipolase™ and Lipolase Ultra™, Lipex™ (Novozymes A/S).

[0034] The method of the invention may be carried out in the presence of 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.

[0035] Cutinases are enzymes which 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*.

[0036] 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 which is herby incorporated by reference. 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.

[0037] Preferred commercial cutinases include NOVOZYM™ 51032 (available from Novozymes A/S, Denmark).

[0038] The method of the invention may be carried out in the presence of 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.

[0039] 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 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.

[0040] 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.

[0041] 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.

[0042] 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 derived from Fusarium oxysporum DSM 2672, or variants thereof.

[0043] 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.).

[0044] Examples of commercial phospholipases include LECITASE™ and LECITASE™ ULTRA, YIELSMAX, or LIPOPAN F (available from Novozymes A/S, Denmark).

[0045] Suitable amylases (alpha and/or beta) include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Amylases include, for example, alpha-amylases obtained from *Bacillus,* e.g. a special strain of *B. licheniformis,* described in more detail in GB 1,296,839, or the *Bacillus* sp. strains disclosed in WO 95/026397 or WO 00/060060.

[0046] Examples of useful amylases are the variants described in WO 94/02597, WO 94/18314, WO 96/23873, WO 97/43424, WO 01/066712, WO 02/010355, WO 02/031124 and PCT/DK2005/000469 (which references all incorporated by reference.

[0047] Commercially available amylases are Duramyl™, Termamyl™, Termamyl Ultra™, Natalase™, Stainzyme™, Fungamyl™ and BAN™ (Novozymes A/S), Rapidase™ and Purastar™ (from Genencor International Inc.).

[0048] Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus, Pseudomonas, Humicola, Fusarium, Thielavia, Acremonium*, e.g. the fungal cellulases produced from *Humicola insolens, Thielavia terrestris, Myceliophthora thermophila*, and *Fusarium oxysporum* disclosed in US 4,435,307, US 5,648,263, US 5,691,178, US 5,776,757, WO 89/09259, WO 96/029397, and WO 98/012307.

[0049] Especially suitable cellulases are the alkaline or neutral cellulases having color care benefits. Examples of such cellulases are cellulases described in EP 0 495 257, EP 0 531 372, WO 96/11262, WO 96/29397, WO 98/08940. Other examples are cellulase variants such as those described in WO 94/07998, EP 0 531 315, US 5,457,046, US 5,686,593, US 5,763,254, WO 95/24471, WO 98/12307 and PCT/DK98/00299.

[0050] Commercially available cellulases include Celluzyme™, Carezyme™, Endolase™, Renozyme™ (Novozymes A/S), Clazinase™ and Puradax HA™ (Genencor International Inc.), and KAC-500(B)™ (Kao Corporation).

[0051] Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Examples of useful peroxidases include peroxidases from *Coprinus*, e.g. from *C. Cinereus*, and variants thereof as those described in WO 93/24618, WO 95/10602, and WO 98/15257. Commercially available peroxidases include Guardzyme™ and Novozym™ 51004 (Novozymes A/S).

[0052] Examples of pectate lyases include pectate lyases that have been cloned from different bacterial genera such as *Erwinia, Pseudomonas, Klebsiella* and *Xanthomonas,* as well as from *Bacillus subtilis* (Nasser et al. (1993) FEBS Letts. 335:319-326) and *Bacillus* sp. YA-14 (Kim et al. (1994) Biosci. Biotech. Biochem. 58:947-949). Purification of pectate lyases with maximum activity in the pH range of 8-10 produced by *Bacillus pumilus* (Dave and Vaughn (1971) J. Bacteriol. 108:166-174), *B. polymyxa* (Nagel and Vaughn (1961) Arch. Biochem. Biophys. 93:344-352), *B. stearothermophilus* (Karbassi and Vaughn (1980) Can. J. Microbiol. 26:377-384), *Bacillus* sp. (Hasegawa and Nagel (1966) J. Food Sci. 31:838-845) and *Bacillus* sp. RK9 (Kelly and Fogarty (1978) Can. J. Microbiol. 24:1164-1172) have also been described. Any of the above, as well as divalent cation-independent and/or thermostable pectate lyases, may be used in practicing the invention. In preferred embodiments, the pectate lyase comprises the amino acid sequence of a

pectate lyase disclosed in Heffron et al., (1995) Mol. Plant-Microbe Interact. 8: 331-334 and Henrissat et al., (1995) Plant Physiol. 107: 963-976. Specifically contemplated pectatel lyases are disclosed in WO 99/27083 and WO 99/27084. Other specifically contemplates pectate lyases derived from *Bacillus licheniformis* is disclosed as in US patent no. 6,284,524 (which document is hereby incorporated by reference). Specifically contemplated pectate lyase variants are disclosed in WO 02/006442, especially the variants disclosed in the Examples in WO 02/006442 (which document is hereby incorporated by reference).

