Title: STABILISATION OF GRANULES COMPRISING ACTIVE COMPOUNDS

Abstract: The present invention relates to a process for preparation of enzyme granules, comprising adding to a granule forming process an aqueous mixture comprising an enzyme and wherein said mixture has a pH of more than 7.
FIELD OF THE INVENTION
The present invention relates to granules comprising an enzyme comprising mixture and wherein said mixture when added to the granule forming process has a pH of more than 7. The present invention further relates to processes for the manufacture of such granules.

BACKGROUND OF THE INVENTION
It is known to the art to incorporate active compounds into dry solid particles or granules and thereby protect the active compound from inactivation and/or protect the environment from the active compound. It is further known to the art to incorporate stabilizers into the active containing particle to protect the active compound against inactivation caused by aggressive materials in the environment.

EP 206,417 discloses enzyme granular compositions comprising an enzyme core and alkaline buffer salt coating.

Measures to prevent the reduction of enzyme activity of enzyme-containing granulated products are disclosed by Japanese Patent Application Laid-Open (kokai) No. 62-79298, which describes an enzyme composition in which the core part containing an enzyme is coated with a protective layer containing an alkaline buffering salt of pH 7-11.

EP 415652 and EP 290223 disclose a bleaching composition comprising coated enzymes wherein the coating may comprise alkaline compounds with a pH above 11 such as sodium silicate.

SUMMARY OF THE INVENTION
One object of the present invention is to provide granules comprising enzymes with increased storage stability.

It has surprisingly been found that it is possible to improve the storage stability of granules comprising enzymes by adjusting the pH of the enzyme mixture added to the granule forming process to more than 7.

The present invention provides thus in a first aspect a method for preparation of an enzyme granule comprising the steps of;

a) preparing an aqueous liquid comprising an enzyme and wherein the aqueous liquid has a pH of more than 7;

b) adding the aqueous liquid of step a) to a granulator apparatus;
c) preparing particles in the granulator apparatus.

The invention further relates to granules obtainable by the above disclosed process, and uses of said granules.

DETAILED DESCRIPTION OF THE INVENTION

5 Definitions

The term "% RH" in the context of the present invention is to be understood as the relative humidity of air. 100% RH is air saturated with water moisture at a fixed temperature and % RH thus reflects the percent moisture saturation of the air.

The term "constant humidity" (in the context of the invention sometimes abbreviated as CH) of a compound or substance is to be understood as the % RH of atmospheric air in equilibrium with a saturated aqueous solution of said compound in contact with the solid phase of said compound, all confined within a closed space at a given temperature. This definition is in accordance with "Handbook of chemistry and physics" CRC Press, Inc., Cleveland, USA, 58th edition, p E46, 1977-1978. Accordingly CH_{20 \degree C} =50% for a compound means that air with a 50% humidity will be in equilibrium with a saturated aqueous solution of the compound at 20\degree C. Accordingly the term constant humidity is a measure of the hygroscopic properties of a compound.

The term "pH" of the enzyme comprising mixture in the context of the present invention is to be understood as the pH of the aqueous enzyme comprising mixture measured at room temperature before adding the enzyme comprising mixture to the granulation process/granulation apparatus.

The term "enzyme comprising mixture" which covers the same as the term "aqueous mixture comprising an enzyme" in the context of the present invention, is to be understood as an aqueous liquid comprising an enzyme. Said mixture may comprise other components than the enzyme.

The "enzyme matrix" in the context of the present invention is to be understood as the matrix comprising the enzyme in the granule. The difference from the "enzyme comprising mixture" is that the "enzyme matrix" is the dry form of the "enzyme comprising mixture" when present in the finished granule, the granule obtained after preparation of the enzyme granule.

30 The term "enzyme concentrate" as used in the context of the present invention is to be understood as a fermentation filtrate which has been processed to increase the concentration of enzyme.
Introduction
The stability of enzymes comprised in granules is influenced by the surrounding environment upon storage, being chemical or physical factors decreasing the stability. Especially humidity is an important factor with regard to stability of granules comprising enzymes; especially high humidity affects the stability negatively.

Generally the pH of enzyme concentrates used in the granulation of enzymes is adjusted to below 6, as it is acknowledged that microbial stability is improved in a slightly acidic environment.

We have surprisingly found it possible to improve the storage stability of enzyme comprising granules significantly by increasing the pH of the aqueous liquid comprising the enzyme which is used in the formulation process of the enzyme granule. It is found that the increasing pH does not harm the enzymes. The residual enzyme active of the granules after storage is significantly improved due to the increase in pH of the liquid comprising the enzyme during formulation of the enzyme granules.

We have further found that by adding an additional moisture barrier such as a salt coating we improve the storage stability even further and achieve even higher residual enzyme activities. The significant improvement is also seen at high relative humidity.

The granule of the invention
The present invention relates to a granule comprising a core and a coating, and wherein said core comprises an enzyme which is added to a granule forming process in form of an aqueous mixture comprising an enzyme and wherein said mixture has a pH of more than 7.

The granule of the invention comprises an enzyme matrix prepared from an aqueous enzyme comprising mixture comprising an enzyme and optionally other granulation materials. The granule may furthermore comprise at least one coating and in particular a salt coating. The enzyme matrix is present in the core of the granule, either applied onto a non-active core particle or as the core itself. In a particular embodiment of the present invention the enzyme layer is applied to a non-active core.
The granule of the invention has an improved storage stability compared to granules prepared with known aqueous mixtures comprising enzymes having a pH of less than 7, such as less than 6.

In a particular embodiment of the present invention the invention relates to a granule comprising a core and a coating, and wherein said core comprises an enzyme which is added to the granulation process in form of an aqueous mixture comprising an enzyme and wherein said enzyme comprising mixture has a pH of more than 7, and wherein the enzyme stability upon storage of the granule is improved compared to an granule prepared with an aqueous mixture comprising an enzyme having a pH of less than 7. In a more particular embodiment the present invention relates to a granule comprising a core and a coating, and wherein said core comprises an enzyme which is added to the granulation process in form of a liquid aqueous mixture comprising an enzyme.

The granule of the invention has an improved residual enzyme activity upon storage measured as an increase of more than 5% after storage at 40°C for 13 weeks in closed jars, such as more than 10%, even more than 20%. In a particular embodiment the residual enzyme stability of the granule is increased more than 50% after storage at 40°C for 13 weeks in closed jars.

In a further embodiment of the present invention the residual enzyme activity of the core particle comprising the enzyme after storage at 40°C for 13 weeks in closed jars is at least 5% such as at least 10%, at least 15%, at least 20%, even at least 30%.

In a particular embodiment of the present invention the residual enzyme activity of the granule of the invention after storage at 40°C for 13 weeks in closed jars is at least 50% such as at least 60%, at least 70%, at least 80%, even at least 90%.

The enzyme comprising mixture

The enzyme comprising mixture, the liquid comprising the enzyme comprises an enzyme and optionally additional granulation materials including a liquid such as water.

In a particular embodiment of the present invention the enzyme comprising mixture is an enzyme concentrate. In another embodiment of the present invention the enzyme comprising mixture is an enzyme concentrate mixed with additional granulation materials.

The enzyme comprising mixture is before the granulation process adjusted to a pH of more than 7, such as more than 8, even more than 9, such as more than 10. In a particular embodiment of the present invention the pH of the enzyme comprising mixture is between 7 and 13. In a more particular embodiment of the present invention the pH of the enzyme comprising mixture is between 8 and 12. In an even further particular embodiment of the present invention the pH of the enzyme comprising mixture is between 9 and 12. In an even more particular em-
bodiment of the present invention the pH of the enzyme comprising mixture is between 9 and 11. In a most particular embodiment of the present invention the pH is between 10 and 11. In a particular embodiment of the present invention the pH of the enzyme comprising mixture is less than 12 such as less than 11.

The alkaline pH of the enzyme comprising mixture may be obtained by adding an alkaline compound to the mixture e.g. sodium hydroxide.

Besides of the enzyme the matrix comprising the enzyme may be constructed in any way or of any material which provides the desired functional properties of the mixture material, e.g. the mixture may consist of materials which allow readily release of the enzyme upon introduction to an aqueous medium. In one preferred embodiment the core particle is constructed of a non-active core with the enzyme comprising mixture absorbed and/or the enzyme comprising mixture applied on the non-active core surface.


Alkaline compounds

Any compound providing a pH above 7 when added to the enzyme comprising mixture may be used to adjust the pH of the enzyme comprising mixture. Suitable compounds may be bases e.g. sodium hydroxide, potassium hydroxide or alkaline buffer salts.

Suitable buffer salts may be potassium bicarbonate, potassium carbonate, tetra potassium pyrophosphate, potassium tripolyphosphate, sodium bicarbonate and sodium carbonate. Other suitable salts may be used.

Enzymes

The enzyme in the context of the present invention may be any enzyme or combination of different enzymes. Accordingly, when reference is made to "an enzyme" this will in general be understood to include one enzyme or a combination of enzymes.

It is to be understood that enzyme variants (produced, for example, by recombinant techniques) are included within the meaning of the term "enzyme". Examples of such enzyme
variants are disclosed, e.g. in EP 251,446 (Genencor), WO 91/00345 (Novo Nordisk), EP 525,610 (Solvay) and WO 94/02618 (Gist-Brocades NV).

Enzymes can be classified on the basis of the handbook Enzyme Nomenclature from NC-IUBMB, 1992), see also the ENZYME site at the internet: http://www.expasy.ch/enzyme/. ENZYME is a repository of information relative to the nomenclature of enzymes. It is primarily based on the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUB-MB), Academic Press, Inc., 1992, and it describes each type of characterized enzyme for which an EC (Enzyme Commission) number has been provided (Bairoch A. The ENZYME database, 2000, Nucleic Acids Res 28:304-305). This IUB-MB Enzyme nomenclature is based on their substrate specificity and occasionally on their molecular mechanism; such a classification does not reflect the structural features of these enzymes.


