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| A CELLULASE WITH REDUCED MOBILITY |

| (57) Abstract |

A cellulytic enzyme preparation comprising a cellulase with reduced mobility, e.g. by increasing the molecular weight or apparent size of the cellulase protein molecule or by insolubilizing or immobilizing the cellulase such as by incorporation of the cellulase component into a gel, by the formation of stable or temporary aggregates with enhanced molecular mass, by rapid immobilization of cellulase protein on insoluble components, by rapid autoimmobilization of the cellulase protein, or by adsorption to an insoluble or soluble carrier, preferably a cellulose-containing carrier of fibrous, microcrystalline or amorphous structure, more preferably a soluble or insoluble polymer, especially a polysaccharide capable of interaction with the enzyme via a cellulose binding domain (CBD) or catalytic domain, or a soluble polycationic cellulose derivative, the cellulase preparation having a much lesser effect or influence on the durability or ageing behaviour of the cellulosic substrate than corresponding unmodified cellulases while at least having as good an effect on the look or feel, when used for treatment of cellulosic fabrics or textiles such as for domestic or industrial laundering or fabric softening as an ingredient of a detergent composition, for bio-polishing or for stone-washing of denim fabric or denim jeans or other dyed fabric or garments.
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A CELLULASE WITH REDUCED MOBILITY

The present invention relates to an enzyme preparation comprising a cellulase with reduced mobility, a detergent composition comprising the enzyme preparation, and a method for obtaining cellulose-containing fibres, textiles or fabrics with sustained durability by treatment with the enzyme preparation.

BACKGROUND OF THE INVENTION

Cellulases or cellulytic enzymes are enzymes involved in hydrolyses of cellulose. In the hydrolysis of native cellulose, it is known that three major types of cellulase enzymes are involved, namely cellbiohydrolase (1,4-β-D-glucan cellbiohydrolase, EC 3.2.1.91), endo-β-1,4-glucanase (endo-1,4-β-D-glucan 4-glucanohydrolase, EC 3.2.1.4) and β-glucosidase (EC 3.2.1.21), Wood et al. (1985).

Cellulases are synthesized by a large number of microorganisms which includes fungi, actinomycetes, myxobacteria and true bacteria but also by plants. Especially endoglucanases of a wide variety of specificities have been identified.

For example, microbial endoglucanases have been described by Beldman et al., 1985, Wei et al., 1992, Persson et al., 1991, and in WO 93/20193, WO 94/14953, and WO 95/02043. Furthermore, Sharma et al., 1991, Ooi et al., 1990, Gilkes et al., 1991, and Dalbøge and Heldt-Hansen, 1994, describe microbial endoglucanases.

A very important industrial use of cellulytic enzymes is the use for treatment of cellulosic textile or fabric, e.g. as ingredients in detergent compositions or fabric softener compositions, for bio-polishing of new fabric (garment finishing), and for obtaining a "stone-washed" look of cellu-

WO 95/02675 discloses a detergent composition comprising two types of endoglucanases which has improved properties with regard to soil removal and colour clarification and, after a limited number of washing cycles, neither damage nor partly degrade the cellulose-containing fabric, e.g. the cotton.

The practical exploitation of cellulolytic enzymes has, to some extent, been set back by the nature of the known cellulase preparations which, along with the benefits described above, may reduce fabric durability. For all domestic or industrial applications of cellulase relating to cellulose-containing fabric, it is highly desirable to use enzyme preparations or compositions capable of providing both sufficient softening, de-pilling, colour clarification and particulate soil removal while at the same time ensuring the durability of the cellulose-containing fabric, e.g. the cotton.

The objective problem is to develop a novel cellulase preparation which - relative to known cellulase preparations - gives just as good or better look and feel of the fibre or fabric (in terms of e.g. soil removal, colour clarification, de-pilling or softening) but affects or influences the durability or ageing behaviour (which may be measurable e.g. in terms of tensile strength loss or pin-holing) to a much lesser extent, when used for treatment of cellulosic fabric or fibres.

SUMMARY OF THE INVENTION

Surprisingly, it has been found that a cellulase preparation comprising a modified cellulase component having reduced mobility has a much lesser effect or influence on the
durability or ageing behaviour of the cellulosic substrate than corresponding unmodified cellulases while at least having as good an effect on the look or feel, when used for treatment of cellulosic fibres or fabric (e.g. washing, softening, bio-polishing or enzymatic stone-washing).

The mobility of the cellulase component may be reduced e.g. by increasing the molecular weight or apparent size of the cellulase protein molecule, or by insolubilizing or immobilizing the cellulase.

The cellulase preparation of the present invention is useful for any known treatment of cellulosic fabrics or textiles such as for domestic or industrial laundering or fabric softening, for bio-polishing or for stone-washing of denim fabric or denim jeans or other dyed fabric or garments.

Accordingly, in further aspects the invention relates to a detergent composition comprising the cellulase preparation, a surfactant and optionally other conventional detergent ingredients; and to a fabric softening composition comprising the cellulase preparation, a perfume or another compound of pleasant fragrance and optionally other conventional fabric softening ingredients.

In yet another aspect, the invention relates to a method of reducing the tendency to tensile strength loss or pin-holing of cellulosic fabric, the method comprising treating the fabric with the cellulase preparation of the present invention.

In yet another aspect, the invention relates to a method of modifying a cellulase component by means of immobilization, insolubilization or increasing the molecular weight or apparent size.

It is to be understood that the present invention provides a cellulase preparation, a detergent composition, a fabric
softener composition and methods which - relative to known preparations, compositions and methods - have increased overall performance on textile fibres or fabric in terms of desirable and undesirable effects of the cellulolytic activity of the enzyme preparation. This may be due to the preparation, composition or method of the invention giving rise to a higher increase in desirable effects than in undesirable effects; or to a higher decrease in undesirable effects than in desirable effects.

DETAILED DESCRIPTION OF THE INVENTION

Without being bound to this theory, it is contemplated that the highly desirable reduced effect or influence of the modified cellulase on the fibre/fabric durability or ageing behaviour is related to a reduced capability of the cellulase protein molecule to penetrate deep inside the cellulosic fibres; and, further, that the susceptibility of the fibres to undesired or non-beneficial cellulase action is at minimum at the initial stages of fibre or fabric swelling when submerged into an aqueous cellulase solution.

In the present specification and claims, the term "cellulase component" denotes an enzyme that hydrolyses cellulose. The cellulase component may be a component occurring in a cellulase system produced by a given microorganism, such a cellulase system mostly comprising several different cellulase enzyme components including those usually identified as e.g. cellobiohydrolases, exo-cellobiohydrolases, endoglucanases, \( \beta \)-glucosidases.

Alternatively, the cellulase component may be a single component, i.e. a component essentially free of other cellulase components usually occurring in a cellulase system produced by a given microorganism, the single component being a recombinant component, i.e. produced by cloning of a DNA sequence encoding the single component and subsequent cell transformed
encoding the single component and subsequent cell transformed with the DNA sequence and expressed in a host, cf. e.g. International Patent Applications WO 91/17243 and WO 91/17244 which are hereby incorporated by reference. The host is preferably a heterologous host, but the host may under certain conditions also be the homologous host.

As used herein, the term "weight of cellulase protein" denotes the weight of the protein constituting a cellulase component.

The term "colour clarification", as used herein, refers to preservation of the initial colours throughout multiple washing cycles by removing fuzz or pills from the surface of garment and/or fabric.

The term "particulate soil removal", as used herein, refers to enhanced cleaning of cellulose-containing fabrics or garment, e.g. cotton, contaminated by particles of soil or of other insoluble matter entrapped by micro-fibrilla spreading out on the fibre surface.

The term "domain", as used herein, is intended to indicate an amino acid sequence capable of effecting a specific task. For example the term "carbohydrate binding domain" or "cellulose binding domain" ("CBD") is intended to indicate an amino acid sequence capable of effecting binding of the enzyme to a carbohydrate substrate, in particular cellulose, and the term "catalytic active domain" ("CAD") is intended to indicate an amino sequence capable of effecting catalytic cleavage and having one or more active sites. A CBD is an example of a non-catalytic domain. CAD's and CBD's may be linked or attached by linking regions. Cf. Trends Biotech No 1., 5, p. 255-261 (1987), and Microbiol. Rev., 55, p. 303-315 (1991).

The term "core enzyme", as used herein, is intended to indicate an enzyme consisting essentially of a single domain, i.e. a catalytic active domain, the core enzyme having no
"tail".

In the present context, the term "immunoreactive" is intended to indicate that the produced protein is reactive with an antibody raised against a native cellulose- or hemicellulose-degrading enzyme.

In the present context, the term "homologue" is intended to indicate a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for the cellulase component with the amino acid sequence in question under certain specified conditions (such as presoaking in 5xSSC and prehybridising for 1 h at ~40°C in a solution of 20% formamide, 5xDenhard't's solution, 50 mM sodium phosphate, pH 6.8, and 50 μg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 100 μM ATP for 18 h at ~40°C). The term is intended to include derivatives of the sequence in question obtained by addition of one or more amino acid residues to either or both the C- and N-terminal of the native sequence substitution of one or more amino acid residues at one or more sites in the native sequence, deletion of one or more amino acid residues at either or both ends of the native amino acid sequence or at one or more sites within the native sequence, or insertion of one or more amino acid residues at one or more sites in the native sequence. It is to be understood that any derivative also hybridizes to the same probe as mentioned above which indicates that the cellulase enzyme derivatives within the scope of the present invention all have the same advantageous activity and effect as the cellulase component having the amino acid sequence in question. Also, any additions or substitutions or deletions or insertions may preferably relate to a relatively limited number of amino acids of the sequence in question, i.e. minor additions, substitutions, deletions or insertions, since it is to be expected that major additions, substitutions, deletions or insertions may result in cellulase components (polypeptides), which do not
fulfil the above-mentioned hybridizing requirement.

The term "mobility", as used herein, refers to the ability of the protein molecule, either solely or in an incorporated form, to change its spacial position in time, reflecting the diffusion (travelling) of the enzyme within the solution, or along the depth profile of cellulose-containing fabric, threads and fibres. Changes in protein molecule mobility may be monitored using several physico-chemical techniques, implying measurement of apparent molecular mass of the cellulase enzyme (e.g. by ultrafiltration or gel-filtration), or the diffusion properties of the protein (electrophoresis, free diffusion).

The term "immobilization", as used herein, refers to the physical or/and chemical modification of the protein molecules resulting in physical trapping of the enzyme in a certain space and in a way enabling the enzyme to retain its activity within these spacial limits during sufficiently extended storage and use.

The term "bio-polishing", as used in the present context, refers to a specific treatment of the fibre or yarn surface, which improves fabric quality with respect to handle and appearance without loss of fabric wettability. The most important effects of bio-polishing can be characterized by less fuzz and pilling, increased gloss/luster, improved fabric handle, increased durable softness and altered water absorbency. Bio-polishing usually takes place in the wet processing of the manufacture of knitted and woven fabrics. Wet processing comprises such steps as e.g. desizing, scouring, bleaching, washing, dyeing/printing and finishing. During each of these steps, the fabric is more or less subjected to mechanical action. In general, after the textiles have been knitted or woven, the fabric proceeds to a desizing stage, followed by a scouring stage, etc. Desizing is the act of removing size from textiles. Prior to weaving on mechanical looms, warp yarns are often coated with size
starch or starch derivatives in order to increase their tensile strength. After weaving, the size coating must be removed before further processing the fabric in order to ensure a homogeneous and wash-proof result. It is known that in order to achieve the effects of bio-polishing, a combination of cellulolytic and mechanical action is required. It is also known that "super-softness" is achievable when the treatment with cellulase is combined with a conventional treatment with softening agents. It is contemplated that use of the modified cellulase and/or enzyme preparation of the invention for bio-polishing of cellulosic fabrics is advantageous. Bio-polishing may be obtained by applying the method described e.g. in WO 93/20278.

As used herein, the term "stone-washing" look refers to fabric which has been mechanically and/or enzymatically treated with the purpose of obtaining a distressed "used and abused" look, which in recent years has become very desirable, particularly in denim clothing. Traditionally, it involves tumbling the fabric with pumice stones while wet for a sufficient period of time so as to let the pumice abrade the fabric thus producing, e.g. in the fabric panels and in the seams in case of clothing items, localized abraded areas of lighter colour. In recent years, "stone-washing" has been carried out by treating the fabric enzymatically, either in combination with pumice or without pumice or in combination with perlite, with a cellulase preparation, e.g. as described in EP-A-0 307 564, EP-A-0 435 876, and International Patent Application PCT/DK94/00360 which all describe the use of cellulolytic enzymes in a "stone-washing" process.

The terms "cellulose-containing fabric" and "cellulosic fabric" are intended to indicate any type of fabric, in particular woven fabric, prepared from a cellulose-containing material, i.e. material containing cellulose or cellulose derivatives such as wood pulp and cotton. In the present context, the term "fabric" is also intended to include garments and other types of processed fabrics.
Examples of cellulosic fabric are cotton, viscose (rayon); ramie; jute; flax (linen); lyocell; all blends of viscose, cotton, ramie, jute or lyocell with other fibres, e.g. animal hair fibres such as wool, alpaca and camel hair, and polymers such as polyester, polyacryl, polyamide and polyacetate.