[0053] Examples of commercially available alkaline pectate lyases include BIOPREP™ and SCOURZYME™ L from Novozymes A/S, Denmark.

[0054] Examples of mannanases (EC 3.2.1.78) include mannanases of bacterial and fungal origin. In a specific embodiment the mannanase is derived from a strain of the filamentous fungus genus Aspergillus, preferably *Aspergillus niger* or *Aspergillus aculeatus* (WO 94/25576). WO 93/24622 discloses a mannanase isolated from *Trichoderma reseei.* Mannanases have also been isolated from several bacteria, including *Bacillus* organisms. For example, Talbot et al., Appl. Environ. Microbiol., Vol.56, No. 11, pp. 3505-3510 (1990) describes a beta-mannanase derived from *Bacillus stearothermophilus.* Mendoza et al., World J. Microbiol. Biotech., Vol. 10, No. 5, pp. 551-555 (1994) describes a beta-mannanase derived from *Bacillus subtilis.* JP-A-03047076 discloses a beta-mannanase derived from *Bacillus* sp. JP-A-63056289 describes the production of an alkaline, thermostable beta-mannanase. JP-A-63036775 relates to the *Bacillus* microorganism FERM P-8856 which produces beta-mannanase and beta-mannosidase. JP-A-08051975 discloses alkaline beta-mannanases from alkalophilic *Bacillus* sp. AM-001. A purified mannanase from *Bacillus amyloliquefaciens* is disclosed in WO 97/11164. WO 91/18974 describes a hemicellulase such as a glucanase, xylanase or mannanase active. Contemplated are the alkaline family 5 and 26 mannanases derived from *Bacillus agaradhaerens, Bacillus licheniformis, Bacillus halodurans, Bacillus clausii, Bacillus* sp., and *Humicola insolens* disclosed in WO 99/64619. Especially contemplated are the *Bacillus* sp. mannanases concerned in the Examples in WO 99/64619 which document is hereby incorporated by reference.

[0055] Examples of commercially available mannanases include Mannaway™ available from Novozymes A/S Denmark.

[0056] Any enzyme present in the composition may be stabilized using conventional stabilizing agents, e.g., a polyol such as propylene glycol or glycerol, a sugar or sugar alcohol, lactic acid, boric acid, or a boric acid derivative, e.g., an aromatic borate ester, or a phenyl boronic acid derivative such as 4-formylphenyl boronic acid, and the composition may be formulated as described in e.g. WO 92/19709 and WO 92/19708.

[0057] In order that the invention may be further understood and carried forth into practice it will be further described with reference to the following non-limiting examples.

Examples

Example 1: Preparation of Swatches

[0058] The following species were used to form "single species" swatches:

Lolium perenne - perennial ryegrass (a low phenolic grass) Festuca *pratensis* - meadow fescue (a high phenolic grass) *Trifolium pratense (red clover)*

[0059] The following species were used to form a six species "multi-species swatch" which comprised six different stains on the same cloth.

[0060] *Lolium perenne* (ryegrass) *Aberglyn* cultivar *Festuca rubra* (red fescue) *Cezanne* cultivar Agrostis (bent grass) *trifolium repens* (white clover) *Aber Dai* cultivar *trifolium repens* (white clover) *Aber Ace* cultivar *Taraxacum officinale* (dandelion)

[0061] All plants were grown under standard greenhouse conditions. The physiology and biochemical composition of cell walls/protoplasts/ etc differs as the plant progresses through its life cycle. For consistency purposes growth under controlled conditions is required. For stain generation, white cotton and polyester fabric was secured between the plates of 2-4cm diameter staining mould. A small bunch of plant leaf material was shaped into a 2-cm ball-shape and rubbed against the fabric in a circular motion for 15 seconds, until a homogeneous stain was obtained. In the case of the multi-species swatches this process was repeated with materials taken from a number of different plants, so as to obtain a single swatch carrying stains from a plurality of different plants.

[0062] Stains were aged in the dark and at room temperature for 3 days prior to washing.

Example 2: Use of the swatches

[0063] Stains were washed in a Tergotometer™ under the conditions sopecified below:

Temperature	= 37°C
Time	= 30min
Agitation	= 100rpm
Wash liquor	= 11
Water Hardness	= 6°FH
Product Dose (Persil Bio/ Non-)	= 6.6g/l
Liquid : Cloth	= 40:1
Rinse	= 2 x 2 mins in 21 demin water (FH6)
Drying	=o/n and at RT/ in the dark
Spectra measurements:	= HunterLab ™ (ΔE)

[0064] The detergent used was Persil™ Bio and non-Bio powder. The 1*a*b and ΔE values of grass stains were obtained before and after wash using a Hunterlab ™ calibrated against clean cotton and polyester fabrics.