The types of enzymes which may be incorporated in granules of the invention include oxidoreductases (EC 1.-.-.-), transferases (EC 2.-.-.-), hydrolases (EC 3.-.-.-), lyases (EC 4.-.-.-), isomerases (EC 5.-.-.-) and ligases (EC 6.-.-.-).

The invention has been found particularly useful for oxidoreductases.

Preferred oxidoreductases in the context of the invention are peroxidases (EC 1.11.1), laccases (EC 1.10.3.2) and glucose oxidases (EC 1.1.3.4)). An Example of a commercially available oxidoreductase (EC 1.-.-.-) is Gluzyme™ (enzyme available from Novozymes A/S).

Further oxidoreductases are available from other suppliers. Preferred transferases are transferases in any of the following sub-classes:

a) Transferases transferring one-carbon groups (EC 2.1);
b) transferases transferring aldehyde or ketone residues (EC 2.2); acyltransferases (EC 2.3);
c) glycosyltransferases (EC 2.4);
d) transferases transferring alkyl or aryl groups, other than methyl groups (EC 2.5); and
e) transferases transferring nitrogenous groups (EC 2.6).

A most preferred type of transferase in the context of the invention is a transglutaminase (protein-glutamine γ-glutamyltransferase; EC 2.3.2.13).

Further examples of suitable transglutaminases are described in WO 96/06931 (Novo Nordisk A/S).

The invention has proven particularly useful for hydrolases, such as phytases. Preferred hydrolases in the context of the invention are: carboxylic ester hydrolases (EC 3.1.1.1) such as lipases (EC 3.1.1.3); phytases (EC 3.1.3.2), e.g. 3-phytases (EC 3.1.3.8) and 6-phytases (EC 3.1.3.26); glycosidases (EC 3.2, which fall within a group denoted herein as “carbohydrases”), such as α-amylases (EC 3.2.1.1); peptidases (EC 3.4, also known as proteases); and other carboxyl hydrolases. Examples of commercially available phytases include Bio-Feed™ Phytase (Novozymes), Ronozyme™ P (DSM Nutritional Products), Natuphos™ (BASF), Finase™ (AB Enzymes), and the Phyzyme™ product series (Danisco). Other preferred phytases include those described in WO 98/28408, WO 00/43503, and WO 03/066847.

The invention is found to be particularly useful for carbohydrases, such as amylases. In the present context, the term “carbohydrase” is used to denote not only enzymes capable of breaking down carbohydrate chains (e.g. starches or cellulose) of especially five- and six-membered ring structures (i.e. glycosidases, EC 3.2), but also enzymes capable of isomerizing carbohydrates, e.g. six-membered ring structures such as D-glucose to five-membered ring structures such as D-fructose.

Carbohydrases of relevance include the following (EC numbers in parentheses):

- α-amylases (EC 3.2.1.1), β-amylases (EC 3.2.1.2), glucan 1,4-α-glucosidases (EC 3.2.1.3),
- endo-1,4-beta-glucanase (cellulases, EC 3.2.1.4), endo-1,3(4)-β-glucanases (EC 3.2.1.6),
- endo-1,4-β-xylanases (EC 3.2.1.8), dextranases (EC 3.2.1.11), chitinases (EC 3.2.1.14), poly-
galacturonases (EC 3.2.1.15), lysozymes (EC 3.2.1.17), β-glucosidases (EC 3.2.1.21), α-
galactosidases (EC 3.2.1.22), β-galactosidases (EC 3.2.1.23), amyl-1,6-glucosidases (EC 3.2.1.33),
- xylan 1,4-β-xylolysidases (EC 3.2.1.37), glucan endo-1,3-β-D-glucosidases (EC 3.2.1.39), α-dextrin endo-1,6-α-glucosidases (EC3.2.1.41), sucrose α-glucosidases (EC 3.2.1.48),
- glucan endo-1,3-α-glucosidases (EC 3.2.1.59), glucan 1,4-β-glucosidases (EC 3.2.1.74),
- glucan endo-1,6-β-glucosidases (EC 3.2.1.75), galactanases (EC 3.2.1.89), arabii-
nan endo-1,5-α-L-arabinosidases (EC 3.2.1.99), lactases (EC 3.2.1.108), chitosanases (EC 3.2.1.132) and xylose isomerasases (EC 5.3.1.5).

Examples of commercially available proteases (peptidases) include Kannase™, Everlase™, Esperase™, Alcalase™, Neutrase™, Durazym™, Savinase™, Ovozyme™, Pyrase™, Pancreatic Trypsin NOVO (PTN), Bio-Feed™ Pro and Clear-Lens™ Pro (all available from Novozymes A/S, Bagsvaerd, Denmark). Other preferred proteases include those described in WO 01/58275 and WO 01/58276.

Other commercially available proteases include Ronozyme™ Pro, Maxatase™, Maxacaï™, Maxapem™, Opticlean™, Propease™, Purafect™ and Purafect Ox™ (available from Genencor International Inc., Gist-Brocades, BASF, or DSM Nutritional Products).

Examples of commercially available lipases include Lipex™, Lipoprime™, Lipopan™, Lipolase™, Lipolase™ Ultra, Lipozyme™, Palatase™, Resinase™, Novozym™ 435 and Lecitase™ (all available from Novozymes A/S).

Other commercially available lipases include Lumafast™ (Pseudomonas mendocina lipase from Genencor International Inc.); Lipomax™ (Ps. pseudoalcaligenes lipase from Gist-Brocades/Genencor Int. Inc.; and Bacillus sp. lipase from Solvay enzymes. Further lipases are available from other suppliers.

Examples of commercially available carbohydrases include Alpha-Gal™, Bio-Feed™ Alpha, Bio-Feed™ Beta, Bio-Feed™ Plus, Bio-Feed™ Wheat, Bio-Feed™ Z, Novozyme™ 188, Carezyme™, Celludast™, Cellusoft™, Cellulzyme™, Ceremyl™, Citrozym™, Denimax™, Dezyme™, Dextrozyme™, Duramy™, Energex™, Finizym™, Fungamy™, Gamanase™, Glucanex™, Lactozym™, Liquezyme™, Maltogenase™, Natalase™, Pentopan™, Pectinex™, Promozyme™, Pulpzyme™, Novamyl™, Termamyl™, AMG™ (Amyloglucosidase Novo), Maltogenase™, Sweetzyme™ and Aquazym™ (all available from Novozymes A/S). Further carbohydrases are available from other suppliers, such as the Roxazyme™ and Ronozyme™ product series (DSM Nutritional Products), the Avizyme™, Porzyme™ and Grindzyme™ product series (Danisco, Finnfeeds), and Natugrain™ (BASF), Purastar™ and Purastar™ OxAm (Genencor).

Other commercially available enzymes include Mannaway™, Pectaway™, Stainzyme™ and Renozyme™.

The present invention has in particular proven useful in connection with the formulation of enzymes exhibiting laccase activity.

Compounds exhibiting laccase activity may be any laccase enzyme comprised by the enzyme classification EC 1.10.3.2 as set out by the Nomenclature Committee of the International Union
of Biochemistry and Molecular Biology (IUBMB), or any fragment derived therefrom exhibiting laccase activity, or a compound exhibiting a similar activity, such as a catechol oxidase (EC 1.10.3.1), an o-aminophenol oxidase (EC 1.10.3.4), or a bilirubin oxidase (EC 1.3.3.5). Preferred laccase enzymes and/or compounds exhibiting laccase activity are enzymes of microbial origin. The enzymes may be derived from plants, bacteria or fungi (including filamentous fungi and yeasts).

Suitable examples from fungi include a laccase derivable from a strain of Aspergillus, Neurospora, e.g., N. crassa, Podospora, Botrytis, Collybia, Fomes, Lentinus, Pleurotus, Trametes, e.g., T. villosa and T. versicolor, Rhizoctonia, e.g., R. solani, Coprinus, e.g., C. cinereus, C. comatus, C. friesii, and C. plicatilis, Psathyrella, e.g., P. condelleana, Panaeolus, e.g., P. papilionaceus, Myceliophthora, e.g., M. thermophila, Schytalidium, e.g., S. thermophilum, Polyporus, e.g., P. pinsitus, Phlebia, e.g., P. radita (WO 92/01046), or Coriolus, e.g., C. hirsutus (JP 2-238885).

Suitable examples from bacteria include a laccase derivable from a strain of Bacillus.

In a particular embodiment of the present invention the laccase is derived from Coprinus, Myceliophthora, Polyporus, Schytalidium or Rhizoctonia. In a more particular embodiment the laccase is derived from Coprinus cinereus, Myceliophthora thermophila, Polyporus pinsitus, Schytalidium thermophilum or Rhizoctonia solani.

The laccase or the laccase related enzyme may furthermore be one which is producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said laccase as well as DNA sequences encoding functions permitting the expression of the DNA sequence encoding the laccase, in a culture medium under conditions permitting the expression of the laccase enzyme, and recovering the laccase from the culture.

In a particular embodiment of the present invention the enzyme is selected from the group consisting of laccases derived from Coprinus, Myceliophthora, Polyporus, Schytalidium and Rhizoctonia. In particular derived from Myceliophthora, Polyporus, Schytalidium and Rhizoctonia mentioned in WO 95/33836, WO 96/00290, WO 95/33837 and WO 95/07988 respectively hereby incorporated by reference.

Determination of Laccase Activity (LACU)

Laccase activity (particularly suitable for Polyporus laccases) may be determined from the oxidation of syringaldazine under aerobic conditions. The violet colour produced is photometered at 530 nm. The analytical conditions are 19 mM syringaldazine, 23 mM acetate buffer, pH 5.5, 30°C, 1 min. reaction time.
1 laccase unit (LACU) is the amount of enzyme that catalyses the conversion of 1.0 mmol syringaldazin per minute at these conditions.

**Determination of Laccase Activity (LAMU)**

Laccase activity may be determined from the oxidation of syringaldazin under aerobic conditions. The violet colour produced is measured at 530 nm. The analytical conditions are 19 mM syringaldazin, 23 mM Tris/maleate buffer, pH 7.5, 30°C, 1 min. reaction time.

1 laccase unit (LAMU) is the amount of enzyme that catalyses the conversion of 1.0 mmol syringaldazin per minute at these conditions.