Specific examples of blended cellulosic fabric are viscose/cotton blends, lyocell/cotton blends, viscose/wool blends, lyocell/wool blends, cotton/wool blends; cotton/polyester blends; viscose/cotton/polyester blends, wool/cotton/polyester blends, flax/cotton blends etc.

The term "desirable effects" or "beneficial action" of cellulase, as used herein, depending on the application refers to:

a. the soil removal from (i.e. enhanced cleaning of) cellulose-containing fabrics or garment, e.g. cotton, contaminated by particles of soil or of other insoluble matter entrapped by micro-fibrils spreading out on the fibre surface;

b. colour clarification of the fabric, i.e. preservation of the initial colours throughout multiple washing cycles by removing fuzz or pills from the surface of garment and/or fabric (laundry wash using cellulase-containing detergent compositions);

c. increased water absorption, softness and subsequent pilling resistance (industrial textile treatment using cellulase bio-polishing compositions);

d. providing a localized variation in colour giving the treated fabrics a "stone-washed" appearance (denim finishing using cellulase denim finishing compositions).

The terms "durability" and "ageing", as used herein, refers to for example the decrease of tensile or tear strength or of breaking energy which may be measurable using the techniques available in textile science, especially those described in textile measurement standards; and to pin-holing.
It should be noted, however, that pin-holing and decrease of tensile or tear strength of fabric is also an unavoidable result of mechanical action due to use/wearing and may further result from damage by a bleaching component, especially if the fabric is contaminated by metallic particles.

The cellulase component present in the enzyme preparation of the present invention may be obtained from a given organism by use of any suitable technique. For instance, a cellulase preparation may be obtained by fermentation of a microorganism and subsequent isolation of a cellulase containing preparation from the fermented broth or microorganism by methods known in the art, but more preferably by use of recombinant DNA techniques as known in the art. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector capable of expressing and carrying a DNA sequence encoding the cellulase component in question, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The component comprised by the cellulase composition of the invention may also be produced by conventional techniques such as produced by a given microorganism as a part of a cellulase system.

The cellulase component to be used according to the present invention may be any cellulase component having cellulytic activity either in the acid, the neutral or the alkaline pH-range. Preferably, the component is a microbial endoglucanase or cellobiohydrolase, preferably of fungal or bacterial origin, which may be derived or isolated and purified from microorganisms which are known to be capable of producing cellulytic enzymes, e.g. species of the genera *Humicola*, *Bacillus*, *Trichoderma*, *Fusarium*, *Myceliophthora*, *Phanerochaete*, *Schizophyllum*, *Penicillium*, *Aspergillus*, and *Geotrichum*.

The derived components may be either homologous or heterologous components. Preferably, the components are homologous. However, a heterologous component, which is derived from a
specific microorganism and is immunoreactive with an antibody raised against a highly purified cellulase component possessing the desired property or properties, is also preferred.

Examples of cellulase components which may be modified according to the present invention are:
A cellbiohydrolase component which is immunoreactive with an antibody raised against a highly purified \(^{70}\)kD cellbiohydrolase (EC 3.2.1.91) derived from *Humicola insolens*, DSM 1800, or which is a homologue or derivative of the \(^{70}\)kD cellbiohydrolase exhibiting cellulase activity. A preferred cellbiohydrolase component has the amino acid sequence disclosed in *Nucleic Acid Research*, vol. 18 (1990), page 668 (De Oliveira, Alzevedo, M. and Radford, A.) or is a variant of said cellbiohydrolase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous therewith; or

an endoglucanase component which is immunoreactive with an antibody raised against a highly purified \(^{50}\)kD endoglucanase derived from *Humicola insolens*, DSM 1800, or which is a homologue or derivative of the \(^{50}\)kD endoglucanase exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO91/17244, Fig. 14A-E or a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous therewith; or

an endoglucanase component which is immunoreactive with an antibody raised against a highly purified \(^{50}\)kD (apparent molecular weight, the amino acid composition corresponds to \(^{45}\)kD with \(2n\) glycosylation sites) endoglucanase derived from *Fusarium oxysporum*, DSM 2672, or which is a homologue or derivative of the \(^{50}\)kD endoglucanase exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No.
WO91/17244, Fig. 13 or is a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90% homologous therewith; or

any of the cellulases disclosed in the published European Patent Application No. EP-A2-271 004, the cellulases having a non-degrading index (NDI) of not less than 500 and being alkalophilic cellulases having an optimum pH not less than 7 or whose relative activity at a pH of not less than 8 is 50% or over of the activity under optimum conditions when carboxy methyl cellulose (CMC) is used as a substrate; the cellulase preferably being selected from the group consisting of alkaline cellulase K (produced by Bacillus sp. KSM-635, FERM BP 1485); alkaline cellulase K-534 (produced by Bacillus sp. KSM-534, FERM BP 1508); alkaline cellulase K-539 (produced by Bacillus sp. KSM-539, FERM BP 1509); alkaline cellulase K-577 (produced by Bacillus sp. KSM-577, FERM BP 1510); alkaline cellulase K-521 (produced by Bacillus sp. KSM-521, FERM BP 1507); alkaline cellulase K-580 (produced by Bacillus sp. KSM-580, FERM BP 1511); alkaline cellulase K-588 (produced by Bacillus sp. KSM-588, FERM BP 1513); alkaline cellulase K-597 (produced by Bacillus sp. KSM-597, FERM BP 1514); alkaline cellulase K-522 (produced by Bacillus sp. KSM-522, FERM BP 1512); CMCase I, CMCase II (both produced by Bacillus sp. KSM-635, FERM BP 1485); alkaline cellulase E-II and alkaline cellulase E-III (both produced by Bacillus sp. KSM-522, FERM BP 1512); or

an endoglucanase component which is immunoreactive with an antibody raised against a highly purified ~43kD endoglucanase derived from Humincola insolens, DSM 1800, or which is a homologue or derivative of the ~43kD endoglucanase exhibiting cellulase activity; a preferred endoglucanase component has the amino acid sequence disclosed in PCT Patent Application No. WO 91/17243, SEQ ID#2 or is a variant of said endoglucanase having an amino acid sequence being at least 60%, preferably at least 70%, more preferably 75%, more preferably at least 80%, more preferably 85%, especially at least 90%
homologous therewith; or
an endoglucanase component which is immunoreactive with an
antibody raised against a highly purified ~60kD endoglucanase
derived from Bacillus latus, NCIMB 40250, or which is a
homologue or derivative of the ~60kD endoglucanase exhibiting
cellulase activity; a preferred endoglucanase component has
the amino acid sequence disclosed in PCT Patent Application
No. WO 91/10732, SEQ ID#7 or is a variant of said endoglucan-
ase having an amino acid sequence being at least 60%, prefer-
ably at least 70%, more preferably 75%, more preferably at
least 80%, more preferably 85%, especially at least 90%
homologous therewith.

In its first aspect, the present invention relates to an
enzyme preparation comprising a cellulase component having
reduced mobility in an aqueous solution which has cellulosic
fibres or fabric submerged therein.

In a preferred embodiment thereof, the mobility of the cellu-
lose component is reduced by adsorption to an insoluble or
soluble carrier. The cellulase may be bound to the carrier
e.g. by covalent binding or by its cellulose binding domain
(CBD), if present.

The adsorption of the cellulase component via its CBD, either
a CBD existing as an integral part of the parent enzyme or a
newly introduced CBD, may advantageously take place on any
soluble or insoluble carrier which CBD has an affinity to.
Preferably such a carrier may be cellulose-containing mate-
rials of fibrous, microcrystalline or amorphous structure,
more preferably a soluble or insoluble polymer, especially a
polysaccharide capable of interaction with the enzyme via CBD
or catalytic domain, or a soluble polycationic cellulose
derivative. Examples of useful carriers are cellulose-con-
taining particles having a mean particle size from 0.01 μm to
100 μm, preferably Avicel™, Vivicel™, Sigmacel™ and chitosan
(with polymeric, oligomeric and monomeric counter-ions of
various hydrophobicity, including amphoteric).

The adsorption may also take place by interaction of the entire cellulase protein (or glyco- or lipoprotein) molecule or by one or more of its definite regions (CAD, CBD, linker) with a carrier providing affinity of binding. The carrier might be one providing a cellulase ion-exchange, hydrophobic, hydrogen-bond, lectin, antibody, metal-chelate, ion-pairing, or any other interactions known in the art of biotechnology. Examples of useful carriers are bentonite, hectorites, Laponite®, silica, zeolite, diatomaceous earth, activated charcoal (e.g. DHP-1), synthetic resins (polystyrene and its derivatives, acryl esters, polyhydroxyalkyl methacrylate, teflon, polypropylene), lignin and derivatives thereof, polysaccharides and derivatives thereof, e.g. methyl cellulose and ethyl cellulose. A preferred carrier is in the form of nano-particles, for example made of isobutyl-2-cyanoacrylate.

It is advisable to check, in a small-scale experiment, whether a particular carrier is suitable for a certain application or use by mixing of the cellulase component and the carrier under favourable conditions and attempting to reverse the binding under harsh conditions imitating real application or use (for example 50°C, pH 7 for bio-polishing, or 40°, pH 10 with ionic/non-ionic surfactant for detergent compositions).

Further, the structure of the cellulase protein molecule may preferably be changed by chemical modification or protein engineering to ensure higher affinity for the most economical carrier selected. The structure of the carrier may be also modified to ensure better adsorption of the cellulase component. As an example porous ceramic material may be pre-treated with polyethyleneimine or a silicone agent.

Covalent binding of the cellulase protein molecule to an
insoluble carrier may be carried out by using techniques well
known in the art. Special efforts may be taken, i.e. by using
the CBD, if present, to orientate the cellulase molecule
prior to the binding to ensure later productive conformation
of immobilized enzyme.

In another preferred embodiment of the invention, the mobili-
ty of the cellulose component is reduced by incorporation of
the cellulase component (the cellulase protein molecule) into
a gel. Such a physical entrapment may be carried out using
techniques well known in the art by generating a gel from
soluble polymers (such as a polysaccharide), for example
agar, xanthan gum, chitosan, locust bean gum and any combina-
tion thereof; oligomers or monomers. Other useful gels are
pectin, Ca-alginate and the mixture of locust bean gum and
carrageenan.

In yet another preferred embodiment of the invention, the
mobility of the cellulose component is reduced by the for-
mation of stable or temporary aggregates with enhanced mole-
cular mass comprising two or more molecules of the cellulase
component (e.g. a dimer or trimer) alone, or one or more
molecules of the cellulase component in combination with
additional molecule(s) of other bio- or man-made polymers,
oligomers or monomers.

The aggregation of several cellulase molecules may be ensured
by modification of the cellulase enzyme either by physical or
chemical methods, or by protein engineering. More specifi-
cally, some or all of the charged amino acid residues on the
cellulase protein surface may be substituted by hydrophobic
amino acid residues. Additionally, the enhanced aggregation
of the enzyme during its thermal denaturation at 80 - 85°C
can be exploited (heat treatment).

The additional polymers for aggregate formation may be selec-
ted among polyelectrolytes, hydrophilic non-charged polymers
and amphoteric polymers, oligomers and monomers. Examples of useful polyelectrolytes and charged oligomers are: polypeptides (e.g. polylysine acid and polyaspartic acid), proteins (including antibodies, more specifically β-lactoglobulin of milk whey; or albumin, soy bean protein, pea protein, potato protein, glut protein), polyethyleneglycol (PEG), polyvinylimidazol (PVI), polyvinylpyrrolidone (PVP), quaternary poly(2-vinylpyridine), polydimethylammonium chloride, polyacrylic acid anions, polystyrene-sulfonate, polyvinylsulfate, various ionized polyacrylamides, methacrylic acid-methylmethacrylate copolymer, methacrylic acid-methacrylate-methylmethacrylate copolymer, cationic hydroxyethyl cellulose, carboxymethyl hydroxyethyl cellulose, cellulose (ether) esters such as those disclosed in JP-A-05227957, e.g. soluble pH-dependant hydroxypropyl methyl cellulose acetate succinate, cellulose acetate phthalate, hydroxypropyl methyl cellulose phthalate, chemically modified soluble chitosan derivatives, in particular chitosan glutamate and N-methylglycolchitosan, sodium alginate, kappa-, lambda- and iota-carrageenan, hyaluronate, heparan sulphate, chondroitin tetra- and octa-sulphate, polyethylenimine, poly(vinylpyrrolidone/dimethylaminoethyl methacrylate), e.g. copolymer 845 (a commercial product from GAF Chemicals), secondary polyamines like Amberlite LA-2 (a commercial product from Röhm and Haas, Inc.); and block polymers and graft polymers thereof, preferably PEG in combination with any of PVP, PVI and chitosan; charged oligosaccharide derivatives, in particular kanamycin. Examples of useful hydrophilic polymers are locust bean gum, guar gum, xanthan gum, quince seed gum, gum arabic, karaya gum, tragacanth gum, glucomannan, arabinogalactan, dextran, pullulan, curdlan, alpha- and beta-cyclodextrin, agarose, gelatine, inulin, pectin, starch, dextrin, polyvinyl alcohol, hydroxyethyl cellulose. Especially effective in aggregate formation are amphoteric oligomers and polymers, the examples of which are: alkylsulfates, alkylsulfonates, LAS, alkyltrimethylammonium bromides, steroidal glycosides, glyco- and phospholipids (e.g. Sophorolipid from Torulopsis sp.), soluble lignin and deriva-
tives thereof, ethyl hydroxyethyl cellulose, methyl hydroxybutyl cellulose, methyl hydroxypropyl cellulose, methyl hydroxyethyl cellulose; and block polymers and graft polymers thereof.