[0065] The results obtained are presented in the tables below:

Table 1

Polyester					**Cotton**				
	mean		**sd**			**mean**		**sd**	
	Non bio	Bio	Non bio	Bio		Non bio	Bio	Non bio	Bio
lolium	32.7	37.3	1.6	1.1	lolium	28.4	33.0	4.6	1.2
festuca	24.2	28.7	1.7	0.9	festuca	27.2	30.6	2.2	0.8
Clover	11.2	30.4	1.5	1.6	Clover	11.9	27.2	1.2	0.7

Table 2

Cotton				
	mean		**sd**	
	non bio	Bio	non bio	bio
ryegrass	28.7	40.3	2.3	2.3
fescue	27.4	42.0	1.3	1.4
argostis	24.4	40.3	1.5	1.5
clover trifolium	29.8	36.8	2.1	2.1
clover trifolium II	31.5	38.7	1.4	2.1
Dandelion	17.7	33.3	2.7	1.3
Polyester				
	mean		**sd**	
	non bio	bio	Non bio	bio
ryegrass	33.1	41.6	2.3	2.3
fescue	29.7	43.3	1.3	1.4
argostis	30.8	36.9	1.5	1.5
clover trifolium	37.3	38.1	2.1	2.1
clover trifolium II	32.0	35.0	1.4	2.1

(continued)

Polyester				
	mean		sd	
	non bio	bio	Non bio	bio
Dandelion	26.5	32.7	2.7	1.3

[0066] Even from these few results it is easy to see the utility of the swatches is exploring how a detergent composition could be improved so as to clean better. The low SD shows good reproducibility. It is also evident that enzymes (present in the bio product) assist in the removal of plant-derived stains.

Claims

1. A swatch of fabric having an area of from 0.25 m^2 to $4x10^{-6}m^2$, said swatch being stained in one or more patches over at least 0.1% of its surface with debris comprising chloroplast material wherein at least 80%wt of the chloroplast material present in a patch is from a single species of green plant other than tea or spinach.

2. A swatch according to claim 1 wherein the fabric is cellulosic, polyester or a mixture thereof.

3. A swatch according to any preceeding claim wherein the remaining 20%wt of the debris is from no more than five, preferably three, preferably one other species.

4. A swatch according to any preceeding claim wherein at least one patch is derived from a single species.

5. A swatch according to any preceeding claim wherein the debris are from one or more species of the genera:

   Agropyron, Agrostis, Axonopus, Bothriochloa, Bouteloua, Fescue, Chamaemelum, Dactyloctenium, Chrysopogon, Deschampsia, Desmodium, Dichanthium, Cynodon, Lolium, Trifolium, Belliss, Taraxacum, Hilaria, Hydrocotyle, Pennisetum, Leptinella, Koeleria, Ischaemum, Eremochloa, Eragrostis, Elymus, Microlaena, Plantago, Paspalum, Eurhynchium, Dichondra, Digitaria, Brachythecium, Polytrias, Pratia, Puccinellia, Ranunculus, Senecio, Distichlis, Holcus, Stenotaphrum, Urochloa, Zoysia, Trisetum and Poa.

6. A swatch according to any preceeding claim wherein the debris are from one or more of the species: *Agrostis canina, Agrostis capillaris, Agrostis* clavata, *Agrostis curtisii, Agrostis gigantea, Agrostis castellana, Agrostis mertensii, Agrostis scabra, Agrostis stolonifera, Agrostis tenuis, Agrostis palustris, Agrostis vineale, Festuca rubra, Lolium perenne, Trifolium repens, Bellis perennis, Taraxacum officinale, Eurhynchium praelongum, Brachythecium rutabulum, Ranunculus repens, Holcus lanatus, Poa trivialis, Poa. pratensis and Poa. Annua.*

7. A method for preparing a swatch according to any of claims 1-7 which comprises the step of contacting a fabric substrate in sheet form having an area greater than $4x10^{-6}m^2$ with chloroplast-containing green plant debris wherein at least 80% of the debris is from a single species of green plant other than tea or spinach.

8. A method for determining the effectiveness of a laundry composition which includes that step of treating swatches according to any of claims 1-7 with the laundry composition.

9. A method according to claim 8 wherein the laundry composition comprises at least one enzyme.

10. A method according to claim 8 wherein the laundry composition comprises a plurality of enzymes.

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EUROPEAN SEARCH REPORT

Application Number
EP 08 16 1667

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
The Hague	7 January 2009	Richards, Michael

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Application Number
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Place of search	Date of completion of the search	Examiner
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