**Inert cores**

Inert core which is the same as non-active cores mean core particles which are substantially enzyme free when added to the process of the invention. Inert cores may be carrier nuclei, placebo nuclei or seeds upon which the mixture comprising the enzyme can be layered. The inert cores may comprise inorganic salts, sugars, sugar alcohols, small organic molecules such as organic acids or salts, minerals such as clays or silicates or a combination of two or more of these.

Enzymes may be absorbed onto/into the inert cores during preparation of the granule.

In a particular embodiment of the present invention the core particle may be prepared by applying the mixture comprising the enzyme onto an inert core.

**Additional granulation materials**

Additional granulation materials to be incorporated in the granule can be binders, polysaccharides, synthetic polymers, waxes, enhancing agents, fillers, fibre materials, enzyme stabilizing agents, solubilising agents, crosslinking agents, suspension agents, viscosity regulating agents, light spheres, chlorine scavengers, plasticizers, pigments, salts, lubricants (such as surfactants or antistatic agents) and fragrances.

The additional granulation materials may be added to the enzyme comprising mixture before adding the mixture to the granule forming process or they may be added separately to the process.

**Binders**

Suitable binders are binders with a high melting point or no melting point at all and of a non waxy nature e.g. polyvinyl pyrrolidin, dextrins, polyvinylalkohol, cellulose derivatives, for example hydroxypropyl cellulose, methyl cellulose or CMC. A suitable binder is a carbohydrate binder such as Dextrin e.g. Glucidex 21D and Avedex W80.
Polysaccharides

The polysaccharides of the present invention may be un-modified naturally occurring polysaccharides or modified naturally occurring polysaccharides.

Suitable polysaccharides include cellulose, pectin, dextrin and starch. The starches may be soluble or insoluble in water.

In a particular embodiment of the present invention the polysaccharide is a starch. In a particular embodiment of the present invention the polysaccharide is an insoluble starch.

Naturally occurring starches from a wide variety of plant sources are suitable in the context of the invention (either as starches per se, or as the starting point for modified starches), and relevant starches include starch from: rice, corn, wheat, potato, oat, cassava, sago-palm, yuca, barley, sweet potato, sorghum, yams, rye, millet, buckwheat, arrowroot, taro, tannia, and may for example be in the form of flour.

Cassava starch is among preferred starches in the context of the invention; in this connection it may be mentioned that cassava and cassava starch are known under various synonyms, including tapioca, manioc, mandioca and manihot.

As employed in the context of the present invention, the term “modified starch” denotes a naturally occurring starch, which has undergone some kind of at least partial chemical modification, enzymatic modification, and/or physical or physicochemical modification, and which - in general - exhibits altered properties relative to the “parent” starch.

Synthetic polymers:

Synthetic polymers may be selected from polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), polyvinyl acetate, polyacrylate, polymethacrylate, poly-acrylamide, polysulfonate, polycarboxylate, and copolymers thereof, in particular water soluble polymers or copolymers.

Waxes

A "wax" in the context of the present invention is to be understood as a polymeric material having a melting point between 25 -150 °C, particularly 30 to100°C more particularly 35 to 85°C most particularly 40 to 75°C. The wax is preferably in a solid state at room temperature, 25°C.

The lower limit is preferred to set a reasonable distance between the temperature at which the wax starts to melt to the temperature at which the granules or compositions comprising the granules are usually stored, 20 to 30°C.

For some granules, e.g. granules used in the detergent industry, a preferable feature of the wax is that the wax should be water soluble or water dispersible, particularly in neutral and alkaline solution, so that when the coated particles of the invention is introduced into an aqueous
solution, i.e. by diluting it with water, the wax should disintegrate and/or dissolve providing a quick release and dissolution of the active incorporated in the particles to the aqueous solution. Examples of water soluble waxes are polyethylene glycols (PEG’s). Amongst water insoluble waxes, which are dispersible in an aqueous solution are triglycerides and oils. For some granules it is preferable that the coating contains some insoluble waxes e.g. feed granules.

The wax composition of the invention may comprise any wax, which is chemically synthesized. It may also equally well comprise waxes isolated from a natural source or a derivative thereof. Accordingly, the wax composition of the invention may comprise waxes selected from the following non limiting list of waxes.

- Poly ethylene glycols, PEG. Different PEG waxes are commercially available having different molecular sizes, wherein PEG’s with low molecular sizes also have low melting points. Examples of suitable PEG’s are PEG 1500, PEG 2000, PEG 3000, PEG 4000, PEG 6000, PEG 8000, PEG 9000 etc. e.g. from BASF (Pluritol E series) or from Clariant or from Ineos. Derivatives of Poly ethylene glycols may also be used.

- Polypropylenes (e.g. polypropylene glycol Pluritol P series from BASF) or polyethylens or mixtures thereof. Derivatives of polypropylenes and polyethylenes may also be used.

- Nonionic surfactants which are solid at room temperature such as ethoxylated fatty alcohols having a high level of ethoxy groups such as the Lutensol AT series from BASF, a C16-C18 fatty alcohol having different amounts of ethyleneoxide per molecule, e.g. Lutensol AT11, AT13, AT25, AT50, AT80, where the number indicate the average number of ethyleneoxide groups. Alternatively polymers of ethyleneoxide, propyleneoxide or copolymers thereof are useful, such as in block polymers, e.g. Pluronic PE 6800 from BASF. Derivatives of ethoxyylated fatty alcohols.

- Waxes isolated from a natural source, such as Carnauba wax (melting point between 80-88°C), Candelilla wax (melting point between 68-70°C) and bees wax. Other natural waxes or derivatives thereof are waxes derived from animals or plants, e.g. of marine origin. Hydrogenated plant oil or animal tallow. Examples of such waxes are hydrogenated ox tallow, hydrogenated palm oil, hydrogenated cotton seeds and/or hydrogenated soy bean oil, wherein the term “hydrogenated” as used herein is to be construed as saturation of unsaturated carbohydrate chains, e.g. in triglycerides, wherein carbon=carbon double bonds are converted to carbon-carbon single bonds. Hydrogenated palm oil is commercially available e.g. from Hobum Oele und Fette GmbH - Germany or Deutche Cargill GmbH - Germany.

- Fatty acid alcohols, such as the linear long chain fatty acid alcohol NAFOL 1822 (C18, 20, 22) from Condea Chemie GMBH - Germany, having a melting point between 55-60°C. Derivatives of fatty acid alcohols.
Mono-glycerides and/or di-glycerides, such as glyceryl stearate, wherein stearate is a mixture of stearic and palmitic acid, are useful waxes. An example of this is Dimodan PM - from Danisco Ingredients, Denmark.

- Fatty acids, such as hydrogenated linear long chained fatty acids and derivatives of fatty acids.
- Paraffines, i.e. solid hydrocarbons.
- Micro-crystalline wax.

In further embodiments waxes which are useful in the invention can be found in C.M. McTaggart et. al., Int. J. Pharm. 19, 139 (1984) or Flanders et. al., Drug Dev. Ind. Pharm. 13, 1001 (1987) both incorporated herein by reference.

In a particular embodiment of the present invention the wax of the present invention is a mixture of two or more different waxes.

In a particular embodiment of the present invention the wax or waxes is selected from the group consisting of PEG, ethoxylated fatty alcohols, fatty acids, fatty acid alcohols and glycerides.

In another particular embodiment of the present invention the waxes are chosen from synthetic waxes. In a more particular embodiment the waxes of the present invention are PEG or non-ionic surfactants. In a most particular embodiment of the present invention the wax is PEG.

Fermentation broth

A fermentation broth in accordance with the invention comprises a biomass e.g. microbial cells and/or cell debris thereof

In a preferred embodiment the fermentation broth comprises at least 10% of the biomass, more preferably at least 50%, even more preferably at least 75% and most preferably at least 90% or at least 95% of the biomass originating from the fermentation. In another preferred embodiment the broth contains 0-31% w/w dry matter, preferably 0-20% w/w, more preferably 0-15% w/w such as 10-15% w/w dry matter, 0% dry matter being excluded from said ranges. The biomass may constitute up to 90% w/w of the dry matter, preferably up to 75% w/w, more preferably up to 50% w/w of the dry matter, while the enzyme may constitute up to 50% w/w of the dry matter, preferably up to 25% w/w, more preferably up to 10% w/w of the dry matter.

The fermentation broth may be purified by filtering such as to obtain a fermentation filtrate.
Enhancing agent

Some enzymes like laccases are often not broad enough in their substrate specificity to be of practical use without introducing also an enhancing agent, which promotes the availability of the substrate e.g. by oxidation of the substrate.

Enhancing agents are particularly useful in combination with laccases for removal of odour e.g. caused by halitosis. In a particular embodiment the granule comprise a laccase and an enhancing agent.

The enhancing agent may be selected from the group consisting of aliphatic, cyclo-aliphatic, heterocyclic or aromatic compounds containing the moiety $>$N-OH. In a preferred embodiment of the invention the enhancing agent is a compound of the general formula I:

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\text{R}^1 \quad \text{R}^2 \quad \text{R}^3 \quad \text{R}^4 \\
\text{[X]} \\
\text{R}^5 \quad \text{R}^6 \\
\text{R}^7 \quad \text{R}^8 \\
\text{R}^9 \quad \text{R}^{10} \\
\text{R}^{11} \quad \text{R}^{12} \\
\end{array}
\]

wherein $R^1$, $R^2$, $R^3$, $R^4$ are individually selected from the group consisting of hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, C$_{1-12}$-alkyl, C$_{1-6}$-alkoxy, carbonyl(C$_{1-12}$-alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonoxy, and salts and esters thereof, wherein the $R^1$, $R^2$, $R^3$, $R^4$ may be substituted with $R^5$, wherein $R^5$ represents hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, C$_{1-12}$-alkyl, C$_{1-6}$-alkoxy, carbonyl(C$_{1-12}$-alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonoxy, and salts and esters thereof; [X] represents a group selected from $(-N=N)$, $(-N=CR^6)_m$, $(-CR^6=N)_m$, $(-CR^7=CR^8)_m$, $(-CR^6=NR^8)_m$, $(-CR^6=N-CHR^8)_m$, $(-CR^6=CHR^7)_m$, $(-CR^6=N-CHR^7)_m$, $(-CR^6=CHR^8)_m$, $(-CR^6=CR^7-CHR^8)_m$, wherein $R^6$, $R^7$, and $R^8$ independently of each other are selected from H, OH, NH$_2$, COOH, SO$_3$H, C$_{1-6}$-alkyl, NO$_2$, CN, Cl, Br, F, CH$_2$OCH$_3$, OCH$_3$, and COOCH$_3$; and m is 1 or 2.