The capability of a cellulase component to form aggregates with heterologous substances may be increased by modifying the cellulase protein molecule by chemical techniques or protein engineering or a combination thereof. More specifically, polypeptide sequences may be introduced into the protein molecule ensuring subsequent binding of biotin (i.e. avidin sequence), polysaccharides, including those secreted by the host used to produce mono-component cellulase, (i.e. corresponding antibody sequence), cationic and amphoteric polymers and oligomers (i.e. amino acid sequences with substitutions resulting in increased local negative charge of the cellulase molecule, more specifically polyaspartate fusions adding 1 - 20 negative charges to the enzyme facilitating the cellulase interaction with polycations, e.g. polyethyleneimine).

Reduced mobility of the cellulase component may also be obtained by combining the formation of aggregates incorporating cellulase protein molecules and heterologous polymers with adsorption of the resulting aggregates on a relevant carrier or support, in particular mineral adsorbants with highly developed surface area, more specifically silica gels or derivatives thereof.

In yet another preferred embodiment, the mobility of the cellulase component may be reduced by rapid auto-immobilization of the cellulase protein on the surface of the cellulose fibres, thereby preventing the cellulase protein molecule from travelling deep into the fibre structure. It is contemplated that this autoimmobilizing property can be obtained by protein engineering of the CBD, if present, or by adding a CBD to the (parent) cellulase component, either N-terminally or C-terminally.
Further, it is contemplated that the mobility of the cellulase component may be reduced by rapid immobilization of cellulase protein on insoluble components present in ready-to-use compositions comprising cellulase. In particular, affinity of cellulase molecule may be ensured by protein engineering or chemical techniques towards e.g. perlite which may be a component of a cellulase composition for enzymatic stone-washing of especially denim, or a zeolite, bentonite or Laponite™ component used in detergent compositions.

In its second aspect, the invention provides a detergent composition comprising the (modified) enzyme preparation of the present invention and a surfactant and optionally other ingredients.

**Surfactant system**

The detergent compositions according to the present invention comprise a surfactant system, wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or ampholytic and/or zwitterionic and/or semi-polar surfactants.

The surfactant is typically present at a level from 0.1% to 60% by weight.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated in such a way that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Preferred systems to be used according to the present invention comprise as a surfactant one or more of the nonionic and/or anionic surfactants described herein.

Polyethylene, polypropylene, and polybutylene oxide conden-
sates of alkyl phenols are suitable for use as the nonionic surfactant of the surfactant systems of the present invention, with the polyethylene oxide condensates being preferred. These compounds include the condensation products of alkyl phenols having an alkyl group containing from about 6 to about 14 carbon atoms, preferably from about 8 to about 14 carbon atoms, in either a straight chain or branched-chain configuration with the alkylene oxide. In a preferred embodiment, the ethylene oxide is present in an amount equal to from about 2 to about 25 moles, more preferably from about 3 to about 15 moles, of ethylene oxide per mole of alkyl phenol. Commercially available nonionic surfactants of this type include Igepal™ CO-630, marketed by the GAF Corporation; and Triton™ X-45, X-114, X-100 and X-102, all marketed by the Rohm & Haas Company. These surfactants are commonly referred to as alkylphenol alkoxylation (e.g., alkyl phenol ethoxylates).

The condensation products of primary and secondary aliphatic alcohols with about 1 to about 25 moles of ethylene oxide are suitable for use as the nonionic surfactant of the nonionic surfactant systems of the present invention. The alkyl chain of the aliphatic alcohol can either be straight or branched, primary or secondary, and generally contains from about 8 to about 22 carbon atoms. Preferred are the condensation products of alcohols having an alkyl group containing from about 8 to about 20 carbon atoms, more preferably from about 10 to about 18 carbon atoms, with from about 2 to about 10 moles of ethylene oxide per mole of alcohol. About 2 to about 7 moles of ethylene oxide and most preferably from 2 to 5 moles of ethylene oxide per mole of alcohol are present in said condensation products. Examples of commercially available nonionic surfactants of this type include Tergitol™ 15-S-9 (The condensation product of \( \text{C}_{11}-\text{C}_{15} \) linear alcohol with 9 moles ethylene oxide), Tergitol™ 24-L-6 NMW (the condensation product of \( \text{C}_{12}-\text{C}_{14} \) primary alcohol with 6 moles ethylene oxide with a narrow molecular weight distribution), both marketed by Union Carbide Corporation; Neodol™ 45-9 (the
condensation product of C_{14}-C_{15} linear alcohol with 9 moles of ethylene oxide), Neodol™ 23-3 (the condensation product of C_{12}-C_{13} linear alcohol with 3.0 moles of ethylene oxide), Neodol™ 45-7 (the condensation product of C_{14}-C_{15} linear alcohol with 7 moles of ethylene oxide), Neodol™ 45-5 (the condensation product of C_{14}-C_{15} linear alcohol with 5 moles of ethylene oxide) marketed by Shell Chemical Company, Kyro™ EOB (the condensation product of C_{13}-C_{15} alcohol with 9 moles ethylene oxide), marketed by The Procter & Gamble Company, and Genapol LA 050 (the condensation product of C_{12}-C_{14} alcohol with 5 moles of ethylene oxide) marketed by Hoechst. Preferred range of HLB in these products is from 8-11 and most preferred from 8-10.

Also useful as the nonionic surfactant of the surfactant systems of the present invention are alkylpolysaccharides disclosed in US 4,565,647, having a hydrophobic group containing from about 6 to about 30 carbon atoms, preferably from about 10 to about 16 carbon atoms and a polysaccharide, e.g. a polyglycoside, hydrophillic group containing from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7 saccharide units. Any reducing saccharide containing 5 or 6 carbon atoms can be used, e.g., glucose, galactose and galactosyl moieties can be substituted for the glucosyl moieties (optionally the hydrophobic group is attached at the 2-, 3-, 4-, etc. positions thus giving a glucose or galactose as opposed to a glucoside or galactoside). The intersaccharide bonds can be, e.g., between the one position of the additional saccharide units and the 2-, 3-, 4-, and/or 6- positions on the preceding saccharide units.

The preferred alkylpolyglycosides have the formula

\[ R^2O(C_6H_{2n}O)_x(glycosyl)_x \]

wherein \( R^2 \) is selected from the group consisting of alkyl, alkylphenyl, hydroxyalkyl, hydroxyalkylphenyl, and mixtures
thereof in which the alkyl groups contain from about 10 to about 18, preferably from about 12 to about 14, carbon atoms; n is 2 or 3, preferably 2; t is from 0 to about 10, preferably 0; and x is from about 1.3 to about 10, preferably from about 1.3 to about 3, most preferably from about 1.3 to about 2.7. The glycosyl is preferably derived from glucose. To prepare these compounds, the alcohol or alkylpolyethoxy alcohol is formed first and then reacted with glucose, or a source of glucose, to form the glucoside (attachment at the 1-position). The additional glycosyl units can then be attached between their 1-position and the preceding glycosyl units 2-, 3-, 4-, and/or 6-position, preferably predominately the 2-position.

The condensation products of ethylene oxide with a hydrophobic base formed by the condensation of propylene oxide with propylene glycol are also suitable for use as the additional nonionic surfactant systems of the present invention. The hydrophobic portion of these compounds will preferably have a molecular weight from about 1500 to about 1800 and will exhibit water insolubility. The addition of polyoxyethylene moieties to this hydrophobic portion tends to increase the water solubility of the molecule as a whole, and the liquid character of the product is retained up to the point where the polyoxyethylene content is about 50% of the total weight of the condensation product, which corresponds to condensation with up to about 40 moles of ethylene oxide. Examples of compounds of this type include certain of the commercially available Pluronic™ surfactants, marketed by BASF.

Also suitable for use as the nonionic surfactant of the nonionic surfactant system of the present invention, are the condensation products of ethylene oxide with the product resulting from the reaction of propylene oxide and ethylenediamine. The hydrophobic moiety of these products consists of the reaction product of ethylenediamine and excess propylene oxide, and generally has a molecular weight
of from about 2500 to about 3000. This hydrophobic moiety is condensed with ethylene oxide to the extent that the condensation product contains from about 40% to about 80% by weight of polyoxyethylene and has a molecular weight of from about 5,000 to about 11,000. Examples of this type of nonionic surfactant include certain of the commercially available Tetronic™ compounds, marketed by BASF.

Preferred for use as the nonionic surfactant of the surfactant systems of the present invention are polyethylene oxide condensates of alkyl phenols, condensation products of primary and secondary aliphatic alcohols with from about 1 to about 25 moles of ethyleneglycol, alkylpolysaccharides, and mixtures hereof. Most preferred are C₆-C₁₄ alkyl phenol ethoxylates having from 3 to 15 ethoxy groups and C₅-C₁₀ alcohol ethoxylates (preferably C₁₀ avg.) having from 2 to 10 ethoxy groups, and mixtures thereof.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula

\[ R^2 - C - N - Z, \]
\[ \| \quad \| \]
\[ 0 \quad R^1 \]

wherein R¹ is H, or R¹ is C₇₋₁₄ hydrocarbyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof, R² is C₅₋₃₁ hydrocarbyl, and Z is a polyhydroxyhydrocarbyl having a linear hydrocarbyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably, R¹ is methyl, R² is straight C₁₁₋₁₅ alkyl or C₁₆₋₁₈ alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants hereof are water soluble salts or acids of the formula RO(A)mSO3M wherein R is an unsubstituted C₁₀-C₂₄ alkyl or hydroxyalkyl group having a C₁₀-C₂₄ alkyl component, preferably a C₁₂-C₂₀ alkyl or hydro-
xyalkyl, more preferably C_{12}-C_{18} alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein. Specific examples of substituted ammonium cations include methyl-, dimethyl, trimethyl-ammonium cations and quaternary ammonium cations such as tetramethyl-ammonium and dimethyl piperdinum cations and those derived from alkylamines such as ethylamine, diethylamine, triethylamine, mixtures thereof, and the like. Exemplary surfactants are C_{12}-C_{18} alkyl polyethoxylate (1.0) sulfate (C_{12}-C_{18}E(1.0)M), C_{12}-C_{18} alkyl polyethoxylate (2.25) sulfate (C_{12}-C_{18}(2.25)M), and C_{12}-C_{18} alkyl polyethoxylate (3.0) sulfate (C_{12}-C_{18}E(3.0)M), and C_{12}-C_{18} alkyl polyethoxylate (4.0) sulfate (C_{12}-C_{18}E(4.0)M), wherein M is conveniently selected from sodium and potassium.

Suitable anionic surfactants to be used are alkyl ester sulfonate surfactants including linear esters of C_{8}-C_{20} carboxylic acids (i.e., fatty acids) which are sulfonated with gaseous SO_{3} according to "The Journal of the American Oil Chemists Society", 52 (1975), pp. 323-329. Suitable starting materials would include natural fatty substances as derived from tallow, palm oil, etc.

The preferred alkyl ester sulfonate surfactant, especially for laundry applications, comprise alkyl ester sulfonate surfactants of the structural formula:
wherein $R^3$ is a C$_6$-C$_{20}$ hydrocarbyl, preferably an alkyl, or combination thereof, $R^4$ is a C$_1$-C$_6$ hydrocarbyl, preferably an alkyl, or combination thereof, and M is a cation which forms a water soluble salt with the alkyl ester sulfonate. Suitable salt-forming cations include metals such as sodium, potassium, and lithium, and substituted or unsubstituted ammonium cations, such as monoethanolamine, diethanolamine, and triethanolamine. Preferably, $R^3$ is C$_{10}$-C$_{16}$ alkyl, and $R^4$ is methyl, ethyl or isopropyl. Especially preferred are the methyl ester sulfonates wherein $R^3$ is C$_{10}$-C$_{16}$ alkyl.

Other suitable anionic surfactants include the alkyl sulfate surfactants which are water soluble salts or acids of the formula ROSO$_3$M wherein R preferably is a C$_{10}$-C$_{24}$ hydrocarbyl, preferably an alkyl or hydroxyalkyl having a C$_{10}$-C$_{20}$ alkyl component, more preferably a C$_{12}$-C$_{18}$ alkyl or hydroxyalkyl, and M is H or a cation, e.g., an alkali metal cation (e.g. sodium, potassium, lithium), or ammonium or substituted ammonium (e.g. methyl-, dimethyl-, and trimethyl ammonium cations and quaternary ammonium cations such as tetramethylammonium and dimethyl piperidinium cations and quaternary ammonium cations derived from alkylamines such as ethylamine, diethylamine, triethylamine, and mixtures thereof, and the like). Typically, alkyl chains of C$_{12}$-C$_{16}$ are preferred for lower wash temperatures (e.g. below about 50°C) and C$_{16}$-C$_{18}$ alkyl chains are preferred for higher wash temperatures (e.g. above about 50°C).

Other anionic surfactants useful for detersive purposes can also be included in the laundry detergent compositions of the present invention. Theses can include salts (including, for
example, sodium, potassium, ammonium, and substituted ammonium salts such as mono-, di- and triethanolamine salts) of soap, C₈-C₂₂ primary or secondary alkanesulfonates, C₆-C₂₄ olefinsulfonates, sulfonated polycarboxylic acids prepared by sulfonation of the pyrolyzed product of alkaline earth metal citrates, e.g., as described in British patent specification No. 1,082,179, C₆-C₂₄ alkylpolyglycolethersulfates (containing up to 10 moles of ethylene oxide); alkyl glycerol sulfonates, fatty acyl glycerol sulfonates, fatty oleyl glycerol sulfates, alkyl phenol ethylene oxide ether sulfates, paraffin sulfonates, alkyl phosphates, isethionates such as the acyl isethionates, N-acyl taurates, alkyl succinamates and sulfosuccinates, monoesters of sulfo succinates (especially saturated and unsaturated C₁₂-C₁₈ monoesters) and diesters of sulfo succinates (especially saturated and unsaturated C₆-C₁₂ diesters), acyl sarcosinates, sulfates of alkyl polysaccharides such as the sulfates of alkylpolyglucoside (the nonionic nonsulfated compounds being described below), branched primary alkyl sulfates, and alkyl polyethoxy carboxylates such as those of the formula RO(CH₂CH₂O)ₖ-CH₂C00-M⁺ wherein R is a C₆-C₂₂ alkyl, k is an integer from 1 to 10, and M is a soluble salt forming cation. Resin acids and hydrogenated resin acids are also suitable, such as rosin, hydrogenated rosin, and resin acids and hydrogenated resin acids present in or derived from tall oil. Alkylbenzene sulfonates are highly preferred. Especially preferred are linear (straight-chain) alkyl benzene sulfonates (LAS) wherein the alkyl group preferably contains from 10 to 18 carbon atoms.

Further examples are described in "Surface Active Agents and Detergents" (Vol. I and II by Schwartz, Perrey and Berch). A variety of such surfactants are also generally disclosed in US 3,929,678, (Column 23, line 58 through Column 29, line 23, herein incorporated by reference).

When included therein, the laundry detergent compositions of the present invention typically comprise from about 1% to
about 40%, preferably from about 3% to about 20% by weight of such anionic surfactants.

The laundry detergent compositions of the present invention may also contain cationic, ampholytic, zwitterionic, and semi-polar surfactants, as well as the nonionic and/or anionic surfactants other than those already described herein.

Cationic detersive surfactants suitable for use in the laundry detergent compositions of the present invention are those having one long-chain hydrocarbyl group. Examples of such cationic surfactants include the ammonium surfactants such as alkyltrimethylammonium halogenides, and those surfactants having the formula:

\[ [R^2(OR^3)_y][R^4(OR^3)_y]_2R^5N^+X^- \]

wherein \( R^2 \) is an alkyl or alkyl benzyl group having from about 8 to about 18 carbon atoms in the alkyl chain, each \( R^3 \) is selected from the group consisting of \(-CH_2CH_2-\), \(-CH_2CH(CH_3)-\), \(-CH_2CH(CH_2OH)-\), \(-CH_2CH_2CH_2-\), and mixtures thereof; each \( R^4 \) is selected from the group consisting of \( C_1-C_4 \) alkyl, \( C_1-C_4 \) hydroxyalkyl, benzyl ring structures formed by joining the two \( R^4 \) groups, \(-CH_2CHOHCHOHCO_8CHOHCH_2OH\), wherein \( R^5 \) is any hexose or hexose polymer having a molecular weight less than about 1000, and hydrogen when \( y \) is not 0; \( R^5 \) is the same as \( R^4 \) or is an alkyl chain, wherein the total number of carbon atoms or \( R^2 \) plus \( R^5 \) is not more than about 18; each \( y \) is from 0 to about 10, and the sum of the \( y \) values is from 0 to about 15; and \( X \) is any compatible anion.

Highly preferred cationic surfactants are the water soluble quaternary ammonium compounds useful in the present composition having the formula:

\[ R_1R_2R_3R_4N^+X^- \] (i)
wherein \( R_1 \) is \( C_4-C_{16} \) alkyl, each of \( R_2 \), \( R_3 \) and \( R_4 \) is independently \( C_1-C_4 \) alkyl, \( C_1-C_4 \) hydroxy alkyl, benzyl, and \(-\text{(C}_2\text{H}_4\text{)}_x\text{H}\) where \( x \) has a value from 2 to 5, and \( X \) is an anion. Not more than one of \( R_2 \), \( R_3 \) or \( R_4 \) should be benzyl.

The preferred alkyl chain length for \( R_1 \) is \( C_{12}-C_{15} \) particularly where the alkyl group is a mixture of chain lengths derived from coconut or palm kernel fat or is derived synthetically by olefin build up or OXO alcohols synthesis.

Preferred groups for \( R_2 \), \( R_3 \) and \( R_4 \) are methyl and hydroxyethyl groups and the anion \( X \) may be selected from halide, methosulphate, acetate and phosphate ions.

Examples of suitable quaternary ammonium compounds of formulae (i) for use herein are:
- coconut trimethyl ammonium chloride or bromide;
- coconut methyl dihydroxyethyl ammonium chloride or bromide;
- decyl triethyl ammonium chloride;
- decyl dimethyl hydroxyethyl ammonium chloride or bromide;
- \( C_{12-15} \) dimethyl hydroxyethyl ammonium chloride or bromide;
- coconut dimethyl hydroxyethyl ammonium chloride or bromide;
- myristyl trimethyl ammonium methyl sulphate;
- lauryl dimethyl benzyl ammonium chloride or bromide;
- lauryl dimethyl (ethenoxyl)\(_4\) ammonium chloride or bromide;
- choline esters (compounds of formula (i) wherein \( R_1 \) is \( \text{CH}_2-\text{CH}_2-\text{O-}\text{C-C}_\text{12-14} \) alkyl and \( R_2R_3R_4 \) are methyl).

\[ \text{\text{CH}_2-\text{CH}_2-\text{O-}\text{C-C}_\text{12-14} \text{ alkyl and } R_2R_3R_4 \text{ are methyl}}. \]

\[ || \]

\[ 0 \]

di-alkyl imidazolines [compounds of formula (i)].

Other cationic surfactants useful herein are also described in US 4,228,044 and in EP 000 224.
When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

Ampholytic surfactants are also suitable for use in the laundry detergent compositions of the present invention. These surfactants can be broadly described as aliphatic derivatives of secondary or tertiary amines, or aliphatic derivatives of heterocyclic secondary and tertiary amines in which the aliphatic radical can be straight- or branched-chain. One of the aliphatic substituents contains at least about 8 carbon atoms, typically from about 8 to about 18 carbon atoms, and at least one contains an anionic watersolubilizing group, e.g. carboxy, sulfonate, sulfate. See US 3,929,678 (column 19, lines 18-35) for examples of ampholytic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such ampholytic surfactants.

Zwitterionic surfactants are also suitable for use in laundry detergent compositions. These surfactants can be broadly described as derivatives of secondary and tertiary amines, derivatives of heterocyclic secondary and tertiary amines, or derivatives of quaternary ammonium, quaternary phosphonium or tertiary sulfonium compounds. See US 3,929,678 (column 19, line 38 through column 22, line 48) for examples of zwitterionic surfactants.

When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.
Semi-polar nonionic surfactants are a special category of nonionic surfactants which include water-soluble amine oxides containing one alkyl moiety of from about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; watersoluble phosphine oxides containing one alkyl moiety of form about 10 to about 18 carbon atoms and 2 moieties selected from the group consisting of alkyl groups and hydroxyalkyl groups containing from about 1 to about 3 carbon atoms; and water-soluble sulfoxides containing one alkyl moiety from about 10 to about 18 carbon atoms and a moiety selected from the group consisting of alkyl and hydroxyalkyl moieties of from about 1 to about 3 carbon atoms.

Semi-polar nonionic detergent surfactants include the amine oxide surfactants having the formula:

\[
\begin{align*}
\text{O} & \\
\uparrow & \\
R^3(\text{OR}^4)xN(R^5)^2 & 
\end{align*}
\]

wherein \(R^3\) is an alkyl, hydroxyalkyl, or alkyl phenyl group or mixtures thereof containing from about 8 to about 22 carbon atoms; \(R^4\) is an alkylene or hydroxyalkylene group containing from about 2 to about 3 carbon atoms or mixtures thereof; \(x\) is from 0 to about 3; and each \(R^5\) is an alkyl or hydroxyalkyl group containing from about 1 to about 3 carbon atoms or a polyethylene oxide group containing from about 1 to about 3 ethylene oxide groups. The \(R^5\) groups can be attached to each other, e.g., through an oxygen or nitrogen atom, to form a ring structure.

These amine oxide surfactants in particular include \(C_{10}-C_{18}\) alkyl dimethyl amine oxides and \(C_8-C_{12}\) alkoxy ethyl dihydroxy ethyl amine oxides.
When included therein, the laundry detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

**Builder system**

The compositions according to the present invention may further comprise a builder system. Any conventional builder system is suitable for use herein including aluminosilicate materials, silicates, polycarboxylates and fatty acids, materials such as ethylenediamine tetraacetate, metal ion sequestrants such as aminopolyphosphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Though less preferred for obvious environmental reasons, phosphate builders can also be used herein.

Suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated zeolite A, X, B, HS or MAP.

Another suitable inorganic builder material is layered silicate, e.g. SKS-6 (Hoechst). SKS-6 is a crystalline layered silicate consisting of sodium silicate (Na₂Si₂O₅).

Suitable polycarboxylates containing one carboxy group include lactic acid, glycolic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycolic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenlegungsschrift 2,446,686, and 2,446,487, US 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates
and citraconates as well as succinate derivatives such as the
carboxymethyloxsuccinates described in British Patent No.
1,379,241, lactoysuccinates described in Netherlands
Application 7205873, and the oxypolycarboxylate materials
such as 2-oxa-1,1,3-propane tricarboxylates described in Brit-
ish Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include
oxydisuccinates disclosed in British Patent No. 1,261,829,
1,1,2,2,-ethane tetracarboxylates, 1,1,3,3-propane
tetracarboxylates containing sulfo substituents include the
sulfosuccinate derivatives disclosed in British Patent Nos.
1,398,421 and 1,398,422 and in US 3,936,448, and the
sulfonated pyrolysed citrates described in British Patent No.
1,082,179, while polycarboxylates containing phosphone
substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include
cyclopentane-cis,cis-cis-tetracarboxylates, cyclopentadienide
pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-
tetracarboxylates, 2,5-tetrahydro-furan-cis, discarboxylates,
2,2,5,5,-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-
hexane - hexacarboxylates and carboxymethyl derivatives of
polyhydric alcohols such as sorbitol, mannitol and xylitol.

Aromatic polycarboxylates include mellitic acid, pyromellitic
acid and the phthalic acid derivatives disclosed in British

Of the above, the preferred polycarboxylates are hydroxy-
carboxylates containing up to three carboxy groups per
molecule, more particularly citrates.

Preferred builder systems for use in the present compositions
include a mixture of a water-insoluble aluminosilicate
builder such as zeolite A or of a layered silicate (SKS-6),
and a water-soluble carboxylate chelating agent such as
citric acid.
A suitable chelant for inclusion in the detergent compositions in accordance with the invention is ethylenediamine-
N,N'-disuccinic acid (EDDS) or the alkali metal, alkaline earth metal, ammonium, or substituted ammonium salts thereof, or mixtures thereof. Preferred EDDS compounds are the free acid form and the sodium or magnesium salt thereof. Examples of such preferred sodium salts of EDDS include Na₂EDDS and Na₃EDDS. Examples of such preferred magnesium salts of EDDS include MgEDDS and Mg₂EDDS. The magnesium salts are the most preferred for inclusion in compositions in accordance with the invention.

Preferred builder systems include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a water soluble carboxylate chelating agent such as citric acid.

Other builder materials that can form part of the builder system for use in granular compositions include inorganic materials such as alkali metal carbonates, bicarbonates, silicates, and organic materials such as the organic phosphonates, amino polyalkylene phosphonates and amino polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated form each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts of from 5% to 80% by weight of the composition. Preferred levels
of builder for liquid detergents are from 5% to 30%.

**Enzymes**

Preferred detergent compositions, in addition to the enzyme preparation of the invention, comprise other enzyme(s) which provides cleaning performance and/or fabric care benefits.

Such enzymes include proteases, lipases, cutinases, amylases, cellulases, peroxidases, oxidases (e.g. laccases).

Proteases: Any protease suitable for use in alkaline solutions can be used. Suitable proteases include those of animal, vegetable or microbial origin. Microbial origin is preferred. Chemically or genetically modified mutants are included. The protease may be a serine 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.

Preferred commercially available protease enzymes include those sold under the trade names Alcalase, Savinase, Primase, Durazym, and Esperase by Novo Nordisk A/S (Denmark), those sold under the tradename Maxatase, Maxacal, Maxapem and Properase by Gist-Brocades, those sold under the tradename Purafect and Purafect OXP by Genencor International, and those sold under the tradename Opticlean and Optimase by Solvay Enzymes. Protease enzymes may be incorporated into the compositions in accordance with the invention at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from
0.01% to 0.2% of enzyme protein by weight of the composition. 

**Lipases:** Any lipase suitable for use in alkaline solutions can be used. Suitable lipases include those of bacterial or fungal origin. Chemically or genetically modified mutants are included.