The term “C$_{1-n}$-alkyl” wherein n can be from 2 through 12, as used herein, represent a branched or straight alkyl group having from one to the specified number of carbon atoms. Typical C$_{1-6}$-alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, iso-pentyl, hexyl, iso-hexyl and the like.

In a more preferred embodiment of the invention the enhancing agent is a compound of the general formula II:
wherein $R^1$, $R^2$, $R^3$, $R^4$ are individually selected from the group consisting of hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, $C_{1-12}$-alkyl, $C_{1-6}$-alkoxy, carbonyl($C_{1-12}$-alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonooxy, and salts and esters thereof, wherein the $R^1$, $R^2$, $R^3$, $R^4$ may be substituted with $R^5$, wherein $R^5$ represents hydrogen, halogen, hydroxy, formyl, carboxy and salts and esters thereof, amino, nitro, $C_{1-12}$-alkyl, $C_{1-6}$-alkoxy, carbonyl($C_{1-12}$-alkyl), aryl, in particular phenyl, sulfo, aminosulfonyl, carbamoyl, phosphono, phosphonooxy, and salts and esters thereof. The enhancing agent may also be a salt or an ester of formula I or II.

Further preferred enhancing agents are oxoderivatives and N-hydroxy derivatives of heterocyclic compounds and oximes of oxo- and formyl-derivatives of heterocyclic compounds, said heterocyclic compounds including five-membered nitrogen-containing heterocycles, in particular pyrrol, pyrazole and imidazole and their hydrogenated counterparts (e.g. pyrrolidine) as well as triazoles, such as 1,2,4-triazone; six-membered nitrogen-containing heterocycles, in particular mono-, di- and triazinanes (such as piperidine and piperazine), morpholine and their unsaturated counterparts (e.g. pyridine and pyrimidine); and condensed heterocycles containing the above heterocycles as substructures, e.g. indole, benzothiazole, quinoline and benzazepine.

Examples of preferred enhancing agent from these classes of compounds are pyridine aldoximes; N-hydroxypyrrrolinediones such as N-hydroxysuccinimide and N-hydroxyphthalimide; 3,4-dihydro-3-hydroxybenzo[1,2,3]triazine-4-one; formaldoxime trimer (N,N',N''-trihydroxy-1,3,5-triazinane); and violuric acid (1,3-diazinan-2,4,5,6-tetronic-5-oxime). Still further enhancing agents which may be applied in the invention include oximes of oxo- and formyl-derivatives of aromatic compounds, such as benzoquinone dioxime and salicylaldoxime (2-hydroxybenzaldehyde oxime), and N-hydroxyamides and N-hydroxyanilides, such as N-hydroxyacetanilide.

Preferred enhancing agents are selected from the group consisting of 1-hydroxybenzotriazole; 1-hydroxybenzotriazole hydrate; 1-hydroxybenzotriazole sodium salt; 1-hydroxybenzotriazole potassium salt; 1-hydroxybenzotriazole lithium salt; 1-hydroxybenzotriazole ammonium salt; 1-hydroxybenzotriazole calcium salt; 1-hydroxybenzotriazole magnesium salt; and 1-hydroxybenzotriazole-6-sulphonic acid.
A particularly preferred enhancing agent is 1-hydroxybenzotriazole.

All the specifications of N-hydroxy compounds above are understood to include tautomeric forms such as N-oxides whenever relevant.

Another preferred group of enhancing agents comprises a -CO-NOH- group and has the general formula III:

in which A is:

and B is the same as A; or B is H or C<sub>1-12</sub>-alkyl, said alkyl may contain hydroxy, ester or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carboxylic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, NH<sub>2</sub>, COOH, SO<sub>3</sub>H, C<sub>1-8</sub>-alkyl, acyl, NO<sub>2</sub>, CN, Cl, Br, F, CF<sub>3</sub>, NOH-CO-phenyl, CO-NOH-phenyl, C<sub>1-4</sub>-CO-NOH-A, CO-NOH-A, COR12, phenyl-CO-NOH-A, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8, R9, R10, R11 and R12 are C<sub>1-12</sub>-alkyl or acyl.

R2, R3, R4, R5 and R6 of A are preferably H, OH, NH<sub>2</sub>, COOH, SO<sub>3</sub>H, C<sub>1-3</sub>-alkyl, acyl, NO<sub>2</sub>, CN, Cl, Br, F, CF<sub>3</sub>, NOH-CO-phenyl, CO-NOH-phenyl, COR12, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8 and R9 are C<sub>1-3</sub>-alkyl or acyl, and R10, R11 and R12 are C<sub>1-3</sub>-alkyl; more preferably R2, R3, R4, R5 and R6 of A are H, OH, NH<sub>2</sub>, COOH, SO<sub>3</sub>H, CH<sub>3</sub>, acyl, NO<sub>2</sub>, CN, Cl, Br, F, CF<sub>3</sub>, CO-NOH-phenyl, COCH<sub>3</sub>, OR7, NR8R9, or COOCH<sub>3</sub>, wherein R7, R8 and R9 are CH<sub>3</sub> or COCH<sub>3</sub>; even more preferably R2, R3, R4, R5 and R6 of A are H, OH, COOH, SO<sub>3</sub>H, CH<sub>3</sub>, acyl, NO<sub>2</sub>, CN, Cl, Br, F, CO-NOH-phenyl, OCH<sub>3</sub>, COCH<sub>3</sub>, or COOCH<sub>3</sub>; and in particular R2, R3, R4, R5 and R6 of A are H, OH, COOH, SO<sub>3</sub>H, CH<sub>3</sub>, NO<sub>2</sub>, CN, Cl, Br, CO-NOH-phenyl, or OCH<sub>3</sub>.

R2, R3, R4, R5 and R6 of B are preferably H, OH, NH<sub>2</sub>, COOH, SO<sub>3</sub>H, C<sub>1-3</sub>-alkyl, acyl, NO<sub>2</sub>, CN, Cl, Br, F, CF<sub>3</sub>, NOH-CO-phenyl, CO-NOH-phenyl, COR12, OR7, NR8R9, COOR10, or NOH-CO-R11, wherein R7, R8 and R9 are C<sub>1-3</sub>-alkyl or acyl, and R10, R11 and R12 are C<sub>1-3</sub>-alkyl; more preferably R2, R3, R4, R5 and R6 of B are H, OH, NH<sub>2</sub>, COOH, SO<sub>3</sub>H, CH<sub>3</sub>, acyl,
NO₂, CN, Cl, Br, F, CF₃, CO-NOH-phenyl, COCH₃, OR7, NR8R9, or COOCH₃, wherein R7, R8 and R9 are CH₃ or COCH₃; even more preferably R2, R3, R4, R5 and R6 of B are H, OH, COOH, SO₂H, CH₃, acyl, NO₂, CN, Cl, Br, F, CO-NOH-phenyl, OCH₃, COCH₃, or COOCH₃; and in particular R2, R3, R4, R5 and R6 of B are H, OH, COOH, SO₂H, CH₃, NO₂, CN, Cl, Br, CO-NOH-phenyl, or OCH₃.

B is preferably H or C₁₋₃-alkyl, said alkyl may contain hydroxy, ester or ether groups; preferably said alkyl may contain ester or ether groups; more preferably said alkyl may contain ether groups.

In an embodiment, A and B independently of each other are:

![diagram](image)

R or B is H or C₁₋₃-alkyl, said alkyl may contain hydroxy, ester or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, NH₂, COOH, SO₂H, C₁₋₃-alkyl, acyl, NO₂, CN, Cl, Br, F, CF₃, NOH-CO-phenyl, CO-NOH-phenyl, COR₁₂, OR7, NR8R9, COOR₁₀, or NOH-CO-R₁₁, wherein R7, R8 and R9 are C₁₋₃-alkyl or acyl, and R₁₀, R₁₁ and R₁₂ are C₁₋₃-alkyl.

In another embodiment, A and B independently of each other are:

![diagram](image)

or B is H or C₁₋₃-alkyl, said alkyl may contain hydroxy or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R2, R3, R4, R5 and R6 independently of each other are H, OH, NH₂, COOH, SO₂H, CH₃, acyl, NO₂, CN, Cl, Br, F, CF₃, CO-NOH-phenyl, COCH₃, OR7, NR8R9, or COOCH₃, wherein R7, R8 and R9 are CH₃ or COCH₃.

In another embodiment, A and B independently of each other are:
or B is H or C₁₋₃-alkyl, said alkyl may contain hydroxy or ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R₂, R₃, R₄, R₅ and R₆ independently of each other are H, OH, COOH, SO₂H, CH₃, acyl, NO₂, CN, Cl, Br, F, CO-NOH-phenyl, OCH₃, COCH₃, or COOCH₃.

In another embodiment, A and B independently of each other are:

or B is C₁₋₃-alkyl, said alkyl may contain ether groups (e.g. wherein the ether oxygen is directly attached to A-N(OH)C=O-, thus including N-hydroxy carbamic acid ester derivatives), and R₂, R₃, R₄, R₅ and R₆ independently of each other are H, OH, COOH, SO₂H, CH₃, NO₂, CN, Cl, Br, CO-NOH-phenyl, or OCH₃.

The terms "C₁₋₁₂-alkyl" wherein n can be from 2 through 12, as used herein, represent a branched or straight alkyl group having from one to the specified number of carbon atoms. Typical C₁₋₃-alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, iso-pentyl, hexyl, iso-hexyl and the like.