Examples of useful lipases include a *Humicola lanuginosa* lipase, e.g., as described in EP 258 068 and EP 305 216, a Rhizomucor miehei lipase, e.g., as described in EP 238 023, a *Candida* lipase, such as a *C. antarctica* lipase, e.g., the *C. antarctica* lipase A or B described in EP 214 761, a Pseudomonas lipase such as a P. alcaligenes and P. pseudoalcaligenes lipase, e.g., as described in EP 218 272, a *P. cepacia* lipase, e.g., as described in EP 331 376, a *P. stutzeri* lipase, e.g., as disclosed in GB 1,372,034, a P. fluorescens lipase, a Bacillus lipase, e.g., a B. subtilis lipase (Dartois et al., (1993), Biochemica et Biophysica acta 1131, 253-260), a B. stearothermophilus lipase (JP 64/744992) and a B. pumilus lipase (WO 91/16422).

Furthermore, a number of cloned lipases may be useful, including the Penicillium camembertii lipase described by Yamaguchi et al., (1991), Gene 103, 61-67), the *Geotricum candidum* lipase (Schimada, Y. et al., (1989), J. Biochem., 106, 383-388), and various Rhizopus lipases such as a R. delemar lipase (Hass, M.J et al., (1991), Gene 109, 117-113), a *R. niveus* lipase (Kugimiya et al., (1992), Biosci. Biotech. Biochem. 56, 716-719) and a *R. oryzae* lipase.

Other types of lipolytic enzymes such as cutinases may also be useful, e.g., a cutinase derived from Pseudomonas *mendocina* as described in WO 88/09367, or a cutinase derived from *Fusarium solani pisi* (e.g. described in WO 90/09446).

Especially suitable lipases are lipases such as M1 Lipase™, Luma fast™ and Lipomax™ (Genencor), Lipolase™ and Lipolase Ultra™ (Novo Nordisk A/S), and Lipase P "Amano" (Amano Pharmaceutical Co. Ltd.).

The lipases are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the
composition, more preferably at a level of from 0.001% to
0.5% of enzyme protein by weight of the composition, even
more preferably at a level of from 0.01% to 0.2% of enzyme
protein by weight of the composition.

5 **Amylases:** Any amylase (a and/or b) suitable for use in
alkaline solutions can be used. Suitable amylases include
those of bacterial or fungal origin. Chemically or
.genetically modified mutants are included. Amylases include,
for example, a-amylases obtained from a special strain of **B.
licheniformis**, described in more detail in GB 1,296,839.
Commercially available amylases are Duramyl™, Termamyl™,
Fungamyl™ and BAN™ (available from Novo Nordisk A/S) and
Rapidas™ and Maxamyl P™ (available from Genencor).
The amylases are normally incorporated in the detergent
composition at a level of from 0.00001% to 2% of enzyme
protein by weight of the composition, preferably at a level
of from 0.0001% to 1% of enzyme protein by weight of the
composition, more preferably at a level of from 0.001% to
0.5% of enzyme protein by weight of the composition, even
more preferably at a level of from 0.01% to 0.2% of enzyme
protein by weight of the composition.

**Cellulases:** Any cellulase suitable for use in alkaline
solutions can be used. Suitable cellulases include those of
bacterial or fungal origin. Chemically or genetically
modified mutants are included. Suitable cellulases are dis-
closed in US 4,435,307, which discloses fungal cellulases
produced from **Humicola insolens**. Especially suitable
cellulases are the cellulases having colour care benefits.
Examples of such cellulases are cellulases described in Euro-
pean patent application No. 0 495 257.

Commercially available cellulases is Celluzyme™ produced by
a strain of Humicola insolens, (Novo Nordisk A/S), and KAC-
500(B)™ (Kao Corporation).

Said cellulases are normally incorporated in the detergent
composition at a level of from 0.00001% to 2% of enzyme
protein by weight of the composition, preferably at a level
of from 0.0001% to 1% of enzyme protein by weight of the
composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Peroxidases/Oxidases: Peroxidase and/or oxidase enzymes are used in combination with hydrogen peroxide or oxygen sources, e.g., percarbonate, perborate, persulfate, hydrogen peroxide, oxygen, etc. They are used for "Solution bleaching", i.e. to prevent transfer of a textile dye from a dyed fabric to another fabric when said fabrics are washed together in a wash liquor, preferably together with an enhancing agent as described in e.g. WO 94/12621 and WO 95/01426. Suitable peroxidases/oxidases include those of plant, bacterial or fungal origin. Chemically or genetically modified mutants are included.

Said peroxidase and/or oxidase enzymes are normally incorporated in the detergent composition at a level of from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level of from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level of from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level of from 0.01% to 0.2% of enzyme protein by weight of the composition.

Mixtures of the above mentioned enzymes are encompassed herein, in particular a mixture of a protease, an amylase, a lipase and/or a cellulase.

The enzyme of the invention is normally incorporated in the detergent composition at a level from 0.00001% to 2% of enzyme protein by weight of the composition, preferably at a level from 0.0001% to 1% of enzyme protein by weight of the composition, more preferably at a level from 0.001% to 0.5% of enzyme protein by weight of the composition, even more preferably at a level from 0.01% to 0.2% of enzyme protein by weight of the composition.
Bleaching agents: Additional optional detergent ingredients that can be included in the detergent compositions of the present invention include bleaching agents such as PB1, PB4 and percarbonate with a particle size of 400-800 microns. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the bleaching agent chosen, one or more bleach activators. When present oxygen bleaching compounds will typically be present at levels of from about 1% to about 25%. In general, bleaching compounds are optional added components in non-liquid formulations, e.g. granular detergents.

The bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches as well as others known in the art.

The bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent.

One category of oxygen bleaching agent that can be used encompasses percarboxylic acid bleaching agents and salts thereof. Suitable examples of this class of agents include magnesium monoperoxyphthalate hexahydrate, the magnesium salt of meta-chloro perbenzoic acid, 4-nonylamino-4-oxoperoxybutyric acid and diperoxynonanoic acid. Such bleaching agents are disclosed in US 4,483,781, US 740,446, EP 0 133 354 and US 4,412,934. Highly preferred bleaching agents also include 6-nonylamino-6-oxoperoxycaproic acid as described in US 4,634,551.

Another category of bleaching agents that can be used encompasses the halogen bleaching agents. Examples of hypohalite bleaching agents, for example, include trichloro isocyanuric acid and the sodium and potassium dichloroisocyanurates and N-chloro and N-bromo alkane sulphonamides. Such materials are normally added at 0.5-10% by weight of the finished product, preferably 1-5% by weight.
The hydrogen peroxide releasing agents can be used in combination with bleach activators such as tetra-acetylene diamine (TAED), nonanoyloxybenzenesulfonate (NOBS, described in US 4,412,934), 3,5-trimethyl-hexanoloxybenzenesulfonate (ISONOBS, described in EP 120 591) or pentaacetylglucose (PAG), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved bleaching effect. In addition, very suitable are the bleach activators C8(6-octanamido-caproyl) oxybenzene-sulfonate, C9(6-nonanamido caproyl) oxybenzenesulfonate and C10 (6-decanamido caproyl) oxybenzenesulfonate or mixtures thereof. Also suitable activators are acylated citrate esters such as disclosed in European Patent Application No. 91870207.7.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in cleaning compositions according to the invention are described in application USSN 08/136,626.

The hydrogen peroxide may also be present by adding an enzymatic system (i.e. an enzyme and a substrate therefore) which is capable of generation of hydrogen peroxide at the beginning or during the washing and/or rinsing process. Such enzymatic systems are disclosed in European Patent Application EP 0 537 381.

Bleaching agents other than oxygen bleaching agents are also known in the art and can be utilized herein. One type of non-oxygen bleaching agent of particular interest includes photoactivated bleaching agents such as the sulfonated zinc and/or aluminum phthalocyanines. These materials can be deposited upon the substrate during the washing process. Upon irradiation with light, in the presence of oxygen, such as by hanging clothes out to dry in the daylight, the sulfonated zinc phthalocyanine is activated and, consequently, the substrate is bleached. Preferred zinc phthalocyanine and a
photoactivated bleaching process are described in US 4,033,718. Typically, detergent composition will contain about 0.025% to about 1.25%, by weight, of sulfonated zinc phthalocyanine.

Bleaching agents may also comprise a manganese catalyst. The manganese catalyst may, e.g., be one of the compounds described in "Efficient manganese catalysts for low-temperature bleaching", Nature 369, 1994, pp. 637-639.

Suds suppressors: Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Silicones can generally be represented by alkylated polysiloxane materials, while silica is normally used in finely divided forms exemplified by silica aerogels and xerogels and hydrophobic silicas of various types. Theses materials can be incorporated as particulates, in which the suds suppressor is advantageously releasably incorporated in a water-soluble or waterdispersible, substantially non surface-active detergent impermeable carrier. Alternatively the suds suppressor can be dissolved or dispersed in a liquid carrier and applied by spraying on to one or more of the other components.

A preferred silicone suds controlling agent is disclosed in US 3,933,672. Other particularly useful suds suppressors are the self-emulsifying silicone suds suppressors, described in German Patent Application DTOS 2,646,126. An example of such a compound is DC-544, commercially available form Dow Corning, which is a siloxane-glycol copolymer. Especially preferred suds controlling agent are the suds suppressor system comprising a mixture of silicone oils and 2-alkyl-alkanols. Suitable 2-alkyl-alkanols are 2-butyl-octanol which are commercially available under the trade name Isofol 12 R.

Such suds suppressor system are described in European Patent Application EP 0 593 841.
Especially preferred silicone suds controlling agents are described in European Patent Application No. 92201649.8. Said compositions can comprise a silicone/silica mixture in combination with fumed nonporous silica such as Aerosil®.

The suds suppressors described above are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

**Other components:** Other components used in detergent compositions may be employed such as soil-suspending agents, soil-releasing agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or nonencapsulated perfumes.

Especially suitable encapsulating materials are water soluble capsules which consist of a matrix of polysaccharide and polyhydroxy compounds such as described in GB 1,464,616.

Other suitable water soluble encapsulating materials comprise dextrins derived from ungelatinized starch acid esters of substituted dicarboxylic acids such as described in US 3,455,838. These acid-ester dextrins are, preferably, prepared from such starches as waxy maize, waxy sorghum, sago, tapioca and potato. Suitable examples of said encapsulation materials include N-Lok manufactured by National Starch. The N-Lok encapsulating material consists of a modified maize starch and glucose. The starch is modified by adding monofunctional substituted groups such as octenyl succinic acid anhydride.

Antiredeposition and soil suspension agents suitable herein include cellulose derivatives such as methylcellulose, carboxymethylcellulose and hydroxyethylcellulose, and homo- or co-polymeric polycarboxylic acids or their salts. Polymers of this type include the polyacrylates and maleic anhydride-acrylic acid copolymers previously mentioned as builders, as
well as copolymers of maleic anhydride with ethylene, methacrylic acid, the maleic anhydride constituting at least 20 mole percent of the copolymer. These materials are normally used at levels of from 0.5% to 10% by weight, more preferably form 0.75% to 8%, most preferably from 1% to 6% by weight of the composition.

Preferred optical brighteners are anionic in character, examples of which are disodium 4,4'-bis-(2-diethanolamino-4-anilino-s-triazin-6-yamino)stilbene-2:2' disulphonate, disodium 4', 4'-bis-(2-morpholino-4-anilino-s-triazin-6-yamino-stilbene-2:2' - disulphonate, disodium 4,4' - bis-(2,4-dianilino-s-triazin-6-yamino)stilbene-2:2' - disulphonate, monosodium 4',4'' - bis-(2,4-dianilino-s-triazin-6 yamino)stilbene-2-sulphonate, disodium 4,4' -bis-(2-anilino-4-(N-methyl-N-2-hydroxyethylamino)-s-triazin-6-yamino)stilbene-2,2' - disulphonate, di-sodium 4,4' -bis-(4-phenoxy-2,1,3-triazol-2-yl)-stilbene-2,2' disulphonate, di-sodium 4,4'bis(2-anilino-4-(1-methyl-2-hydroxyethylamino)-s-triazin-6-yamino)stilbene-2,2'disulphonate, sodium 2(stilbyl-4'--(naphtho-1',2':4,5)-1,2,3, - triazole-2''-sulphonate and 4,4''-bis(2-sulphostyryl)biphenyl.

Other useful polymeric materials are the polyethylene glycols, particularly those of molecular weight 1000-10000, more particularly 2000 to 8000 and most preferably about 4000. These are used at levels of from 0.20% to 5% more preferably from 0.25% to 2.5% by weight. These polymers and the previously mentioned homo- or co-polymeric polycarboxylate salts are valuable for improving whiteness maintenance, fabric ash deposition, and cleaning performance on clay, proteinaceous and oxidizable soils in the presence of transition metal impurities.

Soil release agents useful in compositions of the present invention are conventionally copolymers or terpolymers of terephthalic acid with ethylene glycol and/or propylene glycol units in various arrangements. Examples of such
polymers are disclosed in US 4,116,885 and 4,711,730 and EP 0 272 033. A particular preferred polymer in accordance with EP 0 272 033 has the formula:

\[
(\text{CH}_3(\text{PEG})_{43})_{0.75}(\text{POH})_{0.25}[\text{T-PO}]_{2.8}(\text{T-PEG})_{0.4}]T(\text{POH})_{0.25}((\text{PEG})_{43}\text{CH}_3)_{0.75}
\]

where PEG is \(-(\text{OC}_2\text{H}_4)\)_n-, PO is \((\text{OC}_3\text{H}_6\text{O})\) and T is \((\text{POOC}_2\text{H}_4\text{CO})\).