The term "acyl" as used herein refers to a monovalent substituent comprising a C₁₋₄-alkyl group linked through a carbonyl group; such as e.g. acetyl, propionyl, butyryl, isobutyryl, pivaloyl, valeryl, and the like.

In an embodiment at least one of the substituents R₂, R₃, R₄, R₅ and R₆ of A are H, preferably at least two of the substituents R₂, R₃, R₄, R₅ and R₆ of A are H, more preferably at least three of the substituents R₂, R₃, R₄, R₅ and R₆ of A are H, most preferably at least four of the substituents R₂, R₃, R₄, R₅ and R₆ of A are H, in particular all of R₂, R₃, R₄, R₅ and R₆ of A are H.

In another embodiment at least one of the substituents R₂, R₃, R₄, R₅ and R₆ of B are H, preferably at least two of the substituents R₂, R₃, R₄, R₅ and R₆ of B are H, more preferably at least three of the substituents R₂, R₃, R₄, R₅ and R₆ of B are H, most preferably at least four of the substituents R₂, R₃, R₄, R₅ and R₆ of B are H, in particular all of R₂, R₃, R₄, R₅ and R₆ of B are H.
In particular embodiments according to the invention the enhancing agent is selected from the group consisting of
4-nitrobenzoic acid-N-hydroxyanilide;
4-methoxybenzoic acid-N-hydroxyanilide;
N,N'-dihydroxy-N,N'-diphenylterephthalamide;
decanoic acid-N-hydroxyanilide;
N-hydroxy-4-cyanoacetanilide;
N-hydroxy-4-acetylacetanilide;
N-hydroxy-4-hydroxyacetanilide;
N-hydroxy-3-(N'-hydroxyacetamide)acetanilide;
4-cyanobenzoic acid-N-hydroxyanilide;
N-hydroxy-4-nitroacetanilide;
N-hydroxyacetanilide;
N-hydroxy-N-phenyl-carbamic acid isopropyl ester;
N-hydroxy-N-phenyl-carbamic acid methyl ester;
N-hydroxy-N-phenyl-carbamic acid phenyl ester;
N-hydroxy-N-phenyl-carbamic acid ethyl ester; and
N-hydroxy-N-(4-cyanophenyl)-carbamic acid methyl ester.

Another group of preferred enhancing agents is phenolic compounds (alkylsyringates) of the general formula IV:

\[
\text{\begin{array}{c}
\text{O} \\
\text{B} \\
\text{A} \\
\text{O} \\
\text{C} \\
\text{OH}
\end{array}}
\]

wherein the letter A in said formula denotes be a group such as -D, -CH=CH-D, -CH=CH-
CH=CH-D, -CH=N-D, -N=N-D, or -N=CH-D, in which D is selected from the group consisting of
-CO-E, -SO\textsubscript{2}-E, -N-XY, and -N'-XYZ, in which E may be -H, -OH, -R, or -OR, and X and Y and
Z may be identical or different and selected from -H and -R; R being a C\textsubscript{1}-C\textsubscript{16} alkyl, preferably
a C\textsubscript{1}-C\textsubscript{6} alkyl, which alkyl may be saturated or unsaturated, branched or unbranched and
optionally substituted with a carboxy, sulpho or amino group; and B and C may be the same or
different and selected from C\textsubscript{m}H\textsubscript{2m+1}, where m = 1, 2, 3, 4 or 5.

In the above mentioned general formula IV, A may be placed meta to the hydroxy group
instead of being placed in the para-position as shown.
In particular embodiments of the invention the enhancing agent is selected from the group having the general formula V:

A

\[ \text{OMe} \]

\[ \text{OH} \]

\[ \text{OMe} \]

in which A is a group such as -H, -OH, -CH₃, -OCH₃, -O(CH₂)ₙCH₃, where \( n = 1, 2, 3, 4, 5, 6, 7 \) or 8.

Yet another group of preferred enhancing agents are the compounds as described in general formula VI:

R₂ R₁ R₁₀ R₉

R₃

R₄ R₅ R₆ R₇

R₈

in which general formula A represents a single bond, or one of the following groups: (-CH₂⁻), (-CH=CH⁻), (-NH⁻), (-O⁻), (-C=N-N=N=CH⁻), or (>C=O);
and in which general formula the substituent groups R₁-R₁₁, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, C₁₋₉-alkyl;

which carbamoyl, sulfamoyl, phenyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R₁₂; and which C₁₋₉-alkyl group may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R₁₂;

which substituent group R₁₂ represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, or C₁₋₉-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy or methyl.
and in which general formula R₅ and R₆ may together form a group -B⁻, in which B represents a single bond, one of the following groups (-CH₂⁻), (-CH=CH⁻), (-CH=N⁻); or B represents sulfur, or oxygen.
In particular embodiments of the invention the enhancing agent is selected from the group having the general formula VII:

![Chemical Structure](image)

in which general formula X represents a single bond, oxygen, or sulphur;

and in which general formula the substituent groups R1-R9, which may be identical or different, independently represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, C_{1-8}-alkyl;

which carbamoyl, sulfamoyl, phenyl, and amino groups may furthermore be unsubstituted or substituted once or twice with a substituent group R10; and which C_{1-8}-alkyl group may be saturated or unsaturated, branched or unbranched, and may furthermore be unsubstituted or substituted with one or more substituent groups R10;

which substituent group R10 represents any of the following radicals: hydrogen, halogen, hydroxy, formyl, acetyl, carboxy and esters and salts hereof, carbamoyl, sulfo and esters and salts hereof, sulfamoyl, methoxy, nitro, amino, phenyl, or C_{1-8}-alkyl; which carbamoyl, sulfamoyl, and amino groups may furthermore be unsubstituted or substituted once or twice with hydroxy or methyl.

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**Fillers**

Suitable fillers are water soluble and/or insoluble inorganic salts such as finely ground alkali sulphate, alkali carbonate and/or alkali chloride, clays such as kaolin (e.g. SPESWHITE™, English China Clay), bentonites, talcs, zeolites, chalk, calcium carbonate and/or silicates.

Typical fillers are di-sodium sulphate and calcium-lignosulphonate. Other fillers are silica, gypsum, kaolin, talc, magnesium aluminium silicate and cellulose fibres.

**Fiber materials**

Suitable fiber materials are pure or impure cellulose in fibrous form such as sawdust, pure fibrous cellulose, cotton, or other forms of pure or impure fibrous cellulose. Also, filter aids based on fibrous cellulose can be used. Several brands of cellulose in fibrous form are on the market, e.g.
Cepo™ and Arbocei™ Preferred fibrous cellulose is Arbocei™ BFC200. Also synthetic fibres may be used as described in EP 304331 B1 and typical fibres may be made of polyethylene, polypropylene, polyester, especially nylon, polyvinylformate, poly(meth)acrylic compounds.

Enzyme stabilizing or enzyme protecting agents
Enzyme stabilizing or -protective agents may fall into several categories: alkaline or neutral materials, reducing agents, antioxidants and/or salts of first transition series metal ions. Each of these may be used in conjunction with other protective agents of the same or different categories. Examples of alkaline protective agents are alkali metal silicates, carbonates or bicarbonates which provide a chemical scavenging effect by actively neutralizing e.g. oxidants. Examples of reducing protective agents are salts of sulfite, thiosulfite or thiosulfate, while examples of antioxidants are methionine, butylated hydroxytoluene (BHT) or butylated hydroxyanisol (BHA). Most preferred agents are salts of thiosulfates, e.g. sodium thiosulfate. Also enzyme stabilizers may be borates, borax, formates, di- and tricarboxylic acids and so called reversible enzyme inhibitors such as organic compounds with sulphydryl groups or alkylated or arylated boric acids.

Cross linking agents:
Cross-linking agents such as enzyme-compatible surfactants, e.g. ethoxylated alcohols, especially ones with 10 to 80 ethoxy groups.

Solubilising agents:
The solubility of the granule is especially critical in cases where the coated particle is a component of a detergent formulation. As is known by the person skilled in the art, many agents, through a variety of methods, serve to increase the solubility of formulations, and typical agents known to the art can be found in National Pharmacopoeia's.

Light spheres:
Light spheres are small particles with low true density. Typically, they are hollow spherical particles with air or gas inside. Such materials are usually prepared by expanding a solid material. These light spheres may be inorganic of nature or organic of nature, such as the PM-series (plastic hollow spheres) available from The PQ Corporation. Light spheres can also be prepared from polysaccharides, such as starch or derivatives thereof. Biodac® is an example of non-hollow lightweight material made from cellulose (waste from papermaking), available from GranTek Inc. These materials may be included in the granules of the invention either alone or as a mixture of different light materials.
Suspension agents:
Suspension agents, mediators (for boosting bleach action upon dissolution of the particle in
e.g. a washing application) and/or solvents may be incorporated in the granule.

Viscosity regulating agents:
Viscosity regulating agents may be present in the granule.

Plasticizers:
Plasticizers useful in granules in the context of the present invention include, for example:
polyols such as sugars, sugar alcohols, glycerine, glycerol trimethylol propane, neopentyl gly-
col, triethanolamine, mono-, di- and triethylene glycol or polyethylene glycols (PEGs) having a
molecular weight less than 1000; urea, phthalate esters such as dibutyl or dimethyl phthalate;
thiocyanates, non-ionic surfactants such as ethoxylated alcohols and ethoxylated phosphates
and water.

Pigments:
Suitable pigments include, but are not limited to, finely divided whiteners, such as titanium di-
oxide or kaolin, coloured pigments, water soluble colorants, as well as combinations of one or
more pigments and water soluble colorants.