Also very useful are modified polyesters as random copolymers of dimethyl terephthalate, dimethyl sulfoisophthalate, ethylene glycol and 1-2 propane diol, the end groups consisting primarily of sulphobenzoate and secondarily of mono esters of ethylene glycol and/or propane-diol. The target is to obtain a polymer capped at both end by sulphobenzoate groups, "primarily", in the present context most of said copolymers herein will be endcapped by sulphobenzoate groups. However, some copolymers will be less than fully capped, and therefore their end groups may consist of monoester of ethylene glycol and/or propane 1-2 diol, thereof consist "secondarily" of such species.

The selected polyesters herein contain about 46% by weight of dimethyl terephthalic acid, about 16% by weight of propane - 1.2 diol, about 10% by weight ethylene glycol about 13% by weight of dimethyl sulfobenzoic acid and about 15% by weight of sulfoisophthalic acid, and have a molecular weight of about 3,000. The polyesters and their method of preparation are described in detail in EP 311 342.

Softening agents: Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400898 and in US 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP 0 011 340 and their combination with mono C₁₂-C₁₄
quaternary ammonium salts are disclosed in EP-B-0 026 528 and
di-long-chain amides as disclosed in EP 0 242 919. Other
useful organic ingredients of fabric softening systems
include high molecular weight polyethylene oxide materials as
disclosed in EP 0 299 575 and 0 313 146.

Levels of smectite clay are normally in the range from 5% to
15%, more preferably from 8% to 12% by weight, with the
material being added as a dry mixed component to the
remainder of the formulation. Organic fabric softening agents
such as the water-insoluble tertiary amines or dilong chain
amide materials are incorporated at levels of from 0.5% to 5%
by weight, normally from 1% to 3% by weight whilst the high
molecular weight polyethylene oxide materials and the water
soluble cationic materials are added at levels of from 0.1%
to 2%, normally from 0.15% to 1.5% by weight. These materials
are normally added to the spray dried portion of the
composition, although in some instances it may be more
convenient to add them as a dry mixed particulate, or spray
them as molten liquid on to other solid components of the
composition.

Polymeric dye transfer inhibiting agents: The detergent
compositions according to the present invention may also
comprise from 0.001% to 10%, preferably from 0.01% to 2%,
more preferably form 0.05% to 1% by weight of polymeric dye
transfer inhibiting agents. Said polymeric dye transfer
inhibiting agents are normally incorporated into detergent
compositions in order to inhibit the transfer of dyes from
colored fabrics onto fabrics washed therewith. These polymers
have the ability of complexing or adsorbing the fugitive dyes
washed out of dyed fabrics before the dyes have the
opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye transfer inhibiting agents
are polyamine N-oxide polymers, copolymers of N-vinyl-
pyrrolidone and N-vinylimidazole, polyvinylpyrrolidone
polymers, polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof.

Addition of such polymers also enhances the performance of the enzymes according the invention.

The detergent composition according to the invention can be in liquid, paste, gels, bars or granular forms. Non-dusting granulates may be produced, e.g., as disclosed in US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Examples of waxy coating materials are poly(ethylene oxide) products (polyethyleneglycol, PEG) with mean molecular weights of 1000 to 20000; ethoxylated nonylphenols having from 16 to 50 ethylene oxide units; ethoxylated fatty alcohols in which the alcohol contains from 12 to 20 carbon atoms and in which there are 15 to 80 ethylene oxide units; fatty alcohols; fatty acids; and mono- and di- and triglycerides of fatty acids. Examples of film-forming coating materials suitable for application by fluid bed techniques are given in GB 1483591.

Granular compositions according to the present invention can also be in "compact form", i.e. they may have a relatively higher density than conventional granular detergents, i.e. form 550 to 950 g/l; in such case, the granular detergent compositions according to the present invention will contain a lower amount of "Inorganic filler salt", compared to conventional granular detergents; typical filler salts are alkaline earth metal salts of sulphates and chlorides, typically sodium sulphate; "Compact" detergent typically comprise not more than 10% filler salt. The liquid compositions according to the present invention can also be in "concentrated form", in such case, the liquid detergent compositions according to the present invention will contain a lower amount of water, compared to conventional liquid detergents. Typically, the water content of the concentrated liquid detergent is less than 30%, more preferably less than
20%, most preferably less than 10% by weight of the detergent compositions.

The compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the pretreatment of stained fabrics, rinse added fabric softener compositions, and compositions for use in general household hard surface cleaning operations and dishwashing operations.

The following examples are meant to exemplify compositions for the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention.

In the detergent compositions, the abbreviated component identifications have the following meanings:

**LAS:** Sodium linear C_{12} alkyl benzene sulphonate

**TAS:** Sodium tallow alkyl sulphate

**XYAS:** Sodium C_{1x} - C_{1y} alkyl sulfate

**SS:** Secondary soap surfactant of formula 2-butyl octanoic acid

**25EY:** A C_{12} - C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide

**45EY:** A C_{14} - C_{15} predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide

**XYEZS:** C_{1x} - C_{1y} sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole

**Nonionic:** C_{13} - C_{15} mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an
average degree of propoxylation of 4.5 sold under the tradename Plurafax LF404 by BASF GmbH

CFAA: $C_{12} - C_{14}$ alkyl N-methyl glucamide

TFAA: $C_{16} - C_{18}$ alkyl N-methyl glucamide

Silicate: Amorphous Sodium Silicate ($SiO_2:Na_2O$ ratio = 2.0)

NaSKS-6: Crystalline layered silicate of formula d-$Na_2Si_2O_5$

Carbonate: Anhydrous sodium carbonate

Phosphate: Sodium tripolyphosphate

MA/AA: Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 80,000

Polyacrylate: Polyacrylate homopolymer with an average molecular weight of 8,000 sold under the tradename PA30 by BASF GmbH

Zeolite A: Hydrated Sodium Aluminosilicate of formula $Na_12(AlO_2SiO_2)_{12} \cdot 27H_2O$ having a primary particle size in the range from 1 to 10 micrometers

Citrate: Tri-sodium citrate dihydrate

Citric: Citric Acid

Perborate: Anhydrous sodium perborate monohydrate bleach, empirical formula $NaBO_2 \cdot H_2O$

PB4: Anhydrous sodium perborate tetrahydrate

Percarbonate: Anhydrous sodium percarbonate bleach of empirical formula $2Na_2CO_3 \cdot 3H_2O_2$
TAED: Tetraacetyl ethylene diamine

CMC: Sodium carboxymethyl cellulose

DETPMP: Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Tradename Dequest 2060

PVP: Polyvinyl pyrrolidone polymer

EDDS: Ethylenediamine –N, N'– disuccinic acid, [S,S] isomer in the form of the sodium salt

SudsSuppressor: 25% paraffin wax Mpt 50°C, 17% hydrophobic silica, 58% paraffin oil

Granular Suds: 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form

Sulphate: Anhydrous sodium sulphate

HMWPEO: High molecular weight polyethylene oxide

TAE 25: Tallow alcohol ethoxylate (25)

**Detergent Example I**

A granular fabric cleaning composition in accordance with the invention may be prepared as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount (gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium linear C₁₂ alkyl benzene sulfonate</td>
<td>6.5</td>
</tr>
<tr>
<td>Sodium sulfate</td>
<td>15.0</td>
</tr>
<tr>
<td>Zeolite A</td>
<td>26.0</td>
</tr>
<tr>
<td>Ingredient</td>
<td>Amount</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Sodium nitrilotriacetate</td>
<td>5.0</td>
</tr>
<tr>
<td>Enzyme of the invention</td>
<td>0.1</td>
</tr>
<tr>
<td>PVP</td>
<td>0.5</td>
</tr>
<tr>
<td>TAED</td>
<td>3.0</td>
</tr>
<tr>
<td>Boric acid</td>
<td>4.0</td>
</tr>
<tr>
<td>Perborate</td>
<td>18.0</td>
</tr>
<tr>
<td>Phenol sulphonate</td>
<td>0.1</td>
</tr>
<tr>
<td>Minors</td>
<td>Up to 100</td>
</tr>
</tbody>
</table>

**Detergent Example II**

A compact granular fabric cleaning composition (density 800 g/l) in accord with the invention may be prepared as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>45AS</td>
<td>8.0</td>
</tr>
<tr>
<td>25E3S</td>
<td>2.0</td>
</tr>
<tr>
<td>25E5</td>
<td>3.0</td>
</tr>
<tr>
<td>25E3</td>
<td>3.0</td>
</tr>
<tr>
<td>TFAA</td>
<td>2.5</td>
</tr>
<tr>
<td>Zeolite A</td>
<td>17.0</td>
</tr>
<tr>
<td>NaSKS-6</td>
<td>12.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>3.0</td>
</tr>
<tr>
<td>Carbonate</td>
<td>7.0</td>
</tr>
<tr>
<td>MA/AA</td>
<td>5.0</td>
</tr>
<tr>
<td>CMC</td>
<td>0.4</td>
</tr>
<tr>
<td>Enzyme of the invention</td>
<td>0.1</td>
</tr>
<tr>
<td>TAED</td>
<td>6.0</td>
</tr>
<tr>
<td>Percarbonate</td>
<td>22.0</td>
</tr>
<tr>
<td>EDDS</td>
<td>0.3</td>
</tr>
<tr>
<td>Granular suds suppressor</td>
<td>3.5</td>
</tr>
</tbody>
</table>
Granular fabric cleaning compositions in accordance with the invention which are especially useful in the laundering of coloured fabrics were prepared as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity 1</th>
<th>Quantity 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS</td>
<td>10.7</td>
<td></td>
</tr>
<tr>
<td>TAS</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>TFAA</td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>45AS</td>
<td>3.1</td>
<td>10.0</td>
</tr>
<tr>
<td>45E7</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>25E3S</td>
<td></td>
<td>3.0</td>
</tr>
<tr>
<td>68E11</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>25E5</td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>Citrate</td>
<td>15.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Carbonate</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Citric acid</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Zeolite A</td>
<td>32.1</td>
<td>25.0</td>
</tr>
<tr>
<td>Na-SKS-6</td>
<td></td>
<td>9.0</td>
</tr>
<tr>
<td>MA/AA</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>DETPMP</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Enzyme of the invention</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Silicate</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Sulphate</td>
<td>5.2</td>
<td>3.0</td>
</tr>
<tr>
<td>PVP</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Poly (4-vinylpyridine)-N-Oxide/copolymer of vinylimidazole and vinylpyrrolidone</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Perborate</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Phenol sulfonate</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Water/Minors</td>
<td>Up to 100%</td>
<td></td>
</tr>
</tbody>
</table>

Detergent Example IV

Granular fabric cleaning compositions in accordance with the invention which provide "Softening through the wash"
50

capability may be prepared as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>45AS</td>
<td>10.0</td>
</tr>
<tr>
<td>LAS</td>
<td>7.6</td>
</tr>
<tr>
<td>68AS</td>
<td>1.3</td>
</tr>
<tr>
<td>45E7</td>
<td>4.0</td>
</tr>
<tr>
<td>25E3</td>
<td>5.0</td>
</tr>
<tr>
<td>Coco-alkyl-dimethyl hydroxy-ethyl ammonium chloride</td>
<td>1.4 1.0</td>
</tr>
<tr>
<td>Citrate</td>
<td>5.0 3.0</td>
</tr>
<tr>
<td>Na-SKS-6</td>
<td>11.0</td>
</tr>
<tr>
<td>Zeolite A</td>
<td>15.0 15.0</td>
</tr>
<tr>
<td>MA/AA</td>
<td>4.0 4.0</td>
</tr>
<tr>
<td>DETPMP</td>
<td>0.4 0.4</td>
</tr>
<tr>
<td>Perborate</td>
<td>15.0</td>
</tr>
<tr>
<td>Percarbonate</td>
<td>15.0</td>
</tr>
<tr>
<td>TAED</td>
<td>5.0 5.0</td>
</tr>
<tr>
<td>Smectite clay</td>
<td>10.0 10.0</td>
</tr>
<tr>
<td>HMWPEO</td>
<td>0.1</td>
</tr>
<tr>
<td>Enzyme of the invention</td>
<td>0.10 0.05</td>
</tr>
<tr>
<td>Silicate</td>
<td>3.0 5.0</td>
</tr>
<tr>
<td>Carbonate</td>
<td>10.0 10.0</td>
</tr>
<tr>
<td>Granular suds suppressor</td>
<td>1.0 4.0</td>
</tr>
<tr>
<td>CMC</td>
<td>0.2 0.1</td>
</tr>
<tr>
<td>Water/Minors</td>
<td>Up to 100%</td>
</tr>
</tbody>
</table>

50

Detergent Example V

Heavy duty liquid fabric cleaning compositions in accordance with the invention may be prepared as follows:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS acid form</td>
<td>-</td>
<td>25.0</td>
</tr>
<tr>
<td>Citric acid</td>
<td>5.0</td>
<td>2.0</td>
</tr>
<tr>
<td>25AS acid form</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td>25AE2S acid form</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>25AE7</td>
<td>8.0</td>
<td>-</td>
</tr>
</tbody>
</table>
The enzyme preparation of the invention may be incorporated in concentrations conventionally employed in detergents. It is at present contemplated that, in the detergent composition of the invention, the enzyme preparation may be added in an amount corresponding to 0.00001-1 mg (calculated as pure enzyme protein) of cellulase per liter of wash liquor.