Salts:
The salt may be an inorganic salt, e.g. salts of sulfate, sulfite, phosphate, phosphonate, nitrate,
chloride or carbonate or salts of simple organic acids (less than 10 carbon atoms e.g. 6 or less
carbon atoms) such as citrate, malonate or acetate. Examples of cations in these salt are alkali
or earth alkali metal ions, although the ammonium ion or metal ions of the first transition series,
such as sodium, potassium, magnesium, calcium, zinc or aluminium. Examples of anions
include chloride, bromide, iodide, sulfate, sulfite, bisulfite, thiosulfate, phosphate, monobasic
phosphate, dibasic phosphate, hypophosphite, dihydrogen pyrophosphate, tetraborate, borate,
carbonate, bicarbonate, metasilicate, citrate, malate, maleate, malonate, succinate, lactate,
formate, acetate, butyrate, propionate, benzoate, tartrate, ascorbate or gluconate. In particular
alkali- or earth alkali metal salts of sulfate, sulfite, phosphate, phosphonate, nitrate, chloride or
carbonate or salts of simple organic acids such as citrate, malonate or acetate may be used.
Specific examples include NaH$_2$PO$_4$, Na$_2$HPO$_4$, Na$_3$PO$_4$, (NH$_4$)$_2$HPO$_4$, K$_2$HPO$_4$, KH$_2$PO$_4$,
Na$_2$SO$_4$, K$_2$SO$_4$, KH$_2$SO$_4$, ZnSO$_4$, MgSO$_4$, CuSO$_4$, Mg(NO$_3$)$_2$, (NH$_4$)$_2$SO$_4$, sodium borate,
magnesium acetate and sodium citrate.
The salt may also be a hydrated salt, i.e. a crystalline salt hydrate with bound water(s) of crystallization, such as described in WO 99/32595. Examples of hydrated salts include magnesium sulfate heptahydrate (MgSO\(_4\)(7H\(_2\)O)), zinc sulfate heptahydrate (ZnSO\(_4\)(7H\(_2\)O)), copper sulfate pentahydrate (CuSO\(_4\)(5H\(_2\)O)), sodium phosphate dibasic heptahydrate (Na\(_2\)HPO\(_4\)(7H\(_2\)O)), magnesium nitrate hexahydrate (Mg(NO\(_3\))\(_2\)(6H\(_2\)O)), sodium borate decahydrate, sodium citrate dihydrate and magnesium acetate tetrahydrate.

Lubricant:
As used in the present context, the term "lubricant" refers to any agent, which reduces surface friction, lubricates the surface of the granule, decreases tendency to build-up of static electricity, and/or reduces friability of the granules. Lubricants can also play a related role in improving the coating process, by reducing the tackiness of binders in the coating. Thus, lubricants can serve as anti-agglomeration agents and wetting agents. Examples of suitable lubricants are lower polyethylene glycols (PEGs), ethoxylated fatty alcohols and mineral oils, plant oils and animal oils. The lubricant is particularly a mineral oil or a nonionic surfactant, and more particularly the lubricant is not miscible with the other coating materials.

Salt coating
Enzyme granules are very sensitive to high relative humidity and the granules need further improved means to obtain an acceptable storage stability under such conditions. We have found that we are able of further improving the enzyme stability especially under high humidity by coating the granules with a salt coating. In particular the salt coatings described in WO 00/01793 are found to be useful as a coating in the present invention.

Suitable salts of the present invention are described above in the section additional granulation materials.

Particular suitable salts of the invention are Na\(_2\)HPO\(_4\) (CH\(_{20^\circ\text{C}}\)=95%), Na\(_3\)PO\(_4\) (CH\(_{20^\circ\text{C}}\)=92%), (NH\(_4\))H\(_2\)PO\(_4\) (CH\(_{20^\circ\text{C}}\)=93.1%), KH\(_2\)PO\(_4\) (CH\(_{20^\circ\text{C}}\)=92%), Na\(_2\)SO\(_4\)(CH\(_{20^\circ\text{C}}\)=93%), K\(_2\)SO\(_4\)(CH\(_{25^\circ\text{C}}\)=99%), KH\(_2\)SO\(_4\) (CH\(_{20^\circ\text{C}}\)=86%), ZnSO\(_4\) (CH\(_{20^\circ\text{C}}\)=90%) and sodium citrate (CH\(_{25^\circ\text{C}}\)=86%). In a particular embodiment of the present invention the salt is selected from the group consisting of Na\(_2\)HPO\(_4\), Na\(_3\)PO\(_4\), (NH\(_4\))H\(_2\)PO\(_4\), KH\(_2\)PO\(_4\), Na\(_2\)SO\(_4\), K\(_2\)SO\(_4\), KH\(_2\)SO\(_4\), ZnSO\(_4\) and sodium citrate.

The coating comprises at least 60% w/w, e.g. 65% w/w or 70% w/w of the salt, which preferably may be at least 75% w/w, e.g. at least 80% w/w, at least 85% w/w, e.g. at least 90% w/w or at least 95% w/w.

In a particular embodiment of the present invention the salt coating comprises more than 50% salt, such as more than 70 % salt, such as more than 90% salt, such as 100% salt.
In a particular embodiment of the salt coating was applied in an amount of more than 50% w/w of the enzyme core, such as more than 100% w/w of the enzyme core, even such as more than 200% w/w of the enzyme core.

**Additional coatings**

5 The granules of the present invention may comprise one, two or more additional coating layers.

In a particular embodiment of the present invention the granule comprise at least two coating layers.

Additional coatings may be applied to the granule to provide additional characteristics or properties. Thus, for example, an additional coating may achieve one or more of the following effects:

(i) reduction of the dust-formation tendency of a granule;
(ii) protection of the active compound in the granule against hostile compounds in the surroundings.

15 (iii) dissolution at a desired rate upon introduction of the granule into a liquid medium (such as an acid medium);
(iv) provide a better physical strength of the granule.


In a particular embodiment of the present invention the additional coating is a wax coating, according to US 4,106,991 or EP 0,569,468 which is hereby incorporated by reference. For suitable waxes see the section "Waxes" above. In a particular embodiment of the present invention an additional coating may comprise PEG and/or palm oil.

**Additional Coating materials:**

The coating may comprise additional coating materials such as binders, fillers, fibre materials, enzyme stabilizing agents, solubilising agents, suspension agents, viscosity regulating agents, light spheres, plasticizers, salts, lubricants and fragrances as mentioned in the section "additional granulation materials" above. Further coating ingredients may be pigments.
Preparation of the granules

The present invention also relates to the manufacturing of the granules of the invention. A particular embodiment of the present invention is a method for preparing enzyme granules comprising the steps of:

a) preparing an enzyme comprising aqueous mixture having a pH of more than 7;

b) adding the mixture of step a) to a process for forming particles so as to provide core particles comprising the enzyme;

c) coating the core particle of step b) with at least one coating.

Step c) of the above method is in a particular embodiment performed in a fluid bed apparatus. In a more particular embodiment of the present invention the enzyme comprising mixture in step c) is applied onto an inert core particle.

Another particular embodiment of the present invention includes the method for preparing an enzyme granule comprising the steps of:

a) preparing an aqueous liquid comprising an enzyme, wherein the aqueous liquid has a pH of more than 7;

b) adding the aqueous liquid of step a) to a granulator apparatus;

c) preparing particles in the granulator apparatus.

In a further embodiment the enzyme comprising mixture is adjusted to a pH of more than 8. In an even further embodiment the enzyme comprising mixture is adjusted to a pH of between 9 and 11.

In a particular embodiment of the present invention an aqueous liquid comprising an enzyme is prepared and added to a granulator apparatus and the pH of the aqueous liquid is then adjusted to a pH of more than 7 in the granulator apparatus.

In a particular embodiment of the present invention the particles comprising an enzyme prepared in the granulator apparatus is coated with a coating layer.
In a particular embodiment the coating is a salt coating. In a further embodiment the salt in the salt coating has a constant humidity at 20°C of more than 60%. Even further the salt in the salt coating is selected from the group consisting of NaH₂PO₄, Na₂HPO₄, Na₃PO₄, (NH₄)H₂PO₄, K₂HPO₄, KH₂PO₄, Na₂SO₄, K₂SO₄, KH₂SO₄, ZnSO₄, MgSO₄, CuSO₄, Mg(NO₃)₂, (NH₄)₂SO₄, NaCl, sodium borate, magnesium acetate and sodium citrate.

In a particular embodiment the enzyme is selected from the group consisting of oxidoreductases, cabohydrase and hydrolases. Further the oxidoreductase is a laccase. Even further the laccase is selected from the group derived from Coprinus, Myceliophthora, Polyporus, Scytalidium and Rhizoctoni.

In a particular embodiment of the present invention the preparation of the enzyme granule is taking place in a granulator apparatus. A granulator apparatus is an apparatus wherein any kind of particle comprising an enzyme may be formed.

In a particular embodiment of the present invention the granulator apparatus is selected from the group consisting of fluid bed apparatuses, spray drying apparatuses, fluid bed spray drying apparatuses, extruders, roller compactors and mixer apparatuses.

The enzyme granule may comprise a core and a coating wherein the core comprises the enzyme matrix. In a particular embodiment the core particle is prepared by applying the enzyme comprising mixture onto an inert core particle.

Methods for preparing the core and coating can be found in Handbook of Powder Technology; Particle size enlargement by C. E. Capes; Volume 1; 1980; Elsevier. Preparation methods include known feed and granule formulation technologies, i.e.:

a) Spray dried products, wherein a liquid enzyme-containing solution is atomized in a spray drying tower to form small droplets which during their way down the drying tower dry to form an enzyme-containing particulate material. Very small particles can be produced this way (Michael S. Showell (editor); Powdered detergents; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker).

b) Layered products, wherein the enzyme comprising mixture is coated as a layer around a pre-formed non-active core particle, wherein the enzyme comprising mixture is atomized, typically in a fluid bed apparatus wherein the pre-formed core particles are fluidized, and the enzyme comprising mixture adheres to the core particles and dries up to leave a layer of dry enzyme layer on the surface of the core particle. Particles of a desired size can be obtained this way if a useful core particle of the desired size can be found. This type of product is described in e.g. WO 97/23606
c) Absorbed core particles, wherein rather than coating the enzyme comprising mixture as a layer around the core, the enzyme comprising mixture is absorbed onto and/or into the surface of the core. Such a process is described in WO 97/39116.

d) Extrusion or pelletized products, wherein an enzyme-containing paste is pressed to pellets or under pressure is extruded through a small opening and cut into particles which are subsequently dried. Such particles usually have a considerable size because of the material in which the extrusion opening is made (usually a plate with bore holes) sets a limit on the allowable pressure drop over the extrusion opening. Also, very high extrusion pressures when using a small opening increase heat generation in the active compound paste, which is harmful to the active compound. (Michael S. Showell (editor); *Powdered detergents*; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker)

e) Drilled products, wherein an enzyme powder is suspended in molten wax and the suspension is sprayed, e.g. through a rotating disk atomiser, into a cooling chamber where the droplets quickly solidify (Michael S. Showell (editor); *Powdered detergents*; Surfactant Science Series; 1998; vol. 71; page 140-142; Marcel Dekker). The product obtained is one wherein the active compound is uniformly distributed throughout an inert material instead of being concentrated on its surface. Also US 4,016,040 and US 4,713,245 are documents relating to this technique.

f) Mixer granulation products, wherein an enzyme liquid is added to a dry powder composition of conventional granulating components. The liquid and the powder in a suitable proportion are mixed and as the moisture of the liquid is absorbed in the dry powder, the components of the dry powder will start to adhere and agglomerate and particles will build up, forming granulates comprising the active compound. Such a process is described in US 4,106,991 (NOVO NORDISK) and related documents EP 170360 B1 (NOVO NORDISK), EP 304332 B1 (NOVO NORDISK), EP 304331 (NOVO NORDISK), WO 90/09440 (NOVO NORDISK) and WO 90/09428 (NOVO NORDISK). In a particular product of this process wherein various high-shear mixers can be used as granulators, granulates consisting of enzyme as active compound, fillers and binders etc. are mixed with cellulose fibres to reinforce the particles to give the so-called T-granulate. Reinforced particles, being more robust, release less enzymatic dust.

g) Size reduction, wherein the cores are produced by milling or crushing of larger particles, pellets, tablets, briquettes etc. containing the active material. The wanted core particle fraction is obtained by sieving the milled or crushed product. Over and undersized particles can be re-
cycled. Size reduction is described in (Martin Rhodes (editor); Principles of Powder Technology; 1990; Chapter 10; John Wiley & Sons).

h) Fluid bed granulation. Fluid bed granulation involves suspending particulates in an air stream and spraying a enzyme liquid onto the fluidized particles via nozzles. Particles hit by spray droplets get wetted and become tacky. The tacky particles collide with other particles and adhere to them and form a granule.

i) The cores may be subjected to drying, such as in a fluid bed drier. Other known methods for drying granules in the enzyme industry can be used by the skilled person. The drying preferably takes place at a product temperature of from 25 to 90°C. For some enzymes it is important the cores comprising the enzymes contain a low amount of water before coating with the salt. If water sensitive active compounds are coated with a salt before excessive water is removed, it will be trapped within the core and it may affect the activity of the enzyme negatively. After drying, the cores preferably contain 0.1-10 % w/w water.

Preparation of the salt coating
The salt coating may be applied onto the core granule comprising the enzyme by atomization onto the core granules in a fluid bed, the salt coating may further be applied in vacuum mixers, dragée type coaters (pan-drum coaters), equipment for coating of seeds, equipment comprising rotating bottoms (eks. Roto Glatt, CF granulators (Freund), torbed processors (Gauda) or in rotating fluid bed processors such as Omnitex (Nara).

After applying the salt layer the granule may optionally be dried. The drying of the salt coated granule can be achieved by any drying method available to the skilled person, such as spray-drying, freeze drying, vacuum drying, fluid bed drying, pan drum coating and microwave drying. Drying of the salt coated granule can also be combined with granulation methods which comprise e.g. the use of a fluid bed, a fluid bed spray dryer (FSD) or a Multi-stage dryer (MSD).

Preparation of additional coating
Conventional coatings and methods for applying coatings as known to the art may suitably be used, such as the coatings described in Danish PA 2002 00473, WO 89/08694, WO 89/08695, 270 608 B1 and/or WO 00/01793. Other examples of conventional coating materials may be found in US 4,106,991, EP 170360, EP 304332, EP 304331, EP 458849, EP 458845, WO 97/39116, WO 92/12645A, WO 89/08695, WO 89/08694, WO 87/07292, WO 91/06638, WO
The coating may be prepared by the same methods as mentioned above. The granules obtained can be subjected to rounding off (e.g. spheronisation), such as in a Marumeriser™, or compaction.

Compositions comprising the granules of the invention

The invention also relates to compositions comprising the granules of the invention. The composition may be any composition, but particularly suitable compositions are personal care compositions, cleaning compositions, textile processing compositions e.g. bleaching, pharmaceutical compositions, leather processing compositions, pulp or paper processing compositions, food and beverage compositions and animal feed compositions.

Cleaning compositions includes detergents and anti-microbial compositions. Textile processing compositions includes compositions for enzymatic bleach and/or stone washing of textiles, such as denim. Food and beverage compositions includes enzymatic compositions used in industries producing wine, oils and fats, citrus and juice products, starch and sugar products, alcohols and/or brewed products, soy products and baking flour or dough. The present invention is particularly useful incorporated in cat litter compositions, in diapers, in mouth wash compositions, in chewing gum and chewing mints for removal of odor. The present invention is particularly useful for removal of odor caused by halitosis.

The present invention also encompasses the use of the granules of the invention for odor removal, textile treatment, leather treatment, pulp treatment, paper treatment, food, beverage, hard surfaces and the human or animal body. The granules may also be used in the manufacture of a medicament for treatment of the human or animal body. In a particular embodiment of the present invention the granule or composition of the present invention is used for removal of odor from liquid manure.

The present invention further relates to a method for removal of odour comprising brushing teeth with a composition comprising the granules of the invention. It also relates to a method for removal of odour comprising adding a composition comprising the granules of the invention to a cat tray. Further it relates to a method for removal of odour comprising changing a person with a diaper comprising the granules of the invention. In a particular embodiment it relates to a method for removal of odour from the mouth comprising putting a mint, a chewing gum or mouth wash into the mouth.
EXAMPLES

Example 1

A *Myceliophthora* Laccase granulate was produced in the following manner. 0.525 kg of a liquid concentrate with 20% dry matter and pH 5.5, with an activity of 5275 LAMU/g was sprayed onto 3.5 kg of sodium chloride cores (mean size 315 μm and span 0.94) using a fluid bed type Hüttlin HKC-5-TJ for layering the enzyme onto the cores and for drying. Parameters for the Hüttlin bed were

- Air inlet temperature: 70°C
- Product temperature: 56°C
- Air volume: 450 m³/h
- Atomizing air: 1.5 bar
- Micro clima air: 0.65 bar

A granulate with activity 777 LAMU/g was obtained. Without supplementary coating was the storage stability of the granulate tested per se in closed jars at 25°C up till 26 weeks at 40°C up till 13 weeks and in opens jars exposed to 40°C and 60% relative humidity for 4 weeks. The results from the storage test are listed in table I.

Table I

<table>
<thead>
<tr>
<th></th>
<th>% residual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>25°C closed jars</td>
<td></td>
</tr>
<tr>
<td>40°C closed jars</td>
<td></td>
</tr>
<tr>
<td>40°C /60% RH</td>
<td></td>
</tr>
</tbody>
</table>

Example 2

A series of granulates were made as described in example 1 with the exception that the concentrate was pH adjusted ranging from pH 7 to pH 10 with a 1N NaOH before spraying. The granulates were tested for storage stability as described in example 1 the closed jars results from example 2 listed in table II A-B showing that a significant improvement of the storage stability in closed jars has been reached adjusting the concentrate pH up till 10. At humid conditions 40°C/60% RH all residual activities are below 1% showing that a further barrier for humidity is needed.
Table II A:

<table>
<thead>
<tr>
<th>pH</th>
<th>% residual activity 25°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 weeks</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>23</td>
</tr>
<tr>
<td>pH 7</td>
<td>50</td>
</tr>
<tr>
<td>pH 8</td>
<td>73</td>
</tr>
<tr>
<td>pH 9</td>
<td>74</td>
</tr>
<tr>
<td>pH 10</td>
<td>81</td>
</tr>
</tbody>
</table>

Table II B:

<table>
<thead>
<tr>
<th>pH</th>
<th>% residual activity 40°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>pH 5.5</td>
<td>8</td>
</tr>
<tr>
<td>pH 7</td>
<td>26</td>
</tr>
<tr>
<td>pH 8</td>
<td>29</td>
</tr>
<tr>
<td>pH 9</td>
<td>33</td>
</tr>
<tr>
<td>pH 10</td>
<td>76</td>
</tr>
</tbody>
</table>

Example 3
A series of granulates were made as described in example 1 with the exception that the concentrate was pH adjusted pH 10, pH 10.5 and pH 11 before spraying and that enzyme and eventually salt layer were applied using a GEA Precision Coater fluid bed. The granulate produced from the pH 10.5 concentrate was further applied with a 50% coating (calculated as % of the enzyme loaded core) of sodium chloride. This coating was applied from a 24% water based solution of the salt. The granulates were tested for storage stability as described in example 1 the closed jars results from example 3 listed in table III A-C showing that both temperature and humidity stability have improved at higher pH and with a supplementary salt coating.
Table III A:

<table>
<thead>
<tr>
<th></th>
<th>% residual activity 25°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 weeks</td>
</tr>
<tr>
<td>pH 10</td>
<td>88</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>94</td>
</tr>
<tr>
<td>pH 11</td>
<td>93</td>
</tr>
<tr>
<td>pH 10.5 50% NaCl coating</td>
<td>101</td>
</tr>
</tbody>
</table>

Table III B:

<table>
<thead>
<tr>
<th></th>
<th>% residual activity 40°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>pH 10</td>
<td>82</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>82</td>
</tr>
<tr>
<td>pH 11</td>
<td>83</td>
</tr>
<tr>
<td>pH 10.5 50% NaCl coating</td>
<td>89</td>
</tr>
</tbody>
</table>

Table III C:

<table>
<thead>
<tr>
<th></th>
<th>% residual 40°C/60% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>pH 10</td>
<td>&lt;1</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>pH 11</td>
<td>2</td>
</tr>
<tr>
<td>pH 10.5 plus 50% NaCl coating</td>
<td>82</td>
</tr>
</tbody>
</table>

Example 4

A series of Laccase loaded cores with the concentrate adjusted to pH 10.5 before spraying were made as described in example 1 and further coated with salt as described in example 3 with the exception that the core was sodium sulphate and the coating as well was sodium sul-
phate. The coating was applied in an amount of 50%, 100% and 200% of the enzyme loaded core. The water based coating solution was 26% regarding sodium sulphate and 1.3% regarding dextrin Avedex W80. The temperature of the coating solution was kept between 40-45°C to avoid the formation of Glauber salt. Stability data for this series are listed in table IV A-C.