In its third aspect, the invention provides a fabric softener composition comprising the (modified) enzyme preparation of the present invention and a buffer and optionally other ingredients.

The enzyme preparation of the invention is also useful as an ingredient in ready-to-use compositions for other purposes such as for bio-polishing and enzymatic stone-washing, optionally in combination with other ingredients such as buffers; polymers; abrasives such as perlite; chelating agents etc.

In its fourth aspect, the present invention relates to a method of reducing the tendency to tensile strength loss or pin-holing of cellulosic fabric, the method comprising
treating the fabric with the cellulase preparation of the present invention.

In its fifth aspect, the invention relates to a method of obtaining a cellulase preparation having reduced tendency to cause tensile strength loss or pin-holing of cellulosic fabric when used for treating such fabric, the method comprising reducing the mobility of the cellulase component by means of immobilization, insolubilization or increasing the molecular weight or apparent size of the cellulase protein molecule.
METHODS

The term "activity towards dyed microcrystalline cellulose" as used herein refers to a hydrolytic activity towards microcrystalline cellulose covalently labelled with a light absorbing/fluorogenic compound, e.g. a reactive dye, determined spectroscopically by measuring the liberation of labelled products resulting from hydrolysis under conditions simulating washing conditions with respect to alkaline pH, temperature, duration, agitation and detergent concentrations. The assay is described in WO 95/02675, which is hereby incorporated by reference.

The term "cellulase activity on cellobiose", in terms of $k_{cat}$ (s$^{-1}$), as used herein refers to a coupled assay:

\[
\text{Cellotriose} \rightarrow \text{Glucose} + \text{Cellobiose} \\
(\text{cat.}: \text{cellulase})
\]

\[
\text{Glucose} + O_2 + H_2O \rightarrow \text{Gluconate} + H_2O_2 \\
(\text{cat.}: \text{Glucoseoxidase})
\]

\[
H_2O_2 + \text{ABTS}^R \rightarrow \text{ABTS}^{Ox} \\
(\text{cat.}: \text{Peroxidase})
\]

which is followed spectrophotometrically at 418 nm (maximum absorbance of $\text{ABTS}^{Ox}$ at 418 nm). The assay is described in WO 95/02675, which is hereby incorporated by reference.

**Determination of cellulase activity (S-CEVU)**

The cellulase enzymes hydrolyse CMC, thereby decreasing the viscosity of the incubation mixture.

Determination of the cellulase activity, measured in terms of S-CEVU, was determined according to the method described in
the leaflet AF 302/2-GB, which is available from the Applicant upon request.

The S-CEVU assay quantifies the amount of catalytic activity present in the sample by measuring the ability of the sample to reduce the viscosity of a solution of carboxymethylcellulose (CMC). The assay is carried out at 40°C, pH 7.5 using a relative enzyme standard for reducing the viscosity of the CMC substrate.

The invention is further illustrated by the following non-limiting examples.
EXAMPLE 1
Preparation of 43 kD cellulase autoimmobilized on microcry-
stalline cellulose (Avicel™)

Conditions of autoimmobilization:
1.6 g/l of 43 kD endoglucanase (700 S-CEVU/ml), sodium phosphate buffer, 0.05 M pH 7.5, 100 g/l of Avicel (0.016 g 43 kD endoglucanase/g Avicel), total of volume of 5 liters, rinse with buffer pH 7.5.

43 kD sample:
ultrafiltrate from large-scale fermentation, 40000 S-CEVU/ml (about 100 g/l total protein), specific activity of pure 43 kD component 430 S-CEVU/mg.

Procedure

1. Adsorption for 30 min at 20°C of the mixture described in the table below:

<table>
<thead>
<tr>
<th>Total volume, ml</th>
<th>Avicel (Merck)</th>
<th>43 kD endoglucanase, ml, to 1.6 g/l</th>
<th>0.05 M sodium phosphate buffer, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>500</td>
<td>90</td>
<td>4410</td>
</tr>
</tbody>
</table>

2. Centrifuge at 4000 rpm for 15 min, 5°C. Discard the supernatant.
3. Transfer into a cold room and add further 5 l of 0.12 N sodium phosphate buffer and mix for 30 min at 20°C to rinse
4. Centrifuge at 4000 rpm for 15 min, 5°C
5. Freeze the moist sediment and determine the bound cellulase activity on dyed Avicel under pH 7.5 and 10.
6. Mill (in a food processor such as DITO SAMA K55 at maximum speed for 5 minutes)
Resulting preparation

The cellulase preparation produced using the procedure described above shows the following typical properties:

Share of cellulase bound to Avicel, % of total used 50

10 Protein (mg/g immobilized enzyme preparation) 11.3

Cellulase activity, S-CEVU/g dry Avicel 6000

15 Activity of cellulase on dyed Avicel, % compared to same amount of "free" cellulase protein 100

Cellulase activity extractable by buffer solutions, pH 7 - 10, 1 hour at 40°C, % of total bound 20

Cellulase activity extractable by surfactant solutions, pH 7-10, 1 hour at 40°C, % of total bound 50

EXAMPLE 2
Preparation of 43 kD cellulase autoimmobilized on microcrystalline cellulose with reduced leakage of enzyme from support

Conditions of immobilization:
1.6 g/l of 43 kD cellulase (700 S-CEVU/ml), sodium phosphate buffer, 0.05 M pH 7.5, 100 g/l of Avicel (0.016 g 43 kD endoglucanase/g Avicel), total of volume of 5 liters, rinse with surfactant pH 10.
43 kD sample:
ultrafiltrate from large-scale fermentation, 40000 S-CEVU/ml (about 100 g/l total protein), specific activity of pure 43 kD component 430 S-CEVU/mg.

Procedure
1. Adsorption for 30 min at 20°C of the mixture described in the table below:

<table>
<thead>
<tr>
<th>Total volume, ml</th>
<th>Avicel (Merck)</th>
<th>43 kD endoglucanase, ml, to 1.6 g/l</th>
<th>0.05 M sodium phosphate buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5000</td>
<td>500</td>
<td>90</td>
<td>4410</td>
</tr>
</tbody>
</table>

2. Centrifuge at 4000 rpm for 15 min, 5°C.
3. Transfer supernatant into a cold room and adding 5 l more of 0.12 n sodium phosphate buffer and mixing for 30 min at 20°C to rinse

4. Centrifuge at 4000 rpm for 15 min, 5°C
5. Freeze the moist sediment
6. An extract was made from the commercial powder detergent sold under the trade name Ariel Color™ dissolving it (6.5 g/l) in water at 4°C and removing the insoluble part of the suspension by filtration
7. 250 g of 43 kD immobilized on Avicel (from item 5), were put into 5 l of Ariel Color™ extract and mixed overnight at 4°C
8. The supernatant-1 was decanted, 500 ml 0.6 N sodium phosphate buffer pH 6.8 was added to adjust pH to 7.0 and water up to 5 l.
9. Suspension was mixed at 20°C for 30 min and supernatant-2 was removed by filtration.
10. 5 l of water was added and the suspension mixed again for 30 min to wash away any remains of surfactant.
11. Supernatant 3 was removed by filtration and moist sediment was freeze-dried.

**Resulting preparation**

The cellulase preparation produced using the procedure described above shows the following typical properties:

Share of cellulase bound to Avicel,
% of total used 25

Cellulase activity, S-CEVU/g dry Avicel 3000

Activity of cellulase on dyed Avicel,
% compared to same amount of "free" cellulase protein 100

Cellulase activity extractable by buffer solutions, pH 7 - 10, 1 hour at 40°C,
% of total bound 10

Cellulase activity extractable by surfactant solutions, pH 7-10, 1 hour at 40°C,
% of total bound 20

**EXAMPLE 3**

Preparation of 43 kD cellulase autoimmobilized on fibrous cellulose (BC200™)

**Conditions of autoimmobilization:**
1.6 g/l of 43 kD cellulase (700 S-CEVU/ml), sodium phosphate buffer, 0.05 M pH 7.5, 50 g/l of fibrous cellulose BC200™ (0.032 g 43 kD endoglucanase/g cellulose), total of volume of 2 liters, rinse with buffer pH 7.5.
43 kD sample:
ultrafiltrate from large-scale fermentation, 40000 S-CEVU/ml
/about 100 g/l total protein/, specific activity of pure 43
kD component 430 S-CEVU/mg.

Procedure

1. Adsorption for 30 min at 20°C of the mixture described in
the table below:

<table>
<thead>
<tr>
<th>Total volume, ml</th>
<th>Cellulose BC200</th>
<th>43 kD endoglucanase, ml, to 1.6 g/l</th>
<th>0.05 M sodium phosphate buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2100</td>
<td>100</td>
<td>35</td>
<td>2000</td>
</tr>
</tbody>
</table>

2. Centrifuging at 4000 rpm for 15 min, 5°C
3. Adding 2 l more of 0.12 N sodium phosphate buffer and
mixing for 30 min at 20°C to rinse
4. Centrifuging at 4000 rpm for 15 min, 5°C
5. Freezing the moist sediment
6. Determination of the bound cellulase activity on dyed
Avicel under pH 7.5 and 10.

Resulting preparation

The cellulase preparation produced using the procedure
described above shows the following typical properties:

Share of cellulase
bound to Avicel,
% of total used 28

Cellulase activity,
S-CEVU/g dry Avicel 2000

Activity of cellulase on dyed Avicel,
% compared to same amount of "free" cellulose protein 100

Cellulase activity extractable by buffer solutions, pH 7 - 10, 1 hour at 40°C, % of total bound 20

Cellulase activity extractable by surfactant solutions, pH 7-10, 1 hour at 40°C, % of total bound 50

EXAMPLE 4
Preparation of 43 kD cellulase variants autoimmobilized on microcrystalline cellulose (Avicel™)

Conditions of autoimmobilization:
1.6 g/l of 43 kD cellulase variants (700 S-CEVU/ml), sodium phosphate buffer, 0.05 M pH 7.5, 100 g/l of Avicel (0.016 g enzyme/g Avicel), rinse with buffer pH 7.5.

43 kD variants:
fully described in WO 94/07998, 500 - 40000 S-CEVU/ml, obtained from pilot scale fermentation of products of 43 kD gene modified by site-directed mutagenesis and expressed in Aspergillus oryzae

<table>
<thead>
<tr>
<th>Variant</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>None</td>
</tr>
<tr>
<td>B</td>
<td>N65R, D67R</td>
</tr>
<tr>
<td>C</td>
<td>R158E</td>
</tr>
<tr>
<td>D</td>
<td>R196E</td>
</tr>
<tr>
<td>E</td>
<td>R252L</td>
</tr>
<tr>
<td>F</td>
<td>Y280F</td>
</tr>
</tbody>
</table>

For example N65R denotes substitution of the asparagine in position 65 with argine.
Procedure

1. Adsorption for 30 min at 20°C of the mixture described in the table below:

<table>
<thead>
<tr>
<th>Total volume, ml</th>
<th>Avicel (Merck)</th>
<th>43 kD endoglucanase, ml, to 1.6 g/l</th>
<th>0.05 M sodium phosphate buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>100</td>
<td>up to 700 S-CEVU/ml</td>
<td>up to 1000</td>
</tr>
</tbody>
</table>

2. Centrifuge at 4000 rpm for 15 min, at 5°C
3. Transfer supernatant into a cold room and adding 1000 ml more of 0.12 N sodium phosphate buffer and mixing for 30 min. at 20°C to rinse
4. Centrifuging at 4000 rpm for 15 min, at 5°C
5. Freezing the moist sediment

Resulting preparation

The cellulase preparation produced using the procedure described above show the following typical properties:
### 43 kD variants

<table>
<thead>
<tr>
<th></th>
<th>D</th>
<th>B</th>
<th>F</th>
<th>E</th>
<th>Ref.</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Share of cellulase bound to Avicel, %</td>
<td>71</td>
<td>71</td>
<td>44</td>
<td>41</td>
<td>53</td>
</tr>
<tr>
<td>10</td>
<td>Cellulase activity, S-CEVU/g dry Avicel</td>
<td>7600</td>
<td>6800</td>
<td>5400</td>
<td>4800</td>
<td>4700</td>
</tr>
<tr>
<td>15</td>
<td>Activity of cellulase on dyed Avicel, % compared to same amount of &quot;free cellulase protein&quot;</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>Cellulase activity extractable by buffer solutions, pH 7 - 10, 1 hour at 40°, % of total bound</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>30</td>
<td>Cellulase activity extractable by surfactant solutions, pH 7-10, 1 hour at 40°, % of total bound</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>
EXAMPLE 5
Preparation of 43 kD cellulase incorporated into a gel of chitosan

Conditions:
43 kD - 7.5 g/l (3200 S-CEVU/ml); chitosan - 5 g/l.

43 kD sample:
ultrafiltrate from large-scale fermentation, 40000 S-CEVU/ml (about 100 g/l total protein), specific activity of pure 43 kD component 430 S-CEVU/mg.