Table IV A:

<table>
<thead>
<tr>
<th>Coating</th>
<th>% residual activity 25°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 weeks 26 weeks</td>
</tr>
<tr>
<td>50% Sodium Sulphate</td>
<td>97 91</td>
</tr>
<tr>
<td>100% Sodium Sulphate</td>
<td>99 86</td>
</tr>
<tr>
<td>200% Sodium Sulphate</td>
<td>91 89</td>
</tr>
</tbody>
</table>

Table IV B:

<table>
<thead>
<tr>
<th>Coating</th>
<th>% residual activity 40°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks 8 weeks 13 weeks</td>
</tr>
<tr>
<td>50% Sodium Sulphate</td>
<td>96 76 73</td>
</tr>
<tr>
<td>100% Sodium Sulphate</td>
<td>83 76 75</td>
</tr>
<tr>
<td>200% Sodium Sulphate</td>
<td>89 68 69</td>
</tr>
</tbody>
</table>

Table IV C:

<table>
<thead>
<tr>
<th>Coating</th>
<th>% residual 40°C/60% RH Water up take after 8 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks 8 weeks</td>
</tr>
<tr>
<td>50% Sodium Sulphate</td>
<td>96 91 0.076%</td>
</tr>
<tr>
<td>100% Sodium Sulphate</td>
<td>91 90 0.101%</td>
</tr>
<tr>
<td>200% Sodium Sulphate</td>
<td>82 85 0.087%</td>
</tr>
</tbody>
</table>

Example 5

A Laccase granulate was produced as following described.

3.5 kg of a liquid Laccase concentrate with an activity of 5630 LAMU/g and a pH of 5.5 was sprayed and absorbed onto 15 kg of Cassava cores (particle size 600-700μm) in a 50 liter Lödige mixer as described in (Cassava patent). The wet enzyme loaded granulate was after the absorption dried in a conventional fluid bed.

2 kg of the dry enzyme loaded was following coated in a 5 liter Lödige mixer with a) 80 g PEG 4000 and b) 240 g Kaolin in a manner as described in US 4,106,991.
The storage stability of the coated granulate was tested per se in closed jars at 25°C up till 26 weeks at 40°C up till 13 weeks and in opens jars exposed to 40°C and 60% relative humidity for 4 weeks. The results from the storage test are listed in table V.

Table V

<table>
<thead>
<tr>
<th></th>
<th>% residual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>25°C closed jars</td>
<td></td>
</tr>
<tr>
<td>40°C closed jars</td>
<td>48</td>
</tr>
<tr>
<td>40°C /60% RH</td>
<td>4</td>
</tr>
</tbody>
</table>

Example 6

A series of granulates were made as described in example 5 with the exception that the concentrate was pH adjusted ranging from pH 7 to pH 10 with a 1N NaOH absorption and 2.0 kg of Cassava cores were sprayed with 0.60 kg of adjusted Laccase concentrate in a 5 litre Lödig mixer. A kaolin/PEG4000 coating was applied as described in example 5. The granulates were tested for storage stability as described in example 1 the closed jars results from example 5 listed in table VI A-B showing that a significant improvement of the storage stability has been reached adjusting the concentrate pH to 10. At humid conditions 40°C/60% RH all residual activities are below 1% to slightly above showing that a further barrier for humidity is needed.

Table VI A:

<table>
<thead>
<tr>
<th></th>
<th>% residual activity 25°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13 weeks</td>
</tr>
<tr>
<td>pH 7</td>
<td>62</td>
</tr>
<tr>
<td>pH 8</td>
<td>70</td>
</tr>
<tr>
<td>pH 9</td>
<td>76</td>
</tr>
<tr>
<td>pH 10</td>
<td>83</td>
</tr>
</tbody>
</table>

Table VI B:

<table>
<thead>
<tr>
<th></th>
<th>% residual activity 40°C closed jars</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>pH 7</td>
<td>58</td>
</tr>
</tbody>
</table>
Example 7

A series of Laccase granulates were made as described in example 5 with the exception that the concentrate was pH adjusted pH 10, pH 10.5 and pH 11 before absorption. The enzyme loaded core produced from the pH 10.5 concentrate was applied with a 50% and a 100% coating (calculated as % of the enzyme loaded core) of sodium sulphate alternatively to the kaolin/PEG coating. The coating was applied from a sodium sulphate solution as described in example 4 using the Hüttlin fluid bed for the application as with parameters 3 kg of enzyme loaded cores air inlet temperature 80°C, product temperature 50°C and air volume 500 m³/h. The granulates were tested for storage stability as described in example 1 the closed jars results from example 7 listed in table VII A-C showing that both temperature and humidity stability have improved at higher pH and with a supplementary salt coating.

<table>
<thead>
<tr>
<th>pH</th>
<th>13 weeks</th>
<th>26 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 10</td>
<td>90</td>
<td>86</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>96</td>
<td>83</td>
</tr>
<tr>
<td>pH 11</td>
<td>84</td>
<td>77</td>
</tr>
<tr>
<td>pH 10.5 50% Na₂SO₄ coating</td>
<td>90</td>
<td>77</td>
</tr>
<tr>
<td>pH 10.5 100% Na₂SO₄ coating</td>
<td>104</td>
<td>101</td>
</tr>
</tbody>
</table>

Table VII B:

<table>
<thead>
<tr>
<th>pH</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>13 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 10</td>
<td>83</td>
<td>81</td>
<td>86</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>79</td>
<td>63</td>
<td>51</td>
</tr>
<tr>
<td>pH 11</td>
<td>83</td>
<td>59</td>
<td>43</td>
</tr>
<tr>
<td>pH 10.5 50% Na₂SO₄ coating</td>
<td>82</td>
<td>61</td>
<td>53</td>
</tr>
<tr>
<td>pH 10.5 100% Na₂SO₄ coating</td>
<td>101</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>
Table VII C:

<table>
<thead>
<tr>
<th></th>
<th>% residual activity 40°C/60% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>pH 10</td>
<td>23</td>
</tr>
<tr>
<td>pH 10.5</td>
<td>43</td>
</tr>
<tr>
<td>pH 11</td>
<td>42</td>
</tr>
<tr>
<td>pH 10.5 plus 50% Na₂SO₄ coating</td>
<td>41</td>
</tr>
<tr>
<td>pH 10.5 plus 100% Na₂SO₄ coating</td>
<td>66</td>
</tr>
</tbody>
</table>
CLAIMS

1. A method for preparing an enzyme granule comprising the steps of;
   
a) preparing an aqueous liquid comprising an enzyme and wherein the aqueous liquid has a pH of more than 7;

b) adding the aqueous liquid of step a) to a granulator apparatus;

c) preparing particles in the granulator apparatus.

2. The method according to claim 1, further comprising the step of coating the particles with a coating layer.

3. The method according to claim 1, wherein the pH is more than 8.

4. The method according to claim 1, wherein the pH is between 9 and 12.

5. The method according to claim 2, wherein the coating layer comprises a salt.

6. The method according to claim 5, wherein the salt in the coating has a constant humidity at 20°C of more than 60%.

7. The method according to claim 5, wherein the salt in the coating is selected from the group consisting of NaH₂PO₄, Na₂HPO₄, Na₃PO₄, (NH₄)H₂PO₄, K₂HPO₄, KH₂PO₄, Na₂SO₄, K₂SO₄, KHSO₄, ZnSO₄, MgSO₄, CuSO₄, Mg(NO₃)₂, (NH₄)₂SO₄, NaCl, sodium borate, magnesium acetate and sodium citrate.

8. The method according to claim 1, wherein the enzyme is selected from the group consisting of oxidoreductases, carbohydrases and hydrolases.

9. The method according to claim 8, wherein the oxidoreductase is a laccase.
10. The method according to claim 9, wherein the laccase is selected from the group derived from *Coprinus, Myceliophthora, Polyporus, Scytalidium* and *Rhizoctoni*.

11. The method of claim 1, wherein the aqueous liquid further comprises an enhancing agent.

12. The method of claim 1, wherein the aqueous liquid is an enzyme concentrate.

13. The method of claim 1, further comprising the step of adding additional granulation materials to the granulator apparatus.

14. The method according to claim 1, wherein the aqueous liquid is applied onto an inert core particle in step b).

15. The method according to claim 1, wherein the granulator apparatus in step a) is selected from the group consisting of fluid bed apparatuses, spray drying apparatuses and mixer granulators.

16. A granule obtainable by the process of claims 1 to 15.

17. The granule of claim 16, wherein the enzyme is a laccase and wherein the granule further comprises an enhancing agent.

18. The granule of claim 16, wherein the granule comprises an inert core particle.

19. The granule of claim 16, wherein granule comprises additional granulation materials.

20. A composition comprising the granule according to any of claims 16 to 19.

21. The composition of claim 20, wherein said composition is a chewing gum.

22. The composition of claim 20, wherein said composition is a chewing mint.

23. The composition of claim 20, wherein said composition is a mouth wash composition.

24. The composition of claim 20, wherein said composition is a tooth paste.
25. The composition of claim 20, wherein said composition is a cat litter composition.

26. The composition of claim 20, wherein said composition is a diaper.

27. Use of the granule according to claim 16 or the composition of claim 20, for removal of odour.

28. Use of the granule according to claim 16 or the composition of claim 20, for removal of odour from liquid manure.