Procedure

1. 100 ml of 43 kD sample is diluted by adding 900 ml of water.
2. 2% chitosan solution in water is prepared, the pH is adjusted to 5 by adding acetic acid.
3. 1000 ml of 43 kD solution is mixed with 250 ml of 2% chitosan.
4. Saturated sodium tripolyphosphate aqueous solution is added gradually into the mixture till pH 8.35. Total volume is 1320 ml.
5. The gel formed is separated from supernatant by filtration and cellulase activity on dyed Avicel is determined for initial 43 kD and the gel formed.
6. The gel formed may be used as a cellulase component as it is, or in a freeze-dried form. The resulting dry weight of the gel formed is 4.5 g.
7. Milling.
### Resulting preparation

The cellulase preparation produced using the procedure described above shows the following typical properties:

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Share of cellulase bound to Avicel, % of total used</td>
<td>50</td>
</tr>
<tr>
<td>Cellulase activity, S-CEVU/g dry weight</td>
<td>200000</td>
</tr>
<tr>
<td>Activity of cellulase on dyed Avicel, % compared to same amount of &quot;free&quot; cellulase protein</td>
<td>50</td>
</tr>
<tr>
<td>Cellulase activity extractable by buffer solutions, pH 7 - 10, 1 hour at 40°C, % of total bound</td>
<td>10</td>
</tr>
<tr>
<td>Cellulase activity extractable by surfactant solutions, pH 7-10, 5 min at 40°C, % of total bound</td>
<td>20</td>
</tr>
<tr>
<td>Cellulase activity extractable by surfactant solutions, pH 7-10, 1 hour at 40°C, % of total bound</td>
<td>90</td>
</tr>
</tbody>
</table>
EXAMPLE 6
Preparation of 43 kD cellulase aggregated into aggregates with charged polymer

Conditions:
43 kD - 9.3 g/l (4000 S-CEVU/ml); HPMC acetate succinate - 20 g/l, 0.1 M NaAc buffer pH 6.0.

43 kD sample:
ultrafiltrate from large-scale fermentation, 40000 S-CEVU/ml (about 100 g/l total protein), specific activity of pure 43 kD component 430 S-CEVU/mg.

Procedure
1. 20 gram of hydroxypropyl methyl cellulose acetate succinate (AS-LF, Lot No. 909036, Shin-Etsu Kagaku Kogyo K.K., Tokyo) was dissolved in 900 ml of 0.1 M NaAc buffer pH 6.
2. 100 ml of 43 kD cellulase sample was added, total volume being 1000 ml.
3. The mixture was incubated with mixing at 25°C for 2 hours.
4. The sediment is removed by centrifugation.
5. The supernatant solution was freeze-dried, resulting dry weight is 35 g.

Resulting preparation

The cellulase preparation produced using the procedure described above shows the following typical properties:

Cellulase activity,
S-CEVU/g dry weight 40000

Activity of cellulase on dyed Avicel, % compared to same amount of "free" cellulase protein 70
Cellulase activity extractable by buffer solutions, pH 7 - 10, 5 min at 40\(^\circ\),
% of total bound

10 Example 7
Cellulase pre-adsorbed to Bentonite

A 5.0 \% solution of bentonite (ASB 350) in 0.001 M acetate buffer pH 4.0 is made. 43 kD cellulase (as used in example 1)
is added to final concentration of 1 mg enzyme protein/ml. Almost immediately thereafter, the needed quantity for the
wash can be taken out. Centrifugation followed by activity analysis of supernatant and pellet verifies that all activity
is bound to bentonite.

The test is carried out in Launder-o-meter.

Detergent: European HDG, 6.5 g/l
Volume: 400 ml
pH: 10.0
Temperature: 30\(^\circ\)C
Water
hardness: 2 mM CaCl\(_2\)
Time: 60 min.
Rinse: 5 min.
Cycles: 10
Mechanical effect: 30 steel balls, 6 mm in diameter.
Cellulases: Variant G: 43 kD cellulase-bentonite (see above)
Variant D: 43 kD cellulase-Avicel (from Example 4)
Reference (43 kD cellulase)
Cellulase dosage: 0 - 50 - 100 - 200 - 800 S-CEVU/l
Textile: Knitted fabric, 2 pcs á 4x7 cm
Woven fabric: 6 pcs. app. 5x25 cm.

Evaluation: Panel score of pilling on knitted fabric (High = good)
Tensile strength on woven fabric.
The reference is 0 S-CEVU/l.

Results as Panel Score Units / % Tensile Strength Loss:

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EXAMPLE 9

Preparation of a cellulase granulate

Step 1:
A cellulase containing granulate was produced by mixing in a 50 l Lødige mixer while heating to 30°C.
Mixing time 2 minutes at 145 rpm with the ploughs and a chopper speed of 3000 rpm.

Powder ingredients for 15 kg granulate:
1.50 kg of the enzyme preparation prepared as described in example 1
0.75 kg fibrous cellulose
10.3 kg fine ground sodium sulfate
0.75 kg calcium carbonate
0.90 kg dextrin

Under continuous mixing the following liquid mixture was sprayed thereon:

3.10 kg water
0.80 kg carbohydrate binder

After the spraying the wet mixture was granulated further for 3 minutes and sphere or lens shaped granulates were formed.

Step 2:
The humid granulates were dried at 60°C to a water content of less than 3%.

The dry granulate was sieved to a particle size range between 300 - 1000 μm.

Step 3:
2 kg of the granulate was heated to 75-80°C in a 5 l jacketed Lödige mixer. The temperature was kept constant during the whole coating process. The heated granulate was mixed with 100 g of melted PEG4000, followed by a powdering with 250 g of TiO₂ followed by addition of 20 g of PEG4000. The granulate was finally cooled to room temperature on a fluid-bed and sifted 300-1000 μm.

The produced granulate had an activity of 500 S-CEVU/g.
CLAIMS

1. An enzyme preparation comprising a cellulase component having reduced mobility in an aqueous solution or dispersion of cellulosic fibres or fabric.

2. The enzyme preparation according to claim 1, wherein the cellulase component is adsorbed to an insoluble carrier.

3. The enzyme preparation according to claim 2, wherein the cellulase component is bound to the carrier by covalent binding.

4. The enzyme preparation according to claim 2, wherein the cellulase component comprises a cellulose binding domain (CBD) which is adsorbed to the carrier.

5. The enzyme preparation according to claim 2, wherein one or more regions of the cellulase component are adsorbed to the carrier.

6. The enzyme preparation according to claim 5, wherein the adsorption is mainly due to hydrophobic interaction and the carrier has a hydrophobic or amphiphilic surface.

7. The enzyme preparation according to claim 1, wherein the cellulase component comprises a cellulose binding domain (CBD) which is adsorbed to a soluble carrier.

8. The enzyme preparation according to claim 1, wherein the cellulase component is incorporated into a gel.

9. The enzyme preparation according to claim 1, wherein the cellulase component is present as aggregates of two or more cellulase protein molecules or aggregates of at least one cellulase protein molecule and at least one other molecule.

10. The enzyme preparation according to claim 9, wherein the
molecular structure of the cellulase component is modified by physical or chemical treatment or protein engineering, preferably by substituting one or more charged amino acid residues on the surface of the cellulase protein molecule with hydrophobic amino acid residues, or a combination thereof.

11. The enzyme preparation according to claim 10, wherein the physical treatment is a heat treatment.

12. The enzyme preparation according to claim 1, wherein the cellulose-containing aqueous solution is a washing solution comprising cellulose-containing fibres, fabrics, textiles or garments.

13. The enzyme preparation according to claim 12, wherein the cellulase component is autoimmobilized on the surface of the cellulose-containing fibres, fabrics, textiles or garments.

14. The enzyme preparation according to claim 4, wherein the carrier is selected from the group consisting of cellulose-containing materials having a fibrous, microcrystalline or amorphous structure, preferably cellulose-containing particles having a mean particle size from 0.01 μm to 100 μm, more preferably Avicel™, Vivicel™ or Sigmacel™.

15. The enzyme preparation according to claim 5 or 6, wherein the carrier is selected from the group consisting of compounds providing affinity towards binding, such as ion-exchange, hydrophobic, hydrogen-bond, lectin, antibody, metal-chelate or ion-pairing interaction with cellulase.

16. The enzyme preparation according to claim 15, wherein the carrier is selected from the group consisting of bentonite, hectorites, laponite, silica, zeolites, diatomaceous earth, activated charcoal, synthetic resins, lignin and derivatives thereof, and polysaccharides and derivatives thereof.
17. The enzyme preparation according to claim 7, wherein the carrier is selected from the group consisting of soluble polymers, preferably chitosan.

18. The enzyme preparation according to claim 8, wherein the carrier is selected from the group consisting of polysaccharides and derivatives thereof, preferably chitosan, agar, xanthan gum, locust bean gum and any combination thereof.

19. The enzyme preparation according to any of the claims 9-11, wherein the other molecule is selected from the group consisting of proteins and hydrophilic, amphililic, charged and uncharged polymers and oligomers capable of interacting with the cellulase component.

20. The enzyme preparation according to claim 19, wherein the protein is selected from milk whey proteins, soy bean proteins, pea proteins, albumin, potato proteins and gluten proteins.

21. The enzyme preparation according to claim 19, wherein the hydrophilic polymers are selected from polysaccharides, preferably locust bean gum, guar gum, xanthan gum, quince seed gum, gum arabic, karaya gum, tragacanth gum, glucomannan, arabinogalactan, dextran, pullulan, curdlan, alpha- and beta-cyclodextrin, agarose, inulin, pectin, starch, dextrin, hydroxyethyl cellulose, polyvinyl alcohol.

22. The enzyme preparation according to claim 19, wherein the amphiphilic polymers are selected from hydrophobized polysaccharide derivatives, preferably ethyl hydroxyethyl cellulose, methyl hydroxybutyl cellulose, methyl hydroxypropyl cellulose, methyl hydroxyethyl cellulose, steroidal glycosides, glyco- and phospholipids, soluble lignin and its derivatives, alkylsulfates and alkylsulfonates, LAS, alkyltrimethylammonium bromides and block polymers and graft polymers thereof.
23. The enzyme preparation according to claim 19, wherein the charged polymers and oligomers are selected from the group consisting of polypeptides and proteins, preferably from polyaspartic acid, polylysine acid, polyvinylimidazol (PVI), polyethyleneglycol (PEG), polyvinylpyrrolidone (PVP), quaternary poly(2-vinylpyridine), polyacrylic acid anions, polystyrene-sulfonate, variable ionized polyacrylamide, methacrylic acid-methylmethacrylate copolymer, methacrylic acid-methacrylate-methylmethacrylate copolymer, cationic hydroxyethyl cellulose, carboxymethyl hydroxyethyl cellulose, cellulose (ether) esters such as hydroxypropyl methyl cellulose acetate succinate, cellulose acetate phthalate, hydroxypropyl methyl cellulose phthalate, chemically modified soluble chitosan derivatives such as chitosan glutamate, sodium alginate, kappa-, lambda- and iota-carrageenan, hyaluronate, heparan sulphate, chondroitin tetra- and octa-sulphate, polyethylenimine, poly(vinylpyrrolidone/dimethylaminoethyl methacrylate), secondary polyamines such as Amberlite LA-2, and block polymers and graft polymers thereof, preferably PVP in combination with PEG, PVI in combination with PEG, and chitosan in combination with PEG; and charged oligosaccharide derivatives.

24. The enzyme preparation according to any of the claims 1-23, wherein the cellulase component is a microbial cellulase component, preferably a fungal or bacterial cellulase component.

25. The enzyme preparation according to claim 24, wherein the cellulase component is derivable from a strain of Bacillus, Humicola, Fusarium, Myceliophthora, Phanerochaete, Penicillium, Aspergillus, Schizophyllum or Geotrichum.

26. The enzyme preparation according to claim 24 or 25, wherein the cellulase component is an endoglucanase which is immunoreactive with an antibody raised against a highly purified ~43kD endoglucanase derived from Humicola insolens, DSM 1800, or which is a derivative of the ~43kD endoglucanase
exhibiting cellulase activity.

27. The enzyme preparation according to claim 24 or 25, wherein the cellulase component is an endoglucanase which is immunoreactive with an antibody raised against a highly purified ~60kD endoglucanase derived from Bacillus larytus, NCIMB 40250, or which is a derivative of the ~60kD endoglucanase exhibiting cellulase activity.

28. A detergent composition comprising the enzyme preparation according to any of the claims 1-27 and a surfactant.

29. A fabric softening composition comprising the enzyme preparation according to any of the claims 1-27 and a perfume.

30. A method of reducing the tendency to tensile strength loss or pin-holing of cellulosic fabric, the method comprising treating the fabric with the enzyme preparation according to any of the claims 1-27.

31. A method of obtaining a cellulase preparation having reduced tendency to cause tensile strength loss or pin-holing of cellulosic fabric when used for treating such fabric, the method comprising reducing the mobility of the cellulase component by means of immobilization, insolubilization or increasing the molecular weight or apparent size of the cellulase protein molecule.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/42, C11D 3/386, D06M 16/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N, C11D, D06M

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

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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**&** document member of the same patent family

Date of the actual completion of the international search: 25 Sept 1996

Date of mailing of the international search report: 26-09-1996

Name and mailing address of the ISA/Swedish Patent Office:
Box 5055, S-102 42 STOCKHOLM
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Authorized officer:
Yvonne Siösteen
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<td>(Matsumoto, Tadao et al) 10 November 1987 (10.11.87)</td>
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